# Session T5-K: Theme-5 Keynote Lectures

| Abstract Number | Paper Title & Authors | Included |
|---|---|---|
| 4072 | Modeling and Simulation of Cellular Networks: Different Approaches for Different Problems<br>W Wiechert | Yes |
| 4087 | Biocatalytic synthesis of a biodegradable chelant (S,S-EDDS)<br>J M Woodley, H E M Law | Yes |

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European Congress of Chemical Engineering (ECCE-6)
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# Modeling and simulation of cellular networks: Different approaches for different problems

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## 1. Summary

Systems Biology and Metabolic Engineering are concerned with the unravelling and understanding of the structure and regulation of cellular networks. Modeling and simulation play an important role in these disciplines because they provide predictive tools for an integrative and comprehensive description of the biological system. However, different methods and tools are required for different problems.
Some currently proposed approaches for modelling and simulation of cellular networks are surveyed. It is discussed which approach might be the appropriate depending on the focus of interest, system characteristics, and available data. This results in a variety of mathematical formalisms, methods and tools ranging from particle simulation, over space/time continuous models, to steady state analysis.

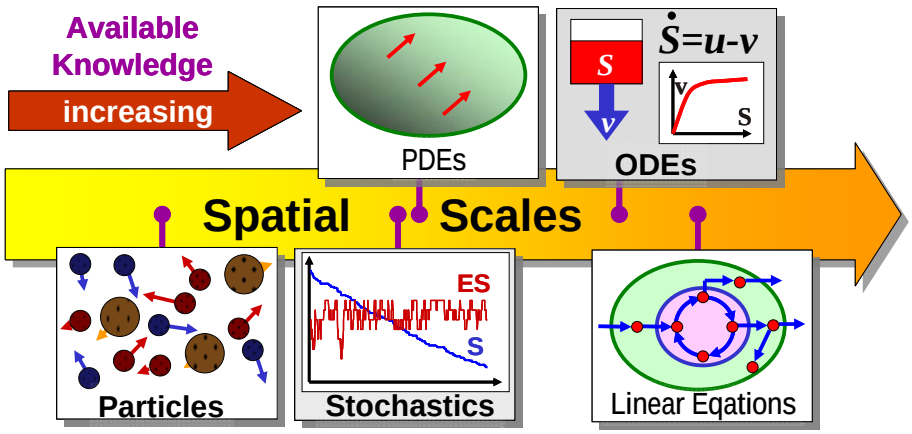


Figure 1: Spatial scales on which different intracellular phenomena are investigated.

## 2. From molecular interactions to whole cells

The function of living cells is determined by complex biochemical networks consisting of many different chemical species interacting in a small and structured spatial domain. This distinguishes the intracellular environment from a classical “well stirred” chemical reaction system. Various approaches for modelling and simulation

of cellular networks have been developed to deal with this new order of complexity. They can be categorized by a walk trough the spatial resolution scales of a living cell:

1. On the smallest length scale the interaction of molecules is governed by molecular motion which can be adequately described by stochastic many particle systems in which molecular collisions cause chemical reactions. Two basic approaches are currently used: Brownian dynamics and cellular automata. Using these methods the effect of spatial substructures on particle diffusion and reaction kinetics can be investigated, although with a high computational effort.
2. In the case of genetic processes, the number of involved molecules can be very low. Cellular behaviour then becomes dominated by stochastic reaction events. In this case it makes sense to take a spatially averaged viewpoint leading to stochastic but spatially homogeneous reaction systems. Several algorithms based on the classical Gillespie algorithm have been developed in recent time to facilitate the efficient simulation of these processes.
3. Another approach is applicable when particle numbers are large and reactions are not diffusion limited. In this case the stochastic effects average and a system description by spatially distributed diffusion reaction equations (i.e. PDEs) is suitable. Particularly, ion diffusion processes can be studied in this way.
4. The simulation of complex reaction networks requires a simplified approach. This, finally, leads to a classical reaction kinetic description of cellular networks in which both time and space fluctuations are averaged. Moreover, alternative reaction kinetic terms are used to reduce the number of parameters.
5. The roughest quantitative representation of cellular networks is obtained from a steady state viewpoint leading to a linear stoichiometric equation system. Since this system does not allow for a unique solution, much effort has been taken in recent years to systematically explore the solution space of this equation system.

Clearly, simulations must be compared with their biological counterparts. The generation of meaningful experimental data is still a bottleneck for modelling. One of the major obstacles is that data must always be taken from the whole system. Moreover, intracellular physico-chemical parameters and the structure of molecular interaction networks are still not completely known.

**References**

Gillespie, D.T., Petzold, L.R., Chapter 16 in: Z. Szallasi, J. Stelling, V. Periwal (eds.), *System Modeling in Cellular Biology*, MIT (2006)

Takahashi, K., Arjunam, S.N.V., Tomita M., FEBS 29342, 2005.

Wiechert, W., (2002) *Journal of Biotechnology*, 94, 37-63.

Wiechert, W. Chapter 11 in: Kholodenko, B.N., Westerhoff, H.V., (eds.) *Metabolic Engineering in the Post Genomic Era*:, Horizon Bioscience (2004).

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# Biocatalytic synthesis of a biodegradable chelant (S,S-EDDS)

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## 1. Summary

This paper describes a method for analysis and evaluation of a biocatalytic process, which we believe to be widely applicable, provided a kinetic model is available. We have illustrated the method using the biocatalytic production of S,S-ethylenediamine-N,N'-disuccinic acid (S,S-EDDS), a non-commodity, biodegradable chelant capable of replacing EDTA. For implementation of this process the focus needs to be on cost reduction. Upstream and downstream processing requirements and required changes to the two enzymes have been identified and quantified. This has led to the definition and evaluation of several alternative process routes. The generic methodology as well as the options for this specific process will be described.

Keywords: biocatalysis, process modelling, process evaluation.

## 2. Extended Abstract

In recent years, industrial and academic interest in biocatalysis to assist in the synthesis of non-pharmaceutical targets has grown, as focus on achieving more sustainable processes has increased (Hatti-Kaul *et al*, 2007). Much has been discussed in the literature and in the development of new industrial processes about the environmentally-compatible reaction conditions (mild temperatures and pressures in an aqueous environment) in many enzymatic and biocatalytic syntheses. Additionally the possibility of creating biodegradable products is attractive from a green standpoint and in this paper we will report work illustrative of this concept using a biocatalytic process for the synthesis of a biodegradable product.

S,S-ethylenediamine-N,N'-disuccinic acid (S,S-EDDS) is a biodegradable, hexadentate chelant with similar chelation ability to EDTA, capable of reducing heavy metal build up in the environment (Schowanek *et al*, 1997). It can be produced biocatalytically from ethylenediamine and fumaric acid by a two step, two enzyme

pathway (Takahashi *et al*, 1999). The biocatalytic route to S,S-EDDS is complex and characterised by competition of fumarase for fumaric acid, inhibition of EDDSase by fumaric acid, low catalyst concentrations from fermentation, a chemical side reaction to the incorrect intermediate enantiomer and equilibrium constants close to unity.

However, although the properties of S,S-EDDS are highly attractive, to be competitive with the synthesis of EDTA, the biocatalytic synthesis needs to meet severe productivity targets. In an attempt to understand what modifications to the catalyst (s) and process are required we have devised a model of the process. This has formed the basis of a sensitivity analysis, which with appropriate process metrics and associated hurdles (such as product concentration, enantiomeric excess, purity, rate of reaction and enzymatic efficiency) gives a method by which various process routes can be analysed and evaluated.

**References**

Hatti-Kaul, R., Tornvall, U., Gustafsson, L. and Borjesson, P., (2007) *Trends in Biotechnology*, 25, 119-124.

Schwanek, D., Feijtel, T.C.J., Perkins, C.M., Hartman, F.A., Federle, T.W. and Larson, R.J., (1997) *Chemosphere*, 34, 2375-2391.

Takahashi, R., Yamayoshi, K., Fujimoto, N. and Suzuki, M., (1999) *Bioscience, Biotechnology and Biochemistry*, 63, 1269-1273.

# Session T5-1: Biochemical Engineering

| Abstract Number | Paper Title & Authors | Included |
|---|---|---|
| 1598 | Steady-State Multiplicity of a Continuous Biofilm Reactor<br>M E Russo, P L Maffettone, A Marzocchella, P Salatino | Yes |
| 2050 | A stoichiometric model for the metabolism of Gluconacetobacter xylinus<br>I H Velasco-Bedrán, U Arechiga-Viramontes, F Lopez-Isunza | No |
| 2504 | Combining Membrane Separation and Fermentation Processes for improved performance<br>J U Rype, A Garde, A Vrang, S Madsen, G Jonsson | Yes |
| 3006 | Template Induced Crystallization for In-Situ Product Removal from biochemical processes<br>J Urbanus, C P M Roelands, J H ter Horst, D Verdoes, P J Jansens | Yes |
| 3388 | Bioprocess Intensification: Application of a Rotating Packed Bed Bioreactor for Mass Transfer Enhancement in Fermentation Systems<br>C D Cartwright, K V K Boodhoo | Yes |

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# Steady-State Multiplicity of a Continuous Biofilm Reactor

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## 1. Summary

A dynamical model of a continuous biofilm reactor is presented. The reactor consists of a three-phase Internal Loop Airlift (ILA) contactor operated continuously with respect to the liquid and gaseous phases, and batchwise with respect to the immobilized cells. The model has been specifically applied to the bioconversion of a phenol-bearing liquid stream by means of an immobilized bacterial strain (*Pseudomonas sp. OX1*) whose metabolic activity toward phenol has been previously characterized. The model embodies the key processes relevant to the reactor performance, with a particular emphasis on the role of biofilm detachment due to combined phenomena promoted by the fluidized state. Results indicate that a finite loading of free microbial cells establishes even under operating conditions that would promote wash out of the suspended biophase in a chemostat. The co-operative/competitive effects of the two biophases (free cells and immobilized biofilm) result in rich bifurcational patterns of the steady state solutions of the governing equations, which have been investigated in the phase plane of the process parameters. Model computations have also been directed to highlight the dynamical patterns of the bioreactor under selected transient operating conditions.

Keywords: Bifurcation, biofilm, detachment rate, dynamics

## 2. Extended Abstract

Biofilm reactors may provide an effective tool to achieve intensification of fermentation-based processes (Qureshi et al., 2005). Important features influencing biofilm reactors performances are: i) the conflicting effects of biofilm growth and detachment, ii) the competition between immobilized and free cells for the carbon source, and iii) the inherent nonlinearity associated with the growth kinetics, which may result in a multiplicity of steady states and periodic phenomena.

The present paper addresses the theoretical characterization of the performance of a three-phase Internal Loop Airlift (ILA) reactor operated with *Pseudomonas* sp. OX1 immobilized on granular carriers (silica sand, pumice stone...) of uniform particle size (Alfieri, 2006). The mathematical model is based on material balances on substrate (phenol) and on free and immobilized cells. The model specifically aims at assessing: a) the multiplicity of steady-states and the bifurcational patterns of the system; b) the co-operative/competitive effects of the two biophases (free cells and immobilized biofilm); c) the dynamical patterns of the bioreactor under selected transient operating conditions.

The model relies on the following main assumptions: 1) the reactor is well-mixed with respect to liquid, gas and solids phases; 3) both free and immobilized cells are characterized by substrate-inhibited growth kinetics (Viggiani et al., 2006), particularly, the kinetics of biofilm cells growth has been assumed equal to that measured for planktonic cells; 4) a linear relationship between the biofilm detachment rate ($r_d$) and its volumetric concentration (Y) has been assumed ($r_d = k_d$ Y); 5) both the resistance to mass transfer in the boundary layer around biofilm pellets and the diffusional resistance across the biofilm are neglected compared with intrinsic reaction kinetics; 4) adhesion of suspended cells onto the carrier surface during the bioprocess is neglected. Mass balance equations on the substrate (S), the biofilm and the free cells (X) extended to the volume of the liquid phase (V), fed continuously at constant volumetric flow rate (Q), are:

substrate $$\frac{dS}{dt} = D \cdot (S_{in} - S) - \mu(S) \cdot \frac{X}{Z_x} - \mu(S) \cdot \frac{Y}{Z_y} \quad (1)$$

free cells $$\frac{dX}{dt} = -D \cdot X + \mu(S) \cdot X + r_d \quad (2)$$

biofilm $$\frac{dY}{dt} = \mu(S) \cdot Y - r_d \quad (3)$$

where D(=Q/V) is the dilution rate, $S_{in}$ the substrate inlet concentration, $\mu(S)=\mu_{max}S/(K_m+S+S^2/K_i)$ the specific cells growth rate, $Z_x$ and $Z_y$ the biomass yields.

Solutions of eq.s (1)-(3) have been reported in the phase-space of design variables (S,X,Y) and show multiplicity of steady states depending on D, $k_d$ and $S_{in}$. According to the literature (Tang and Fan, 1987; Tijhuis et al., 1994) the growth of stable biofilm is prevented when D is too small to give rise to wash-out of the suspended cells.

An important feature of the steady operation of the reactor at large D is that, even when wash out of the freely immobilized phase is promoted by large Q, a suspended biophase is always present as free cells and is continuously renewed by detachment of the biofilm. On one hand, the persistence of a freely suspended biophase even under wash-out conditions enhances the productivity of the bioreactor. On the other hand, this feature implies that competition of free and immobilized cells for the common substrate cannot be ruled out simply by operating at large dilution rate. This, in turn, might negatively affect the development of a stable biofilm loading that would be required for highly intensified operation of the bioreactor. These two features have to be carefully balanced by proper selection of operating conditions, and more specifically by tuning the biofilm detachment rate.

**References**

Alfieri F., Ph. D. Thesis, Università degli Studi di Napoli *Federico II*, DdR "Scienze Biotecnologiche" - XVIII ciclo (2006)

Chisti M. Y., **Airlift Bioreactors**, Elsevier, New York (1989)

Gjaltema A, van der Marel N., van Loosdrecht MCM, Heijnen JJ., *Biotech. and Bioeng.* (1997), 55, 880-889.

Qureshi N., Annous B.A., Ezeji, T.C., Karcher, P., Maddox, I.S., (2005) *Microbial Cell Factories*, 4(24).

Tang W.-T. and Fan L.S. *AIChE J.* (1987), 33, 239-249.

Tijhuis L, van Loosdrecht MCM, Heijnen JJ. *Biotech. and Bioeng.*(1994),28, 595-608.

Viggiani A., Olivieri G., Siani L., Di Donato A., Marzocchella A., Salatino P., Barbieri P., Galli E., (2006) *J. of Biotechnology*, 123, 464-477

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# Combining Membrane Separation and Fermentation Processes for improved performance

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## 1. Summary

Fermentation processes are often inhibited by a build-up of either products or by-products thereby limiting the maximum product concentration which can be obtained during the fermentation. Inhibitors limit and influence the quality of biomass concentration which can be obtained thereby decreasing the production rate.
The patented electro-membrane separation process, REED, provides a membrane technology for removing organic acid inhibitors from live fermentation broth, while retaining cells, proteins, sugars and most other feed components [1].
The ability of the Reverse Electro-Enhanced Dialysis (REED) separation system to extract and control growth inhibiting organic acids (e.g. lactic acid) during lactic acid bacteria (LAB) fermentations has yielded significant boosts to productivity and product yield in various fed-batch fermentation setups.

Keywords: fermentation, membrane separation, integrated processes, electrodialysis

## 2. REED fermentation

In a typical REED fermentation setup, a side-stream from the fed-batch fermenter is taken out and pushed through a REED membrane unit, where inhibiting organic acid ions (e.g. acetate, lactate) are replaced by hydroxide ions, which act to neutralise the acid. The depleted ferment is then returned to the fermenter. The driving separation force of the REED system is an electrical current, which does not add sizeable shear stress to the microorganisms compared to pressure-driven membrane processes as ultra- and micro-filtration. Non-charged components as substrate are not affected and the membranes are too dense for cells and charged proteins to escape the ferment.
The result is a fed-batch fermentation sustaining constant growth at a stable concentration of organic acids below the inhibition level.

An obvious application for this system is production of microorganisms/biomass, where productivity increases 10-fold. Another successful application is expression of

recombinant protein by a gene-modified LAB strain, developed by Danish contract research organisation Bioneer [2]. In normal batch fermentation, the protein expression is limited between the time where the fermentation reaches the lactate level inducing expression and the time the lactate level inhibits further cell growth. Through the REED system, the fermentation can be sustained at constant lactate level, optimal for protein expression at continuous biomass growth practically indefinitely. This leads to many-fold increases in biomass and protein yield as demonstrated in figure 1, where cell density is measured by optical density ($OD_{600}$).

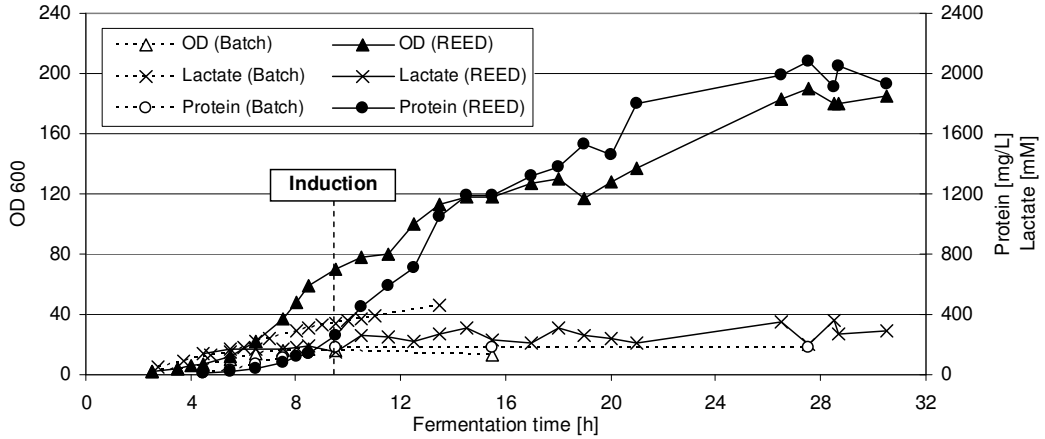


Figure 1. Comparison between standard fed-batch fermentation (Batch) and REED fermentation for production of recombinant protein.

In the standard fed-batch fermentation, biomass growth and protein expression ceased when lactate reached 400mM after 8-10 hours at OD 17 and 180 mg/L protein. The REED fermentation maintained lactate concentration below 150 mM until biomass had reached OD 75, then lactate concentration was increased to an optimal expression level at 300mM (induction) and maintained at this level. Biomass kept increasing to OD 185 and protein concentration reached above 2 g/L. Both fermentations started with 1 L of starting medium, but the REED fermentation was able to consume significantly more concentration feed solution. In total, the standard fed-batch fermentation produced 200 mg of the recombinant protein, while the REED fermentation in comparison yielded in total 7.2 g of the same protein.

**References**

[1] Garde, A., (2002) *Production of lactic acid from renewable resources using electrodialysis for product recovery*, ISBN 87-90142-84-5

[2] Bredmose, L. *et al*, (2001) "Development of a Heterologous Gene Expression System for use in Lactococcus lactis", O.-W. Merten, et al. (eds.), *Recombinant Protein Production with Prokaryotic and Eukaryotic Cells*, p. 269-275, Kluwer Academic Publishers (Netherlands)

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# Template Induced Crystallization for *In-Situ* Product Removal from biochemical processes

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## 1. Summary

*In-situ* separation techniques are developed to increase productivity in sustainable biological processes. Template Induced Crystallization (TIC) aims for selective separation of the product and easy regeneration of the auxiliary phase. A method is developed to screen for templates reducing the induction time of crystallization. Results show that certain templates enhance crystallization of the model compound. Future work is focused on designing an ISPR process based on TIC.

Keywords: sustainable processing, *In-Situ* Product Removal (ISPR), Template Induced Crystallization (TIC), screenings methodology, high throughput equipment.

## 2. Extended Abstract

Nowadays biological processes are replacing conventional processes for the production of (fine) chemicals, for the reasons of specificity, efficiency, to reduce environmental unfriendly wastes or the absence of chemical routes. However, in many processes driven by fermentation, the products have inhibitory or toxic effects on the productivity and growth of the biomass. In order to overcome these constraints the product should be removed from the bioreactor as soon as it is formed. This is achieved by application of a form of process integration called *In-Situ* Product Removal (ISPR)[1]. As a result the productivity and efficiency of the biological process are improved. Additionally, downstream processing is potentially simplified by reducing the total number of downstream processing steps.

Within the framework of introducing a toolbox of ISPR separation techniques[2], it is our objective to develop an integrated process of fermentation and *in-situ* crystallization using the principle of Template Induced Crystallization (TIC). With this technique compounds are selectively separated from the broth by crystallization on templates. The templates are particles that are introduced in the broth and subsequently removed. After their removal, templates are regenerated for reuse while the product is further purified (see figure 1).

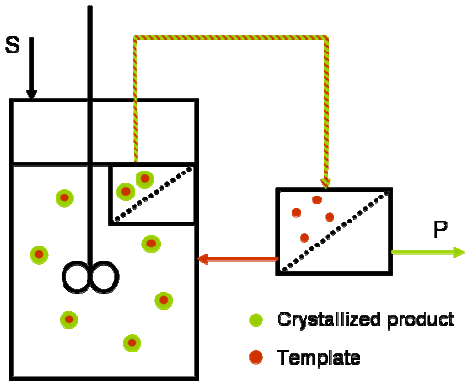


Figure 1: Schematic overview integrated process

Templates enable crystallization at low supersaturations because the introduced surface lowers the nucleation work. Although the TIC principle is generic, the templates have to be specific for each compound. Crucial for the development of this technique is therefore the availability of a generic screening methodology for TIC. With this methodology templates are evaluated upon their potential to promote crystallization. The methodology comprises metastable zone width determination, induction time measurements, pH-stat experiments and desupersaturation curve measurements. The screening method is implemented in robotized equipment for parallel experimentation.

For the model compound cinnamic acid, the associated species becomes supersaturated at low pH. The equilibrium reaction below indicates that upon crystallization of associated cinnamic acid, the pH will increase.

$$CA^{-}_{(aq)} + H^{+}_{(aq)} \Leftrightarrow CAH_{(aq)} \Leftrightarrow CAH_{(s)} \downarrow$$

In figure 2, the pH is plotted versus time. A solution of cinnamic acid is titrated with HCl to a pH (~ 4) such that the system is slightly supersaturated (within the meta-stable zone). At $t_0$, HCl is injected to increase the supersaturation such that nucleation is initiated. After HCl is injected, an increase in pH can be observed, as a result of crystal-lization of cinnamic acid. The induction time significantly decreases with the use of $TiO_2$ tem-plates. This is an indication for the effectiveness of this template.

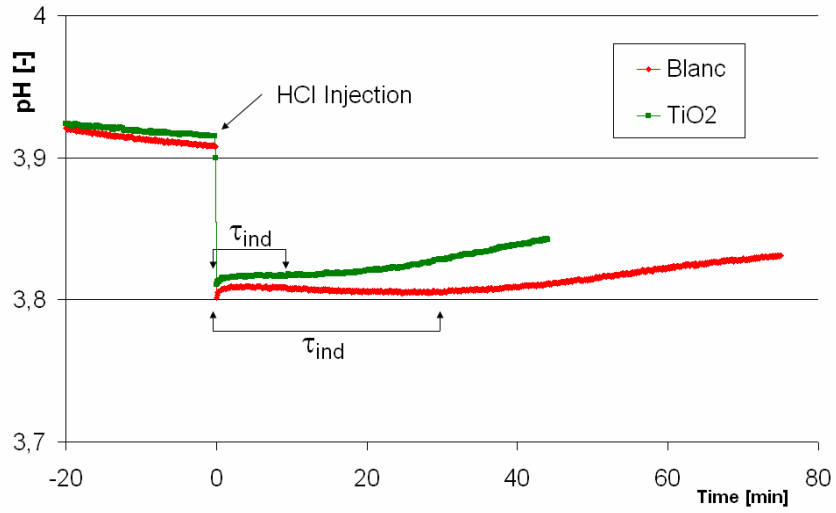


Figure 2: pH-shift crystallization of cinnamic acid. Red graph represents blanc experiment (without templates). Green graph represents experiment with $TiO_2$ templates.

## 3. Future Work

Future work is focused on designing an ISPR process based on TIC. For this purpose, 1) properties of templates will be modified such that their recovery from the broth is facilitated and also 2) regeneration of templates, for example using organic solvents, needs to be exploited.

### References

1. Lye, G. J. and Woodley, J. M., (1999) *Trends in Biotechnology*, 17, 395-402.
2. J.A. Vente, J. van Erkel, 'In-situ product recovery in bioconversions', in Proceedings of the 6th International Conference on Process Intensification, P. Jansens, A. Stankiewicsz, A. Green (editors), Delft, The Netherlands, (2005).

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# Bioprocess Intensification: Application of a Rotating Packed Bed Bioreactor for Mass Transfer Enhancement in Fermentation Systems

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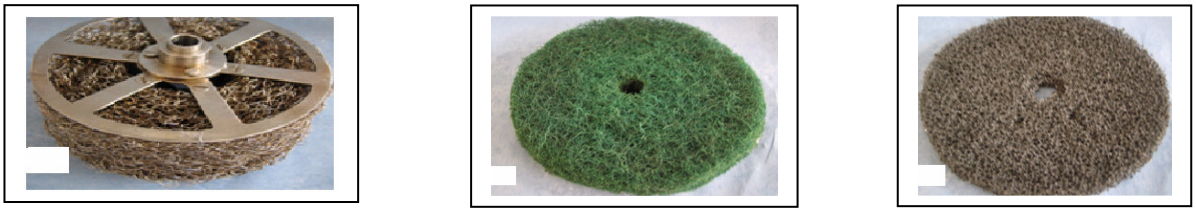
## 1. Summary

Aerobic fermentation systems tend to be limited by oxygen mass transfer, affecting the microbial culture and ultimately the reactors productivity. Our research proposes the use of a rotating packed bed of porous material that produces small oxygen bubbles and therefore increases the oxygen mass transfer within the reactor system. Preliminary studies conducted have shown that a knitted wire mesh can produce double the volumetric mass transfer ($K_La$) compared to the standard Rushton impeller. The present work reports on further mass transfer studies in air/water, air/water-glycerol, and fermentation systems. This paper looks at oxygen bubble size produced by each impeller with the use of a Phase Doppler Anemometery (P.D.A) system, comparing this with previous research conducted with digital photography.

*Keywords: Bioprocess Intensification, Oxygen Mass Transfer, Rotating Packed Bed,, Porous mesh*

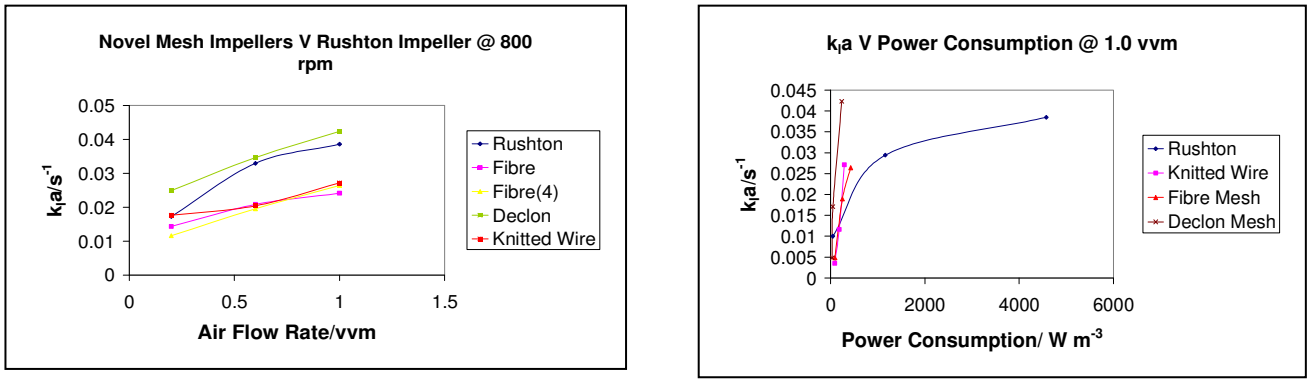
## 2. Extended Abstract

The present work reports on further mass transfer experiments conducted in air-water and air-water/glycerol systems using a range of porous mesh materials as shown in Figures 1 (a)-(c). It is seen from Figure 2 (a) that the $K_La$ obtained at 800 rpm impeller speed using the Declon mesh is the highest at all aeration rates tested. We believe the improvements observed in the oxygen transfer rate with the porous mesh systems are attributable to smaller oxygen bubbles being formed and sustained in the reactor, thereby increasing the interfacial area available for mass transfer. Power consumed by the rotated beds has also been measured using a torque meter. Figure 2(b) demonstrates that has been shown that the porous impellers consume less power to give similar $K_La$ when compared to the Rushton design. This indicates that the power consumed by porous mesh is directed at enhancing the mass transfer of oxygen into the liquid medium instead of the power being wasted in vortex formation when the double Rushton design is employed.



(a) (b) (c)

**Figure 1:** Porous mesh impellers (a) Knitted Wire Mesh, (b) Fibre Mesh, (c) Declon Mesh




**(a)** **(b)**

**Figure 2: (**a) Effect of aeration rates on $K_La$ for all the porous materials tested compared with a conventional double Rushton turbine impeller in air-water system; (b) $K_La$ vs power consumed by rotating bed of porous materials for air-water system compared with double Rushton turbine

Application of the porous mesh impellers to an *Escherichia coli* K12 batch fermentation system is also being investigated and preliminary results indicate higher biomass concentrations achieved with the knitted wire mesh under certain conditions in comparison with the double Rushton turbine. The effect of the viscosity of the fermentation broth will also be investigated by means of a second batch fermentation system utilising *Xanthomonas campestris*. The *X.campestris* fermentation produces a highly viscous Non-Newtonian fermentation system; it is known that a higher viscosity will lead to a reduction in $K_La$. This may be overcome to a certain extent with application of the novel porous impellers.

Experimental work is currently underway to measure the size of the oxygen bubbles produced by the novel impeller systems using the Phase Doppler Anemometery (PDA) technique. Preliminary studies using digital photography have shown that, in a water-like viscosity medium, the novel impellers produce smaller oxygen bubbles. It is envisaged that the PDA system will provide a more accurate model of the bubble distribution.

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# Session T5-2: Design, Analysis & Control of Fermentation Processes

| Abstract Number | Paper Title & Authors | Included |
|---|---|---|
| 796 | Non-linear Modeling Of Kefir Grains Growth Curve<br>M Tramšek, A Goršek | Yes |
| 1235 | Determination of Immobilized Enzyme Apparent Kinetic Parameters in Packed-Bed Reactors: Presentation of a New Methodology<br>A R Özdural, C Webb | Yes |
| 1336 | A model based nonlinear observer for simultaneous state and disturbance estimation in continuous anaerobic digesters<br>G Savvoglidis, C Kravaris, K Stamatelatou, G Lyberatos | Yes |
| 1452 | A Mathematical Model for the Growth of Aspergillus niger in a Solid-State Fermentation.<br>I Reyes-Ocampo, C Castillo-Araiza, F Lopez-Isunza | Yes |
| 4065 | Product Optimization in a Fed-batch Fermentation Processes<br>A A Ahmad, B N A F A Samad, C K A Hamid | Yes |

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# Non-linear modeling of kefir grains growth curve

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## 1. Summary

The main objective of this research is non-linear modeling of growth curve during kefir grains batch propagation. For this purpose some laboratory experiments were performed in an RC1 reaction calorimeter provided data for growth curve construction. Afterwards, we fitted several sigmoid growth models and compared their biological parameters. Finally, we established the statistically most appropriate growth model for prediction of biomass increase during kefir grains batch propagation in milk under selected bioprocess conditions.

Keywords: kefir grains, batch propagation, growth models, statistical analysis

## 2. Extended Abstract

Kefir grains consist of complex symbiotic microflora, containing more than 35 probiotic bacteria which are entrapped in a water-soluble polysaccharide matrix and have proven highly beneficial to humans. They are primarily used as natural culture starters in traditional large scale kefir fermentation processes. Over recent years, it has been established that their variegated microbial composition also enables applications in bread production as baker's yeast (Plessas *et al.*, 2005), volatile aroma compounds production (Beshkova *et al.*, 2003) and ethanol production using immobilized kefir yeast cells (Athanasiadis *et al*., 1999). Owing to their potential commercial applications, special attention should be focused on their production, using traditional batch cultivation in milk with low biomass increase. Kefir grains batch propagation is a inherently very complex process, thus it is of critical importance for its further improvement, (optimization, monitoring and controlling) to develop models that provide an accurate description of kefir grains growth curve.
Therefore, the aim of the present study was to model a growth curve during kefir grains batch propagation using traditional cultivation in fresh high temperature pasteurized (HTP) whole fat cow's milk. Several non-linear sigmoidal growth models (Logistic, Gompertz and Richards) (Zwietering *et al.*, 1990) and values of their biological parameters were compared to describe the growth curve using experimental

data regarding time-dependent kefir grains increase. These experiments were performed in an RC1 reaction calorimeter under selected bioprocess conditions (temperature, $\vartheta = 24$ °C, rotational frequency of the stirrer, $f = 90$ (1/min), initial kefir grains mass concentration, $\gamma_{KG,0} = 75$ g/L and glucose mass concentration, $\gamma_G = 20$ g/L). The models were compared statistically by using six statistical indicators, i.e. standard error, *SE*, coefficient of the variation, *CV*, adjusted coefficient of the determination, $R_{adj}^2$, root mean squared error, variance ratio, *F*, predicted residual error sum of squares, *PRESS*, and t-statistic value, *t-st*.

The graphical results of change in the logarithm of relative kefir grains mass versus batch propagation time, show that all the proposed models have a good predictive ability and, therefore, satisfactorily fit the data obtained from the experimental measurements. The maximum specific growth rates and asymptotic values proposed by all three models are very similar, meanwhile, lag phase times and batch propagation times at maximum specific growth rate are markedly different. Statistically, the $R_{adj}^2$ values were almost equal (variation ±0.4 %) for all models. Therefore, this statistic indicator can not be used as a single criterion for growth model ranking. Furthermore, the *RMSE* and *PRESS* values of the Logistic model are smaller, compared to the Gompertz and Richards ones. Therefore, it is statistically less reliable for describing the kefir grains growth curve. Further comparison shows that Gompertz, compared to the Richards model has greater *F* value. The relative difference is more than 63 %. Moreover, it also has greater *t-st* values for all kefir grains growth's biological parameters. Finally, on the basis of the applied statistical indicators, we established that the kefir grains growth curve during traditional batch propagation in fresh HTP whole fat cow's milk, under selected bioprocess conditions, can be statistically described most successfully by the Gompertz growth model.

The presented results are specific for the selected bioprocess conditions, for used propagation medium and initial kefir grains culture. It is also well-known that microbial compositions of milk and kefir grains vary considerably over time and are dependent on age and storage conditions. Therefore, these conclusions cannot be presented as general for all kinds of kefir grains propagations. In spite of this, the procedure given in this research can be used to find the best model describing batch kefir grains growth under different experimental and propagation setups.

## References

Plessas, S., Pherson, L., Bekatorou, A. Nigam, P. and Koutinas, A. A., (2005) *Food Chemistry*, 93,585-589.

Beshkova, D. M., Simova, E. D., Frengova, G. I., Simov, Z. I. and Dimitrov, P. Zh., (2003) *International Dairy Journal*, 13,529-535.

Athanasiadis, I., Boskou, D., Kanellaki, M. and Koutinas, A. A., (1999) *Journal of Agricultural and Food Chemistry*, 47,4474-4477.

Zwietering, M. H., Jongenburger, I., Rombouts, F. M. and Vant't Riet, K., (1990) *Applied and Environmental Microbiology*, 56,1875-1881.

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# Determination of immobilized enzyme apparent kinetic parameters in packed-bed reactors: Presentation of a new methodology

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## 1. Summary

An explicit equation is proposed for the determination of immobilized enzyme apparent Michaelis-Menten kinetics parameters in packed-bed reactors. The mathematical analysis is based on recycling systems, and yields a new technique. Glucose decomposition reaction in a recirculation system with immobilized glucose oxidase on a weak base ion exchanger resin was envisaged. The experimental system is composed of a reservoir and a packed-bed immobilized enzyme reactor. It was concluded that the proposed explicit equation successfully predicts $K_m$ values.

Keywords: enzymatic reactors, recirculation systems, immobilized enzyme.

## 2. Extended Abstract

In this work we focus our attention on the extension of Özdural-Alkan methodology, which was originally used in the membrane separations [1] and later in the first order packed-bed catalytic reactions [2], to the explicit determination of packed-bed immobilized enzyme apparent kinetic parameters. Eq (1) gives generally used Michaelis–Menten kinetics for soluble enzymes.

$$v = \frac{v_{\max}[S]}{K_m + [S]} \qquad (1)$$

where v is the initial reaction rate of enzyme catalyzed reaction, S is the substrate conc. $v_{max}$ is the maximum reaction rate of enzyme catalyzed reaction and $K_m$ is the Michaelis–Menten constant. The kinetic parameters for the immobilized enzyme may be different from those of the suspended enzyme. Furthermore, due to the difference in hydrodynamic conditions, the immobilized enzyme apparent kinetic parameters determined through batch wise stirred reactor experiments usually do not reflect the apparent kinetic parameters of immobilized enzymes in packed-bed reactors. In order to determine the apparent kinetic coefficients in continuous mode operating packed-bed enzymatic reactors, via established techniques, simultaneous measurement of the

concentration differences between the substrate inlet and outlet flows are required [3], where it is likely to come across with very small substrate concentration differences. Thus the measurement of the substrate concentration differences between the inlet and outlet streams can be difficult and inaccurate. The present method is free from such limitations and, for the model development; a packed-bed immobilized enzyme reactor with a recycle system is envisaged. The maximum reaction rate is retrieved by the following equation for recirculation systems [4].

$$v_{\max} = -\frac{mV}{M} \qquad (2)$$

M is the mass of biocatalyst in fixed-bed, m is slope of the reservoir conc. versus time data of high conc. experiments, and V is the substrate reservoir fluid volume. For low substrate concentrations it can be assumed that Michaelis–Menten kinetics can be approximated by first-order kinetics [5], where application of Özdural-Alkan methodology [1] leads to the derivation of the following explicit equation to be used for $K_m$ determination.

$$K_m = v_{\max}\left\{\frac{\varepsilon}{(1-\varepsilon)\rho_p}\frac{1}{\tau_{bed}}\ln\left[\frac{\left(\frac{S_{out}|_t}{S_o}\right)^{-\frac{\tau_{bed}}{t}}}{\ln\left(\frac{S_{out}|_t}{S_o}\right)^{\frac{\tau_{res}}{t}}+1}\right]\right\}^{-1} \qquad (3)$$

$\tau_{bed}$ and $\tau_{res}$ are the residence time of packed-bed reactor and the reservoir respectively, $S_{out}$ is the reservoir concentration for low concentration experiments and $S_0$ is the reservoir initial concentration, $\varepsilon$ is the bed porosity. The experimental system is composed of a reservoir and a packed-bed immobilized enzyme reactor. The experimental data of glucose decomposition reaction in a recirculation system with immobilized glucose oxidase on a weak base ion exchanger resin was used for $K_m$ value calculations through Eq (3). It was concluded that the proposed Eq (3) successfully predicts $K_m$ values with the use of a single reservoir concentration measurement and the corresponding time data, as long as it is employed in the range of low substrate concentrations. Furthermore the apparent $K_m$ values calculated by the employment of Eq. 3 for various recirculation rates were in excellent agreement with those obtained via other techniques [4] that make no reference to Eq. 3. Here the $K_m$ values were found to be $0.5\times10^{-4}$ and $0.6\times10^{-4}$ mMol cm$^{-3}$ for the recirculation rates of 75 and 25 cm$^3$ min$^{-1}$ respectively, regardless of the reservoir substrate conc. vs t data couple employed in Eq. 3 for a certain recirculation rate.

**References**

[1] Özdural, A.R. and Alkan, A., (2003) *Journal of Membrane Science*, 223, 49-57.
[2] Özdural, A.R., Alkan, A. and Webb, C., (2006) *Chemical Engineering Journal*, 118, 17-22.
[3] Lilly, M.D., Hornby, W.E. and Crook, E.M., (1966) *Biochemical Journal*, 100, 718–723.
[4] Özdural, A.R., Tanyolaç, D., Demircan, Z., Boyacı, I.H., Mutlu, M. and Webb, C., (2001) *Chemical Engineering Science*, 56, 3483–3490.
[5] Narsimhan, G., (1981) *Chemical Engineering Journal*, 22, 101–105.

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# Determination of immobilized enzyme apparent kinetic parameters in packed-bed reactors: Presentation of a new methodology

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where v is the initial reaction rate of enzyme catalyzed reaction, S is the substrate conc. $v_{max}$ is the maximum reaction rate of enzyme catalyzed reaction and $K_m$ is the Michaelis–Menten constant. The kinetic parameters for the immobilized enzyme may be different from those of the suspended enzyme. Furthermore, due to the difference in hydrodynamic conditions, the immobilized enzyme apparent kinetic parameters determined through batch wise stirred reactor experiments usually do not reflect the apparent kinetic parameters of immobilized enzymes in packed-bed reactors. In order to determine the apparent kinetic coefficients in continuous mode operating packed-bed enzymatic reactors, via established techniques, simultaneous measurement of the

concentration differences between the substrate inlet and outlet flows are required [3], where it is likely to come across with very small substrate concentration differences. Thus the measurement of the substrate concentration differences between the inlet and outlet streams can be difficult and inaccurate. The present method is free from such limitations and, for the model development; a packed-bed immobilized enzyme reactor with a recycle system is envisaged. The maximum reaction rate is retrieved by the following equation for recirculation systems [4].

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$\tau_{bed}$ and $\tau_{res}$ are the residence time of packed-bed reactor and the reservoir respectively, $S_{out}$ is the reservoir concentration for low concentration experiments and $S_0$ is the reservoir initial concentration, $\varepsilon$ is the bed porosity. The experimental system is composed of a reservoir and a packed-bed immobilized enzyme reactor. The experimental data of glucose decomposition reaction in a recirculation system with immobilized glucose oxidase on a weak base ion exchanger resin was used for $K_m$ value calculations through Eq (3). It was concluded that the proposed Eq (3) successfully predicts $K_m$ values with the use of a single reservoir concentration measurement and the corresponding time data, as long as it is employed in the range of low substrate concentrations. Furthermore the apparent $K_m$ values calculated by the employment of Eq. 3 for various recirculation rates were in excellent agreement with those obtained via other techniques [4] that make no reference to Eq. 3. Here the $K_m$ values were found to be $0.5\times10^{-4}$ and $0.6\times10^{-4}$ mMol cm$^{-3}$ for the recirculation rates of 75 and 25 cm$^3$ min$^{-1}$ respectively, regardless of the reservoir substrate conc. vs t data couple employed in Eq. 3 for a certain recirculation rate.

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[2] Özdural, A.R., Alkan, A. and Webb, C., (2006) *Chemical Engineering Journal*, 118, 17-22.
[3] Lilly, M.D., Hornby, W.E. and Crook, E.M., (1966) *Biochemical Journal*, 100, 718–723.
[4] Özdural, A.R., Tanyolaç, D., Demircan, Z., Boyacı, I.H., Mutlu, M. and Webb, C., (2001) *Chemical Engineering Science*, 56, 3483–3490.
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# A Mathematical Model for the Growth of *Aspergillus niger* in a Solid-State Fermentation.

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## Summary
*Aspergillus niger* is an important mould for the production of citric acid that grows in liquid as well as in solid state fermentation (SSF). The former presents serious limitations when substrate concentration (glucose) is above 50 g/L, however this is not the case in SSF where this fungi can grow on glucose concentration up to 250 g/L. To study the radial growth of a colony of *A. niger* in SSF, an experimental and theoretical work has been performed, and a mathematical model for this has been developed. The model accounts for a modified Monod kinetics and describes mass transport by diffusion through the solid substrate as well as within the colony, and fungal growth. Experiments were carried out in a Petri dish, using different glucose concentration (50- 250 g/l), as carbon source, the radial growth was measured at different periods in time, and in all cases the morphometrical parameters: diameter and average length of the hypha ($d_h$, $L_{av}$), were measured by image analysis. Model simulations in the range of glucose concentration used in this study showed good agreement with experimental data, which allowed the estimation of the kinetic parameter in the Monod expression ($\mu_{max}$), and was used to infer the axial and radial glucose concentration gradients within solid medium. The results of the simulations suggest that the growth is affected by the glucose concentration, and the morphometrical parameters have a direct relation with the value obtained for $\mu_{max}$.

**Key words**: *Aspergillus niger*, solid-substrate fermentation, fungal radial growth, mathematical model, morphometrical parameters.

## Extended Abstract
The present model considers the symmetrical radial growth of an *A. niger* colony, and it is modeled with a modified expression of the Monod equation. Growth is due to a single carbon source consumption, which is transported by diffusion through the solid media. The transport of the glucose in the colony occurs simultaneously in the axial and radial directions through a water film that surrounds the mycelia and allows the nutrients transport. The radial growth of the colony, due the microbial growth, is modelled like a moving boundary problem. *Aspergillus niger* (C28B25) from the *Universidad Autónoma Metropolitana-Institut pour le Recherche et D´eveloppement*'s fungi collection, was grown on a solid medium with of glucose as the carbon source with a ratio C/N=12. The colony diameters were measured and it was found that *A. niger* colonies are nearly circular, and during the exponential growth its apparent rate of increase is linear for all glucose concentrations. In this work hyphal length and diameter were measured, for the hyphae located on the expansion zone; the data showed an inverse relation among these with the

initial carbon source; also, Larralde (1996) showed that the hyphal average diameter decreases as the glucose concentration increases.

The model used in this work gives good predictions of the rate of increase of *A. niger* colony, as shown in Fig. 1. Other results show that radial changes in the biomass density are negligible in the centre of the colony and they increase linearly in the mycelia periphery, as it was also show by Olsson (1994). Perpendicular grow of the colony is rather small, due to the fact that the mycelial preferentially grows (radially) on the solid surface.

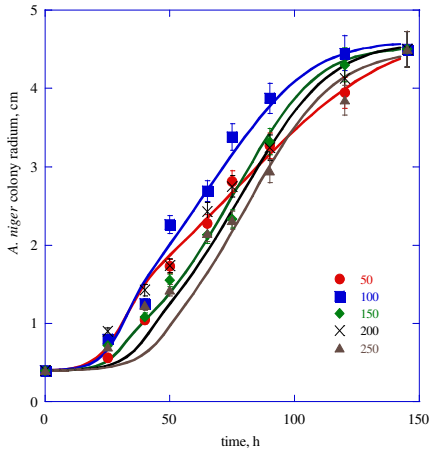


Figure1. Radial evolution of *A. niger* colony.

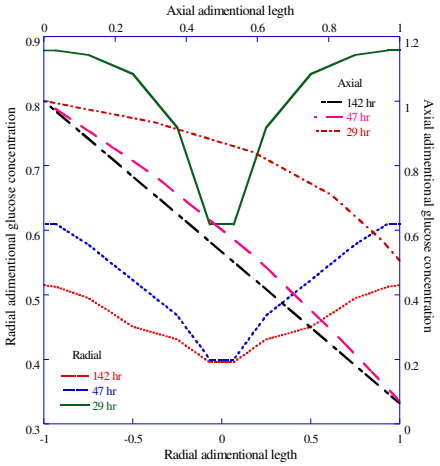


Figure 2. Radial and axial profiles of the glucose concentration in the solid surface.

Predictions of the radial concentration profiles of glucose in the solid media show that the highest concentration is always at the periphery. The rate of glucose consumption is larger at the centre of the colony and in a region below it, as shown in Fig. 2. These predictions are in agreement with those reported by Robson et. al. The shapes of the radial profiles are very similar for all initial glucose concentration (IGC). The maximum growth rate was estimated for several values of IGC, and it was found that there is an inverse relationship between IGC and the values for $\mu_{max}$ (for 50 g/L, $\mu_{max}$=0.112 $h^{-1}$; for 250 g/L, $\mu_{max}$=0.0527 $h^{-1}$); showing also the relationship of the hyphal morfometric properties with IGC.

## References

Larralde–Corona C. P. *PhD. Thesis (in Spanish) UAM-Iztapalapa* (1996)
Trinci A.P.J. (1971) *Journal of General Microbiology*, 67, 325 – 344.
Olsson S. (1994) *Experimental Mycology*, 18, 33 – 47.
Robson G.D. *et. al.* (1987) *Journal of General Microbiology*, 133, 361 – 367.

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# Product Optimization of a Fed-batch Fermentation Processes

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## 1. Summary

This paper discusses the development of constrained optimization strategies for a fed-batch penicillin fermentation process. To facilitate the study, a mathematical model of the system is developed based on published materials, and simulated using MATLAB software. Good agreement is obtained when the results are validated against published work. To provide on-line estimates of the difficult to measure penicillin concentration, Partial Least Squares model is employed. Using these estimates, good control of product concentration is established thus enabling it to be implemented as part of product concentration control loop. Further improvements are introduced using dynamic optimization aiming at increasing the achievable product concentration while satisfying all process constraints. Two strategies are considered. These are optimal control policy using direct-shooting algorithm and unconstrained Dynamic Matrix Control (DMC). From this two optimization approaches, it is possible to estimate the optimal operating conditions as substrate feed rate so that the systems presents high performance within threshold value limit. The result also revealed that the use of DMC approach is superior the direct shooting method in term of the penicillin concentration as well as penicillin purity. Results obtained in this study have exposed the potentials of dynamic optimization schemes in improving the product purity in a fed-batch fermentation process.

Keywords: penicillin, partial least squares, direct shooting method, dynamic matrix control

## 2. Extended Abstract

Many industrially important fermentation processes for the production of antibiotics, amino acids, vitamins and enzymes are carried out in a fed-batch mode in which a feed stream containing substrate and/or nutrients is fed into the fermenter during the course of an otherwise batch operation [1].

An unstructured model for a fed-batch Penicillin G fermentation process has been developed based on the extension mechanistic model of Bajpai and Reuβ (1980) [2]. This mathematical model includes additional input variables like feed flow rate of substrate, pH, temperature, aeration rate, agitation power as well as output variables such as $CO_2$ evolution and heat generation terms. The model was simulated in MATLAB environment. Good agreement was established between the results obtained in this work and those published in the literature.

The issue of on-line measurement of difficult to measure quality variables such as penicillin fermentation was addressed. In practice, it is the lack of robust on-line sensors for some of these key fermentation variables has been a significant obstacle for the implementation efficient process control [3]. Since it is desirable to be able to optimize fermentation operation, this weakness must therefore be overcome. This can either be done through off-line analyses that are highly human dependent or making use of on-line inferential estimation strategy. The latter was adopted in this study and an inferential estimator based on Partial Least Squares (PLS) model has been developed to provide reliable prediction of the unmeasured quality variables. The results obtained proved the good capability of the estimator to perform in various operating conditions, thus enabling it to be implemented as part of product concentration control loop.

The success in providing reasonably accurate estimation of important fermentation variables opened the opportunity for process optimization. Dynamic optimization of process operation can be established using several approaches. The optimal control policy using direct shooting method and single step ahead DMC has been developed, aiming at optimizing the end of the batch penicillin concentration. From this two optimization approaches, it is possible to estimate the optimal operating conditions as substrate feed rate so that the systems presents high performance within threshold value limit. The result also showed that the single step ahead DMC approach is superior the direct shooting method in term of the penicillin concentration as well as penicillin purity.

**References**

[1] Parulekar, S.J., and Lim, H.C. (1995). *Advances in Biochemical Engineering and Biotechnology*, 32:207-258.

[2] Birol, G., Űndey, C. and Cinar, A. (2002). *Computers and Chemical Engineering*. 26:1553-1565.

[3] Zhang, H. and Lennox, B. (2004). *Journal of Process Control.* 14(1): 41-50.

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# Session T5-3: Bio-transformation in the Laboratory and in Large Scale Production

| Abstract Number | Paper Title & Authors | Included |
|---|---|---|
| 1645 | Saccharomyces cerevisiae Morphology under Hyperbaric Gases<br>M A Z Coelho, J A P Coutinho, M Mota, E C Ferreira, I Belo | yes |
| 1647 | Evaluation of mass transfer enhancement in lipase production by Yarrowia lipolytica in a multiphase system<br>P Amaral, M Martins, I Marrucho, M R Leão, J Coutinh, MA Coelho | Yes |
| 1865 | Micro-Enzyme-Membrane Reactor – a tool for assessing enzyme activity and stability under continuous reaction conditions<br>D H Mueller, M A Liauw, W Hempelmann, L Greiner | Yes |
| 2773 | Steps towards the rational design of an immobilized biocatalyst with improved process stability<br>I Dib, J Nahalka, B Nidetzky | Yes |
| 2835 | Enzymatic production of lactobionic acid: From laboratory to pilot scale<br>M Nordkvist, L Hua, P M Nielsen, J Villadsen | Yes |
| 3022 | Cell array preparation in flow-type microchip by using photoresponsive polymer substrate<br>J Edahiro, K Sumaru, Y Tada, S Sugiura, T Takagi, T Shinbo, Y Yoshimi, T Kanamori | Yes |

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# *Saccharomyces cerevisiae* Morphology under Hyperbaric Gases

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## 1. Summary

The effects of hyperbaric stress on the morphology of *Saccharomyces cerevisiae* were studied in batch cultures under pressures between 0.1 MPa and 0.6 MPa and different gas compositions (air, $O_2$, $N_2$, or $CO_2$), covering aerobic and anaerobic conditions. A method using automatic image analysis for cell classification based on their morphology was applied to experimental data. Cell viability was assessed through the Methylene Blue staining method and the percentages of viable and non-viable cells were also estimated using digital image processing. The results show that the effect of pressure on cell activity strongly depends on the nature of the gas used for pressurization. While nitrogen and air to a maximum of 0.6 MPa of pressure were innocuous to yeast, oxygen and carbon dioxide pressure caused cell inactivation, which was confirmed by the reduction on the number of budding cells with time and also a decrease in the average cell size (0.6 MPa $CO_2$). A model taking into account cell viability reveals the opposing effects between oxygen availability and the baric and oxidative stresses present on the system. It is shown that cell viability in general is not constant during the experiments but strongly depends on the environment.

Keywords: *Saccharomyces cerevisiae*, pressure, viability, image processing analysis, hyperbaric stress

## 2. Extended Abstract

The yeast *Saccharomyces cerevisiae* is one of the most important microorganisms employed in industry. Growth rate, mutation, and environmental conditions affect yeast size and shape distributions but, in general, the influence of spatial variations in large-scale bioreactors is not considered. Many differences found between laboratory and industrial behavior can be partially explained by different environmental conditions, especially when gas solubility is an important parameter, since it is a function of the local position within the reactor. As a consequence, analysis of pressure effects in cell physiology and morphology must be considered. Both $O_2$ and $CO_2$ partial pressures are directly involved in yeast metabolism, the former being an essential nutrient for cell respiration whereas $CO_2$ is a product of cellular metabolic activity. Thus, batch studies with pure air, oxygen, nitrogen or carbon dioxide were performed, covering aerobic and anaerobic conditions often used in fermentation technology. Besides metabolic analysis of hyperbaric stress on yeast cells, a further insight was made into cell morphology and viability changes caused by different gases at moderate pressures.

*Experimental conditions: S. cerevisiae* ATCC 32167 was grown in a medium comprising 0.4 g $l^{-1}$ $MgSO_4 \cdot 7H_2O$, 2.0 g $l^{-1}$ $(NH_4)_2SO_4$, 5.0 g $l^{-1}$ $KH_2PO_4$, 1.0 g $l^{-1}$ yeast extract, and 5.0 g $l^{-1}$

glucose (pH 4.0). Hyperbaric experiments were carried out in a 600-ml stainless steel reactor (Parr 4563; Parr Instruments) at 30°C and 400 rpm. Compressed pure gas was continuously sparged into the culture medium at a flow-rate of 1 l $min^{-1}$ (measured at standard conditions of pressure and temperature). The reactor was connected to different bottles containing the pure gases (air, $O_2$, $N_2$ or $CO_2$). The operating pressure was set by manipulation of the pressure of the inlet gas and the regulatory valve position in the exit gas line. The reactor was equipped with a pressure transducer to monitor total internal pressure. The initial yeast concentration was 0.2 g $l^{-1}$. Cell concentration was estimated through optical density (620 nm), previously correlated to dry weight determination. Glucose was measured by the 3,5-dinitrosalicylic acid method. Ethanol was quantified by HPLC. Cell viability was determined through the Methylene Blue staining method and the amount of viable and non-viable cells were estimated using digital image processing. Further details about images acquisition and analysis procedure can be found in Coelho *et al.* (2004) and Coutinho *et al.* (2005).

*Main Results and Discussions:* The effect on cell growth of pressure up to 0.6 MPa strongly depends on the nature of the gas used for pressurization. An increase in air pressure to 0.6 MPa leads to a considerable increase (52%) in cell growth rate. On the other hand, the influence of an oxidative environment determined by a pure oxygen atmosphere of 0.3 MPa or above, drives the cell response in the opposite direction. Furthermore, an increase in oxygen pressure from 0.3 MPa to 0.5 MPa induced a drastic decrease (90%) in cell growth rate. Due to cell inactivation, no ethanol was produced, and glucose remained in the medium at a concentration near to the initial value. Under anaerobic conditions, different effects were also obtained for $N_2$ and $CO_2$ environments. An increase in $N_2$ pressure to 0.6 MPa did not affect cell growth, and slightly enhanced ethanol production. Thus, as far as fermentation process are concerned, the size of the reactor and local variations in pressure due to hydrostatic liquid pressure (to a maximum of 0.6 MPa), should not interfere with process performance if nitrogen is used as sparging gas. On the other hand, a $CO_2$ environment of 0.6 MPa inhibited the fermentation process.

The morphology of the cells seems to be intimately correlated with their physiological state. Since no significant increase in the percentage of bud cells over time was obtained for $O_2$ and $CO_2$ at high pressure, it can be stated that these conditions lead to inhibition of cell division. Moreover, experimental conditions with high substrate consumption rates led to a decrease in bud cell percentage. Under $CO_2$ and $O_2$ at high pressure, the bud cell percentage remained constant until the end of the process, indicating that cells were kept in the lag phase. In spite of the similarity between the effects of high-pressure $O_2$ and $CO_2$ on bud formation, a cell size decrease was found in the case of the final culture exposed to 0.6 MPa $CO_2$.

The cell viability analysis along batch cultivation reveals a complex pattern relating opposite influences of the increase in oxygen content and pressure. In fact, the pressure increase leads to a favorable effect in cell viability until pressures of about 1.0 MPa that can be related to an increment in oxygen availability at high pressure. At pressures higher than 1.0 MPa, the advantages of the increment in oxygen content are offset by the oxidative stress. For anaerobic conditions an increase of $N_2$ pressure did not affect cell viability at pressures up to 0.6 MPa. The modeling approach proposed describes the effects of barometric pressures of different gases (air, $O_2$ or $N_2$) on the loss of cell viability, showing that the common assumption that cell viability is constant during the fermentation may hold in some cases but, in general, is not correct.

## References

Coelho M.A.Z., *et al.* (2004) *Applied Microbiology and Biotechnology*, 66, 318-324, 2004.
Coutinho J.A.P., *et al.* (2005) *Journal of Chem. Technol. & Biotechnol.*, 80, 872–877.

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# Evaluation of mass transfer enhancement in lipase production by *Yarrowia lipolytica* in a multiphase system

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## 1. Summary

An alternative way to overpass the problem of oxygen limitation in aqueous aerobic fermentation is to add a second water-immiscible phase in which oxygen has a greater solubility, such as haemoglobin, hydrocarbons and perfluorocarbons (PFC). The present work shows that perfluorodecalin has a great effect in lipase production from *Yarrowia lipolytica*, with a 23 times increase in lipase productivity with the addition of 20% (v/v) PFC in 1 $dm^3$ flaks agitated at 250 rpm (Amaral *et al.*, 2006). A variation in the PFC volume fraction permitted to observe that the benefit of PFC is maximized at 0.2. The influence of oxygen transfer coefficient ($k_La$) on lipase production from *Y. lipolytica* in the presence of perfluorodecalin was investigated using a 2 $dm^3$ bioreactor. The intrinsic factor determining cell growth and lipase production was oxygen transfer rate (OTR) rather than DO concentration. Improvements in OTR, either by aeration, agitation or PFC concentration, resulted in an increase in lipase yield.

Keywords: *Yarrowia lipolytica*, mass transfer, perfluorocarbon, oxygen, multiphase system

## 2. Extended Abstract

Perfluorocarbons (PFCs) are petroleum-based compounds stable, chemically inert and the oxygen solubility in PFC's is 10-20 times higher than that in pure water (Riess and Le Blanc, 1982). *Yarrowia lipolytica* is a strictly aerobic organism with ability to provide a wide spectrum of important products into the medium, for example: lipases, citric acid and unicellular proteins. Several works in literature have shown the dependence of lipase production from *Y. lipolytica* (Alonso, 2001) and others microorganism (Chen *et al*., 1999; Gulati *et al.*, 2000) with the amount of oxygen available and it has been proved that the addition of perfluorodecalin in the culture medium also benefits the productivity of this enzyme (Amaral *et al*., 2006).

*Experimental conditions*: The influence of oxygen transfer coefficient ($k_La$) on lipase production from *Y. lipolytica* in the presence of perfluorodecalin was investigated at 29ºC in a 2 $dm^3$ bench fermenter (New Brunswick Sci. Inc., USA), using 1.0 or 1.5 $dm^3$ medium volume. Stirring speeds of 100-350 rpm and airflow rates (Q) of 0.5-2.0

$dm^3$/min were used. The volumetric coefficient of oxygen transfer was determined by the dynamic gassing out method (Bandyopahyay and Humphrey, 1967).

*Main Results and Discussions*: Figure 1 shows that perfluorocarbon volume fraction ($\phi_v$) influences $k_La$. For both agitation speeds studied, and aeration rate above 1.0 $dm^3$/min the $k_La$ reaches a maximum at 20 % (v/v) PFC. It seems that PFC benefits the oxygen transfer rate until a certain concentration, beyond which it modifies the rheological behavior of the medium increasing its viscosity and, consequently, affecting the oxygen transfer. Additionally, the raise in agitation speed promoted a higher effect of PFC in oxygen transfer.

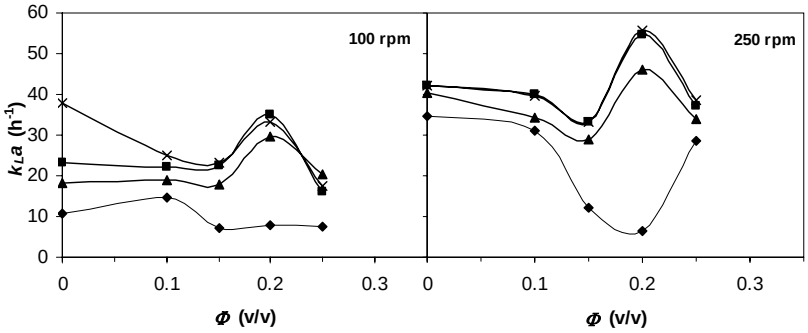


Figure 1: Influence of PFC volume fraction (%) on the $k_La$ of the bioreactor with water, agitated at 100 and 250 rpm, at different aeration rate, $dm^3$/min.: (◆) 0.5; (▲) 1.0; (■) 1.5; (x) 2.0.

The $\phi_v$ of 0.2 was chosen for the production of lipase by *Y. lipolytica* in the same fermenter and kla was measured during *Y. lipolytica* exponential growth. When agitation speed was 250 rpm, $k_La$ raised slightly, also as lipase production (Table 1). As the PFC was not well dispersed at 250 rpm with the cells, the agitation speed was raised to 350 rpm. Table 1 shows that in this condition $k_La$ raised much more with addition of PFC as well as lipase and biomass production. This demonstrates that $k_La$ influences both lipase activity and biomass production.

Table 1: Maximum lipase activity for experiments in 2 $dm^3$ bench fermenter with *Y. lipolytica* and $k_La$ during exponential growth.

| Agitation | 0% PFC | | | 20% PFC | | |
|---|---|---|---|---|---|---|
| | $k_La$ ($h^{-1}$) | Lipase Activity (U/l) | Biomass (mg d.w/ml) | $k_La$ ($h^{-1}$) | Lipase Activity (U/l) | Biomass (mg d.w/ml) |
| 250 rpm | 4.9 | 632 | 9.8 | 6.5 | 1625 | 13.8 |
| 350 rpm | 11.2 | 685 | 11.2 | 26.3 | 5310 | 16.5 |

**References**

Amaral, P.F.F., Rocha-Leão, M.H.M., Marrucho, I.M., Coutinho, J.A.P, Coelho M.A.Z., (2006) *J Chem Technol Biotechnol*, 81, 1368–1374.
Bandyopahyay, B. and Humphrey, A. C., (1967) *Biotechnol Bioeng*, 9, 533-544.
Chen, J., Wen, C. and Chen, T., (1999) *Biotechnol. Bioeng*., v.62, n.3, p.311-315.
Cho, M.H. and Wang, S.S., (1988) *Biotechnol Lett*, 10(12), 855-860.
Elibol, M. and Mavituna, F., (1999) *Biochem Eng J*, 3, 1-7.
Gulati, R., Saxena, R.K. and Gupta, R.., (2000) *Proc. Biochem*., v.36, p.149-155.
Riess, J.G. and Le Blanc, M., (1982) *Pure Appl. Chem.*, v.54, p.2383-2406.

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# Micro-Enzyme-Membrane Reactor – a tool for assessing enzyme activity and stability under continuous reaction conditions

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## 1. Summary

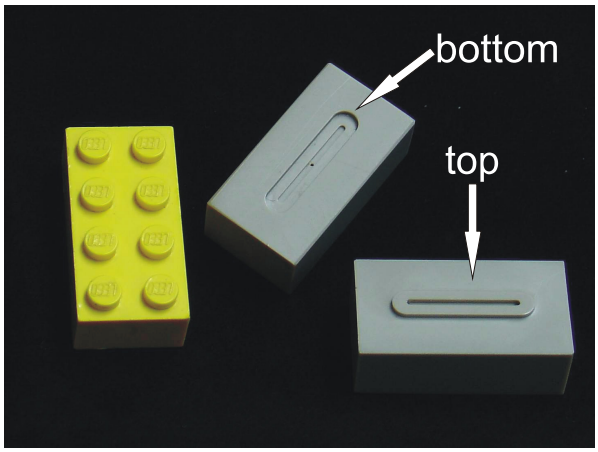
Enzyme testing under continuous conditions is often constrained by the amount of biocatalyst available at early stages of process development. We established the Micro-Enzyme-Membrane Reactor with a reactor volume minimised down from 10mL to 200µl

Keywords: micro reactor, membrane reactor, enzyme catalysis, cofactor generation

## 2. Extended Abstract

The Enzyme-Membrane-Reactor (EMR) has proven to be a reactor concept which is applicable to the continuous production of fine chemicals without the need for laborious immobilisation protocols. However, evaluation of (bio-)catalyst stability under realistic process conditions is often hampered by the limited availability of enzyme and/or starting materials.

Therefore, we developed a Micro-Enzyme-Membrane Reactor (MEMR) with a volume of less than 200µL that allows evaluation of continuous conditions at early stage process development. Following previous set ups a new design with increased membrane area to volume ratio allowing for variable volumes and different flow characteristics was developed.




A range of reactions utilising a variety of different enzymes was carried out and monitored online by chromatography or spectrometry. Among them is the enantioselective reduction of acetophenone to phenylethanol by various alcohol dehydrogenases. Apart from its model character enantiopure phenylethanol is a common intermediate in organic synthesis e.g. fragrances and pharmaceuticals. The enzymes will be compared for enzymatic activity and stability under continuous reaction conditions. Another reaction of current interest is the reductive production of NADH from $NAD^+$ with malate dehydrogenase (MDH). Results will be presented for this new method for cofactor generation and regeneration.

**References**

D. Müller, *Diplomarbeit, FH Aachen* (2005)

D. H. Müller, M. A. Liauw, L. Greiner, Chemical Engineering & Technology, (2005), 12, 1569-1571

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# Steps towards the rational design of an immobilized biocatalyst with improved process stability

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## 1. Summary

The implementation of biocatalysis in large-scale industrial processes is widely seen as a major step towards a greener chemistry. However, many potentially interesting biocatalysts fail to meet the requirements of an industrial process, especially concerning operational stability. Immobilization techniques are widely used to overcome these limitations. The effects of immobilization on activity and stability of the enzymes have, however, rarely been studied in detail. Lacking mechanistic understanding, rational improvement of current immobilization techniques thus remains a distant prospect. We have used D-amino acid oxidase from *Trigonopsis variabilis* (*Tv*DAO) as an example of a biocatalyst of industrial relevance to study and dissect the stabilizing effects in various immobilized enzyme forms. Different immobilization techniques have been used: covalent immobilization of the enzyme on an epoxy-activated support; encapsulation in semipermeable microcapsules; and directed immobilization via an affinity tag. Using a mechanistic model of the inactivation process, we have performed a detailed kinetic analysis of the thermal inactivation of free and immobilized *Tv*DAO forms. This comparative study was complemented by experiments in a miniaturized reactor system under operational and process-near conditions. Dissecting and understanding the various beneficial effects of immobilization on *Tv*DAO stability will provide a mechanistic tool for rational stabilization of this and possibly other industrially important enzymes for improved process performance.

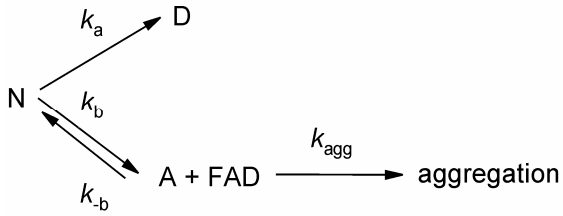
Keywords: biocatalysis, enzyme immobilization, (operational) stability, D-amino acid oxidase

## 2. Extended Abstract

The implementation of biocatalysis in large-scale industrial processes is widely seen as a major step towards a greener chemistry. Biocatalysts offer several advantages as compared to their chemical counterparts and a number of biocatalytic processes has been developed also on large scale. However, many potentially interesting enzymes fail to meet the requirements of an industrial process, especially concerning operational stability. Enzyme immobilization techniques have been used for several decades to overcome these limitations. Immobilization facilitates downstream processing and allows for the repetitive use of the enzyme or its use in continuous reactor systems. Furthermore enzymes are usually stabilized through immobilization. However, these beneficial effects are often overcompensated by the high costs of immobilization and a severe loss in specific enzyme activity. Mass transport phenomena limiting – among other effects – the activity of the biocatalyst as well as the underlying effects of stabilization have clearly been underexplored so far. Lacking mechanistic understanding, rational improvement of current immobilization techniques remains a distant prospect.

We have used D-amino acid oxidase as an example of a biocatalyst of industrial relevance to study and dissect the stabilizing effects in different immobilized enzyme forms. Immobilized D-amino acid oxidase from the yeast *Trigonopsis variabilis* (*Tv*DAO) is used in a multi-ton-per-year biocatalytic process for the conversion of cephalosporin C to 7-amino cephalosporanic acid, a valuable building block for semisynthetic cephalosporin antibiotics. Although *Tv*DAO is considered to be a comparably robust $O_2$-dependent biocatalyst, its operational stability is not fully satisfactory and the overall economics of the above-mentioned process could significantly benefit from an increase in total turnover numbers.

Different immobilization techniques have been used in this study: covalent immobilization of the enzyme on an epoxy-activated support; encapsulation in semipermeable microcapsules [1]; and directed immobilization via an affinity tag [2]. Using a mechanistic model of the inactivation process [3, 4], we have performed a detailed kinetic analysis of the thermal inactivation of free and immobilized *Tv*DAO forms. This comparative study was complemented by experiments in a miniaturized reactor system under operational and process-near conditions. Dissecting and understanding the various beneficial effects of immobilization on *Tv*DAO stability will provide a mechanistic tool for rational stabilization of this and possibly other industrially important enzymes for improved process performance.




Scheme 1: Proposed mechanism of thermal inactivation of *Tv*DAO. Symbols: N, native enzyme; D, (partly) unfolded enzyme that lacks activity but has FAD bound; A, apo-enzyme (inactive). Rate constants describe partial unfolding ($k_a$), reversible cofactor release ($k_b$, $k_{-b}$) and aggregation ($k_{agg}$).

### References

[1] Nahalka J., Dib I., and Nidetzky B., (2007) *Biotechnol Bioeng*, submitted.
[2] Dib I., Stanzer D., Nidetzky B., (2007) *Appl Environ Microbiol*, 73(1), 331-333.
[3] Dib I., Slavica A., Riethorst W., Nidetzky B., (2006) *Biotechnol Bioeng*, 94(4), 645-654.
[4] Slavica A., Dib I., Nidetzky B., (2005) *Appl Environ Microbiol*, 71(12), 8061-8068.

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# Enzymatic production of lactobionic acid: From laboratory to pilot scale

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## 1. Summary

Oxidation of lactose to lactobionic acid by a *Microdochium nivale* carbohydrate oxidase was studied both in small scale and in pilot-scale equipment. The kinetics of the reaction was studied in a 1 L bioreactor at constant pH and temperature. Catalase was included in all reactions to avoid inhibition and deactivation of the oxidase by hydrogen peroxide. At pH 6.4 and 38°C, $K_m$ for lactose was 0.066 mM, $K_m$ for oxygen was 0.97 mM, while the catalytical rate constant, $k_{cat}$, was 94 $s^{-1}$. Surprisingly, we found that the operational stability of the oxidase was dependent on pH of the base used for neutralization. Furthermore, the reaction was studied in a pilot-scale reactor of 600 L working volume using a rotary jet head for mixing and mass transfer (Nordkvist et al., 2003). Compared to mechanically stirred systems, high values of the volumetric mass transfer coefficient, $k_La$, were obtained.

Keywords: lactobionic acid; oxidase kinetics; enzyme deactivation; mixing; mass transfer.

## 2. Extended Abstract

Lactose solution in the form of whey is produced in an annual quantity of 145 million tons of which only approximately 60% is utilized. Consequently, new methods for production of value-added products derived from lactose are desired. One such product is lactobionic acid. Currently, lactobionic acid and its salts are mainly used in high-price specialty products, e.g. as an ingredient in solutions used for organ stabilization. Lactobionic acid (and its salts) has the potential to become a bulk chemical provided that the price becomes competitive with e.g. citric acid. It could be used as a biodegradable builder in washing or cleaning detergents and in various food technology applications, e.g. as an ingredient in pizza-type cheese. Novozymes A/S has recently cloned a carbohydrate oxidase from *Microdochium nivale* and expressed it in *Fusarium venenatum* (Xu et al., 2001). The carbohydrate oxidase can oxidize several mono-, oligo-, and polysaccharides using oxygen as terminal electron acceptor. Oxidation of lactose by this enzyme has been investigated both in laboratory-scale and pilot-scale equipment.

*2.1. Kinetic investigation in laboratory scale*

$K_m$ for lactose, obtained by a traditional enzymatic assay, was very low, 0.066 mM at pH 6.4 and 38°C. The effect of oxygen on the enzymatic rate of reaction and on the operational stability of the enzyme was studied in a 1 L stirred tank reactor with control of pH, temperature, and dissolved oxygen (Nordkvist et al., 2007). Due to the negative influence of the byproduct hydrogen peroxide on the oxidase, catalase was added in excess to remove the byproduct, also providing extra oxygen. The Michaelis constant for oxygen was high (0.97 mM) compared to the solubility of oxygen (0.21 mM) from air at 38°C and 1 bar. The catalytical rate constant, $k_{cat}$, was 94 $s^{-1}$. Surprisingly, the study showed that mixing is very important even in a 1 L bioreactor stirred by 2 Rushton turbines revolving at 1000 RPM. Thus, the oxidase deactivated due to gradients in pH when 2 M NaOH was used for neutralization even though the medium was buffered with 50 mM phosphate buffer. This was not the case when the weak base ammonia was used for neutralization. The results are highly important for applications of potentially pH-sensitive enzymes.

*2.2. Scale-up*

On the basis of the experiments in 1 L scale, experiments were performed in a 600 L reactor equipped with a rotary jet head for mixing and oxygen transfer (Hua et al., 2007). Deactivation could also be avoided in this system when ammonia was used for neutralization. The dissolved oxygen tension was constant throughout the tank for a given set of operating conditions, indicating that liquid mixing was sufficiently good to avoid oxygen gradients in the tank. Relative to mechanically stirred systems, high values of the volumetric mass transfer coefficient were obtained, especially at low values of the specific power input and the superficial gas velocity.

*2.3. Further optimization*

Through further optimization it is expected that lactobionic acid can be produced at a price that is attractive for e.g. the food industry. Currently, inhibition and deactivation of the oxidase by hydrogen peroxide and the effect of hydrogen peroxide on the catalase is under investigation to determine the optimal quantity of catalase used in a given reaction. By the use of rotary jet heads it will be easy to retrofit existing tanks in the food industry for production of lactobionic acid since the rotary jet heads can be installed without welding, without reinforcing the tanks, and without using mechanical seals.

## References

Hua, L., Nordkvist, M., Nielsen, P. M., Villadsen, J. (2007). Scale-up of enzymatic production of lactobionic acid using the rotary jet head system. Biotechnol. Bioeng. (accepted).

Nordkvist, M., Grotkjaer, T., Hummer, J. S., Villadsen, J. (2003). Applying rotary jet heads for mixing and mass transfer in a forced recirculation tank reactor system. Chem. Eng. Sci. **58**:3877-3890.

Nordkvist, M., Nielsen, P. M., Villadsen, J. (2007). Oxidation of lactose tolactobioinic acid by a *Microdochium nivale* carbohydrate oxidase: Kinetics and operational stability. Biotechnol. Bioeng. (accepted).

Xu, F., Golightly, E. J., Fuglsang, C. C., Schneider, P., Duke, K. R., Lam, L., Christensen, S., Brown, K. M., Jørgensen, C. T. & Brown, S. H. (2001). A novel carbohydrate:acceptor oxidoreductase from *Microdochium nivale*. Eur. J. Biochem. **268**: 1136-1142.

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# Cell array preparation in flow-type microchip by using photoresponsive polymer substrate

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## 1. Summary

In order to develop a cell array chip, which enables high throughput assay of pharmacological effect of bioactive substances, we examined a novel layout of micro cell chip, in which living cells were arranged in the prescribed locations in microchannels. For the purpose, we developed a photoresponsive culture substrate on which cell adhering region can be prepared by local light irradiation, by using both a photochromic polymer containing 6-nitrospiropyran, and polyethyleneglycol. Onto the photoresponsive culture substrate, polydimethylsiloxane sheet having four parallel microchannels was attached to construct a flow-type microchip. In each microchannel of the microchip, cell domains of CHO-K1 cells were arranged separately through the local light irradiation and following cell seeding.

Keywords: photoresponsive culture substrate, adhering animal cell, cell array chip, high-throughput assay

## 2. Extended Abstract

In recent years, cell array chips have been studied actively aiming at effective analysis of pharmacologic effect of bioactive substances. In order to make such analysis more efficient, we examined a novel layout of micro cell chip, in which living cells were arranged in the prescribed locations in microchannels. We expect that the micro cell array chip enables multiple *m* (reagents) x *n* (cell species) assay (Fig.1).

For the purpose, we developed a functional culture substrate on which cell

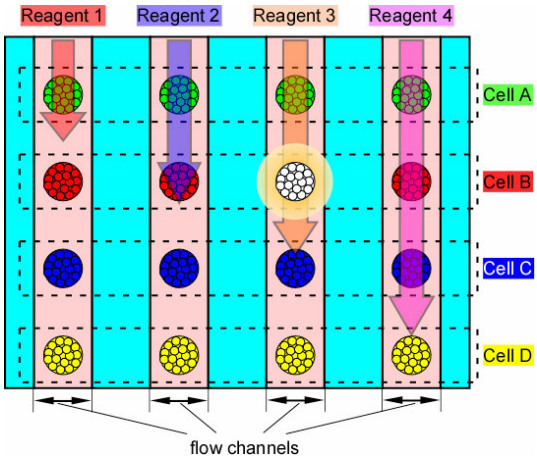


Figure 1 Scheme of multiple (reagents and cell species) assay based on the flow-type cell array chip.

adhering region can be prepared by local light irradiation, by using a photochromic polymer, polymethylmethacrylate modified with 6-nitrospiropyran, and polyethyleneglycol (PEG), which has a strong hindering effect on cell adhesion. When ultraviolet light (UV) with the wavelength of 365 nm was irradiated on the photoresponsive culture substrate in water, PEG was lost through the dissolution into water due to change in the interaction with the photochromic polymer. [1)] In this condition, adhering animal cells can be attached only at the UV-irradiated region (Fig. 2). In addition, we also confirmed that cell adhering region can be appended in the non-adhering region by UV irradiation even after cell seeding. Therefore, this technique enables immobilization of cell domains of different kinds of cells separately through repetitive local light irradiation and following cell seeding.

Onto the photoresponsive culture substrate, we attached a polydimethylsiloxane (PDMS) sheet having four parallel flow-channels (width 400 μm, depth 200 μm), and constructed a channel-type microchip. In each microchannel of the microchip, cell adhering regions were prepared by local UV irradiation and cell suspension of CHO-K1 cells were introduced. The microchip was incubated for 24 h at 37 ºC in 5% $CO_2$ in humidified air. After the incubation, each microchannel was washed evenly by culture medium to remove non-adhering cells. Figure 3 shows that cell domains of CHO-K1 cells were arranged separately in each microchannel. From the results in this study, we expect that the technique enables the construction of cell array chip (Fig. 1) in which different kinds of cells were arranged separately in the microchannels.

## References

1. Tada Y, et al. (2006) *Journal of Applied Polymer Science*, 100, 495-499.

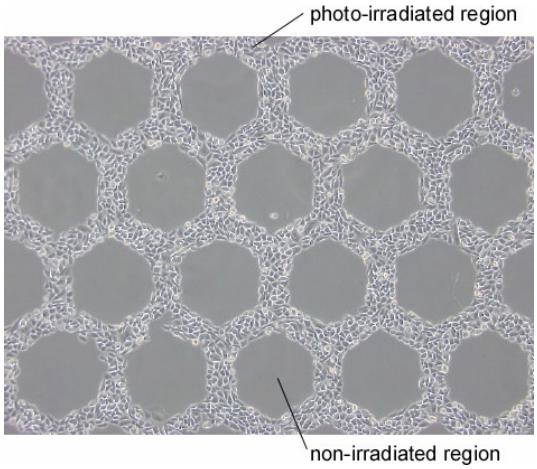


Figure 2 Patterning of CHO-K1 cells based on the photoresponsive culture substrate.

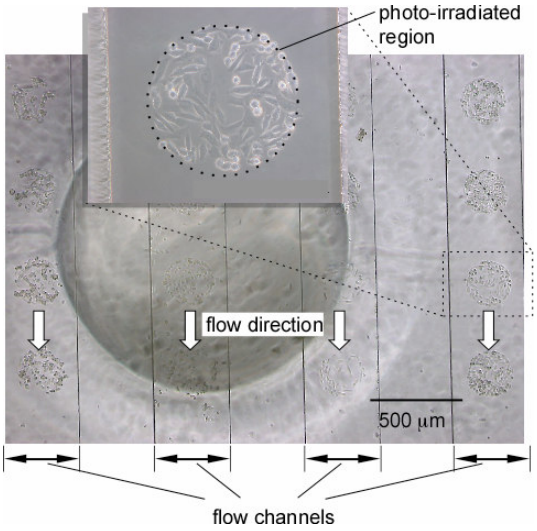


Figure 3 Cell domains of CHO-K1 cells immobilized separately in each flow-channel of the microchip.

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# Session T5-P: Integration of Life Sciences & Engineering - Poster

| Abstract Number | Paper Title & Authors | Included |
|---|---|---|
| 237 | Enzymatic synthesis of lactulose from lactose and fructose by commercial β_ Galactosidase from Klyveromyces lactis<br>F Zokaee A, H Fattahi M, B Bonakdarpoor, S A R Hashemi | Yes |
| 289 | Varied Properties of Hepatitis-Delta Virus-like Particles Produced by Baculovirus-transduced Mammalian Cells<br>Y Chiang, J Wu, K Wang, Y Hu | Yes |
| 333 | Characterization of a biosurfactant produced by psuedomonas fluorescens<br>M Abouseoud, A Yataguene, R Maachi, A Amrane | Yes |
| 838 | Measurement of the activated sludge acclimation in a bi-substrate environment<br>F López, A Abad, M J Palomo, J Navarro-Laboulais | Yes |
| 854 | Kinetics of metabolism during growth of the hydrogen producing bacterium Ruminococcus albus on glucose<br>Ntaikou I, Gavala H N and Lyberatos G | Yes |
| 902 | Biodesulfurization of DBT in model oil by resting cell of Pseudomonas putida CECT5279. process enhancement<br>R A del Aguila, K Boltes, P Leton, A Rodriguez, R Rosal, J A Perdigon and E Garcia-Calvo | Yes |
| 1086 | Product Inhibition of Cellulases during Enzymatic Hydrolysis of the Pre-Treated Ligno-Cellulose<br>P Andrić, K Dam-Johansen, P A Jensen, A Meyer | Yes |
| 1273 | Membrane-Attached Biofilm Reactor Behavior Under Different Flow Velocity for the Treatment of Synthetic Waste Water<br>M González-Brambila, F López-Isunza | Yes |
| 1349 | Dynamic modeling of the acrylic acid synthesis from renewable resources<br>B H Lunelli, R Filho, E C V de Toledo | No |
| 1649 | Characterization of an Extracellular lipase from Yarrowia lipolytica<br>A I S Brígida, P F Amaral, L R Gonçalves, M A. Z Coelho | Yes |
| 1669 | Development of a Horseradish peroxidase-based Flow Injection Amperometric Biosensor for the Determination of Phenolic Compounds<br>S Korkut, M Y Can, E Erhan | Yes |
| 1795 | Glucose Uptake in Electrically Stimulated Cultures of Saccharomyces cerevisiae<br>O Q F Araújo, A A C Oliveira, C O Torres, M H Rocha-Leão, I C P Margarit, M A Z Coelho | Yes |
| 1797 | Fructooligosaccharides production from sucrose by | Yes |

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| | Aspergillus sp. N74 in a hybrid bioreactor<br>S Oscar, G Felipe, G Diana, SEdelberto, C Luis | |
| 1896 | Production of 1,3-propanediol using Klebsiella oxytoca NRTL B-199 growing cells: Medium composition optimization using Taguchi method<br>Galdeano C, Zazo M, Santos V E, Garcia J L, Garcia-Ochoa F | Yes |
| 1902 | Oxygen transfer in small scale animal cell culture reactors: comparison of two reactors by experimental and numerical methods<br>N Barbouche, E Guedon, A Marc, E Olmos | Yes |
| 1908 | Biodesulfurization of Alkylated Dibenzothiophenes using Whole Cells of Pseudomonas putida CECT 5279: Comparison of Substrate Consumption Rates<br>A B Camara, J Calzada, A Alcon, V E Santos, F Garcia-Ochoa | Yes |
| 1945 | Oxygen mass transfer to emulsions in bubble column contactor<br>Gómez-Díaz D b, Gómes N, Teixeira J A, Belo I | Yes |
| 1988 | Continuous enzymatic epoxidation of methyl oleate-Process development and -optimisation<br>F Pontzen, D Herzog, D H Mueller, M A Liauw, L Greiner | Yes |
| 2205 | Oxygen transfer, mixing time and gas holdup characterization in a hybrid bioreactor<br>S Oscar, G Felipe, G Diana, S Edelberto, C Luis | |
| 2300 | Statistical optimization of lipase catalyzed enantioselective production of 1-phenyl 1-propanol by response surface methodology<br>A Soyer, E Bayraktar, Ü Mehmetoglu | Yes |
| 2404 | Polymerization of D,L-lactide and glycolide in supercritical carbon dioxide and in bulk<br>R Mazarro , A Lucas , I Gracia , J F Rodríguez | Yes |
| 2454 | Coupling of Pervaporation system with Fermentation Process<br>S Eslami, A Aroujalian, B Bonakdarpour, A Raeesi | Yes |
| 3137 | Development of Continuous Culture Microbioreactors<br>D Schäpper, KV Gernaey, A E Lantz, S Stocks, N Szita | Yes |
| 3168 | Development of large scale dynamic metabolic model of Penicillium chrysogenum using linlog kinetics<br>I E Nikerel, R M P Blankestijn, W M Gulik, W A Winden, P J T Verheijen, J J Heijnen | Yes |
| 3468 | Optimization of bioreactor performance for the oxidation of ferrous sulfate using Taguchi approach<br>S M Mousavi, A Jafari, S Yaghmaei, M Vossoughi, I Turunen | No |
| 3279 | Using Lie algebra to assess the parameters identifiability and to perform experimental design<br>F P Davidescu, S B Jørgensen | Yes |
| 3336 | Investigating the interactions between nonionizing radiation and living system by studying of the dielectric properties of phantom materials<br>D Acierno, A A Barba, M d'Amore | Yes |
| 3607 | Three-dimensional measurements in the baffle region of a turbulently stirred tank<br>C Galletti, S Pintus, E Brunazzi | Yes |
| 3800 | Toxicity of nanoparticles on mammalian cells<br>B Han, M N Karim | Yes |

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| 3957 | Bubble formation in a forced loop reactor<br>A Fadavi | Yes |
| 4153 | Analysis of the microbial membrane bioreactor working at the reagents separation<br>A T Holownia | Yes |
| 4154 | Thermolysine adsorption on membrane at parallel flow of retantate<br>A T Holownia, A Noworyta | Yes |

Book of Abstracts
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# Enzymatic synthesis of lactulose from lactose and fructose by commercial β_ Galactosidase from *Klyveromyces lactis*

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## 1. Summary

In this research, hydrolysis and transgalactosylation reactions in lactulose synthesis from lactose and fructose by commercial β-galactosidase from *Klyveromyces lactis* were studied. At first stage, lactose and lactulose conversion to the hydrolysis products was investigated. More than 90% of lactose and 75% of lactulose were consumed with addition of 100 µl of enzyme stock solution (3000 U/ml) to the 100g of 5%(w/w) sugar solutions in 40°C and pH=6.7 after 1 and 3hr respectively. Then transgalactosylation reaction for synthesis of lactulose in the presence of lactose as galactosyl donor and fructose as an acceptor was studied. While Lactose concentration was kept constant in 10% (w/w), by varying fructose concentration of 10, 20, 30%(w/w), maximum lactulose yield of 12.2% related to the initial lactose concentration was achieved by adding of 400 µl of enzyme stock solution (3000 U/ml) to the 100g of sugar solution containing 30%(w/w) fructose in 40°C and pH=6.7 after 120 minutes.

Keywords: Lactulose, Enzymatic Synthesis, Transgalactosylation, β-galactosidase, *Klyveromyces lactis*

## 2. Extended Abstract

Lactulose (4-O-β-D-galactopyranosyl-D-fructose) is a synthetic ketose disaccharide that can be obtained from lactose by chemical synthesis. Because lactulose cannot be decomposed by human digestive enzymes, it is classified as an indigestible oligosaccharide. Lactulose specifically promotes the intestinal proliferation of *Bifidobacterium*, which is known to be a very important humanizing factor in infant formula and is added to commercial infant formula products and various milk products as a prebiotic. Lactulose is used in pharmaceuticals as a gentle laxative and to treat hyperammonemia.

Lactulose is currently manufactured by chemical synthesis involving the alkaline isomerization of lactose. The process includes expensive separation and purification steps to remove byproducts. The formation of a considerable percentage of hard-to-separate colored byproducts leads to low lactulose yield. Lactulose is also produced using complex reagents such as aluminate and borate; however, this process is unsatisfactory from an industrial standpoint, due to the need to eliminate the aluminate and borate. To overcome the disadvantages of lactulose production by various types of chemical synthesis, an alternative method such as a biocatalysis process with clean production and easy purification should be developed.

In this research, we have developed a simple system for the enzymatic synthesis of lactulose by commercial β_ galactosidase from *Klyveromyces lactis*. The reaction catalyzes by β_ galactosidase in the presence of lactose as a galactosyl doner and fructose as an acceptor leads to the synthesis of transgalactosylation compound (lactulose) in addition to the hydrolysis products.

At first stage, we have studied the effect of the conditions such as reaction time and enzyme amount for lactose and lactulose conversion to the hydrolysis products. More than 90% of lactose and 75% of lactulose were consumed with addition of 100 µl of enzyme stock solution (3000 U/ml) to the 100g of 5%( w/w) sugar solutions in 40°C and pH=6.7 after 1 and 3hr respectively.

Then we have studied the effect of the reaction time, enzyme amount and acceptor concentration on the lactulose synthesis reaction.

The presence of fructose decreased the kinetics of lactose conversion, so more amount of enzyme solution (400µl) were used for 100g of reaction mixture at 40°C and pH=6.7. While lactose concentration was kept constant in 10% (w/w), by varying fructose concentrations of 10, 20, 30%(w/w), maximum lactulose yield related to the initial lactose concentration was 8.7, 10.1 and 12.2% after 60, 100 and 120 minutes respectively.

## References

Mizota, T., Tamura, Y., Tomita, M., Okonogi, S., "Lactulose as a sugar with physiological significance", Bulletin of the IDF", 212, pp. 69-76, 1987.

Zokaee, F., Kaghazchi, T., Zare, A., Soleimani, M. ,"Isomerization of lactose to lactulose-study and comparison of three catalytic systems", "Process Biochemistry", 37, pp. 629-635. 2002.

Davis, B. G.; Hancock, S. M., "The uses of glycoprocessing enzymes in synthesis". In Carbohydrates; Elsevier Academic Press: London, U.K. pp 385-426, 2003.

Nakkharat, Ph., Kulbe, K. D., Yamabhai, M., Haltrich, D., "Formation of galacto-oligosaccharides during lactose hydrolysis by a novel β-galactosidase from the moderately thermophilic fungus Talaromyces thermophilus". "Biotechnology journal", 1, 2006.

Roy, I., Gupta M. N., "Lactose hydrolysis by Lactozym TM immobilized on cellulose beads in batch and fluidized bed modes", "process Biochemistry", 39, pp. 325-332, 2003.

Petzelbauer, I., Zeleny, R., Reiter, A., Kulbe, K. D., Nidetzky, B., "Development of an ultra-high-temperature process for the enzymatic hydrolysis of lactose: II. Oligosaccharide formation by two thermostable β-glycosidases", "Biotechnology Bioengineering", 69, pp.140-149, 2000.

Vaheri, M., Kauppinen, V.,"The formation of lactulose (4-O- β - galactopyranosylfructose) by β –galactosidase", "Acta Pharmacutica Fennica", 87, pp.75-83, 1978.

Mayer, J., Conrad, J., Klaiber, I., Lutz-What, S., "Enzymatic production and complete nuclear magnetic resonance assignment of the sugar lactulose", "Agricultural and Food Chemistry", 52, pp.6983-6990, 2004

Lee, YJ., Kim, CS., Oh, DK. "Lactulose production by β-galactosidases in permeabilized cells of KluyVeromyces lactis", "Applied Microbiol Biotechnology", 64, pp.787-793, 2004.

Book of Abstracts
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# Varied Properties of Hepatitis-Delta Virus-like Particles Produced by Baculovirus-transduced Mammalian Cells

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## 1. Summary

Hepatitis delta virus-like particles (HDV VLP) may be a promising vaccine candidate against hepatitis delta virus, which causes severe acute liver inflammation or chronic liver diseases. Currently there is no effective vaccine or diagnostic reagent for HDV due to the lack of a proper method to efficiently produce HDV VLP. To mass produce HDV VLP, we constructed recombinant baculoviruses Bac-GD expressing large-hepatitis delta antigen (L-HDAg) and Bac-GS2 expressing hepatitis B surface antigen (HBsAg). In this study, we demonstrated that co-transduction of mammalian cells led to co-expression of both proteins, as well as secretion of HDV VLP. However, we found that the ratios of virus dosages led to changes in VLP properties. A high dosage ratio of Bac-GD to Bac-GS2 led to higher L-HDAg expression relative to HBsAg, which in turn resulted in the incorporation of more L-HDAg into VLP, larger particle size, and larger density. A model correlating the VLP properties and the relative baculovirus dosage is proposed.

Keywords: hepatitis delta virus, baculovirus, virus-like particle, vaccine

## 2. Extended Abstract

In this study, we used Bac-GD expressing large-hepatitis delta antigen (L-HDAg) and Bac-GS2 expressing hepatitis B surface antigen (HBsAg) to co-transduce BHK cells to produce the HDV VLP. We found that the co-transduction led to successful production and secretion of the HDV VLP into the medium. To manipulate the VLP composition, we attempted to vary the relative dosage of both viruses, represented by the relative multiplicity of transduction (MOT). The BHK cells were transduced with Bac-GD and Bac-GS2 at MOT ratios (Bac-GD: Bac-GS2) of 20:10, 5:10 and 2:10. Table 1 shows that as the relative dosage of Bac-GD and Bac-GS2 decreased from

20:10 to 2:10, the relative production of L-HDAg and HBsAg decreased as well, although the total production of both proteins increased. This led to the incorporation of more HBsAg into VLP, as the percentage of L-HDAg to HBsAg (D/B ratio) in the particles decreased. In accordance, the particle size distribution varied from 20-90 nm for MOT 20:10 to 20-50 nm for MOT 2:10, indicating that the particles became smaller in response to the decreased L-HDAg incorporation. This shift in particle composition, further affected the density distribution of the particles. In summary, this study demonstrated that the composition, and hence the properties, of HDV VLPs could be manipulated via the manipulation of relative expression levels of structure proteins.

Table 1 Summary of the VLP properties at different MOT

| MOT ratio (Bac-GD:Bac-GS2) | HBsAg[*1] ($ng/10^7$ cell) | L-HDAg[*2] ($ng/10^7$ cell) | HBsAg VLP[*3] (%) | HDV VLP[*4] (%) | D/B ratio[*5] | Size[*6] (nm) | Density[*7] ($g/cm^3$) |
|---|---|---|---|---|---|---|---|
| 20:10 | 31.3 | 39.4 | 5.8 | 94.2 | 1.25 | 20-90 | 1.27 |
| 5:10 | 71.4 | 53.9 | 10.1 | 89.9 | 0.98 | 20-60 | 1.25 |
| 2:10 | 85.8 | 59.9 | 21.2 | 78.8 | 0.89 | 20-50 | 1.24 |

[*1] HBsAg represents the total HBsAg that was secreted to the extracellular medium for the synthesis of HBsAg particles (22 nm) and HDV VLP.
[*2] L-HDAg represents the total L-HDAg that was secreted to extracellular medium for the synthesis of HDV VLP.
[*3] HBsAg VLP represents the empty particles self-assembled from HBsAg alone with a diameter range of 20-25 nm.
[*4] HDV VLP represents the empty particles that were formed from the interactions of HBsAg and L-HDAg. with a diameter range of 25-90 nm.
[*5] D/B ratio represents the weight ratio of L-HDAg/HBsAg in the HDV VLP.
[*6] Size represents the diameter distribution of all sorts of particles.
[*7] Density represents the average density of VLPs.

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# CHARACTERIZATION OF A BIOSURFACTANT PRODUCED BY *PSEUDOMONAS FLUORESCENS*

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## 1. Summary

The production of a biosurfactant by *Pseudomonas fluorescens* Migula 1895-DSMZ was studied. Cultures conditions involving variations in carbon and nitrogen sources and different C:N ratios were examined at constant temperature and pH, with the aim of increasing productivity in the process. Biosurfactant synthesis was followed by measuring surface tension and emulsifying index E24. The best results were obtained when using olive oil and ammonium nitrate as carbon and nitrogen sources respectively with a C:N ratio of 10.
The production of biosurfactant was growth associated as indicated by the growth and biosurfactant production kinetics. The surface tension was reduced to below 32 dyne/cm and with emulsification index E24 of 65% in 36 to 48 hours . The properties of biosurfactant that was separated by acetone precipitation were investigated. It showed stability during exposure to high temperatures (up to 120 °C for 15 minutes), high salinity (5-10% NaCl) and a wide range of pH (4-9). The biosurfactant had a good foaming, emulsifying and antimicrobial activities. Analysis by biochemical test (rhamnose test) and infrared spectroscopy (FTIR) showed that the biosurfactant was a rhmnolipid-type in nature.

Keywords: Biosurfactant, Production, Factors, Optimization, Characterization.

## 2. Extended Abstract

Some of the advantages of biosurfactants over synthetic ones include lower toxicity, biodegradability, selectivity, specific activity at extreme temperatures, pH and salinity, the possibility of their production through fermentation, their potential applications in environmental protection and management, crude oil recovery, as antimicrobial agents in health care and food processing industries [Banat et al., 2000;

Kosaric, 1992].

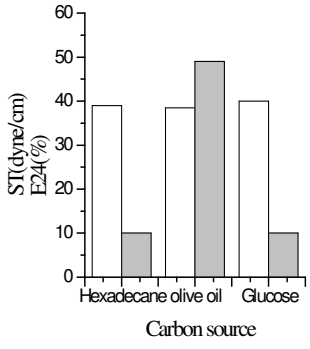


Fig.1: Influence of carbone source on the variation of surface tension ST and emulsification index E24 during biosurfactant synthesis by *Pseudomonas fluorescens* with $NH_4Cl$ as nitrogen source

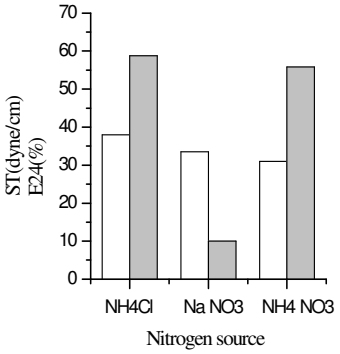


Fig.2: Influence of nitrogen source on the variation of surface tension ST and emulsification index E24 during biosurfactant synthesis by *Pseudomonas fluorescens* with olive oil as carbon source (2% v/v)

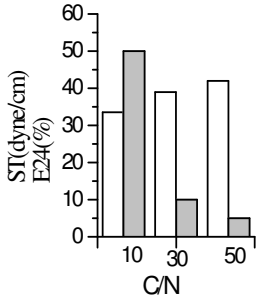


Fig.3: Influence of C/N ratio on the variation of surface tension ST and emulsification index E24 during biosurfactant synthesis by *Pseudomonas fluorescens* with olive oil as carbon source (2% v/v) and $NH_4NO_3$ as nitrogen source

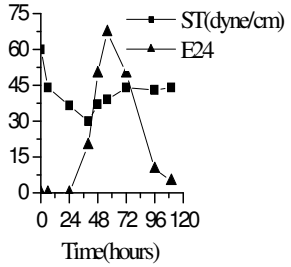


fig.4: Kinetics of biosurfactant production by *Pseudomonas fluorescens*. with optimized media : olive oil(C) 2%; $NH_4No_3$ (N)1g/l; C/N=10

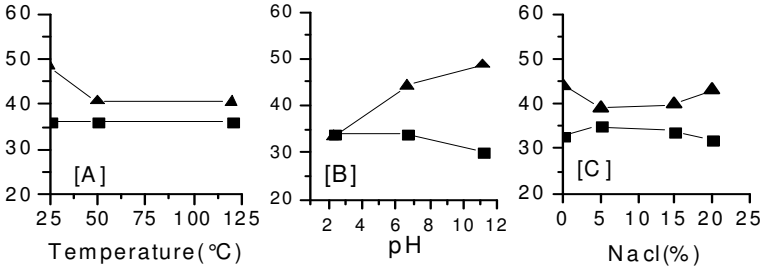


fig.5: Effect of temperature[A], pH[B] and salinity(Nacl=[C] on biosurfactant activity.
Surface tension ST(dyne/cm)
Emulsification index E24(%)

## References

Banat, I.M.; Makkar, R.S. and Cameotra, S.S., (2000). Potential commercial applications of microbial surfactants. *Appl. Microbiol. Biotechnol.*, 53: 495-508

Kosaric, N., (1992). Biosurfactants in industry. *Pure & Appl. Chern.*, Vol. 64, No. 11, pp. 1731-1737

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# Measurement of the activated sludge acclimation in a bi-substrate environment.

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## 1. Summary

Wastewater effluents in WWTP are characterized by their variability with time and substrate complexity. Biological sludge is able to adapt to these changing conditions for organic matter elimination in plant reactors. This work is focused on describing the experimental and data analysis method used to study the biokinetic parameters evolution in microorganisms acclimation [1]. The evolution of biokinetic parameters is done as a function of successive substrate injections in order to study the adaptation process of the biomass to the substrate.

Keywords: acclimation, activated sludge, bi-substrate, repirometric techniques, biokinetic parameters.

## 2. Extended Abstract

The experimental data was obtained with a hybrid respirometer [2], the aerobic reactor is a vessel of 3.8 L coupled to a respiration chamber of 0.1 L. The temperature of the reactors was kept at 20ºC. The dissolved oxygen present in both reactors is continuously recorded by two independent oxymeters and their values acquired by a computer. Biomass, coming from an urban wastewater treatment plant, is acclimated to OECD synthetic wastewater and has been kept constant with ratio $S_0/X_0$ about 0.05 g COD/g TSS. Respirometric measurements with initial single substrate (ethanol, sodium acetate and sodium propionate) and a mixture of two substrates (ethanol+sodium acetate and ethanol+sodium propionate) at different concentrations are carried out.
In order to explain the evolution of the biokinetic parameters, a bi-substrate mathematical model has been proposed for the respirometer experiments. In this model, the dissolved oxygen is used as a state variable [3] and there is also a confidence interval reduction because error in dissolved OD measurement is smaller

than in OUR data. We assume that both substrates elimination is carried out independently by the biomass. In these equations, "1" is applied to aerobic reactor control volume, "2" to respiration chamber and "a" and "b" to utilized substrates. "$S_{ij}$ is substrate concentration and $C_i$ is oxygen concentration.

$$\dot{S}_{1a}(t) = \frac{q}{V_1}(S_{2a} - S_{1a}) - \frac{1}{Y_a}\mu_{\max a}\frac{S_{1a}}{K_{Sa} + S_{1a}}X_0 \qquad S_{a1}(0) = S_{0a}$$

$$\dot{S}_{2a}(t) = \frac{q}{V_2}(S_{1a} - S_{2a}) - \frac{1}{Y_a}\mu_{\max a}\frac{S_{2a}}{K_{Sa} + S_{2a}}X_0 \qquad S_{2a}(0) = 0$$

$$\dot{S}_{1b}(t) = \frac{q}{V_1}(S_{2b} - S_{1b}) - \frac{1}{Y_b}\mu_{\max b}\frac{S_{1b}}{K_{Sb} + S_{1b}}X_0 \qquad S_{1b}(0) = S_{0b}$$

$$\dot{S}_{2b}(t) = \frac{q}{V_2}(S_{1b} - S_{2b}) - \frac{1}{Y_b}\mu_{\max b}\frac{S_{2b}}{K_{Sb} + S_{2b}}X_0 \qquad S_{2b}(0) = 0$$

$$\dot{C}_1(t) = \frac{q}{V_1}(C_2 - C_1) - \frac{1-Y_a}{Y_a}\mu_{\max a}\frac{S_{1a}}{K_{Sa} + S_{1a}}X_0 - \frac{1-Y_b}{Y_b}\mu_{\max b}\frac{S_{1b}}{K_{Sb} + S_{1b}}X_0$$
$$- rO_2\big|_{endo} + k_L a(C^* - C_1) \qquad C_1(0) = C_{sat}^{WW}$$

$$\dot{C}_2(t) = \frac{q}{V_2}(C_1 - C_2) - \frac{1-Y_a}{Y_a}\mu_{\max}\frac{S_{2a}}{K_{Sa} + S_{2a}}X_0 - \frac{1-Y_b}{Y_b}\mu_{\max b}\frac{S_{1b}}{K_{Sb} + S_{1b}}X_0 - rO_2\big|_{endo} \qquad C_2(0) = C_{sat}^{WW}$$

The results for single substrate experiments show a continuous increase of the specific growth rate constant probably due to a change in the enzymatic activity of the cells within the substrate injections. Additionally, the calculated heterotrophic yield remains constant along the experimental series which confirms that the mechanism of the substrate assimilation does not change with the experiments. Finally, the changes observed in the saturation coefficient could be explained considering a modification of the transport properties of the substrate into the biomass floc. When the bi-substrate media is analysed, the calculated biomass yields are similar to the single substrate experiments and the specific growth rate variations are slower than the single ones. As the experiments advance, there is a bigger difference between the experimental values and the proposed model (Fig. 1). These results suggest that the oxygen rate uptakes are not a linear combination of substrate consumption rates.

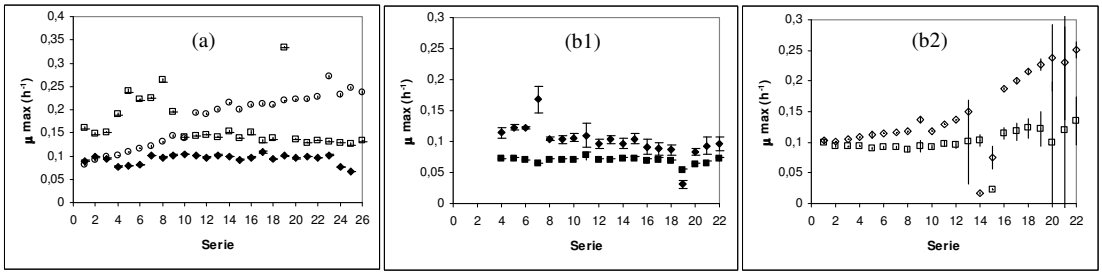


Figure 1: Biokinetic parameters evolution. (a) Single substrate: (o)Ethanol, (♦) Propionate, ( ) Acetate. (b) bi-substrate medium, (b1) (♦)Ehtanol, (■) Acetate. (b2) (◇)Ethanol, ( ) Propionate

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## References

1. Carvalho, G. Novais, J.M., Vanrolleghem, P.A., (2002) *Wat. Sci. Tech.*, 45, 345-353.
2. P.A. Vanrolleghem, (1998) *Wat. Sci. Tech.*, 12, 237-246.
3. Navarro-Laboulais, J., López F., Torregrosa, J.I., Cardona, S.C., Abad, A., (2006) *Journal of Mathematical Chemistry*, On line first. DOI: 10.1007/s10910-006-9153-2

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# Kinetics of metabolism during growth of the hydrogen producing bacterium *Ruminococcus albus* on glucose

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## 1. Summary

Batch and continuous stirred tank reactor (CSTR) experiments were carried out using glucose as carbon source in order estimate the growth and metabolism kinetics of *Ruminococcus albus*, an important hydrogen-producing, fibrolytic bacterium of the rumen. The main metabolic products detected were acetic and formic acid as well as ethanol. The effect of the hydrogen partial pressure ($P_{H2}$) on the yield and degradation rate of formic acid, a metabolite directly associated with the production of hydrogen, was investigated.

Keywords: *Ruminococcus albus*, glucose, hydrogen, kinetics

## 2. Extended Abstract

*Ruminococcus albus* is an important fibrolytic, hydrogen producing bacterium of the rumen (Bryant, 1959), that has been widely studied for its cellulolytic properties (Ohmiya *et al*., 1985, 1987, 1989). However, little is known about the metabolic paths through which $H_2$ is produced from pure cultures of *R. albus*, and about the factors affecting the distribution of its metabolic products. It has already been reported that the amount of hydrogen, and in particular its partial pressure in continuous cultures of *R. albus* may influence the yields of the main metabolic products i.e. acetate and ethanol (Iannoti *et al.*, 1973), but no particular model has been proposed for describing the metabolism of the microorganism. Miller and Wolin (1973) and Pavlostathis *et al*. (1988) reported the presence of formate among the final products of *R. albus* pure cultures as well. Formate is considered to be an important intermediate of *R. albus* metabolism, which as shown from previous experiments degrades to carbon dioxide and $H_2$ (Ntaikou *et al*., 2005). Thus formate degradation kinetics seem to be one of the key factors for the sufficient $H_2$ production.
The aim of the present study was, to estimate the growth kinetics of *R. albus* and to describe its metabolism and the interactions between the metabolic products by simulating the results from batch and CSTR cultures using mathematic equations. In

all cases glucose was used as carbon source. Batch experiments with different initial substrate concentration and different initial hydrogen partial pressure ($P_{H2}$) were carried out in order to investigate substrate inhibition and the effect of $P_{H2}$ to the metabolic shift. Microbial growth was described using Monod kinetics, taking into account the inhibition factor for lower pH values (Batstone *et al*. 2002), as well as the inhibition factor for non-competitive substrate inhibition (Ierusallimsky 1967). Acetate and ethanol production were assumed to occur simultaneously, by direct sugar consumption and the $H_2$ final yield was reversely connected to the accumulation of ethanol. Formate was also considered to be produced by direct sugar consumption, and subsequently to break down to hydrogen and carbon dioxide following first order kinetics. The degradation rate of formate was shown to be strongly influenced by $H_2$ partial pressure. Consequently $H_2$ was considered to be produced during both sugar consumption and formate degradation. Subsequently, the CSTR performance at different hydraulic retention times, at steady state was simulated. The model predicted the CSTR performance quite well.

## References

Batstone D.J., Keller J., Angelidaki I., Kalyuzhnyi S.V., Pavlostathis S.G., Rozzi A., Sanders W.T.M., Siegrist H. and Vavilin V.A. *Anaerobic Digestion Model No 1*, IWA Publishing, UK. (2002)

Bryant M. P. (1959) Bacterial species of the rumen. *Bacteriol. Rev.* 23,125–153.

Dehority B.A. (1973) Hemicellulose degradation by rumen bacteria. *Fed. Proc.* 32, 1819-1825.

Ianotti E.L., Kafkewitz D., Wolin M.J. and Bryant M.P. (1973) Glucose fermentation products of *Ruminococcus albus* grown in continuous culture with *Vibrio succinogenes*: changes caused by interspecies transfer of $H_2$. *J Bacteriol*. 114, 1231-1240.

Ierusalimsky N.D. Bottle-necks in metabolism as growth rate controlling factors. In : Powell E.O. Evans C.G.T., Strange R.E., Tempest D.W. (eds), *Microbial Physiology and continuous culture , 3rd International Symposium.* Her Majestry's Stationery Office, London. (1967)

Miller T. and Wolin M.J. (1973) Formation of hydrogen and formate by *Ruminococcus albus. J Bacteriol.* 116, 836-846.

Ntaikou I, Gavala H.N., Kornaros M. and Lyberatos G. (2005) Hydrogen production from sweet sorghum biomass using *Ruminococcus albus.* Proceedings of the 9th International Conference on Environmental Science & Technology, Rhodes, Greece, 27 August–1 September 2005, 1125-1130

Ohmiya K., Maeda K., and Shimizu S. (1987) Purification and properties of endo-b-1,4-glucanase from *Ruminococcus albus*. *Carbohydr. Res.* 166,145–155.

Ohmiya K., Nagashima K., Kajino T., Goto E., Tsukada A. and Schimizu S. (1988) Cloning of the cellulase gene from *Ruminococcus albus* and its expression in *Escherichia coli*. *Appl. Environ. Microbiol*. 54, 1511–1555.

Ohmiya K., Shiral M., Kurac Y.and Shimizu S. (1985) Isolation and properties of b-glucosidase from *Ruminococcus albus*. *J. Bacteriol.* 161, 432–439.

Pavlostathis S., Miller T. and Wolin M.J. 1988Fermentation of insoluble cellulose by continuous cultures of *Ruminococcus albus. Appl. Env. Microbiol.* 54, 2655-2659.

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# BIODESULFURIZATION OF DBT IN MODEL OIL BY RESTING CELL OF P*seudomonas putida* CECT5279. PROCESS ENHANCEMENT

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## 1. Summary

Biodesulfurization (BDS) can be used as complementary technology to traditional hydrodesulfurization (HDS) process for sulfur reduction in fossil fuel. One of the mayor problems of BDS is the lower desulfurization yield obtained due to the low solubility of organosulfur compounds in water phase where microorganisms survive. In this work, the use of chemical surfactants largely increase the process yield conducted in resting cell operation mode.

Keywords: biodesulfurization, dibenzothiophene, resting cell, surfactant.

## 2. Extended Abstract

Sulfur dioxide emission through fossil fuel combustion is the mayor contributor to the acid rain and air pollution (Monticello, 2000). For environmental protection, the sulfur in diesel oil will have to be lower than 10 ppm by 2010 in European Union and the USA.

By biodesulfurization is possible to remove recalcitrant compounds to conventional hydrodesulfurization, usually heterocyclic organosulfur compounds like dibenzothiophene (DBT) (Rhee et.al., 1998). Several aerobic bacterial strains have been used to selective desulfurization of DBT via the so-called 4S pathway; in witch 2-hydroxybiphenyl (2HBP) is the final product free of sulfur. In this work, biodesulfurization of DBT in a model oil solution were conducted by resting cells of *Pseudomonas putida* CECT 5279, a GMO.

The efficiency of a biodesulfurization largely depends on the bioavailability of the DBT in the aqueous phase, where the microorganism exists. The solubility of DBT, a

highly hydrophobic compound and their capability to be transported into bacterial cells, is probably the rate limiting step of the biodesulfurization process in biphasic liquid medium.

Several assays were conducted to study the effect of the addition of ethanol as co-solvent, and some non ionic surfactant on desulfurization yield. The resting cell reactions were conducted in orbital incubator, at 30 ºC of temperature and 250 rpm of agitation speed. In all cases the liquid medium contains 50 mg/L of DBT dissolved in hexadecane (organic phase) and HEPES buffer at pH 8 (aqueous phase) in 1:1 volume proportion. The biocatalyst was produced by growing in agitated tank bioreactor (Biostat B, Braun) following a standardized protocol (Martin et.al., 2004). Cells was collected by centrifugation in the late logarithmic phase, conserved in saline serum-glycerol 1:1 volume proportion and stored at -80ºC until use. In all desulfurization assays, the biomass concentration was always 15 g/L of dry cell and the reaction time was 24 hours.

Emulsions were separated by centrifugation to determine DBT and resulting products concentration. The organic and aqueous phases were analyzed by HPLC-UV. The results indicate that the addition of ethanol or surfactant mixed o not with the alcohol, increase remarkably the desulfurization yield. Moreover, the polysorbates Tween 20 and Tween 85, can be used without any problems of separation or cell disruption

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**References**

1. Martin, A.B., Alcon, A., Santos, V.E., Garcia-Ochoa, F. (2004). *Energy Fuels*, 18, 851-857.
2. Monticello, D. J., *Curr.Opin. Biotechnol*. 11 (2000) 540-546.
3. Rhee, S.K., Chang, J.H., Chang, Y.K., Chang, H.N. (1998). *App. Environm. Microbiol*. 64, 2327-2331.

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# Product Inhibition of Cellulases during Enzymatic Hydrolysis of the Pre-Treated Ligno-Cellulose

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## 1. Summary

It is known that cellulase enzymes can be inhibited by intermediate or final hydrolysis products, cellobiose and glucose, respectively. The objective of this study is to quantify commercial cellulases product inhibition rates during the enzymatic hydrolysis of ligno-cellulose, at high sugar concentration levels, which are associated with a high dry matter contents.

Keywords: enzymatic hydrolysis, cellulases, product inhibition, ligno-cellulose, bio-reactor

## 2. Extended Abstract

The use of cellulose degrading enzymes (cellulases) for the hydrolysis of ligno-cellulosic biomass, as part of bio-ethanol process, is a promising, but also a very difficult and challenging task. Due to the problems with the inefficient stirring of the highly viscous feed mixture (pre-treated biomass and enzymes), crystalline structure of cellulose (resistant to enzymes action) and enzymes de-activation (by product inhibition, un-productive adsorption to lignin or shear stress), the application of cellulases in hydrolysis reactor is considered inefficient, and gives rise to the high contribution of enzymes costs to the overall bio-ethanol processing costs.
It is known that all three groups of enzymes found in cellulases (cellobiohydrolases, endoglucanases, β-glucosidases) can be inhibited by intermediate or final hydrolysis products, cellobiose and glucose, respectively. In particular, cellobiohydrolases are strongly and β-glucosidases very strongly inhibited by cellobiose, and glucose, respectively.
The consequence of product inhibition is a significant loss in cellulose degrading activity and quantification of cellulase inhibition rate provides a useful tool in the hydrolysis reactor design. In general, there has been a lack of information on inhibition of commercially available enzymes acting on pre-treated ligno-cellulosic substrates, at high sugar concentrations, which are associated with a high dry matter contents.
The objective of this study is to quantify commercial cellulase (Celluclast®1.5L supplemented with Novozym®188) product inhibition rates during the enzymatic

hydrolysis, at high sugar concentration levels (up to 250 g/L), and investigate the possibility of reducing the inhibition. The product inhibition is studied in continuously stirred batch reactor by either addition of pure sugars or by sugar removal from the hydrolysis reactor with a diafiltration membrane. The results are analyzed in terms of glucose and cellobiose concentration and glucose yield, and compared with the same results from continuously stirred batch reactor without added or removed sugars.

The enzymatic hydrolysis experiments are done with wheat straw hydro-thermally pre-treated in 3 steps (60, 180, 195$^{o}$C; 15, 10, 3 min, respectively) in a pilot plant operated by DONG A/S (Elsam). Cellulase activity measurement is done using standard FPU assay. Biomass compositional analysis is performed in accordance with NREL procedure. The reaction is performed at 50$^{o}$C and pH=5, using enzymes Celluclast®1.5L and Novozym®188, produced by Novozymes A\S (enzyme loading 8 FPU/$g_{substrate}$ and 13 CBU/$g_{substrate}$, respectively). The reaction mixture is analyzed for glucose and cellobiose concentration using Dionex HPLC system.

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# Membrane-Attached Biofilm Reactor Behavior Under Different Flow Velocity for the Treatment of Synthetic Waste Water

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## 1. Summary

This study compares three recirculation mass flow rates in a membrane-attached biofilm reactor (MABR), to assess the impact of interfacial mass transfer on the treatment of a synthetic wastewater. A single-tube MABR, connected to a reservoir with full recirculation, was used to measure the reduction of sodium acetate in water by supplying oxygen through the membrane and simultaneously bubbling air to the residual water in the tank. The decreases of substrate and oxygen concentration were measured along the batch operation. A heterogeneous dynamic model using Monod kinetics with dual limitation substrate was employed to predict the observed evolution of substrate and dissolved oxygen concentrations. The model accounts for the counter-diffusion of substrate and oxygen as well as for the bioreaction within the biofilm (BF). It also predicts biomass growth and the production of extra cellular polymers, which in turn causes the BF to growth. Transport and kinetic parameters, estimated from previous experiments (González-Brambila, et al., 2006), were used, and the interfacial mass transport coefficients were estimated specifically for this study, by comparing those calculated from the Blasius´s solution to the boundary layer equations, and other empirical equations. The model successfully predicts concentration measurements for the different sets of experiments, and it was found that the increase of the mass flow rate recirculation in the MARB enhance substrate consumption. Prediction of transient profiles of substrate and oxygen concentration and reaction rates inside the BF along the batch time are presented and discussed.

Keywords: biofilm, mathematical modelling, attached-membrane biofilm reactor, mass transfer coefficient.

## 2. Extended Abstract

The objectives of this work are: 1) to compare three different mass flow rates in a MABR in terms of the substrate consumption for the wastewater treatment; 2) to find a relationship between the bulk liquid velocity and the mass transfer coefficient in the liquid-BF interface; and 3) to make a theoretical study to explain the behavior of an activated sludge BF in a MABR when the flow velocity is changed.
Biofilms are being specifically studied in the treatment of wastewater because they offer a higher biomass concentration than the conventional process used nowadays. The amount of biomass can be up-to ten times that of the conventional activated-sludge reactor process (Casey et al., 1999)

The model employed in this study assumes that the BF is a two phases system: the liquid within pores and channels, and the clusters conformed by extracellular polymers that contain the cells, an that the concentration within the BF is different to that in the bulk external liquid outside of the BF (Lewandowski, et al., 1994). The proposed model describes the convective mass transfer between the bulk liquid and: 1) the BF clusters and 2) the liquid in pores and channels; and also assumes a convective mass transfer between the liquid in pores and channels and the BF clusters. The mass transport within the clusters takes place by diffusion according with Fick´s Law. The microorganisms live in the BF are trapped in the clusters and then the substrates are consumed only in the solid phase of the BF.

In this work the relationship between the bulk liquid velocity and the interfacial mass transfer coefficient was studied, and it was found that the mass transfer coefficient at the bulk liquid-BF interface based on the Blasius´s solution to the boundary layer equations (Schlichting, 1968) provided the best fit. The experimental results were used to estimate the mass transfer coefficient with the pseudo-heterogeneous model and the predicted values of the coefficients found were very similar to those calculated with the Blasius´s equation: $k_l = \frac{1.66 u_\infty}{Re^{1/2} Sc^{2/3}}$.

Figure 1 shows the measured consumption of substrate in the external liquid of the MARB at three different mass flow rates, and the corresponding growth reaction rate predicted with the model inside the BF are shown in Figure 2.

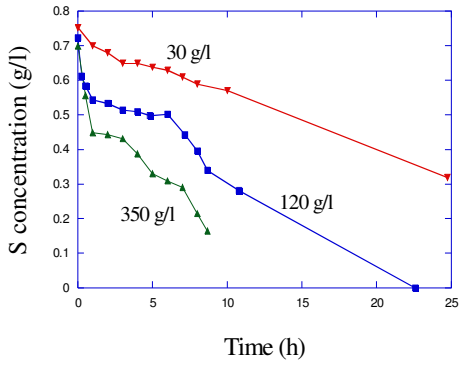


Figure 1. Comparison of substrate consumption for three recirculation fluxes

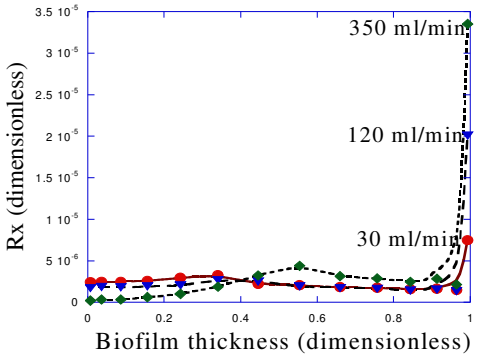


Figure 2. Comparison of Rx inside the BF for three recirculation fluxes.

## References

Casey E, Glennon B, Hamer G. (1999). Biotechnology & Bioengineering, 62, 183-192.
Cussler, E. *Diffusion: mass transfer in fluid systems*. Cambridge University Press, New York, (1989).
Lewandowski, Z., Characklis W.S (1994). Biotechnology & Bioengineering, 38, 877-882.
González-Brambila, M., Monroy, O., López-Isunza, F. (2006). Chemical Engineering Science, 61, 5268-5281.
Schlichting, H., *Boundary layer theory*. McGraw Hill, New York, (1968)

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# Characterization of an Extracellular lipase from *Yarrowia lipolytica*

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## 1. Summary

Studies of an extracellular lipase from *Yarrowia lipolytica* IMUFRJ, produced under submerged fermentation, were realized. The best results of enzyme activity in the p-nitrophenyl laurate hydrolysis were obtained at 37 °C and pH 7. Thermal stability was studied at 25 ºC, 37 ºC and 60 ºC. It became completely inactive after incubation for 15 minutes at 60 ºC but was quite stable at 25 ºC and 37 ºC. The half-lives were 154.37h, 105.34h and 0.058h at 25 ºC, 37 ºC and 60 ºC, respectively. Those results are not very different from the obtained using a commercial *Candida antarctica* lipase B (half-lives of 100.16h for 37 ºC and 0.097h for 60 ºC). Regarding storage stability at -10 ºC, *Y. lipolytica* lipase was very stable, keeping 100% of residual activity after seven months of assay. Considering p-nitrophenyl laurate as substrate, the enzyme exhibited a $K_m$ and $V_{max}$ of 0.207 mM and 0.031 μM/mL/min, respectively.

Keywords: *Yarrowia lipolytica* IMUFRJ, lipase, characterization, p-nitrophenyl laurate, hydrolysis reaction

## 2. Extended Abstract

Lipases (EC 3.1.1.3) are enzymes that catalyze the hydrolysis of ester bonds, especially triglycerides with long chains and water-insoluble substrates, and can be found in animals, plants and microorganisms. In organic media, these enzymes catalyze reactions such as esterification, interesterification and transesterification and, therefore, they have become one of the most widely used enzymes in organic synthesis and various industrial applications (detergent, food, oleochemical, pulp and paper industries and resolution of chiral drugs). Lipases from different sources have distinct properties, such as specificity, stability and optimal operational conditions. Moreover, different conditions of lipase production may alter the enzyme properties, for instance, molecular weight, specificity and isoelectric point. Therefore, the aim of this study was to determine the main characteristics of a lipase produced by *Yarrowia lipolytica* IMUFRJ 50682 (Baía de Guanabara isolated strain, Brazil), under submerged fermentation, due to its great importance for different applications as catalysts in chemical processes.

The enzyme production was carried out in a growth medium with the following composition: peptone (0.64%, w/v), glucose (2%, w/v), yeast extract (1%, w/v) and perfluorodecalin (20%, v/v), according to Amaral *et al.* (2006). Fermentation was carried out for up to 48 h at 25 ºC in a bioreactor maintaining 250 rpm and with a 1.5 $dm^3$/min of oxygen outflow. After 48 h, the cells were separated by centrifugation (5.000 rmp x 15 min) at 10 ºC in a refrigerated centrifuge (SIGMA, 2K15). The supernatant was used for enzyme assay and characterization studies. The activity of lipase was assayed by incubating, at 37 ºC, 0.2 mL of enzyme solution with 1.8 mL of 560 μM p-nitrophenyl laurate (pNP-laurate) dissolved in 50mM potassium-phospate buffer (pH 7.0). The reaction was followed for 100 seconds in a spectrophotometer (HACH, DR/4000U) at $\lambda$= 410 nm.

The extract of lipase produced showed a specific activity at 22.4 U/ g protein. The characterization studies under pH 3-10 (at 37°C) and temperature 25-55 ºC (at pH 7) in the p-nitrophenyl laurate hydrolysis activity revealed that the enzyme is active in a pH range of 7-9, with a maximum lipase activity at pH 7, and between temperatures of 25-55ºC with an optimum temperature for the lipase activity at 37 ºC. These values of optimum pH and temperature were similar to those reported in literature for other *Y. lipolytica* lipases (Corzo and Revah, 1999; Fickers *et al*., 2006).

To determine the lipase stability in *Y. lipolytica* extract, the enzyme was incubated in at 25 ºC, 37 ºC and 60 ºC and the residual activities were measured under standard conditions. It became completely inactive after incubation for 15 minutes at 60 ºC but was quite stable at 25 ºC and 37 ºC. The obtained half-lives were 154.37 h, 105.34 h and 0.058 h at 25 ºC, 37 ºC and 60 ºC, respectively. These results are in agreement with those obtained using a commercial *C. antarctica* lipase B (half-lives of 100.16 h and 0.097 h for 37 ºC and 60 ºC, respectively). Regarding storage stability at -10ºC, *Y. lipolytica* lipase was very stable, keeping 100% of residual activity after seven months of assay.

Finally, the effect of substrate pNP-laurate concentration (0.05 to 3.00 mM) on the initial reaction rates of hydrolysis was assayed by using the standard enzyme activity assay. The experimental data obtained at low substrate concentration obey classical Michaelis-Menten kinetics. For substrates concentrations higher than 1.50 mM, a slight substrate inhibition effect is observed. The Michaelis-Menten constant ($K_m$) and maximum velocity ($V_{max}$) for the reaction with pNP-laurate as substrate were calculated modeling experimental data by Lineweaver Burk plot. The calculated $K_m$ and $V_{max}$ were 0.207 mM and 0.031 μM/mL/min for *Y. lipolytica* lipase. For *C. antarctica* lipase B (CALB), $K_m$ and $V_{max}$ obtained were 0.183 mM and 0.041 μM/mL/min, respectively. Based on $K_m$ values, it can be assumed that *Y. lipolytica* lipase and CALB have similar affinities for pNP-laurate.

## References

Amaral, P. F. F., Vidal, A. P., Peixoto, T., Leão, M. H. R., Coutinho, J. A. P. Coelho, M. A. Z. (2006) *World Journal of Microbiology & Biotechnology*, 23, 339-344.

Corzo, G., Revah, S. (1999) *Bioresource Technology*, 70, 173-180.

Fickers, P, Ongena, M., Destain, J., Weekers, F., Thonart, P. (2006) *Enzyme and Microbial Technology*, 38, 756-759.

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# Development of a Horseradish peroxidase-based Flow Injection Amperometric Biosensor for the Determination of Phenolic Compounds

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## 1. Summary

An amperometric biosensor has been developed for the quantitative estimation of catechol and *p*-benzoquinone in aqueous solution. The enzyme, *horseradish peroxidase* was chemically immobilized on a polyglutaraldehyde modified polypyrolle polymer film, electrochemically prepared onto a glassy carbon electrode. The enzyme electrode system was constructed in a continuous flow cell. The electrode showed a wide linear range for the *p*-benzoquinone between 2.5 -750 µM (r=0.997). Detection limit was found to be 2 µM for *p*-benzoquinone. Optimum hydrogen peroxide concentration and pH for the system were found to be 1.5 mM and 7, respectively. Sensitivities at various flow rates range between 100-200 nA/mM for catechol and 30-100 nA/mM for *p*-benzoquinone. The biosensor response time for each phenolics was found to be 3 s at 1mL/min flow rate. The standard deviation was calculated as $\pm 2.3\times10^{-9}$ for the repetitive analysis of 1 mM *p*-benzoquinone recorded intervals in a day. $K_m^{app}$ values for *p*-benzoquinone and catechol at various flow rates range between 0.077-0.468 mM and 0.112-0.709 mM, respectively. The electrode lost 41 % of its initial activity at the end of 40 th day when it was used everyday.

Keywords: İmmobilized horseradish peroxidase; Flow injection; *p*-benzoquinone; Catechol; Amperometric biosensor; Polypyrrole.

## 2. Extended Abstract

Phenol is used in the production of a large variety of aromatic compounds, pharmaceuticals, fertilizer, paint, textiles, drugs and plastics, at the same time, the compounds have high toxicity to humans when present above certain concentration limits [1]. For phenol determination various spectrometric and chromatographic methods are in common use. Instead of these conventional methods, biosensors could be a cheap and easy alternative, getting increasing attention in the literature [2,3]. Biosensors have recently attracted much interest [4,5,6]. In this study, all electrochemical experiments were performed by using a CHI Model 800B electrochemical analyzer. The flow-injection system comprised a HPLC pump model GBC LC1120, adjusted to deliver carrier buffer (monobasic-dibasic potassium phosphate buffer, 100 mM, pH 7) at a flow of 1 mL/min, an injection valve

(Shimadzu) for sample introduction to the carrier, provided with a 1 mL loop and a flow cell with three electrode system included a glassy carbon electrode used as PGA/PPy electropolymerized working electrode, a Pt wire counter electrode and Ag/AgCl (3M NaCl) reference electrode. Amperometric experiments were conducted at a potential of -50mV.

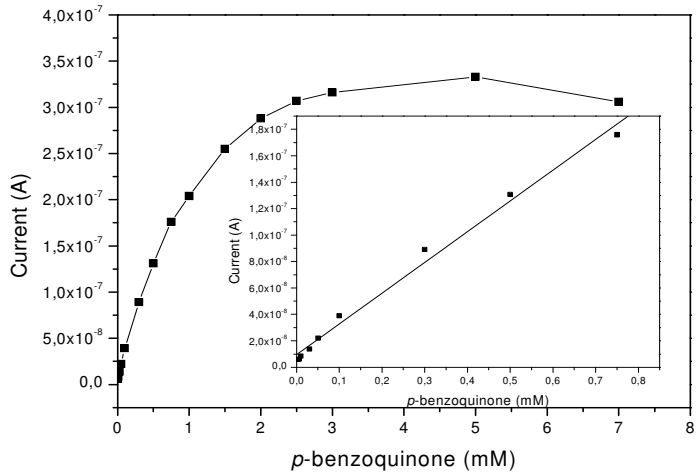


**Fig. 1.** Calibration curve of the biosensor; applied potential, -50 mV. Inset: the linear relationship between amperometric response with concentration of 1.5 mM hydrogen peroxide and various concentrations of *p*-benzoquinone from 2.5 µM to 0.75 mM .

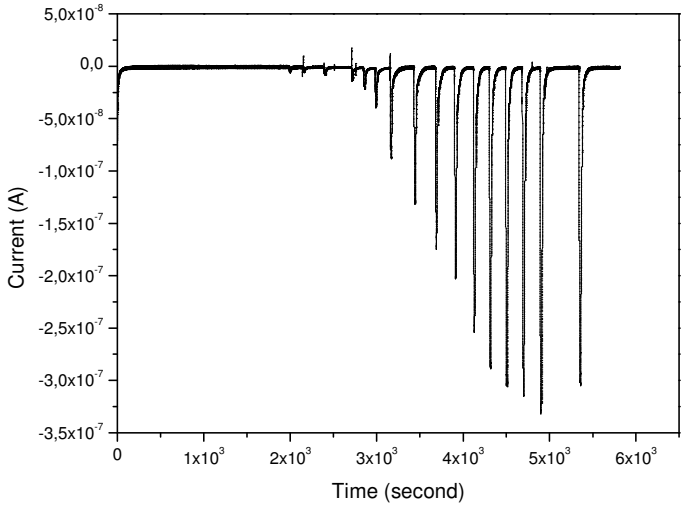


**Fig. 2.** Flow injection peaks at various concentrations of *p*-benzoquinone range between 2.5 µM- 7 mM.

## References

[1] S. Timur, L. D. Sete, N. Pazarlioglu , R. Pilloton, A. Telefoncu, *Process Biochem.* 39 (2004) 1325.
[2] J. Metzger, M. Reiss, W. Hartmeier, *Biosens. Bioelectron*. 13 (1998) 1077.
[3] S. Topçu, M. K. Sezginturk, E. Dinckaya, *Biosens. Bioelectron*. 20 (2004) 592.
[4] G. Chiti, G. Marrazza,, M. Macsini, *Analytica Chimica Acta* 427 (2001) 155.
[5] F. Pérez, I. Tryland, M. Mascini, L. Fiksdal, *Anal. Chim. Acta* 427 (2001) 149.
[6] C. Capannesi, I. Ilaria Palchetti, M. Macsini, A. Parenti, *Food Chemistry* 71 (2000) 553.

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# Glucose Uptake in Electrically Stimulated Cultures of *Saccharomyces cerevisiae*

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## 1. Summary

Glucose uptake stimulation by electric field was investigated in cultures of *Saccharomyces cerevisiae*. Experiments were carried in sequential batches, cut to half of the initial volume when cell growth was approaching the deaceleration phase. Original volume and initial glucose concentration were restored and 0.75 V was applied to the cell suspension, under aerobic and non-aerobic conditions. Growth rate in the second batch increased 20% under non-aerobic conditions as compared to control experiments, and sugar uptake rate increased 100% due to electrical stimulation, in aerated conditions, and 26% increase, under non-aerobic conditions. Results suggest that electrical stimulation may increase the activity of transport enzymes and cell membrane permeation facilitating sugar uptake. Furthermore, applied potential promoted oscillatory behavior in intracellular glycogen concentration superior to the oscillation pattern observed without electrical stimulation, which implies in higher energy and carbon source storage for the cells, showing that these cells are under stress conditions, which lead to robust cellular system.

Keywords: Electric field; yeast; metabolism

## 2. Extended Abstract

The yeast *Saccharomyces cerevisiae* shows rapid fermentation of sugars to ethanol and carbon dioxide. It is widely known that glucose and other sugars exert a repressive effect on the synthesis of a variety of enzymes. On the other hand, glucose may also exert enhancement of enzyme synthesis. The former are mitochondrial enzymes or enzymes linked to cellular respiration and the second are enzymes linked to fermentative metabolism.

The external environment and its changes strongly influence the physiological behavior of yeast. Tight control of the sugar concentration at low threshold values prevents fermentation while high sugar concentrations trigger the catabolite repression, with a decrease of the biomass yield. Of special interest to control metabolic fluxes and direct the production to the aimed application (e.g., biomass or ethanol) the relationship between glucose uptake and cell cycle is of particular interest. Furthermore, mastering ways of stimulating a culture through exogenous variables is interesting in the development of biotechnological processes.

*Experimental conditions:* *Saccharomyces cerevisiae S288C* (ATCC 26108, α GLC mal gal 2) was preserved at 4 $^{o}$C in YPD agar medium. Inocula were grown in YPD (1% yeast extract, 2% peptone and 2% glucose) along 15h at 28 $^{o}$C and 160 rpm. The inocula was introduced into the bioelectrochemical reactor containing modified YPD medium (0.64% peptone, 1% yeast extract, 2% glucose, conductivity 1.74 mS/cm and pH=6.5) in order to obtain an initial cell concentration around 0.75 mg d.w./mL. Experiments were carried using sequential batches with substrate depletion were carried. The batch cut volume was set at 50% of the previous batch final volume and new media was added in order to achieve the total volume and the initial substrate concentration (0.5% glucose). In cell growth experiments, the value of applied potential was 0.75 VSCE, based on Araujo *et al.* (2004).

Measurements of cell density were carried at 570nm. Glucose was measured by glucose oxidase method and glycogen determination followed the method presented in Becker (1978). Samples for morphological analysis were taken at three points in the growth curve: at the initial batch time, within the medium change, and at the final batch time. The cell classification and population morphological analysis were carried by the assessment of budding, non-budding and total cells through the digital image analysis procedure described by Coelho *et al.* (2004).

*Main Results and Discussions:* Table 1 presents the obtained values for the maximum specific growth rate (μ) and for the glucose consumption (-dS/dt) in the second batch for both experiments, with and without aeration. It is possible to notice that the application of electric potential lead to a 20% increase in μ value in those experiments carried without aeration. Considering glucose consumption, different profiles were observed according to the aeration condition: an increase of 100% in (-dS/dt) was found for aerobic condition while only 26% fold could be achieved in the system without aeration.

Table1: Specific growth rate (μ) and glucose consumption (-dS/dt)

| | **μ ($h^{-1}$)** | **-dS/dt (g/L)** |
|---|---|---|
| **With aeration** | | |
| without potential | 0.37 | 0.65 |
| With potential | 0.36 | 1.37 |
| **Without aeration** | | |
| without potencial | 0.19 | 0.95 |
| with potencial | 0.23 | 1.20 |

Glycogen accumulation was enhanced (around 2-fold) by the electrical stimulation triggering a regulatory cell mechanism that induces oscillation. This mechanism balances carbon uptake and catabolic flux, using glycogen as a cell energetic buffer. With respect to the cell duplication, both conditions demonstrated that the potential application can induce a higher number of cells entering in the cell cycle (through scar counting method with the aid of calcofluor staining) as presented in Figure 1. This fact may indicate cell cycle acceleration or lower critical size for duplication and is in accordance to the higher budding cell fraction observed under potential application (Table 2).

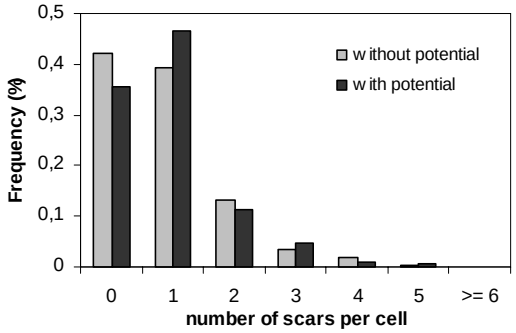


**Table2:** Budding Cell Fraction (%)

| | Batch cut | Final |
|---|---|---|
| **With aeration** | | |
| without potential | 49.5 | 64.6 |
| With potential | 51.2 | 16.1 |
| **Without aeration** | | |
| without potential | 34.5 | 63.5 |
| with potential | 66.2 | 39.1 |

## References

Araujo O.Q.F. *et al.* (2004) *Braz. J. Microb.*, 35, 97-103.
Becker, J.U. (1978) *Anal. Biochem.*, 86: 56.
Coelho M.A.Z., *et al.* (2004) *Applied Microbiology and Biotechnology*, 66, 318-324, 2004.

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# Fructooligosaccharides production from sucrose by *Aspergillus sp.* N74 in a hybrid bioreactor

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## 1. Summary

Batch fructooligosacharides (FOS) production by fructosyltransferase from *Aspergillus sp. N74* using whole cells was studied. The biomass production and enzymatic reaction were carried out in a hybrid reactor (airlift mechanically agitated). To evaluate the fructosyltransferase activity two biomass concentrations (6 and 9.5 g $L^{-1}$) of *Aspergillus sp* N74 mycelia were employed. For each biomass concentration the reaction time for the batch operation was 26h. FOS yield in batch operation for 6 g $L^{-1}$ biomass concentration was 69%, while the highest FOS yield was 76% for 9.5 g $L^{-1}$ biomass concentration in the first 4h of reaction. Results showed the design reactor and the native strain *Aspergillus sp.* N74 as an industrial alternative for the fructooligosaccharides production.

**Keywords:** Fructosyltransferase, Fructooligosccharides, *Aspergillus sp*., Hybrid reactor

## 2. Extended Abstract

### 2.1 Enzymatic Reaction and Analysis

Biomass production and enzymatic reaction were carried out at batch operation in a bench scale hybrid reactor (airlift mechanically agitated) which airlift draft tube was conformed by a stainless steel filtration module with a pore size of 20 μm and a no-conventional geometry. The reactor design allows changing the fungi growth media for the sucrose solution without the biomass exposure to the environment. Two biomass concentrations 6 and 9.5 g $L^{-1}$ of *Apergillus sp.N74* mycelia were employed to evaluate the fructosyltransferase activity. Conditions for the enzymatic reaction were pH 5.5, temperature 60$^{o}$C and initial sucrose concentration 70% (w/v); in the reactor, the superficial air velocity and agitation rate were 0.012 m $s^{-1}$ and 450 rpm respectively, the reaction time was 26 h. Carbohydrates were analysed by HPLC with a refractive index detector (Waters 410), each sample was characterized in two different columns; first by a Sugar-Pak® column for the sucrose, fructose and glucose analysis and then by a Shodex™ column for the fructooligosaccharides analysis.

## 2.2 Results and discussion

The composition of the produced fructooligosaccharides showed a dependence upon the reaction time and biomass concentration (Fig. 1). In fact, 1-kestose and nystose were the FOS produced for the 6 g $L^{-1}$ biomass concentration, while for the 9.5 g $L^{-1}$ biomass concentration was synthesized besides 1-β-fructofuranosyl nystose (FFN) during the reaction. FOS yield in batch operation for 6 g $L^{-1}$ biomass concentration was 69% (43% 1- kestose and 26% nystose), while for the the 9.5 g $L^{-1}$ biomass concentration the highest FOS yield was 76% (43% 1-kestose, 29% nystose and 4% 1-β-fructorianosyl nystose) in the first 4 h of the reaction but at the end of the reaction time (~ 26h) the FOS yield decrease to 57% (18% 1-kestose, 33% nystose and 6% 1-β-fructofuranosyl nystose). This phenomenon could be caused by the enzyme hydrolytic activity over the FOS and its ability to form sucrose from free glucose and fructose (Hirayama *et al.* 1989, Song and Jacques 1999). It is noticed that at 9.5 g $L^{-1}$ biomass concentration the control of the reaction time is very important to get a considerable sucrose bioconversion to FOS and handle their composition.

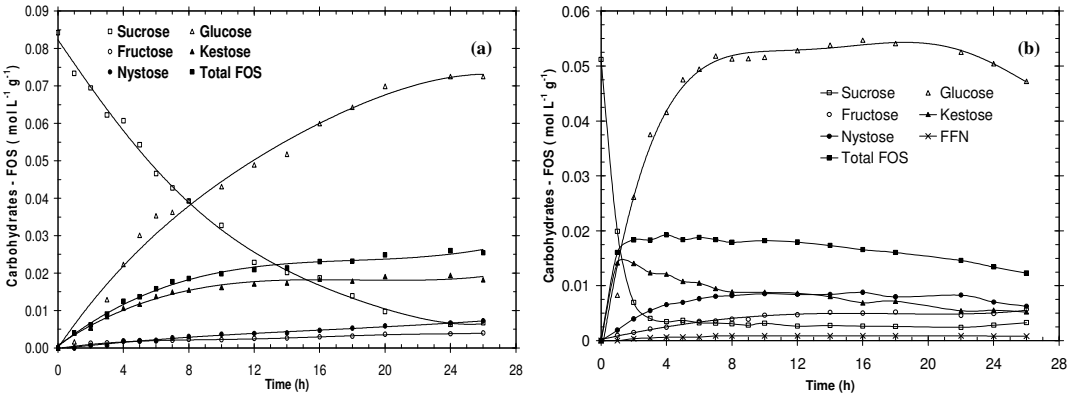


**Figure 1.** Concentration profiles for carbohydrates and FOS gotten with a biomass concentration of 6 (a) and 9.5 g $L^{-1}$ (b).

In conclusion, the designed reactor and fructosyltransferase from the native strain *Aspergillus sp*. N74 can be considered as an industrial alternative for the fructooligosaccharides production, in contrast to reports with *Aspergillus orizae* CFR 202 in a 10-L reactor (Sangeetha *et al.* 2005), with FOS yield of 52% in 18h reaction time under similar conditions (60% sucrose solution, pH 5.5 and 55°C).

### References

Hirayama, M., Sumi, N. and Hidaka, H. (1989). Purification and properties of a fructooligosaccharide-producing β-fructofuranosidase from *Aspergillus niger* ATCC 20611. *Agric. Biol. Chem.* 53: 667–673.

Sangeetha, P.T., Ramesh, M.N. and Prapulla, S.G. (2005), Maximization of fructooligosaccharide production by two stage continuous process and its scales up. *J. Food Eng.* 68, 57-64.

Song, D.D. and Jacques, N.A. (1999). Purification and enzymic properties of the fructosyltransferas of *Streptococcus salivarius* ATCC 25975. *Biochem. J.*, 341:285–291

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# Production of 1,3-propanediol using *Klebsiella oxytoca* NRTL B-199 growing cells: Medium composition optimization using Taguchi method

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## 1. Summary

In this work, the microbial conversion of glycerol to 1,3-PD with *Klebsiella oxytoca* has been studied. The determination and optimization of the medium composition has been carried out using the Taguchi method.

Keywords: glycerol fermentation, 1,3-propanediol, *Klebsiella oxytoca,* medium optimization

## 2. Extended Abstract

***Introduction:*** The recent development of a new polyester called poly (propylenterephtalate), with unique properties for the fiber industry has increased the attention towards the production of 1,3-propanediol (1,3-PD), which has this and other applications in cosmetics, foods, lubricants and medicines.

This compound, 1,3-PD, can be produced by two methods: chemical and biotechnological synthesis. The latter is more attractive because it is carried out using milder operational conditions than the first one and it does not generate toxic by-products. Moreover, the microbial process can use glycerol as substrate, a very suitable compound because it abounds in the market due to the increase of biodiesel production in which it is the main by-product. Glycerol can be naturally fermented into 1,3-PD by bacteria belonging to the genera *Klebsiella, Clostridia, Citrobacter* and *Enterobacter* under anaerobic or microaerobic conditions (Chen et al., 2003). In the biological production of 1,3-PD, several by-products produced (such as acetic acid, ethanol, 2,3-butanediol, etc.) decrease the yield in 1,3-PD.The highest yields are reported when bacteria of genera *Klebsiella* are employed (Huang et al, 2002) .

The aim of this work is to identify the optimal medium composition which maximize both the growth of *K. oxytoca* and the production of 1,3-PD.

***Materials and Methods:*** The microorganism used in this study has been *Klebsiella oxytoca* NRTL B-199.

The Taguchi method was applied to determine and optimize the nutrients requirements, taking into account both responses biomass growth and glycerol utilization into 1,3-PD production. This method facilitates identifying the influence of individual factors by establishing the relationships among variables and operational conditions and finally to establish the performance at the optimum levels obtained with a few experiments predicted by the use of the orthogonal array designs (Taguchi 1986).

The experimental program was designed for four factors (glycerol, phosphate and MgCl concentrations; and the K:Na ratio) at three levels as shown in Table 1 with orthogonal array layout of L9 (nine experimental trials). Experiments were carried out growing *K. oxytoca* at 30 ºC, in an orbital shaker at 250 r.p.m. during 24h. Cell growth was monitored as Optical Density at 600 nm. The concentration of glycerol, 1,3-PD, and the other by-products were determined by HPLC using an Aminex HPX-87H column and a refractive index detector.

| Factor / Level | Glycerol (g/L) | K/Na | $PO_4^{-3}$ (g/L) | Mg (mM) |
|---|---|---|---|---|
| 1 | 20 | 25/75 | 1.5 | 0.5 |
| 2 | 40 | 50/50 | 4.5 | 1 |
| 3 | 80 | 75/25 | 10 | 2 |

Table 1. Factors and levels taken into account by the Taguchi method for the optimization of medium composition

***Results and Conclusions:*** The results obtained after the application of the Taguchi method to the experimental data show a clear influence of the chosen factors on both the biomass growth and the 1,3-PD production. The optimal medium composition finally determined was: 40 g/L glycerol, 50:50 K:Na, 4,5 g/L $PO_4^{3-}$ and 1mM de MgCl

The glycerol, 1,3-PD and biomass concentration evolutions employing the cited medium are shown in Figure 1.

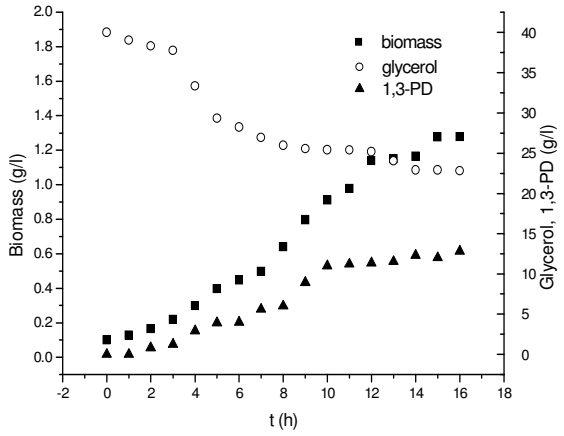


Figure 1.Time course experimental results obtained employing the optimized medium composition.

## References

- Chen, X.; Xiu, Z; Wang, J.; Zhang, D; Xu, P., (2003). *Enz. Microb. Technol.* **33**, 386-394.
- Huang, H.; Gong, S.Cheng; Tsao, G.T. (2002) *Appl. Biochem. Biotechnol.* **98-100**, 687-691.
- Taguchi, G. "Introduction to Quality Engineering", (1986). Ed. Asian Prod. Org., Tokyo, Japan.

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# Oxygen transfer in small scale animal cell culture reactors: comparison of two reactors by experimental and numerical methods

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## 1. Summary

Scale-up of animal cell culture reactors for the production of therapeutic molecules is still a major scientific challenge. Indeed, a strong coupling exists between aeration efficiency, hydrodynamic stress, cellular responses and mixing performance. This study is dedicated to the measurement of oxygen transfer coefficients in two small scale animal cell culture reactors. The results are then confronted to numerical simulations (VOF) for a better understanding of the complex phenomena encountered in these systems.

Keywords: animal cell culture, bioreactor, aeration, CFD, oxygen transfer

## 2. Extended Abstract

Nowadays, animal cells such as Chinese Hamster Ovary cells are widely used as biocatalysts for the production of therapeutic molecules (monoclonal antibodies, recombinant proteins). Large-scale culture of these cells is performed in bioreactors whose volume keeps increasing. Shear stress distributions and volumetric oxygen transfer coefficient ($k_La$) are two key parameters for a successful scale-up of these reactors. This scale-up is complex as these parameters are strongly coupled (an increase in agitation rate promotes oxygen transfer but may be lethal to the cells). Moreover only few studies dedicated to true cell culture reactors and none to oxygen transfer in spinner flasks are found in the literature. One of our previous studies (Barbouche et al., 2007) has shown the positive effects of hydrodynamic stress on the quantity of cells produced, as long as these ones remain lower than a critical value. Nevertheless, it is necessary to quantify the contribution of the simultaneous improvement of the oxygen transfer to clearly establish a correlation between cell behaviour and reactor hydrodynamics. To do this, experimental methods of $k_La$ measurements have been combined with numerical simulations for the prediction of the $k_La$ evolution with the agitation rate and with the culture system used.

Two reactors have been studied. A 250 mL spinner flask and a 1.4 L agitated and sparged bioreactor. For the first one, a surface aeration is encountered; for the second system, both surface aeration and gas sparging coexist. The liquids studied are PBS and Protein-Free-BDM culture medium. No oxygen probe can be placed in the spinner flask so the $k_La$ is measured by the sulphites method. In the reactor, a Mettler-Toledo oxygen probe allows the use of the dynamic method. The agitation rates respectively vary from 40 to 300 rpm and from 80 to 1000 rpm. When only surface aeration is encountered, Computational Fluid Dynamics (CFD) consisting in a Volume Of Fluid approach (VOF) are used to calculate precisely the evolution of the free surface shape when the agitation rates are increased. As no gas sparging is considered in the calculations, the Euler-Euler approach is not provided. Shape deformations, and consequently the interfacial area $a$, are compared and linked to $k_La$ experimental results. Moreover, the simulations provide the volumetric and surface turbulent energy dissipation rate, which are helpful for the knowledge of surface and bulk turbulence.

Concerning the spinner flask, our results show that, under a critical agitation rate of 160 rpm approximately, no changes of $k_La$ are observed. Beyond this value, the free surface becomes wavy and a central vortex exists. The oxygen transfer is then appreciably improved. This is confirmed by our VOF numerical simulations which precisely predict this transition of the free surface from a quiet to a wavier aspect. For the reactor, gas sparging efficiency can rapidly be surpassed by surface aeration if agitation rates become higher than 150 rpm. Indeed, with these conditions, oxygen bubbles are transferred to the liquid bulk by surface turbulence. Once again, our VOF simulations allow the predictions of this transition from a non-interpenetrating two-phase flow to a bubbly gas-liquid flow.

At last, our study establishes an original and generalized correlation for surface aeration which links the volumetric oxygen transfer coefficient to the turbulent energy dissipation rate whatever the reactor design. This correlation will be useful for a better knowledge and a better description of the complex interactions between cell physiology and reactor hydrodynamics.

## References

Barbouche, N., Olmos, E., Guedon, E., Marc, A.,(2007). Coupling between cell kinetics and CFD to establish a physio-hydrodynamic correlation in various stirred culture systems. 20th meeting of the European Society of Animal Cell Technology, June 17-20, Dresden, Germany.

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# Biodesulfurization of Alkylated Dibenzothiophenes using Whole Cells of *Pseudomonas putida* CECT 5279: Comparison of Substrate Consumption Rates

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## 1. Summary

In this work the biodesulfurization of 4 and 4,6-alkyl-derivatives of dibenzothiophene has been studied. The biodesulfurization is carried out using whole cells of a GMO – *Pseudomonas putida* CECT 5279- The variables studied were: the alkyl derivative nature and the cell age; the results show a clear influence of both variables on the substrate consumption rates.

Keywords: biodesulfurization, alkyl-dibenzothiophenes, *Pseudomonas putida*, resting cells

## 2. Extended Abstract

***Introduction:*** Nowadays, the massive consumption of fossil fuels in our society has become an important environmental problem. Therefore, more and more restrictive legal limitations have been imposed in order to control sulphur emissions in combustion processes. Many technologies have been proposed (Babich and Moulijn, 2003), among all these, deep hydrodesulphurization (HDS) has been the most extensively employed (improved catalysts, advanced reactor design, combination with distillation, etc.). However, European Union has fixed for 2009 a maximum sulphur content of 10 p.p.m. in diesel fuel (ED, 2003), this fact demands severe operation conditions if HDS is used, which could affect fuel properties. Since 90's, biodesulfurization (BDS) has been proposed as an alternative technology, which joined with a previous HDS process, can solve these problems. This technique, consisting on employing micro-organisms, both wild and genetically modified (GMO) as catalysts, allows to degrade sulphur aromatic molecules using mild conditions of temperature and pressure, avoiding C-C bond breakdown, and preserving fuel characteristics. Most of BDS studies have used dibenzothiophene (DBT) as model compound. Nevertheless, many of the recalcitrant compounds to HDS are alkyl-DBTs.

The aim of this work is the study of the alkyl-DBTs consumption rates compared to the DBT desulfurization rate under the same conditions.

***Materials and Methods:*** a GMO, *Pseudomonas putida* CECT 5279, has been used as biocatalyst for BDS process. As well as DBT, the substrates employed have been alkyl-DBT compounds (4-methyl and 4, 6-dimethyldibenzothiophene). It is assumed that these alkyl-DBTs are metabolized by means of a similar metabolic pathway to that usually admitted for DBT, called 4S route (Olfield et al., 1997).

The cells employed as biodesulfurizing catalyst were obtained in a 2 L stirred tank bioreactor using a BSM medium (Martin et al., 2004), at 30 ºC, 1 L/L/min of air flow rate and 200 rpm of stirrer speed (Martin et al., 2005). Cells for carrying out BDS resting cells assays were collected at different growth times, corresponding to the different growth phases. BDS resting experiments were conducted in an orbital shaker at 30 ºC and 210 rpm, using 250 mL Erlenmeyer flasks containing 40 mL HEPES medium. Different sulphur substrates (DBT and Me-DBTs) were used alone and combined as binary mixtures. In this last case, the biomass concentration used was doubled because of the double substrate concentration. Biomass was monitored by absorbance measurements at 600 nm. HPLC-UV-diode Array (with a C-8 Supercosil, 3μm, 150 x 4.6 mm column) was employed to analyze the evolution of all intermediates compounds of the 4S route.

***Results and Conclusions:***
Experimental results show that the more alkylated the substrate is, the more difficult the degradation results as it can be observed in the Figure. There is a clear influence of cell age in the substrate consumption rates. Cells with a bigger growth time achieve quicker alkyl-dibenzothiophenes degradation. It was also observed that two isomers forms of Me-HBP (final product of BDS route) are generated through the 4S route.

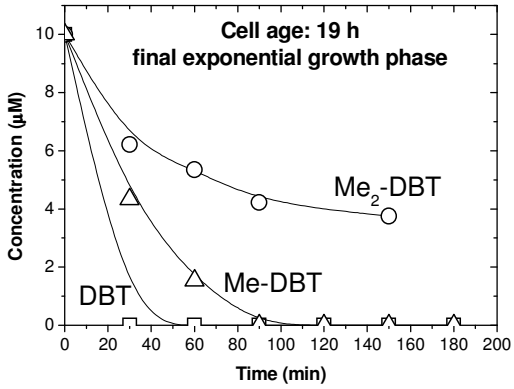


Figure 1: Evolution of the three sulphur substrates.

**References**

Babich, I. V. and Moulijn, J. A. (2003), *Fuel* **82**, 607-631.
European Directive (2003/17/CE).
Olfield, C.; Pogrebinsky, O.; Simmonds, J.; Olson, E. S. and Kulpa, C. F., (1997) *Microbiology* **143**, 2961-2973.
Martin, A. B.; Alcon, A.; Santos, V. E. and Garcia-Ochoa, F. (2004), *Energy & Fuels* **18**, 851-857
Martin, A. B.; Alcon, A.; Santos, V. E. and Garcia-Ochoa, F. (2005), *Energy & Fuels* **19**, 775-782.

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# Oxygen mass transfer to emulsions in bubble column contactor

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## 1. Summary

Present work includes an exhaustive study about the oxygen absorption process by the analysis of volumetric mass transfer coefficient and the corresponding value of interfacial area between gas phase and liquid heterogeneous medium generated in a rectangular bubble contactor. The system studied has been composed by water, methyl ricinoleate and Tween 80, since it is the base of the medium used for an aroma production from the biotransformation of ricinoleic acid by the yeast *Yarrowia lipolytica*.

Keywords: oxygen mass transfer, absorption, bubble column, biphasic systems

## 2. Extended Abstract

Nowadays, the understanding of gas mass transfer by absorption processes when a heterogeneous system is employed as absorbent medium is an important tool with growing interest. Gas-liquid-liquid systems are encountered in many reactions, such as in homogeneous biphasic catalysis and in numerous biotechnological processes based on the development of microorganisms within a biphasic medium (Pulido-Mayoral et al, 2004). Oil-in-water emulsions are found in aerobic culture media where an inert hydrophobic compound is used with the purpose of improving oxygen transfer rate (Amaral et al., 2006) from the gas to the medium, but also in bioprocesses where the oil phase is the substrate of the biological reaction (Aguedo et al, 2005).
The influence of Tween80 presence and concentration upon interfacial area and mass transfer coefficient has been studied and the experimental results indicate that an increase in surfactant concentration in the liquid phase produces a clear increase in the value of bubble diameter that produces a decrease in interfacial area. Also, using the interfacial area values, mass transfer coefficient could be calculated observing a continuous decrease in its value due to the reduction in liquid surface renewal caused by the accumulation of surfactant molecules at gas-liquid interface.
Using the highest Tween80 concentration and adding different quantities of methyl ricinoleate to the liquid phase, the gas-liquid interfacial area has been determined by

the same photographic method previously employed. The experimental results obtained are shown in figure 1. The bubbles diameter distribution for the different liquid phases employed indicates that the presence of methyl ricinoleate in liquid phase produces a decrease in bubbles diameter that is related with a decrease in interfacial area. This behaviour is due to the reduction in the free liquid surface surfactant concentration by the transference to organic medium. This reduction in Tween80 concentration in aqueous medium produces the previously commented increase in bubbles diameter.

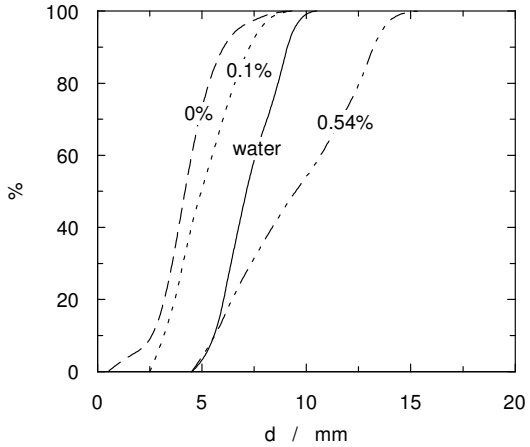


Figure 1. Influence of methyl ricinoleate concentration upon bubble size distribution. Medium section. $Q_g$ = 0.5 L·min$^{-1}$. [Tween80] = 0.093% (v/v).

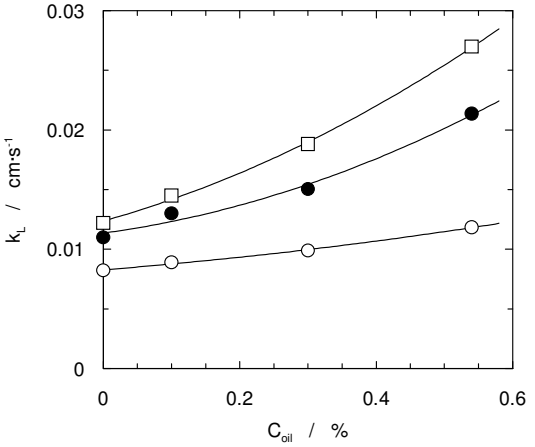


Figure 2. Influence of methyl ricinoleate upon mass transfer coefficient. (○) $Q_g$ = 0.25 L·min$^{-1}$; (●) $Q_g$ = 0.5 L·min$^{-1}$; (□) $Q_g$ = 0.7 L·min$^{-1}$.

The interfacial area values determined employing the bubble size distribution shown in figure 1 were employed to calculate the mass transfer coefficient that inform about the mass transfer process without the influence of interfacial area upon oxygen absorption. Figure 2 shows a resume of obtained mass transfer coefficient data and the influence of organic phase concentration and gas flow-rate. Both variables produce an increase in mass transfer coefficient, in both cases due to the increase in the liquid surface renewal. In the case of gas flow-rate, it produces an increase in turbulence while methyl ricinoleate presence decreases the surfactant surface concentration implying that the reduction in surface renewal is lesser than the corresponding to the system without methyl ricinoleate.

**References**

Pulido-Mayoral, N., Galindo, E. (2004) *Biotechnology Progress*, 20, 1608-1613.

Aguedo, M., Gomes, N., Garcia, E. E., Waché, Y., Mota , M., Teixeira, J. A., Belo I. (2005) *Biotechnology Letters*, 27, 1617-1621.

Amaral P.F.F., Rocha-Leão, M.H.M, Marrucho, I.M., Coutinho, J.A.P., Coelho, M.A.Z. (2006) *J. Chem. Technol. Biotechnol.*, 81:1368–1374.

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# Continuous enzymatic epoxidation of methyl oleate- Process development and -optimisation

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## 1. Summary

In order to establish enzyme catalysis in the field of lubrification based on biodegradable renewable ressources the lipase-catalysed epoxidation of methyl oleate is studied. In contrast to chemical catalysts the used lipase is highly selective. A continuous process without addition of any organic solvent was built up using a fluidized bed reactor. The focus of this work lies on the process optimisation and the improvement of the catalyst performance.

Keywords: enzyme catalysis, process development, lubrification, solvent free

## 2. Extended Abstract

Since the interest in renewable raw materials and biodegradable products is increasing the collaborative research centre (SFB 442 Environmentally Friendly Tribosystems by Suitable Coatings and Fluids) deals with the development and investigation of environmentally friendly high performance tribosystems. For this purpose the derivatives of natural oils and fats have proven to be beneficial in terms of toxicity and biodegradability. [1]

In this context the enzymatic epoxidation of oleic acid methylester (OME) is studied (figure 1). This reaction is the initial step in the synthesis of the low-viscosity lubricant Hydroxy-isobutoxystearic acid methyl ester (HISM).

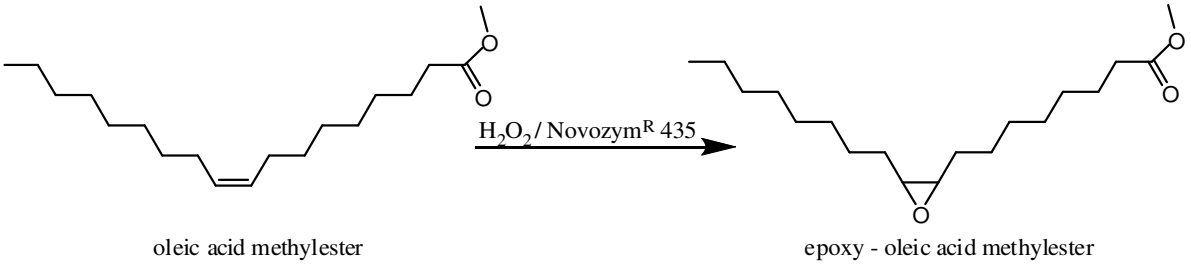


figure 1: enzymatic epoxidation of oleic acid methyl ester

The enzymatic epoxidation is carried out in a multiphase system containing two liquid phases (OME and an aqueous 35% hydrogen peroxide solution) and one solid phase (immobilised enzyme) without the use of any additional organic solvent.
The used catalyst is the polyacrylate-supported lipase B from *Candida antarctica* (commercially available as Novozym 435® from *Novo Nordisk*). [2] In the presented reaction the unsaturated fatty acid ester is converted into the corresponding epoxidised fatty acid ester. This reaction proceeds via the formation of a peroxy fatty acid in situ (known from literature as the enzymatic perhydrolysis of esters with $H_2O_2$ and a lipase). [3],[4]
The research is focused on the development and optimisation of a continuous process and the improvement of the performance of the polymer-supported enzyme. The reaction is carried out in a fluidised bed reactor to improve the mass transfer between the three phases. A scheme of the reactor is shown in figure 2. [5]

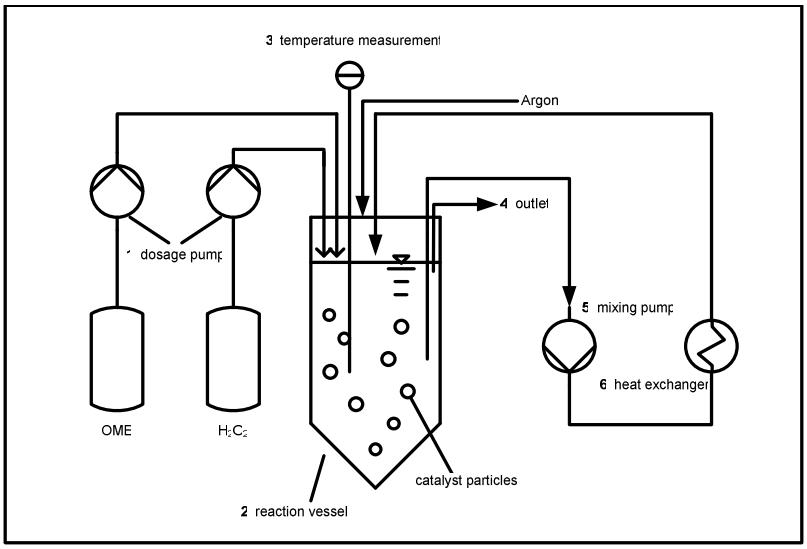


figure 2: scheme of the fluidised bed reactor

In this work the enzyme stability could be increased by an accurate choice of reaction conditions such as amount of hydrogen peroxide and temperature. The observed half life times could be increased from 4 to 188 hours. Results of selected continuous reaction runs will be presented and discussed in terms of productivity, selectivity and catalyst stability.

**References**

[1] Wagner, H.; Luther, R.; Mang, T. and Gassner, T., (2001) *Appl. Catal. A: General*, 221, 429-442.
[2] Björkling, F.; Godtfredsen, S.E. and Kirk, O., (1991) *Trends in Biotechnology*, 9, 360-363.
[3] a) Warwel, S. and Rüsch gen. Klaas, M., (1995) *J. Mol. Cat. B*, 1, 29-35.
b) Rüsch gen. Klaas, M. and Warwel, S., (1997) *J. Mol. Cat. A*, 117, 311-319.
[4] Rüsch gen. Klaas, M. and Warwel, S., *Recent Developments in the Synthesis of Fatty Acid Derivatives*, AOCS Press, Champaign, Illinois (1999).
[5] Pontzen, F., *diploma thesis*, RWTH Aachen (2006).

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# Oxygen transfer, mixing time and gas holdup characterization in a hybrid bioreactor

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## 1. Summary

The global oxygen transfer coefficient ($k_La$), gas holdup (ε) and mixing time were characterized in a hybrid bioreactor (mechanically agitated airlift) using water and culture medium under different agitation and aeration conditions. Correlations for gas holdup and $k_La$ were achieved in the bubbly and coalesced bubble flow, getting predictions within ±15% of maximum deviation. The oxygen transfer and mixing performance was enhanced due to the use of mechanically agitation; however, the oxygen transfer efficiency did not increase for all the agitation and aeration conditions. The designed reactor is shown as a novel alternative for aerobic fermentations in order to get high cell density cultures and for operations where is required to get biomass for further assays without an environment exposure. This bioreactor can be considered as a prototype for the design and hydrodynamic characterization of a larger bioreactor.

Keywords: Bioreactor, Airlift reactor, Mechanically agitated.

## 2. Extended Abstract

### 2.1 Reactor size and measurements

Measurements were made in a concentric agitated airlift reactor with stirrer rates between 0 and 450 rpm and superficial gas velocities (referred to the riser area $U_{Gr}$) between 0 and 0.012 m $s^{-1}$. The draft-tube was conformed by a filtration module in stainless steel with a pore size of 20 μm and an irregular geometry in order to increase the filtration area (area-volume ratio 0.036 $m^2$ $L^{-1}$); it was located 0.05 m above the bottom of the reactor and had an in internal equivalent diameter of 0.09 m and 0.015 m height. Agitation was made with two Rushton turbines, the 6-bladed turbines, 0.075 m in diameter were placed at the bioreactor vessel centerline. 0.050 m was the vertical distance between the impellers and the lower impeller was located 0.085 m from the bottom of the tank. The bioreactor vessel was rounded bottom, 0.17 m in diameter and its overall height was 0.32 m, it was sparged in the concentric zone through a perforated pipe ring sparger (4 holes of 0.0015 m in diameter located on one concentric sparger). The riser and downcomer area ratio (Ar/Ad) was 0.83. For all experiment the static liquid height was 0.22 m. Mixing time, gas holdup and $k_La$ were

determined by the acid tracer technique, the volume expansion method and the dynamic gassing-in method respectively. Each rehearsal was made by triplicate.

## 2.2 Results and discussion

The mechanical agitation may or may not improve the mixing time and the oxygen transfer on both test fluids, nevertheless was observed a decrease in the oxygen transfer efficiency, a similar behavior was reported by Chisti and Jauregui-Haza (2002) in a hybrid reactor with a different impeller and geometrical configuration. The highest oxygen transfer efficiencies were gotten at 100 and 300 rpm with a superficial gas velocity of 0.008 m $s^{-1}$. Although, culture media solids (1.4%$^{W}/_{V}$) decreased up to 20% the global oxygen transfer coefficient, they did not have any incidence over the mixing time. Bubbly flow regime persisted until a gas velocity of <0.008 m $s^{-1}$ for both fluids. At higher aeration rates, the coalesced bubble flow (churn turbulent flow) occurred. For the proposed reactor, correlations for gas holdup ($\varepsilon$) and $k_La$ as function of the agitation speed (N, $s^{-1}$), concentration of solids ($C_s$, $\%^{P}/_{V}$), superficial gas velocity ($U_{Gr}$, m $s^{-1}$) or specific pneumatic power ($P_G/V_L$, W $m^{-3}$) and specific mechanic power ($P_M/V_L$, W $m^{-3}$) were gotten for the bubbly (Ec.1, 3) and coalesced bubble flow (Ec.2, 4), with a $r^2 > 94\%$).

$$\varepsilon = \left[0.00155208 - 5.72964\text{x}10^{-5}\ C_s\right]\frac{P_G}{V_L}^{0.189013 + 0.215991\ N^{0.255007}} \quad (1)$$

$$\varepsilon = \left[0.0288281 - 0.00170792\ C_s\right]\frac{P_G}{V_L}^{-0.132772 - 0.227202\ N^{-0.334161}} \quad (2)$$

$$K_La = \left[-0.966334 + e^{-0.0012331(C_s - 49.4487)} + 61.7874\left[\frac{P_M}{V_L}\right]^{1.10928}\right] U_{Gr}^{\ 0.798673 + 0.0844382\ N} \quad (3)$$

$$K_La = \left[0.000144644 + -2.2671\text{x}10^{-5} C_s + 0.0876241\left[\frac{P_M}{V_L}\right]^{1.12264}\right] U_{Gr}^{\ -0.60065 + 0.09531 N} \quad (4)$$

The theoretical consideration of a $k_La$ plot against the gas holdup ratio $\varepsilon/(1-\varepsilon)$ is expected to be linear in any sparged bioreactor, irrespective of the fluid used and the prevailing flow regime (Chisti, 1989). In the designed hybrid reactor, the dependence between $k_La$ and the holdup ratio for the evaluated stirrer and aeration rates showed to be higher than the unit in the range from 1.3 to 2.6, possibly by the geometry of the designed filtration module. In conclusion, a better oxygen transfer is expected than in a conventional airlift bioreactor, which is an advantage for a high cell density aerobic culture.

## References

Chisti, Y. (1989). Airlift bioreactor. (Elsevier, New York)

Chisti, Y. and Jáuregui-Haza U.J. (2002). Oxygen transfer and mixing in mechanically agitated airlift bioreactor. *Biochem. Eng. J*. 10, 143 -153.

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# Statistical optimization of lipase catalyzed enantioselective production of 1-phenyl 1-propanol by response surface methodology

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## 1. Summary

The optically active 1-phenyl 1-propanol is used as chiral building block and synthetic intermediate in pharmaceutical industries. The aim of this study is to systematically obtain a model of factors that would yield enantiomeric ratio (ee) for resolution of 1-phenyl 1-propanol. Independent variables such as substrate concentration ($x_1$), molar ratio of acyl donor to the substrate ($x_2$), amount of enzyme ($x_3$), temperature ($x_4$) and stirring rate ($x_5$) were optimized using Response Surface Optimization (RSM). The dependent variable selected was ee (Y). Maximum ee was obtained as 91 % at the substrate concentration of 323 mM, a molar ratio of acyl donor to substrate of 0.8, amount of enzyme of 156 mg, temperature of 47$^{o}$C and stirring rate of 166 rpm.
Keywords: Enantioselectivity, 1-phenyl 1-propanol, kinetic resolution, response surface methodology

## 2. Extended Abstract

The different forms of enantiomeric products can cause quite different biological effects. Chiral drugs, agrochemicals, food additives and fragrances are classes of compounds with high economic and scientific potential. Therefore, during the last decade there has been an increased interest in using enzymes for asymmetric synthesis and kinetic resolution to obtain pure enantiomers. Lipases successfully resolve chiral secondary alcohols. Lipase activity and selectivity are strongly influenced by the medium used for desired reaction (Theil 2000).
In this study, the effect of the reaction conditions on the enantioselective transesterification of 1-phenyl 1-propanol catalyzed by Novozyme 435 has been investigated. Izooktan and vinyl laurate have been identified as the best solvent and acyl donor, respectively. Optimization of reaction condition for maximization of enantioselective production of 1-phenyl 1-propanol was studied using RSM.
Conventional methods of optimization, changing one parameter at a time and keeping the other parameters constant are the time consuming methods and do not give information of the mutual interaction. Statistical methods provide an alternative

methodology to optimize a particular process by considering mutual interactions among the variables and give an estimate of the combined effect of these variables on final result (Murthy et al 2000). ee were determined by HPLC on a chiralcel OB-H column. RSM was applied using "Design Expert" software (6.0, Stat-ease Inc., Minneapolis, USA). The substrate concentration (8.34-325 mM), molar ratio of acyl donor to substrate (0.8-3.2), amount of enzyme (40-160 mg), temperature (16-64$^{o}$C) and stirring rate (31-270 rpm) were the critical components of the reaction condition optimized. Experiments were carried out in 10 ml closed vessel with 3 ml working volume. The response surface equation to predict ee % in above range of critical component is

$$y=71.41+7.93x_1-1.37x_2-2.21x_3+3.22x_4+0.80x_5-8.69x_1^2+0.065x_2^2-3.03x_3^2-5.77x_4^2-4.71x_5^2-7.19x_1x_2+5.91x_1x_3+4.55x_1x_4+2.09x_1x_5-1.47x_2x_3+0.66x_2x_4-0.78x_2x_5-1.78x_3x_4-1.84x_3x_5-0.84x_4x_5 \quad (1)$$

The significance of each coefficient was determined by F-value and Prob>F values which are listed in Table 1. Values of Prob>F less than 0.0500 for any factor in analysis of variance (ANOVA) indicates significant effect of corresponding factors on the response. This implies that the first order and quadratic main effects of substrate concentration (Prob>F value<0.0001) are more significant than other effects. Among the interactions, those between substrate and acyl donor/substrate mol ratio ($x_1x_2$) is highly significant while the interactions between substrate and amount of enzyme ($x_1x_3$) also shows some effects on ee.

Table 1.least square fit of the parameter estimates

| Variable | Parameter estimate | F | Prob>F | Variable | Parameter estimate | F | Prob>F |
|---|---|---|---|---|---|---|---|
| Intercept | 71.41 | | < 0.0001 | $x_1x_2$ | -7.19 | 13.95 | 0.0008 |
| $x_1$ | 7.93 | 23.19 | 0.4132 | $x_1x_3$ | 5.91 | 9.50 | 0.0045 |
| $x_2$ | -1.37 | 0.69 | 0.1891 | $x_1x_4$ | 4.55 | 5.59 | 0.0249 |
| $x_3$ | -2.21 | 1.81 | 0.0529 | $x_1x_5$ | 2.09 | 1.19 | 0.2835 |
| $x_4$ | 3.22 | 4.07 | 0.6321 | $x_2x_3$ | -1.47 | 0.59 | 0.4495 |
| $x_5$ | 0.80 | 0.23 | < 0.0001 | $x_2x_4$ | 0.66 | 0.12 | 0.7344 |
| $x_1^2$ | -8.69 | 35.69 | 0.9649 | $x_2x_5$ | -0.78 | 0.17 | 0.6864 |
| $x_2^2$ | 0.065 | 1.97E-003 | 0.0461 | $x_3x_4$ | -1.78 | 0.86 | 0.3602 |
| $x_3^2$ | -3.03 | 4.34 | 0.0004 | $x_3x_5$ | -1.84 | 0.93 | 0.3439 |
| $x_4^2$ | -5.77 | 15.74 | 0.0030 | $x_4x_5$ | -0.84 | 0.19 | 0.6629 |
| $x_5^2$ | -4.71 | 10.49 | | | | | |

Maximum Y (91%) was obtained by employing the optimum reaction conditions which were obtained by solving Eq. (1) using Microsoft Excell Software; a substrate concentration of 323 mM, a molar ratio of acyl donor to substrate of 0.8, amount of enzyme of 156 mg, temperature of 47$^{o}$C and stirring rate of 166 rpm.

## References

Murthy MSRC, Swaminathan SK, Rakshit SK and Kosugi Y. (2000) *Bioprocess Engineerring,*22, 35-39.

Theil, F., (2000) *Tetrahedron,* 56, 2905-2919.

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# Polymerization of D,L-lactide and glycolide in supercritical carbon dioxide and in bulk

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## 1. Summary

The production of bioabsorbable polymeric microparticles for their use in the controlled release of medicines using a new technique based on supercritical technology has been achieved. For this purpose the ring-opening co-polymerization of D,L-lactide and glycolide with zinc (II) 2-ethylhexanoate ($ZnOct_2$) as catalyst was carried out in bulk and in supercritical carbon dioxide ($scCO_2$). The comparison of the experimental data showed the different reaction patterns that the same polymerization reaction follows depending on the conditions, supercritical or in bulk. The obtaining of kinetic parameters in bulk conditions allows us to interpret better the mass transfer issues relating with the polymerization in $scCO_2$.

Keywords: Poly(D,L-lactide-co-glycolide), biodegradable polymers, supercritical carbon dioxide, zinc (II) 2-ethylhexanoate, ring-opening polymerization.

## 2. Extended Abstract

Polyglycolide (PGA), polylactide (PLA) and their co-polymers have been widely used as biodegradable materials in medical applications for years. Nowadays they are receiving special attention as controlled drug delivery material carriers owing to the fact that there are lots of possibilities to control of the polymers biodegradation rate. For medical applications, the presence of toxic substances from the production process in the final product is crucial. Recently, there has been an increasing interest in the synthesis and the processing of these biomaterials using supercritical carbon dioxide as reaction media.

In order to understand the physical nature of the process and consequently to propose the accurate set of equations to describe properly the polymerization it is necessary to know the kinetic of polymerization of both monomers, the reactivity ratios of the monomers and the influence of the initiator using $ZnOct_2$ as catalyst first in bulk conditions and after that in $scCO_2$ varying pressure and temperature.

In figure 1, it is possible to observe how the presence of an initiator improves the polymerization rate and increases the Mw achieved. The lineal dependence of Mw on time at lower reaction times shown in this figure means there are not side reactions.
The reactivity ratios $r_i$ of D,L-lactide and glycolide have been obtained by the Feinemann-Ross equation with $ZnOct_2$ at 130 ºC and with a molar ratio monomers/catalyst of 100. The values obtained have been $r_G$ 4.68 and $r_L$ 0.10 for glycolide and lactide respectively. It means that the active centre of the glycolide prefers almost fifty times the homo-polymerization to the co-polymerization. This could explain that the first samples obtained in bulk polymerization had two different polymer phases, one of these soluble in tetrahydrofuran (THF) enriched in lactide and the other one insoluble enriched in glycolide. In some of the experiments in $scCO_2$ we observed the same different phases but the morphology of the no soluble phase was defined.

The Mw values obtained in $scCO_2$ are always lower than in bulk polymerization as we can see in figure 2. To the same reaction conditions the Mw achieved at 2 hours in bulk is near 60,000 g $mol^{-1}$ while in $scCO_2$ is lower than 7,000 g $mol^{-1}$.

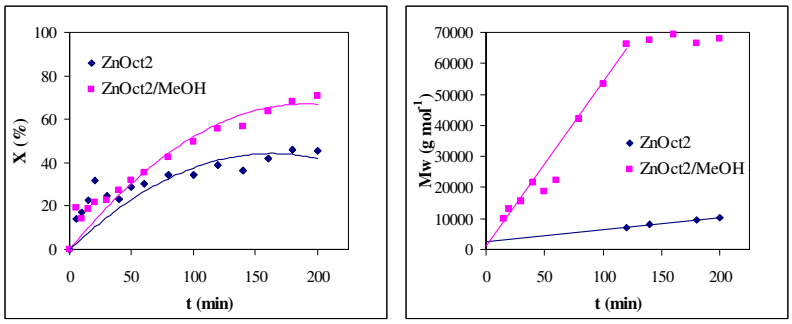


Figure 1: Conversion and Mw as a function of time to bulk polymerization using $ZnOct_2$. Study about the effect of the presence of an initiator. Monomers/catalyst molar ratio: 500, D,L-lactide/glycolide molar ratio: 4, T: 130 ºC.

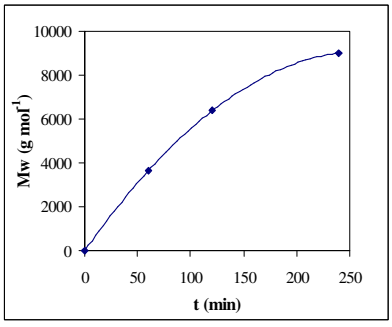


Figure 2: Mw as a function of time to polymerization in $scCO_2$ using $ZnOct_2$ as catalyst. Monomers/catalyst molar ratio: 100, D,L-lactide/glycolide molar ratio: 4, T: 130 ºC.

## References

Mazarro, R., Lucas, A. de, Gracia, I., Rodríguez, J. F., (In press) *J. Biomed. Mater. Res., Part B.*

Asandei, A.D., Erkey, C., Burgess, D.J., Saquing, C., Saha, G. and Zolnik, B.S., (2005) *Mater. Res. Soc. Symp. Proc.*, 845, AA5.7.1- AA5.7.6.

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# Coupling of Pervaporation system with Fermentation Process

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## Abstract

The ethanol fermentation process using beet molasses as the feedstock has been studied. The fermentation process which was coupled with a membrane separation unit (Pervaporation) was compared to a conventional batch process. Ethanol was produced by *Saccharomyces Cerevisiae* ATCC 9763 and recovered by pervaporation system using a composite polydimethylsiloxane (PDMS) membrane with 10 µm thickness and 0.0132 $m^2$ surface area. Initial sugar concentration was adjusted to 60g/l and fermentation lasted for 120 hours.
Sugar consumption, ethanol production, cell growth and also Flux and Selectivity of membrane were measured by a function of time. Sugar conversion, ethanol productivity and cell yield in the coupled system were %94.25, 0.69 $\frac{gr}{lit.hr}$ and 0.107 $\frac{gr\ cell}{gr\ sugar\ utilized}$ respectively. Also average flux and selectivity of 0.204 $\frac{kg}{m^2 \times hr}$ and 7.993 respectively were achieved.

Keywords: Ethanol fermentation, Pervaporation, PDMS, *Saccharomyces Cerevisiae*

**Fig.1 Ethanol production curve**

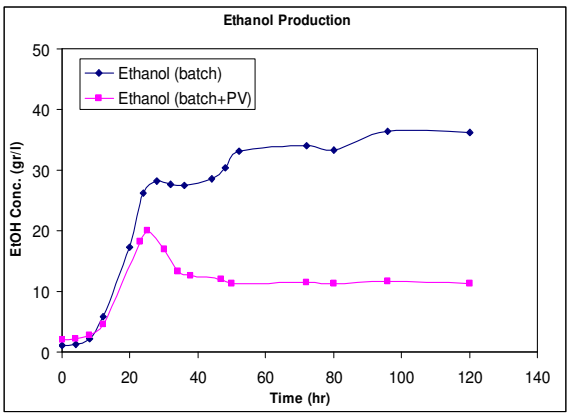



**Fig.2 Sugar consumption curve**

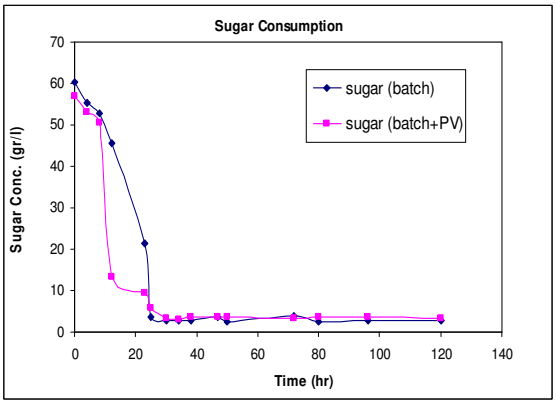



**Fig.3 Cell growth Curve**

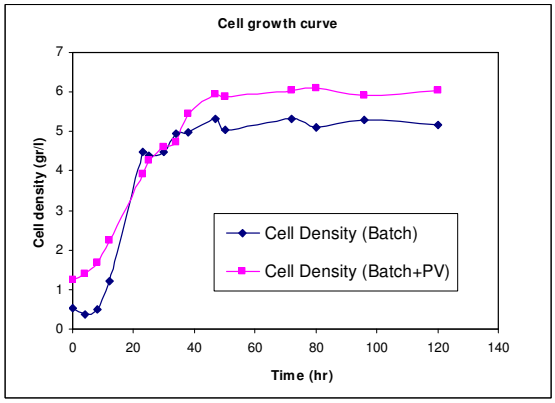
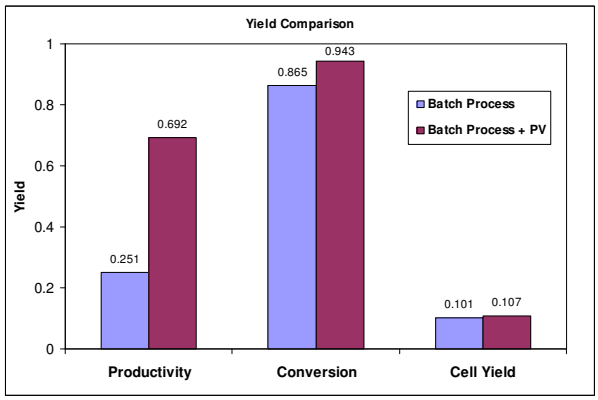



**Fig.4 Yields Comparison**




## References


1. Abdolreza aroujalian, Kaled Belkacemi, Stephen J. Davids, Ginette Turcotte, Yves Pouliot ***" Effect of residual sugars in fermentation broth on Pervaporation flux and selectivity for ethanol"*** Desalination 193 (2006) 103-108
2. Feng Xianshe and Robert Y. M. Huang, ***"Liquid separation by Membrane Pervaporation: A Review"*** Ind. Eng. Chem. Res. 1997, 36, 1048-1066
3. Friendl A. Qureshi N. and Maddox S., ***"Continuous Acetone-Butanol-Ethanol (ABE) Fermentation Using Immobilized Cell of Clostridium acetobutylicum in a Packed Bed Reactor and Integration with Product Removal by Pervaporation",*** Biotechnology and Bioengineering Vol. 38, 518-527 (1991)
4. Kueir- Rarn Lee, Min-Yu teng, Tsung-Neng Hsu, Juin –Yih Lai , ***"A study on Pervaporation of aqueous ethanol solution by modified polyurethane membran***e" , Journal of Membrane Science 162 (1999) 173-180
5. Lewandowska M. , kujawski W. ***" Ethanol Production from lactose in a fermentation/pervaporation system"*** , Journal of Food engineering (2006)
6. Leland M Vane, ***" A Review of Pervaporation for product recovery from biomass fermentation processes"*** Journal og hemical technology and Biotechnology, vol. 80, Issue 6, 603-629
7. Shin-Ichi nakao, Fumiyo Saitoh, Tomoko Asakura, Kiyoshi Toda and Shoji Kimura, "Continuous Ethanol Extraction By Pervaporation From a Membrane bioreactor" Journal of membrane science, 30, 1987, 273-287.

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# Development of Continuous Culture Microbioreactors

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## 1. Summary

Microbioreactors offer a variety of advantages over conventional cultivation techniques which result amongst others in high-throughput screening, lower cost and on-line measurements. A membrane-aerated, polymer-based microbioreactor allowing continuous culture, steady-state cultivations is presented. The reactor has integrated mixing and heating, and allows for on-line measurement of OD, DO, and pH, to monitor yeast (*Saccharomyces cerevisiae*) cultivations. Integrated flow control will enable step changes in the dilution rate to force the culture from one steady-state to the other, thus leading to dynamic information on the behavior of the culture.

Keywords: microbioreactor, continuous culture, *Saccharomyces cerevisiae*, membrane aeration

### 2. Extended Abstract

In industrial fermentation processes, starting up a new production is usually preceded by a tremendous research effort in which for example the productivity of different candidate production strains is compared (=screening). Ranking of strains according to productivity resulting from the screening phase is crucial in selecting strains for full-scale production. Initially, screening is done in microtiter plates or by using shake flask cultures. Experiments are rather easy to set up, but only batch experiments are possible. The information gained per experiment is limited, and typically only end-point measurements are performed. The ranking of strains resulting from these experiments does sometimes not reflect results at industrial scale, for example because these batch type processes are characterized by more extreme forms of

nutrient excess/limitation compared to the fed-batch processes used at larger scales. In a later stage of production process development, experiments are performed in bench scale reactors to investigate the influence of process conditions on productivity. Bench scale reactors (typically with a volume of 1 to 10 L) have the advantage that they allow on-line measurements, and they are flexible since they can be operated in batch or fed-batch, but also as a continuous culture. However, the effort needed to prepare, operate and subsequently clean bench scale reactors is vast.

Microbioreactors offer the possibility to circumvent many of the above-mentioned problems: (1) Scaling out microbioreactors to systems with many parallel reactors allows for high-throughput screening; (2) The working volumes are very small (μL to mL range), keeping costs for culture media low; (3) On-line measurements are possible for the most important culture variables (optical density (OD), dissolved oxygen (DO), pH); (4) The reactors can be fabricated from polymers, thus making them disposable after use which greatly reduces assembly, cleaning and sterilisation efforts, thereby further reducing cost; (5) Finally, for batch type microbioreactors, the fermentation variables compare favorably with bench scale reactors, which indicates that the right culture physiology can be maintained at small vs. larger scale, thus, making the technology commercially viable.

This work reports on the development of a continuous culture microbioreactor platform that can perform experiments with yeast (*Saccharomyces cerevisiae*). Compared to a batch experiment, the continuous culture has the advantage that steady-state conditions can be achieved. Additionally, it should be possible to induce step changes in the dilution rate, forcing the culture from one steady-state to the other with continuous measurement of the important culture variables, thus leading to dynamic information on the behavior of the culture under well-controlled experimental conditions.

The microbioreactor – with a volume of 100 μL – is fabricated out of the polymers poly(methylmethacrylate) (PMMA) and poly(dimethylsiloxane) (PDMS). Dissolved oxygen (DO) and pH are both measured with fluorescent sensor spots. Optical density (OD) is measured in the reactor itself, and in the outflow channel. Contrary to conventional bench-scale reactors, microbioreactors are designed to work bubble-free: aeration is done through a semi-permeable PDMS membrane. The oxygen transfer rates achievable in the microbioreactors are compared with results for bench-scale systems. Proper mixing is essential for good cultivation results in the microbioreactors, since substrate gradients might lead to a varying (location dependent) metabolic state of the culture. In the projected volume range, convective mixing is difficult to achieve due to the small Reynolds numbers. On the other hand, the volume is too large to be able to rely on diffusion alone. Different methods for mixing the reactor contents are currently investigated. Finally, results of *S. cerevisiae* cultivations performed in the microbioreactor will be reported and compared to literature data.

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# Development of large scale dynamic metabolic model of *Penicillium chrysogenum* using linlog kinetics

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## 1. Summary

In an attempt to get more insights on the dynamics of the biological system by making use of large scale mathematical models, this contribution reports our current progress on developing large scale kinetic model of our intensively studied model organism *Penicillium chrysogenum*. To overcome several challenges in the construction of such a large model (e.g. lack of experimental data, pitfalls of the traditional kinetic modeling), we compiled several sources of information (e.g. biological, thermodynamics, reaction kinetics) and reported the stages of this model building procedure. Briefly, we started from the previously published stoichiometric network and after extensive literature and database search, we have postulated the possible effectors for each reaction. To manage the resulting complex network, first we reduced the number of compartments by making the appropriate adjustments on the reactions, and further performed *a priori* model reduction, based on time-scale analysis. We believe such model building framework will create a basis for the systematic analysis of biological systems.

Keywords: large scale kinetic modelling, *Penicillium chrysogenum*, short term dynamic perturbation data, model reduction, linlog kinetics.

## 2. Extended Abstract

Biological systems present a complexity beyond intuitive comprehension and to obtain a better understanding of the behaviour of the living organisms, we now use large scale dynamic mathematical models. From a system biology perspective, these models should not only describe the kinetic behaviour of metabolic reaction networks that feature metabolite-enzyme interactions (allosteric feedback or feed forward), inter-compartmental transport, and cofactor coupling, but, they should also ultimately allow combining several pathways (horizontal modeling) and/or “omic” levels (vertical modeling) in the cell. However, currently by far most of the available models are limited to only one pathway and one “omic” level.

In this work, we present a large scale kinetic metabolic model of *Penicillium chrysogenum* which is intensively studied in our laboratory. Although still focusing on one level (i.e. metabolome), such a model aims encompassing all the major pathways present in the organism. In constructing the kinetic model, we first considered the stoichiometric network presented in van Gulik et al. (2000), which consists of 188 metabolites and 167 reactions located in 3 compartments (cytosol, mitochondria and peroxisome) and postulated a kinetic expression for each of the reactions. We used approximative linlog kinetics for the rate expressions, which allowed us to represent the enzyme-metabolite kinetic interactions by an elasticity matrix. Information on the presence and absence of mass action and allosteric enzyme kinetic information was obtained from literature survey and database search. The final values of the elasticities needed to be estimated by fitting the model to the available short term kinetic response data.

We encountered two major limitations on measurements of metabolites: (1) measurement of a metabolite at all and (2) compartmentation, i.e. measurements of metabolites/reactions that are present in multiple compartments (Nasution et al., 2006). To deal with the compartmentation problem, we reduced the system size to 96 metabolites and 82 reactions within one compartment, by lumping using insights gained both from biochemical knowledge and from data recently published by our group on short term kinetic responses of primary metabolism of Penicillium chrysogenum (Nasution et al., 2006). The limited number of available measurements was dealt by data-driven model reduction while applying the parameter estimation scheme to the large model. To estimate the kinetic parameters, we followed the methodology presented in (Nikerel et al., 2006) in which the theory was applied to a small example system.

We present the results and considerations in reducing the model from 3 to one compartment and from 167 to about 50 reactions. As such, the model describes central metabolism (glycolysis, TCA cycle, pentose phosphate pathway, storage material pathways) amino acid production and nucleotide pathways and product pathways (the biosynthesis of PenG), and represents the time dependent dynamic perturbation data presented in Nasution et al., (2006). From the results, it is concluded that the linlog modeling framework facilitated model reduction and parameter identification of this complex system.

**References**

van Gulik, W.M. and de Laat, W. T. A. M. and Vinke, J. L. and Heijnen, J. J. (2000) *Biotechnology and Bioengineering*, 68(6), 602-618.

Nasution, U. and van Gulik, W.M. and Proell, A. and van Winden, W.A. and Heijnen, J.J. (2006), *Metabolic Engineering*, 8(5), 395-405.

Nikerel, I.E. and van Winden, W.A. and van Gulik W.M. and Heijnen, J. J. (2006) *BMC Bioinformatics*, 7:540.

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# Using Lie algebra to assess the parameters identifiability and to perform experimental design

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## 1. Extended abstract

The increasing interest in producing expensive fine chemicals in the pharmaceutical industry using biochemical synthesis motivates this paper. To develop a purely enzymatic synthesis of complex molecules from inexpensive substrates, large reaction networks are necessary. One way to achieve such a functional network is by using a System of Biotransformations (SBT). The SBT is based on a microorganism's metabolic network containing the synthesis path including cofactor regeneration reactions down to the desired product, which most often is an intermediate in the metabolic network. Expression of the enzymes catalyzing reactions from this intermediate are turned off prior to the extraction i.e the genes are knocked-out. The SBT is used as cell free extract in the production phase. In order to identify the bottlenecks, to describe them qualitatively and subsequently to optimize the productivity of the SBT a dynamic model with good long term predictions properties over the operating window is necessary. Since the SBT is a system of high dynamics and complexity, it may be modeled based upon physical knowledge. In order to validate such models it is, however, necessary to assess the parameter identifiability and to design experiments.
In addressing the identifiability problem one has to determine which input variables should be varied and which outputs to be measured during the experiments in order to be able to render all the model parameters identifiable. In a general setting, one can ask, when all the possible inputs are varied and the possible outputs measured which are the parameters that can be identified. Once the minimal set of inputs and outputs which render the maximum number of parameters identifiable has been chosen, that is, qualitative experimental design has been performed, the next step is to design experiments which aims at improving the statistical quality of the model parameter estimates. During this step the idea is to determine how to manipulate the inputs and to measure the outputs chosen during the previous phase, that is, to do quantitative experimental design.
The identifiability analysis method for qualitative experimental design used in this contribution aims at addressing the theoretical identifiability of the parameters and is based on generating series [1]. First, the original state space description is converted

into a series expansion based on the Lie derivatives where the model output (the measured states) can be expanded in generating series with respect to inputs and time around an initial time [1]. The coefficients of the series are used to form systems of algebraic equations. At a minimum, a system of algebraic equations equal to the number of the parameters occurring in model needs to be formed using the coefficients of the series. The system of equations needs to be solved analytically. If a combination of equations can be solved uniquely for the parameter set, then the set is theoretically identifiable. For qualitative experimental design, more states and perturbation of additional input variables related to the model are included and the analysis repeated.

**References**

1. Walter, E. and Pronzato, L. (1996) *Mathematics and Computers in Simulation*, 42, 125–134

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# Investigating the interactions between nonionizing radiation and living system by studying of the dielectric properties of phantom materials

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## 1. Summary

Many researches have been done to elucidate the interaction between nonionizing electromagnetic fields (EM) and living systems. The protection from electronic devices leakages (mobile antennas, personal computer, industrial and domestic appliances) and, on the other hand, the use of EM in medical applications can be designed or improved only understanding the mechanism(s) of interactions involved. Crucial point of any investigations on any deleterious effects or induced benefits effects is to quantify the specific absorption rate (SAR) of the biological systems, i.e. to study their dielectric behaviour during electromagnetic fields exposure. Currently, the tools used to evaluated the interactions are experimental measurements, by thermographic methods or dielectric spectrometry, and numerical simulation procedures. In many experimental investigations dielectric properties measurements of human tissue are carried out *in vivo* on accessible parts of the body, *in vitro* on freshly excised tissue and in tissues-equivalent materials. Tissues-equivalent or phantom compounds are materials (liquid, solid or semi-solid gel mixtures) with dielectric and thermal properties similar to biological systems. The interactions between tissues and applied EM are studied to evaluate possible biological hazards [1.] as well as to determine useful medical applications such as the heating patterns produced in diathermy and hyperthermia for the treatment of cancer [2.].

Keywords: dielectric properties, phantom materials, electromagnetic fields

## 2. Extended Abstract

### *2.1. Materials and methods*

Formulation of the phantom materials must fulfill some requirements: these have to be easier to obtain than the living tissues which they simulate; their production processes have to be simple and inexpensive; their characteristic have to be known, reproducible and constant.
Recipes and methodologies to formulate and to prepare phantom materials are reported in literature [3.], [4.]. In this work the development of materials which mimic

the behaviour of biological tissues are performed used saline solutions, sugar, polyacrilamide (PAAM), polyethylene powder, hydroxethyl cellulose (HEC) and gel agent (Agar) ingredients.
The interactions between tissues-equivalent and applied EM are determined via dielectric spectroscopy using a network analyzer (HP8753ES, *Agilent Technologies*), equipped with a coaxial probe system (HP85070B, *Agilent Technologies*). The measurements are performed by pressing the open end of the coaxial probe against the samples. Dielectric constant and loss factor are then measured in the frequency range 0.4÷6.0 GHz.

*2.2. Results and discussion*

The first step in the study of interactions between tissues and applied EM is to evaluate the intensity of the interactions (dielectric properties) and then to determine the role of correlated parameters such as frequency, penetration depth, distribution of the absorbed energy. Measurements of dielectric properties (in the range frequency 400 MHz - 6 GHz) of formulated phantom compounds are reported in Fig.1. The EM interaction responses of these latter are compared with dielectric constant of human tissues (from literature data [4.][5.]).

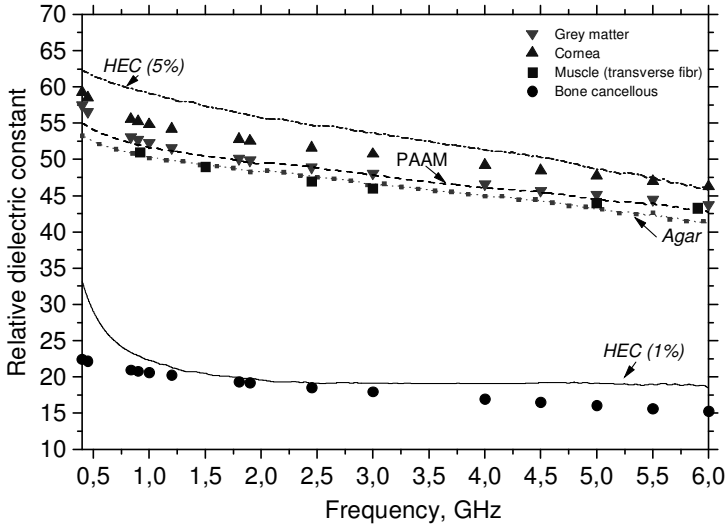


Fig. 1 Comparison between dielectric properties of phantom compounds (curves) and human tissues (grey matter, cornea and bone dielectric constant data from FCC web site http://www.fcc.gov/fcc-bin/dielec.sh; muscle from [4.]).

The EM phantom technology is a powerful tool to study heating of body tissues by nonionizing radiation (radiofrequencies/microwave) energy. As a matter of fact, phantom compounds are composite materials and their behaviour under EM fields can be studied with the classic methods of lossless and lossy dielectric systems. Thus it is possible to simulate human tissues with appropriate models or mixing formula.

## References

1. Adair, E.R., Petersen, R.C., (2002) *IEEE TRANSACTIONS ON MICROWAVE THEORY AND TECHNIQUES*, 50, 3, 953-962
2. Kowalski, M., Behnia, B., Webb A.G., Jin, J..M., (2002) *IEEE TRANSACTIONS ON MICROWAVE THEORY AND TECHNIQUES*, 49, 11, 1229-1241
3. Kainz, W., Alesch, F., Chan, D.D., (2003) *BioMedical Enginering OnLine*, 2:11
4. Neelakanta, P.S., *Handbook of Electromagnetic Materials*, CRC Press Boca Raton FL (1995)
5. Gabriel, S., Lau, R.W., Gabriel C., (1996) *Phys. Med. Biol*., 41, 2251-2269

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# Three-dimensional measurements in the baffle region of a turbulently stirred tank

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## 1. Summary

Three-dimensional laser Doppler Anemometry measurements of the turbulent flow field in the vicinity of the baffles in a vessel agitated with a Rushton turbine have been performed. The measurements have provided simultaneously all three components of the instantaneous velocity, and thus information on all elements of the Reynolds stress and anisotropy tensors in addition to data on flow field and turbulence levels.

Keywords: stirred vessel, baffle, Reynolds stress, turbulence, 3-D laser anemometry

## 2. Extended Abstract

It is well established that the knowledge of the mean flow field is not sufficient for the optimisation of mixing in stirred vessels and substantial information on turbulence characteristics is considered necessary. However, most of the turbulence data available in stirred tanks have focused on the impeller region (Derksen et al., 1999, Escudiè and Linè, 2003) and little information can be found about other regions of the vessel (see also Galletti et al., 2004).
This work presents an experimental investigation of turbulence characteristics both upstream and downstream of the baffles. The literature on baffles in not so exhaustive despite baffles have been confirmed to play a role in mixing which is as important as that of the agitator. Baffles promote mixing by dumping the swirling motion generated by the rotation of the impeller, and effects are substantial as, for instance, the pumping number of a Rushton turbine dramatically decreases by approximately 60% when removing the baffles. In addition recent LES simulations have indicated high values of the dissipation rate of the kinetic energy in the region close to the baffles (Micheletti et al., 2004). However, baffles may have negative effects on mixing especially for viscous fluids because they can promote the formation of dead regions upstream and downstream of them and it is important to evaluate the extent of those regions.

3-dimensional velocity measurements have been performed with a laser Doppler anemometer (LDA) in a vessel stirred with a D/T = 0.33 Rushton turbine. The LDA operated in orthogonal side scattered mode. All the measurements were taken in coincidence mode so that the three instantaneous velocity components were measured simultaneously; such a 3-D configuration ensures high spatial resolution and it is the only system which can accurately correct all errors associated with the velocity bias.
The LDA acquisition system was also synchronised with a shaft encoder in order to obtain phase-resolved data. Mean and turbulence levels were estimated, these latter were used to evaluate the extent of the dead regions near the baffles. All six Reynolds stresses were determined.
A detailed description of turbulence stresses is important for all those applications where the vessel contains particles or droplets of size similar to the size of the turbulent eddies. This is likely to happen in bioreactors where the biological entities have been observed to be shear-sensitive to varying degrees; for instance some bacteria, yeast and fungi can be relatively tolerant to high shear environments whereas other organisms require low shear rate for their viability. The effects of shear stresses on biological particles have been discussed extensively in literature (Papagianni, 2004) and they go from their death to sub-lethal effects, e.g. modulation of their metabolism. In biological applications stirred tanks are often preferred to other equipments such as airlift reactors, because of their low capital and operating costs. However, the agitator and other internal fittings, i.e. baffles, produce high shear stresses. Most of the experimental studies on the shear response of biological organisms have been conducted in laboratory equipments such as parallel plates or cylindrical tubes which are very different from the equipments used in practice. Therefore it is important to assess the shear levels which are present in real stirred tanks in order to choose the most appropriate agitation rates to enhance mixing without damaging the bacterial population. Finally, knowledge of Reynolds stresses may help validating advanced CFD approaches, e.g. LES and high order turbulence models in RANS.

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**References**

Derksen, J.J.; Doelman, M.S.; Van den Akker, H.E.A, (1999) *Exp. Fluids*, 27, 522.

Escudiè, R.; Linè, A., (2003) *AIChE J.*, 49, 585.

Galletti, C.; Brunazzi, E.; Pintus, S.; Paglianti, A.; Yianneskis, M., (2004) *Chem. Eng. Res. Des.*, 82, 1214.

Micheletti, M., Baldi, S., Yeoh, S.L., Ducci, A., Papadakis, G., Lee, K.C., Yianneskis, M., (2004) *Chem. Eng. Res. Des.*, 82, 1188.

Papagianni, M., (2004). *Biotechnology Advances* 22, 189.

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# Toxicity of nanoparticles on mammalian cell

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## Summary

The cytotoxicity of fullerene C60 particles on two mammalian cell lines, i.e., the Chinese hamster ovary (CHO) cells and the MDCK (NBL-2) cells, has been investigated. Although innate fullerene particles have a very low solubility in DI water, these particles can be dissolved in the tetrahydrofuran (TFH) solvent at a great value. Further, the dissolved fullerene particles in the TFH solvent could be extracted into a DI water solution at a significantly increased solubility. The formation of fullerene particle aggregates is believed to be the cause of the increased solubility. Results presented here show that, once the concentration of the fullerene aggregates reaches a certain level, the cells start to die. The critical dosage $LD_{50}$, which is defined as the lowest fullerene concentration, which presented in a given cell culture, results in a 50% cell death within 24 hours, has been determined. Further, both the fullerene concentration and incubation time have negative effect on the cell mortality. These results will help to better understand the side effects of fullerene particles, in mammalian cells.

## Extended Abstract

Nanoscience and nanotechnologies have been widely recognized to have huge potential to affect drug development, water decontamination, development of information and communication technologies, and the production of stronger and lighter materials. It has been estimated that, the annual value for all nanotechnologies-related products, including information and communication technologies, will reach $1 trillion by 2011 – 2015. Therefore, it is not surprising that the nanotechnology related research has been attracting rapidly and continuously increasing investments from both the governments and businesses in many parts of the world. However, concerns about the possible side effects of the extensive application of the nanotechnology onto various aspects, including the ecosystem, human health, food and agricultural industries, have been raised recently.

The fullerenes, which were first discovered in 1985 by researchers at Rice University, are a family of carbon allotropes. They are molecules composed entirely of carbon, in different shapes. Spherical fullerenes are sometimes called buckyballs. Among all the spherical fullerene variants, the $C_{60}$ variant has been extensively studied. Large production of fullerene particles has been successfully achieved.

Frontier Carbon Corporation, the world's first fullerene mass production company, reached its fullerene production capacity at 40 tons per year in 2003, and plans to increase its capacity to 300 tons per year in the future.

Although fullerene C60 has been thought in theory to be relatively inert, several researches have demonstrated that aggregated fullerene $C_{60}$, often termed as nano $C_{60}$, have negative effect on various animals, organs and microorganisms. For example, Oberdörster reported that uncoated fullerenes can cause oxidative damage and depletion of total glutathione (GSH) level in vivo in an aquatic species [1]. Zhu et al. found that fullerene $C_{60}$ could lead two aquatic species, daphnia and fathead minnow, to die [2]. Fortner et al. shown that prokaryotic exposure to nano-$C_{60}$ at relatively low concentrations is inhibitory, indicated by lack of growth and decreased aerobic respiration rates [3]. However, only very few studied have been conducted to investigate the cytotoxicity of fullerene $C_{60}$ on mammalian cells. Isakovic et al. reported that pure fullerene $C_{60}$ suspension was at least three orders of magnitude more toxic than water-soluble polyhydroxylated fullerene on mouse L929 fibrosarcoma, rat C6 glioma, and U251 human glioma cell lines [4].

In this study, two mammalian cell lines, Chinese hamster ovary (CHO) cells and MDCK (NBL-2) cells have been spiked with fullerene aqueous solution to investigate the cytotoxicity of fullerene particles on these two cell lines. CHO cell line has been widely used in the biotechnology/pharmaceutical industry to produce various recombinant proteins. And MDCK cell line is also frequently used to produce influenza viruses. As nanoparticles, including fullerene particles, are more and more applied in various industries, this study aims to evaluate the possible cytotoxicity of fullerene particles by exemplifying with those two frequently used cell lines. Some results are shown below.

Results:

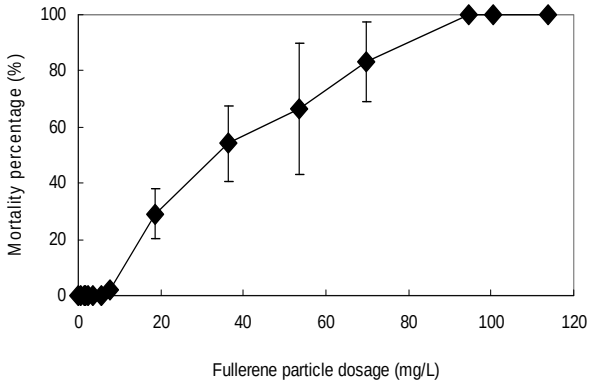


Figure 1: The mortality percentage of CHO cells as a function of the fullerene dosage.

## References:

1. Oberdorster E. Environmental Health Perspectives, 2004, 112: 1058-1062.

2. Zhu SQ, Oberdorster E, Haasch ML. Marine Environ. Research, 2006, 62: S5-S9.

3. Fortner JD, Lyon DY, Sayes CM, Boyd AM, Falkner JC, Hotze EM, Alemany LB, Tao YJ, Guo W, Ausman KD, Colvin VL, Hughes JB. Environ Science and Technol, 2005, 39: 4307-4316.

4. Isakovic A, Markovic Z, Todorovic-Markovic B, Nikolic N, Vranjes-Djuric S, Mirkovic M, Dramicanin M, Harhaji L, Raicevic N, Nikolic Z, Trajkovic V. Toxicol Sciences, 2006, 91 (1): 173-183.

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# Bubble formation in a novel forced loop reactor

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## 1. Summary

In order to have a uniform bubble size, effect of liquid velocity was utilized in a better manner with a new gas liquid sparger. A nearly uniform bubbles were produced. Bubble size was measured by photographic method. Bubble size of two different liquid flow rates $Q_L$= *1* and *2* ($m^3$/h) were compared. The distribution of bubbles was normal and the majority of bubbles had a diameter of more than *2.5* mm, the amount of bubble between *2.5-5* mm is higher at higher liquid flow rate. The increase liquid flow rate, increase the detachment of bubbles formed on sparger holes, resulting a decrease in Sauter mean bubble diameter.

Keywords: Bubble size, forced circulation loop reactor, jet loop reactor, airlift reactor, gas holdup

## 2. Extended Abstract

A uniform radial profile of the gas holdup decreases bubble-bubble collisions. Airlift reactors suffer serious drawbacks due to this fact that the large bubbles in reactors disengage from the vessel. The size of bubbles affect gas hold up and mass transfer (link et al., 2004, Chisti, 1989) resulting even impact of cell metabolism in bioreactor (Russel et al., 1995). Many investigations have been done to improve the bubble size distribution in reactors (Ellenberger& Krishna, 2003, Krichnavaruk & Pavasant, 2002, Tung et al., 1998).
The reactor consists of a liquid vessel and a sparger which explained somewhere else (Fadavi, 2004). The liquid and gas phases were introduced to the reactor from the individual passages (*1)* and (*2)* respectively. Gas enters the mixing tube *(8)* via orifices (*6)* which are located on the surface of the conical surface of the gas sparger *(4).* Liquid enters the mixing tube through the static mixer *(3)*, which forms a conical high turbulent flow of liquid (*5)* in the entrance of the mixing tube. Bubbles which were built up on the conical surface of the gas sparger were swept immediately from the conical surface. Size of bubbles depends on the gas and liquid flow rate; i.e. higher liquid flow rate and lower gas flow rate, smaller bubbles and better bubble distribution were observed. The two phase flow that is formed will be homogenous through the mixing tube and then is directed to the venturi entrance of the riser (*13).* This shape of entrance causes the two phase flow to have higher velocity relative to

the riser, so the entering liquid with high velocity entrains the content of the downcomer, which results in an increase of the liquid circulation and mixing**.**

Gas and liquid flow rate changed from $1.3x10^{-4} \geq Q_G \geq 4.5x10^{-4}$ $m^3/s$ and $Q_L=1$ and $Q_L=2$ $m^3/h$ respectively. Bubbles size were measured by photographic method using AutoCAD software.

Results of statistical analyses are shown that the amount of bubble between 2.5-5 mm is higher at $Q_L=2$ ($m^3$ /h) relative to that of $Q_L=1$($m^3$ /h), while the amount of bubbles which are greater than 5 mm is lower at $Q_L=2$ relative to that of $Q_L=1$($m^3$ /h), and therefore a uniform radial profile of gas holdup with lower bubble size is expected to exist in a higher liquid flow rate due to higher liquid velocity and existing turbulence.

The Sauter mean diameter decreases with an increasing liquid flow rate. This can be explained in this way: the frequency of bubble generation at the orifices of the gas sparger increases with an increase of the liquid flow rate, i.e., bubbles with small diameter were swept immediately from the gas sparger. Additionally, existing disturbances favor the disintegration of large bubbles to smaller ones.

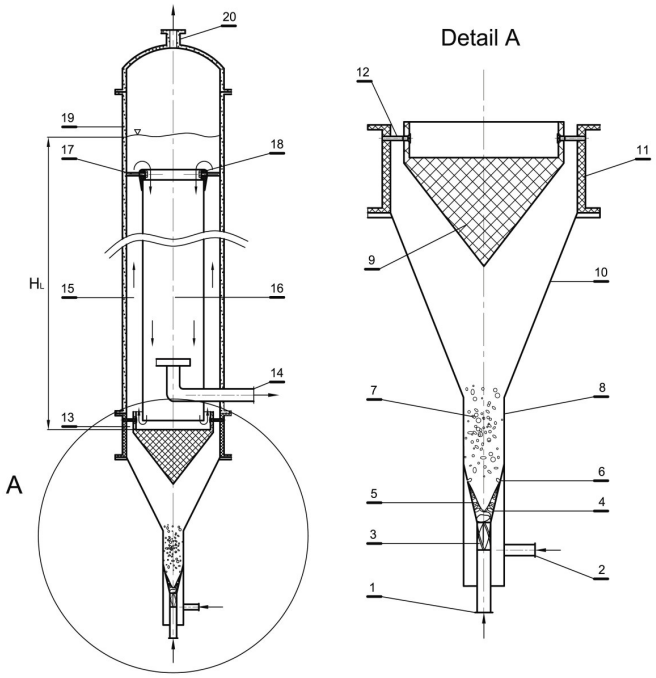


Fig. 1. Details of the reactor: 1) liquid inlet; 2) gas inlet; 3) static mixer; 4) gas sparger; 5) conical liquid input zone; 6) orifice; 7) two-phase mixture; 8) mixing tube; 9) guiding cone; 10) diffuser; 11) support; 12) screw; 13) riser venturi entrance; 14) liquid outlet; 15) riser; 16) downcomer; 17) screw; 18) draft-tube support; 19) reactor vessel; 20) gas outlet.

## References

Chisti, M. Y., *Airlift Bioreactors,* Elsevier Applied Science, New York, (1989).

Ellenberger, J. and Krishna, R., (2003) *Chemical Engineering Science*, 58, 705 – 710.

Fadavi, A., (2004) *Circulation reactor*. Patent application SK4135U, Industrial Property Office of Slovak Republic.

Krichnavaruk, S. and Pavasant, P., (2002) *Chemical Engineering Journal* 89, 203–211.

Linek, V., Kordač, M., Fujasová, M. and Moucha, M., (2004) *Chemical Engineering and Processing*, 43, 1511–1517.

Russell, A., B., Thomas C., R., and Lilly M., D., (1995) *Bioprocess Engineering*, 12, 71-79.

Tung H.l., Tu C.C., Chang Y.Y., and Wu W.,T., (1998) *Bioprocess Engineering* 18, 323–328.

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# Analysis of the microbial membrane bioreactor working at the reagents separation

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## 1. Summary

Selecting appropriate values of the streams leaving microbial membrane bioreactor has an important influence on biomass concentration, i.e. a parameter which has the greatest effect on the bioreactor efficiency. For that reason the parameter $\Psi$ called as the intensification coefficient of a microbial membrane bioreactor was introduced to the proposed model. The second important parameter of the model is called as substrate permeability coefficient ($\eta_s$). Selection of a proper pair of parameters $\Psi$ and $\eta_s$ is a typical optimization task which could be accomplished after formulating an optimization criterion.

Keywords: microbial membrane bioreactor, membrane separation, model of process, substrate conversion degree

## 2. Extended Abstract

The main task of a membrane reactor is to hold up a mass of biocatalyst in the reaction zone. This is obtained by using a membrane with selectivity chosen according to its quantity. In the case of a microbiological transformation in which microbial cells play the role of a catalyst, there is a characteristic effect of biomass growth (an autocatalytic reaction). Due to this, in order to ensure steady-state conditions, part of the biocatalyst mass must be removed ($Q_3$) continuously from the reaction zone. For this reason, two separate streams removed from the reaction zone (Fig. 1) are a characteristic element of microbiological membrane bioreactor and influence on the biomass concentration in reaction zone. For that reason the parameter $\Psi$ called as the intensification coefficient of a microbial membrane bioreactor was introduced to the proposed model.

$$\Psi = \frac{1}{1 - \frac{Q_6}{Q_1}} \tag{1}$$

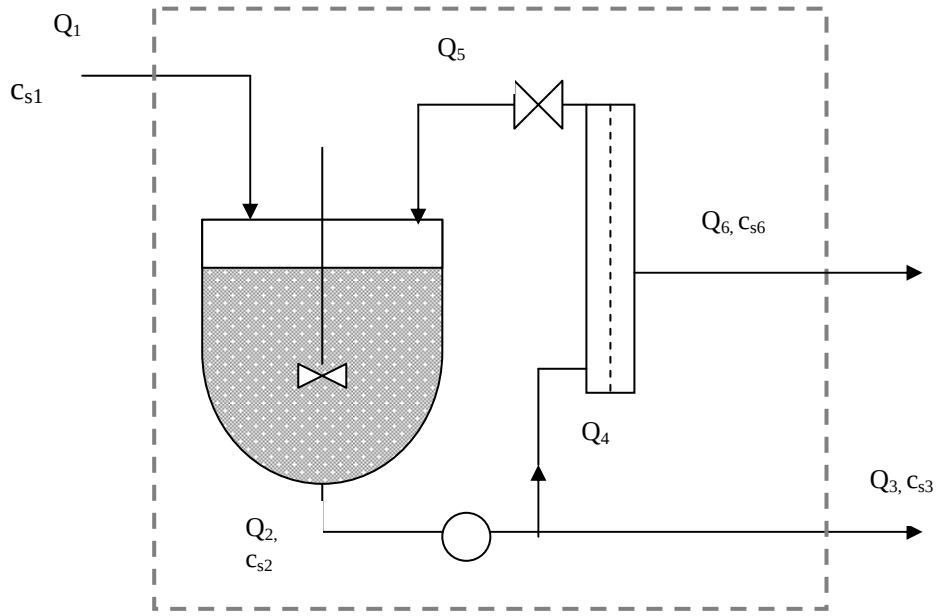


Figure 1: Scheme of microbial membrane bioreactor.

At an identical residence time in CSTR and in the one with a separation module (at other parameters being constant) due to the higher cell concentration in the membrane bioreactor the relation of substrate conversion degrees in these reactors is given by the equation

$$\frac{\alpha_{bm}}{\alpha_{clas}} = \frac{1-\frac{1}{c_{s,1}^{*}}\cdot\frac{1}{\mu_{max}\cdot\tau\cdot\Psi-1}}{1-\frac{1}{c_{s,1}^{*}}\cdot\frac{1}{\mu_{max}\cdot\tau-1}} \qquad (2)$$

An increase of the intensification coefficient ($\Psi$) induces a substantial increase of the conversion degrees, specially strong influence being observed for low concentrations of the raw material ($c_{s,1}^{*}$). This is related to the fact that to these concentrations correspond low reaction degrees in the classical bioreactor and the range of intensification in this case is broad. Also for this reason more evident is the intensification at a lower value of $\mu_{max} \cdot \tau$. The effect of the intensification coefficient is significant in the range of $\Psi$ from 1 to 3.

When the membrane bioreactor is applied it is also very well when the reaction substrate is also hold up by membrane. A parameter that expresses this phenomenon, in this paper is called as substrate permeability coefficient ($\eta_s$). Its value has a linear influence on substrate conversion degree ($\alpha_{bm}^{\#}$):

$$\alpha_{bm}^{\#} = \frac{Q_1\cdot c_{s1} - Q_3\cdot c_{s3} - Q_6\cdot c_{s6}}{Q_1\cdot c_{s1}} = \frac{c_{s1} - c_{s2}\cdot[1-(1-\eta_s)\cdot(1-\frac{1}{\Psi})]}{c_{s1}} \qquad (3)$$

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# Thermolysine adsorption on membranes at parallel flow of retentate

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## 1. Summary

Enzyme surface concentration and permeate stream obtained in the membrane module with a dynamically formed biocatalyst layer were determined experimentally using two different, polymeric membranes. Correlations were formulated to calculate these values. It was found that their variability range was so big that when controlling the transmembrane pressure and turbulence of a retentate stream, the mass of used biocatalyst in membrane bioreactor could be distributed in a controlled way between both reaction zones (with the native and adsorbed enzyme).

Keywords: enzymatic membrane bioreactor, enzyme adsorption, dynamic membrane, enzyme surface concentration, parallel flow

## 2. Extended Abstract

In practical solutions of the membrane modules, a circulating stream flows parallel to the membrane surface. This implies the presence of shear stresses which tear off the biocatalyst deposited on the membrane from its surface. Hence, the main parameter which determines distribution of the biocatalyst mass in membrane bioreactor between both reaction zones is hydrodynamics of the stream flowing along the membrane surface. It is obvious that the higher is turbulence of the stream flowing past a membrane deposited in the module of given geometry, the smaller mass of the used biocatalyst will be kept on its surface. It is easy to control stream circulation, so distribution of the biocatalyst mass in the both bioreactor zones and hence a total reaction effect can be dynamically controlled.
Sorption of the selected enzyme (thermolysine) was carried out on the surface (A=10 $cm^2$) of capillary membranes from polysulfone 1700 NT LCD and polieterosulfone E6020P (IBIB, Warsaw) at the temperature 50ºC. Prior to measurements, the membrane module was rinsed each time with deionized water and next with 0.1 M phosphate buffer, pH 7.0. In this buffer also a solution of thermolysine was prepared at the concentration 0.08, 0.16, 0.28 and 0.82 g $l^{-1}$ in subsequent experiments. The degree of enzyme immobilisation as a function of retentate flow corresponding to Re

number ranging from 33 to 2464 and transmembrane pressure ranging from 0.036 to 0.152 MPa was investigated. During each experiment the permeate flux was also measured.

As results from experiments the surface concentration of the biocatalyst and the permeate flux changed several times with the change of turbulence of the retentate stream and difference pressure. The changes are so significant that they form a set which can be successfully used to control the bioreactor operation. An additional factor which has a very strong influence on the biocatalyst distribution is the specific surface of the applied membrane that causes a linear increase of mass fraction of the biocatalyst deposited on the membrane.

No models describing relationships between surface concentration of a catalyst bound with the membrane and process parameters of membrane separation are known. Thus, basing on the analysis of relations obtained experimentally, the following correlation was proposed:

$$\frac{x}{c} = Z_1 \Delta P^{Z_2} - Z_3 \,\mathrm{Re} \qquad (1)$$

where: x- enzyme surface concentration (g $m^{-2}$), c – enzyme concentration in solution (g $l^{-1}$), $\Delta P$ – pressure difference (MPa), Re – Reynolds No., $Z_1$, $Z_2$, $Z_3$– constants.

Correlation describing an influence of turbulence of retentate stream, surface concentration and pressure difference on permeate flux (j) was also proposed:

$$j = \frac{\Delta P}{R_m + R_x} = \frac{\Delta P \cdot 10^{-2}}{R_m + b \cdot x} \qquad (2)$$

where: j- permeate flux ($m^3$ $m^{-2}$ $s^{-1}$), $R_m$, $R_x$- resistance of membrane and enzyme layer (N $s^{-1}$), b- constant

For the tested system the obtained constants are presented in Table 1.

Figure 1: The constant values of Eq. (1) and (2).

| membrane | $Z_1$ | $Z_2$ | $Z_3$ | $R_m$ | b |
|---|---|---|---|---|---|
| polysulfone | 127.0 | 1.0 | 0.0033 | 179.0 | 37.0 |
| polieterosulfone | 55.0 | 1.0 | 0.0073 | 64.0 | 32.2 |

As results from above values of constants, in the case of the enzyme deposited on the surface of the polysulfone membrane influence of retentate turbulence (Re) is two times lower than in the case of the enzyme deposited on the surface of the polietrosulfone membrane. This membrane causes lower resistance and what was expected the resistance of membrane layer (constant b) in both cases is similar.

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# Session S-5D: Meals - Convenience, Gastronomy & Quality (Food-1a)

| Abstract Number | Paper Title & Authors | Included |
|---|---|---|
| 2423 | Non-crystalline formulation of lipophilic, surface active and hardly soluble active agents in water-dispersible carrier systems<br>R Engel, H Schubert, H P Schuchmann | Yes |
| 3865 | Structural engineering of dairy products<br>R Ipsen | Yes |
| 3896 | Interactions between macro-molecules and starter cultures in the cheese curd using antibody phage display technique and bioimaging<br>Z Duan | No |
| 3975 | Quality of yogurts prepared from UHPH-treated milk<br>M Serra, A J Trujillo, B Guamis, V Ferragut | Yes |
| 4020 | Functional properties of soft wheat (Triticum aestivum L.) grains: lignan profiles of conventional and old varieties from Italy<br>G. Dinelli, I Marotti, S Bosi, A Bonetti, S Benedettelli, A S Carretero | Yes |
| 4050 | Does the morphology of fat crystals have impact on the rheologial properties of milk fat?<br>L Wiking, I Foubert, V D Graef, M Rasmussen, K Dewettinck | Yes |
| 4058 | An investigation of new product introductions in the Danish food industry: survey findings<br>D Baker | Yes |
| 4099 | Health through Convenience: The Technological Challenge<br>J A Nissen | Yes |

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# Non-crystalline formulation of lipophilic, surface active and hardly soluble active agents in water-dipersible carrier systems

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## 1. Summary

The formulation of lipophilic, surface active and hardly soluble active agents in water-based food- or pharmaceutical systems turns out to be problematic both in terms of the achievable dose-response as well as the concentration of the active agents in the formulation. Since lipophilic solutions of most active agents are known to yield the highest dose-responses, the aim of the study was to produce highly disperse, water-dispersible systems of solved, supersaturated active agents in order to combine a high dose response with both applicability in water-based food- and pharmaceutical systems as well as high concentrations of the active agents. By the example of phytosterols as lipophilic, surface active and hardly soluble active agents, it is demonstrated how active agents with these properties can be formulated in food-grade oil-in-water-emulsions, liposomes and micellar systems with the scope of the development of functional foods.

Keywords: formulation, water-dispersible, lipophilic active agents, carotenoids, phytosterols

## 2. Extended Abstract

Many lipophilic active agents like Carotenoids or Phytosterols are insoluble in water and not sufficiently soluble in vegetable oils. Additionally, they can exhibit surface active behaviour leading, in the case of Phytosterols, to undesired interfacial crystallisation.
Nevertheless Phytosterols are of high interest, as they can significantly reduce high serum cholesterol levels and hence the risk for cardiovascular diseases. The dose-response, however, is strongly dependent on their formulation. Crystalline phytosterols exhibit the weakest dose-response. With higher dose-responses of phytosterol fatty acid esters currently applied in commercial functional foods, they still do not reach the level expected from a study for free, dissolved phytosterol molecules.
Up to a concentration of 30 % with regard to the dispersed phase, Phytosterols could be stabilised in a non-crystalline state in sub-micron oil droplets of an o/w-emulsion

by means of a high temperature emulsification process combined with a suitable emulsifier system of at least one oil- and one water-soluble emulsifier. Oil droplet diameter was reduced to the range of 100 nm. The emulsion proved to be stable over a period of 60 days as well as during the passage of the stomach until reaching the intestine, which is the site of activity of the Phytosterols.

Liposomes, resembling cell membranes where phytosterols occur naturally, also represent a suitable water-dispersible carrier system for non-crystalline phytosterols and could be loaded with up to 30 % of phytosterols following the film-processing method.

Some surface-active substances are suitable for the production of phytosterol loaded micelles in a watery phase, as well. Until now, only up to 2.5 % phytosterols could be solubilised in mixed micelles. Here, the stability of the micelles turned out to depend on both the Phytosterol- and the dispersed phase concentration.

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# Structural engineering of dairy products

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## 1. Summary

Treatments affecting the molecular properties and supramolecular organization of dairy product constituents reflect in the final product quality. Examples of such treatments include application of specific enzymes (phospholipase, serine protease) or ingredients (microparticulated whey protein, carrageenan, exopolysaccharide-producing lactic acid bacteria).

Keywords: dairy products, structural engineering, enzymes, ingredients

## 2. Examples of structural engineering of dairy products

Phenomena on the molecular or colloidal level can have profound influence on final dairy product quality and treatments affecting the molecular properties and supramolecular organization of various constituents will reflect in the quality and utilization of manufactured products.

*2.1: Manufacture of nanotubules from whey protein*

Hydrolysis of the whey protein alpha-lactalbumin with a specific serine protease has been shown to result in regular nanotubes of approximately 20 nm in outer diameter and reaching several micrometers in length[1]. Tubular assembly depends on the concentration of protein, as this determines how far the hydrolysis proceeds. Possible applications of alpha-lactalbumin nanotubes include use as a viscosifyer and gelling agent but also pharmaceutical utilization (such as targeted drug release) and use in nanotechnology can be envisioned.

*2.2: Use of phospholipase in manufacture of cheese*

Phospholipase treatment of milk for production of Mozzarella can reduce fat losses in whey and cooking water and increase cheese yield[2]. The microstructure of the resultant cheese is very similar to control cheese as is flowability, stretchability and browning. The observed yield improvement is presumably a result of improved emulsification and water-holding capacity as a consequence of the released lysophospholipids present in the curd

*2.3: Application of different carrageenans in dairy deserts*

Using either kappa-, iota- or hybrid-carrageenan in dairy deserts results in different microstructures, as well as having major influence on rheology and melting behaviour[3]. All three types of carrageenan interact with the casein micelles in milk, but the impact of the interaction on the total gel properties varies with the type of carrageenan used.

*2.4: Microparticulation of whey proteins*

Microparticulated whey protein has been shown to be capable of making low-fat stirred yoghurt significantly more creamy than a full-fat control sample[4]. The microparticulated whey protein particles probably do not actively promote structure formation and the observed sensory effect is hence more subtle, in that it affects the oral breakdown path in a similar way to homogenized fat globules or it may simply be matter of having approximately the same particle size[5].

*2.5: Effect of exopolysaccharides in fermented milk structure*

Exopolysaccharides (EPS) produced by starter cultures have a major effect on the texture properties of yoghurt[6]. Yoghurts made with EPS-producing cultures exhibit greater mouth thickness, higher ropiness and tend to be creamier than yoghurts made with non-EPS- producing cultures, which in contrast has higher gel firmness and reduced spontaneous syneresis. Texture variations within yoghurts made with different EPS-producing cultures can be attributed to differences in the produced EPS and to whether the EPS actively interacts with the protein network or not.

## 3. Conclusion

Small changes in molecular structure, even for minor constituents in milk, can have major impact on the interactions determining important quality parameters, including sensory quality, as well as yield.

## References

1. Ipsen, R., Otte, J., Qvist, K.B. 2001 Molecular self-assembly of partially hydrolysed alpha-lactalbumin resulting in strong gels with a novel microstructure. J. Dairy Res. 68 277-286

2. Lilbæk, H.M., Broe, M.L., Høier, E., Fatum, T.M., Ipsen, R., Sørensen, N.K. 2006 Improving the Yield of Mozzarella Cheese by Phospholipase Treatment of Milk J. Dairy Sci. 89 4114–4125

3. Arltoft, D., Ipsen, R., Madsen, F., de Vries, J. 2007 Interactions between Carrageenans and Milk Proteins: A Microstructural and Rheological Study Biomacromol. 8 729-736

4. Janhøj, T., Petersen, C.B., Frøst, M.B. & Ipsen, R. 2006 Sensory and rheological characterization of low-fat stirred yoghurt, J. Text. Stud. 37 276–299

5. Janhøj, T. & Ipsen, R. 2006 Effect of Pre-heat treatment on the functionality of microparticulated whey protein acid milk gels, Milchwiss. 61131-34

6. Folkenberg, D.M., Dejmek, P., Skriver, A., Guldager, H.S. & Ipsen R. 2006 Sensory and rheological screening of exopolysaccharide producing strains of bacterial yoghurt cultures Int. Dairy J. 16 111-118

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# Evolution of acid gels from UHPH-treated milk during cold storage

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## 1. Summary

The effects of ultra-high pressure homogenisation (UHPH) on cow's milk were investigated and its suitability for yogurt manufacturing was compared with the conventional process currently applied in the yogurt industry. This study included determination of titrable acidity, determination of syneresis, textural and rheological evaluation of gels. In order to follow the evolution of yogurts during the storage at refrigeration temperature (4-6ºC), the analysis were carried-out weekly (1, 7, 14, 21 and 28 days).

Keywords: Ultra-high pressure homogenisation, yogurt, texture, rheology

## 2. Extended Abstract

Milk for yogurt manufacture is normally homogenized (15-20 MPa) and heat treated at >85ºC) for microbial reduction and physico-chemical modifications. These changes result in yogurt with increased stability, consistency and body texture and decreased whey separation. In recent years, ultra high pressure homogenization (UHPH) has been developed. The increase in pressure causes a droplet size reduction of the obtained emulsions and this improves product shelf life. In addition, the application of UHPH leads to temperatures close to pasteurization, which combined with the mechanical treatment, is an effective method for microbial inactivation.
The aim of this work was to study the evolution during cold storage of some physico-chemical parameters of gels from UHPH-treated milk compared to gels prepared following the conventional process currently applied in the industry.
Prior to yogurt manufacture milk was subjected to UHPH treatments at 200 and 300 MPa at 40ºC, using a high pressure homogenizer (Stansted, FPG 11300). Control gels were obtained from heat-treated milk at 90ºC, 90s, homogenized at 15 MPa and fortified with 3% of SMP (HT+SMP). After UHPH or heat-treatment, samples were inoculated with *Streptococcus termophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (DVS YF-3331, Chr. Hansen, Horshölm, Denmark) and incubated at 43 ± 2ºC for 4 h. All physical parameters were measured weekly at 1, 7, 14, 21 and 28 days after manufacture.

Yogurt acidity, syneresis and texture analysis were performed as described by Serra et al. (2007). To determine the viscoelastic properties of yogurt frequency sweep tests were performed 4ºC using 20 mm parallel serrated plates in a a rheometer ThermoHaake RS1 (ThermoHaake GmbH, Karlsruhe, Germany.
Results obtained are shown in Figure 1.
Titrable acidity was higher in yogurts from HT+SMP than in all those from UHPH-treated milks all along the storage period and slightly increased during the cold storage in all treatments as a result of the persistent metabolic activity of starters. As expected, G' was in all cases higher than G'',showing the predominant elastic character of yogurts over the viscous behaviour. As shown in Figure1 all the rheological parameters were higher for HT+SMP-yogurts than for both types of UHPH-yogurts, and increased until day 14 of storage. When measuring syneresis forced by centrifugation, UHPH gels presented higher water holding capacity (WHC) values than HT+SMP gels. During the storage time, WHC values tend to increase with ageing of the gels. In the case of textural analysis, UHPH gels were the firmness ones, although firmness values hardly increased during cold storage in all treatments performed.

## References

Lucey, J.A., Teo, T.C., Munro, P.A. & Singh, H. (1998) *Food Hydrocolloids,* 12, 159

Popper, L. & Knorr, D.(1990). *Food Technology*, 84-89.

Serra, M, Trujillo, A.J., Quevedo, J.M., Guamis, B. & Ferragut, V. (2007) *International Dairy Journal,* 17, 782-790.

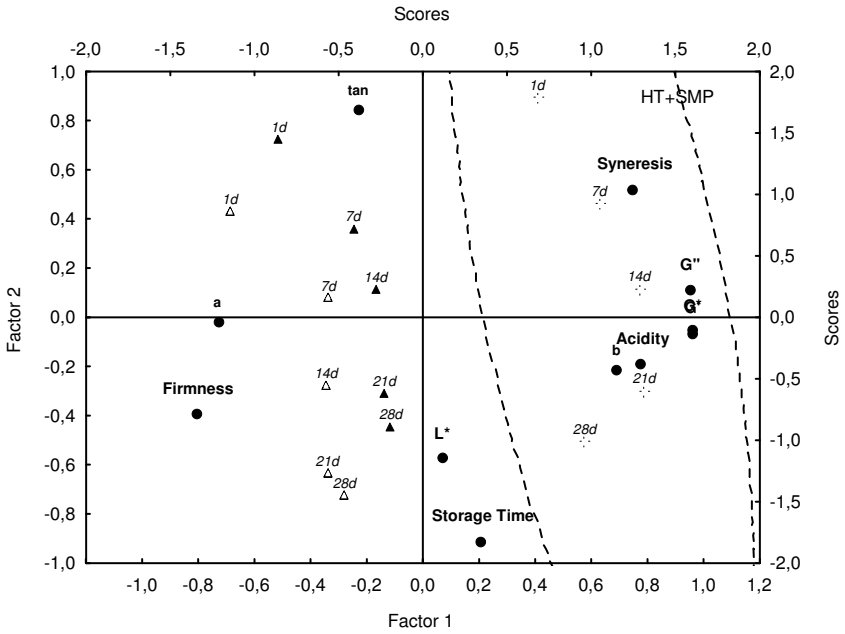


**Figure 1:** PCA distribution of physical properties of gels from UHPH (▲200 MPa; Δ 300 MPa) and heat-treated milk (HT+SMP) during storage.

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# Functional properties of soft wheat (*Triticum aestivum* L.) grains: lignan profiles of conventional and old varieties from Italy

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## 1. Summary

The health-promoting effects of whole-grain consumption have been attributed in part to their unique phytochemical contents and profiles. Wheat is an important component of the human diet; however, little is known about the phytochemical profiles of different wheat varieties, especially of old wheats. The objectives of this study was to investigate the distribution of lignans, a class of phytochemicals with proved health benefit effects, of 4 conventional and 6 ancient Italian soft wheat varieties. Results highlighted the high content and unique composition in lignans of old varieties suggesting their uses into a wide range of regular and specialty food products naturally enriched with health-promoting compounds.

Keywords: soft wheat, conventional cultivars, old varieties, lignans, phytochemicals

## 2. Extended abstract

Numerous lines of research have categorized the cereals, within the context of a balanced diet, as having a protective function in human health. This important function is partly attributed to the unique phytochemical content of grains (Thompson, 1994). Recent studies on the health benefits of functional products from wheat have become increasingly more focused on the importance of introducing phytochemicals through the use of different varieties, particularly ancient wheats (Abdel-Aal and Wood, 2004). At present most of the wheats grown are hybrids resulting from domestication and breeding processes aimed mainly at creating varieties with particular characters, i.e. high yielding and better disease resistance. However, these hybrids may lack some of the other unique properties of the ancestral wheats. Among functional compounds residing in cereal grains, lignans has recently gained much attention in many scientific research areas, especially for their broad spectrum of biological activities (bacterial-static, anti-mitotic, anti-viral and antioxidant properties) (Adom and Liu, 2002). To the best of our knowledge, little is known about the varietal differences in phytochemical profiles, particularly of

lignans, of different wheat varieties, which ultimately influence the associated nutritional and health benefits of wheat and wheat products.
The aim of the present research was to investigate the distribution and the qualitative profile of lignans in four conventional (Bolero, Mieti, Eureka, Nobel) and six old soft wheat varieties (Verna, Sieve, Andriolo, Inallettabile, Gentil rosso aristato., Gentil rosso mutico) from Italy. Significant differences were observed in total lignan content between conventional (2.6 ± 0.2 mg lignans/kg dry seed weight) and old varieties (5.0 ± 1.3 mg lignans/kg dry seed weight). Capillary electrophoresis-mass spectrometry (CE-MS) qualitative analyses highlighted remarkable difference between conventional and old cultivars for the type of aglycon lignans and for the number of glucosidic forms. In particular, three lignan aglycon forms, namely arctigenin, isolariciresinol and syringaresinol, were exclusively identified in old wheat varieties. Principle coordinates analysis of lignan qualitative data allowed the visualization of clusters of relatedness amongst old and conventional cultivars. Conventional cultivars appeared as a compact cluster sharply separated from old varieties. Relevant inter-varietal distances among old soft wheat cultivars were observed (Figure 1).

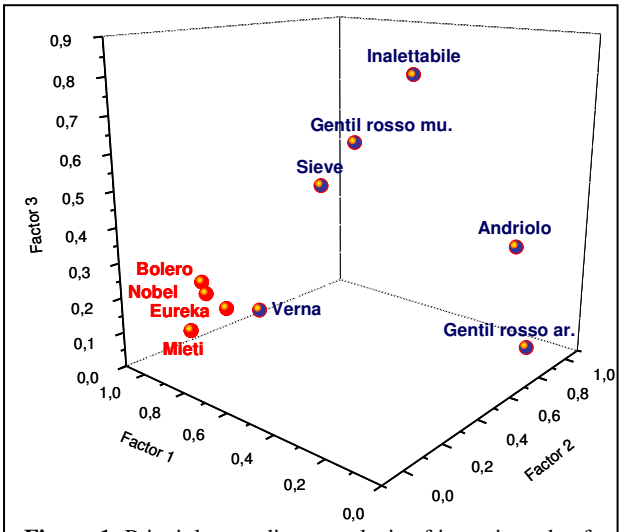


**Figure 1**: Principle coordinate analysis of investigated soft wheat varieties based on lignan qualitative data.

On the whole, results highlighted that investigated old soft wheat varieties may represent a rich source of genetic diversity, especially with regard to functional properties. The unique composition in biologically active compounds of the old cultivars may suggest to expand their uses into a wide range of regular and speciality food products, distinguished by their added value, based on health properties. Studies are in progress in order to enrich the phytochemical profile with other classes of functional compounds (carotenoids, ferulic acid, tocotrienols, tocopherols) in investigated soft wheat varieties as well as in durum wheat (*Triticum durum* L.) conventional and old cultivars.

## References

Thompson, L. U., (1994) *Critical Review of Food Science and Nutrition,* 34, 473-497.
Abdel-Aal, E-S. M. and Wood, P., *Specialty grains for food and feed*, AACC Press, Washington DC, USA, (2004).
Adom, K. K. and Liu, R. H., (2002) *Journal of Agricultural and Food Chemistry*, 50, 6182–6187.

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# The effects of fat crystal morphology on rheological properties of milk fat

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## 1. Summary

Texture of many fat rich foods is highly based on the nature of fats. In a lot of products with a high fat content, fat is present in a semi solid state and structured in a fat crystal network. The morphology of fat crystals can be affected by cooling rate. The shape of the fat crystal clusters can be spherulitic, feather-like, blade- or needle-shaped. Few attempts have been made to establish relationships between the morphology and size of fat crystals and the rheological properties of fat. In the present study, the morphology of fat crystals was monitored with confocal laser scanning microscopy. Upon different cooling rates and temperature fluctuations, the size of the milk fat crystals change. These images will be linked with oscillatory rheologial measurements. This will give insight to understand and design the texture of fat-based food products.

Keywords: Milk fat, rheology , confocal laser scanning microscopy, fat crystals.

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# An investigation of new product introductions in the Danish food industry: survey findings

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## Summary

This paper reports on a firm-level empirical study of new food product introductions. It aims to measure features of new product introduction and uses associated data about the firms. The paper addresses the Danish food industry, which although thought to be highly innovative at an industry and organisational level, has not been examined at the firm level in this connection. Finally, the paper targets the role of government and its means and potential to bring about change.

## Extended abstract

New products have been afforded considerable recent importance at the industry and firm level, as well as being an accelerant of regional development. The food industry has until recently been viewed as a consumer of new technologies, with innovation that is limited to applications that come from beyond its borders. However, new interpretations of product innovation, along with organisational change in the food marketing chain, reveal a more dynamic and creative picture. The current paper uses a database from a recent survey of Danish firms (131 valid responses, a 30% response rate) in food retail, processing and distribution to investigate the introduction frequency and form of new products, their place in branding and overall strategy, and firms' perceived barriers to such introductions. Past empirical investigation of innovation and new product introduction in the food industry has been limited by a shortage of metrics and reliable data. Further, very little firm-level data has been used, with the preference being for case-studies at executive level, or a focus on the products.

In the current paper, surveyed firms produce more new products in 2005 than in 2000. They also remove more products, but the net effect is of an increase in numbers of products in the market. This result is not strongly associated with stage of chain, commodity sector or location.

In terms of branding profile and "newness" of products, the pattern of forms taken by new products is little changed between 2000 and 2005. It also bears little resemblance to that predicted from the literature. Some 40% of new food products in the survey are "truly new" and eth great majority are introduced under existing, as opposed to

new, brands. There has been a marked increase in the proportion of new products that are introduced as retailers' own-label brands.

Against the conventional wisdom, product introduction cycles appear to have slowed down in the interval studied (2000-2005). Products seem to be lasting longer on eth market and the processes of introduction are slowing down. However, these results vary across sectors and phases of the product development process, and according to firms' vertical integration status and the concentration of the markets in which they operate.

Firms' impressions of the barriers to new product development feature a plurality of opinion that no such barriers are significant. Key barriers identified (mostly regulatory) differ from those proposed in recent studies, particularly food industry concentration.

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# Health through Convenience: The Technological Challenge

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## 1. Summary

Guidelines for healthy eating stress the importance of an increased consumption of vegetables; however, this is difficult to achieve for those among the population who depend on getting their daily meals through food service. The problems are primarily rooted in inadequate technology and sub-optimal supply chain management. It is argued that there is a need for improved heat-treated vegetable meal components. The issues to be discussed in connection herewith are 1) technologies for heat treatment and distribution, where a new process opens up for an improved quality of frozen vegetables; 2) the potential advantages of distributing vegetable components in a partially frozen state; 3) the potentials of texturing partially frozen, stir-fried vegetables, allowing new vegetable-rich meal components to be made for fast food use.

Keywords: health, convenience, vegetables, food service

## 2. Extended Abstract

A prevalent and undisputed advice in guidelines for healthy eating is to increase the consumption of vegetables. This advice complies well with emerging trends in gastronomic circles of lifting the variety of traditional vegetables from their humble position of *garniture* to become a characteristic element of the meal. Consumers, who take most of their meals through food service (canteens and fast food outlets) and/or generally prefer to buy ready-made meals, are, however, in practice faced with a limited availability of meals which are both attractive and rich in vegetables. A major reason is that vegetables are generally labour-intensive to prepare from the raw, thus encouraging professionals in food service to use semi-prepared vegetables as components in the meals. However, it has been realised that this demand is difficult to meet with existing products (Mikkelsen 2004). The same message is conveyed to the author from international producers of high-class ready-made meals.

There are several barriers to increase the consumption of vegetables in food service, including fast food and ready-made meals. These barriers are *not* lack of knowledge

of the health-promoting advantages of achieving this goal; the barriers are a complex of challenges rooted in inadequate technology and sub-optimal supply chain management. The latter issue has recently been discussed in relation to hospital kitchens (Engelund et al. 2007). The technological issues to be discussed – partly based on own experimental work – are:

1) Technologies for heat treatment and distribution are inadequate. Many consumers, in particular men and the elderly, often find raw vegetables too hard and bulky to be attractive, and a brief heat treatment can reduce bulkiness and soften the plant tissue while maintaining an appealing texture and appearance. However, many heat treated vegetables have a short shelf life even when distributed cold (e.g. loss of vitamin C), and they are difficult to handle. Freezing is one solution, but the loss of water binding capacity is a major drawback for the later use in meals, in particular in cold buffets or as components in vegetarian pies. A new continuous stir-frying process can reduce the tendency to drip loss and preserve an attractive texture also after thawing (Adler-Nissen 2007); data and examples will be given.

2) Critical convenience properties in food service. The vegetable components should be easy to distribute and handle, and portion sizes should be flexible to give the kitchen manager freedom to compose the final meals. Conventional freezing in loose weight to $-18^{o}$C solves these problems, but lack of storage capacity and too long thawing times are major drawbacks. An alternative solution proposed is partial freezing and distribution in the cold chain instead. Theoretical calculations indicate that this is feasible provided that the logistic possibilities for short distribution and delivery times are utilised.

3) Potential technologies for making vegetable-rich meal elements for fast food. In fast-food outlets, raw cut vegetables usually fit poorly into bread-based fast foods meant for eating “on the go”, because the vegetables are bulky, stiff or watery and there is a risk of spilling. It has recently been observed that the mechanical properties of stir-fried vegetables in a partially frozen state are not that of a brittle solid but exhibit a considerable plasticity that allows shaping, texturing and / or precision cutting. In preliminary experiments stir-fried vegetable sticks were mechanically disintegrated and assembled into solid burger-like pieces which could be fried conventionally. Additional treatments like rolling may increase the versatility of these components further.

**References**

Adler-Nissen, J. (2007) *J. Food Engineering*, in print.

Engelund, E.H., Lassen, A. and Mikkelsen, B.E. (2007) *Nutrition & Food Science*, 37, 90-99.

Mikkelsen, B. E. (ed.) (2004) *Aktøranalyse indenfor cateringsektoren i Øresundsregionen*, Øresund Food Network, Copenhagen.

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# Session S-5Dd: Meals - Convenience, Gastronomy & Quality (Food-1b) – 5 min oral presentation plus poster presentation

| Abstract Number | Paper Title & Authors | Included |
|---|---|---|
| 3835 | Characterization of onion (Allium cepa L.) by-products as food ingredients with antioxidant and antibrowing properties<br>E Roldán, C S Moreno, B D Ancos, M P Cano | Yes |
| 4061 | Influence of pH, NaCl and protein composition on the melting performance of casein gels made from concentrated skim milk<br>A O Karlsson | No |
| 4063 | Differences in aroma pattern of individual fruits of three apple cultivars<br>M A Petersen, L Poll, T B T Andersen | Yes |
| 4068 | Product designed packaging of fresh-cut fruit and vegetables<br>M Edelenbos | No |
| 4109 | Molecular gastronomy – cooking of soups and stocks<br>P S Nielsen | No |

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# Characterization of onion (*Allium cepa* L.) by-products as food ingredients with antioxidant and antibrowning properties

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## 1. Summary

The objective of this research was to characterize by-products -juice, paste and bagasse- from two Spanish onion cultivars -'Figueres' and 'Recas'- that have been stabilized by temperature -freezing, pasteurization and sterilization- in order to evaluate their potential use in the food industry as natural food ingredients, sources of antioxidants and antibrowning bioactive compounds. This study triggered the choice of one onion by-product with good characteristics as antioxidant ingredient. Total phenols were determined spectrophotometrically (Vinson et al, 1998) and total quercetin was determined by High Performance Liquid Chromatography (HPLC) following the method of Hertog et al (1992) with minor modifications. Antioxidant activity was determined by the measurement of the $DPPH^{\bullet}$ radical scavenging (Sánchez-Moreno et al, 1998). Poliphenol Oxidase (PPO) inhibition assay was measured spectrophotometrically following the method of Kim et al (2005) with minor modifications. The results showed that process 'Recas' cultivar onion wastes to obtain paste as a by-product and stabilize onion by-products with mild treatments as pasteurization offered better characteristics in order to develop potential antioxidant food ingredients. From this study we could conclude that there is a real possibility of using onion wastes to obtain natural food ingredients with technological (antibrowning) and functional characteristics. These onion surplus and wastes could be used to obtain by-products as food ingredients in the design of functional food rich in phenolic compounds and fructooligosaccharides, interesting bioactive compounds for their proven relation with health.

Keywords: onion by-products, bioactive compounds, antioxidant, antibrowning, food ingredients

## 2. Extended Abstract

'Figueres' and 'Recas' onions were supplied by an onion industry CEBACAT in Lleida (Catalonia). Their processing and stabilization was held in The National Center for Food Technology and Safety (CNTA) in San Adrián (Navarra) and their analysis were performed in Instituto del Frío, Consejo Superior de Investigaciones Científicas

(CSIC) (Madrid). Inferring from the analysis performed, we conclude that pasteurized 'Recas' paste was the optimum onion by-product for its potential development as an antioxidant ingredient. It showed a moderate high bioactive composition (total phenols, 329.77 ± 83.49 mg Clorogenic Acid Equivalents (EAC)/100 g dry weight (dw) (**Figure 1**); and total quercetin, 195.17 ± 7.27 mg/100 g dw (**Figure 2**)). This paste was more effective as radical scavenger than pasteurized 'Recas' bagasse or juice, 15.20% and 72.17%, respectively (**Figure 3**). Moreover, its inhibitory effect towards avocado PPO was higher (53.49%) than that found in pasteurized 'Figueres' paste (32.82%) (**Figure 4**).

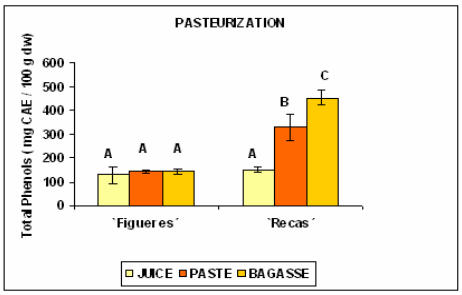


**Figure 1**

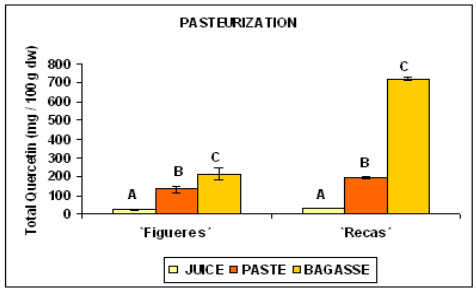


**Figure 2**

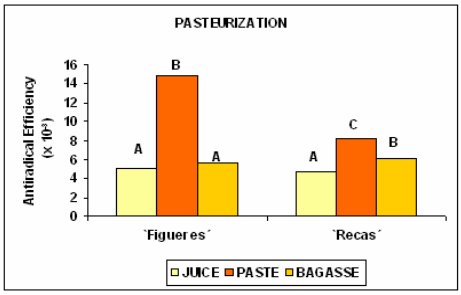


**Figure 3**

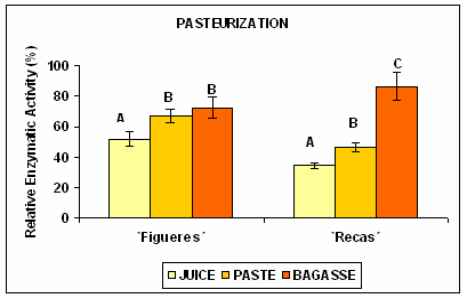


**Figure 4**

## References

Hertog, M. G. L., Hollman, P. C. H. and Venema, D. P., (1992) Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *Journal of Agricultural and Food Chemistry*, 40, 1591-1598.

Kim, M. J., Kim, C. Y. and Park, I., (2005) Prevention of enzymatic browning of pear by onion extract. *Food Chemistry*, 89, 181-184.

Sánchez-Moreno, C., Larrauri, J. A. and Saura-Calixto, F., (1998) A procedure to measure the antiradical efficiency of polyphenols. *Journal of the Science of Food and Agriculture*, 76, 270-276.

Vinson, J. A., Hao, Y., Su, X. H. and Zubik, L., (1998) Phenol antioxidant quantity and quality in foods: vegetables. *Journal of Agricultural and Food Chemistry*, 46, 3630-3634.

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# DIFFERENCES IN AROMA PATTERN OF INDIVIDUAL FRUITS OF THREE APPLE CULTIVARS

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## 1. Summary

From each of three cultivars (‘Elshof’, ‘Holsteiner Cox’ and ‘Ingrid Marie’) 36 apples were analysed for content of potential aroma compounds. The apples were picked at maturity in four zones on the trees (top, bottom, east, west). As the apples were analysed individually, it is possible to determine relevant measures of quality variability, and sampling strategies for different types of quality characterisation and cultivar identification studies are discussed.

Keywords: aroma pattern, positional effect, quality variability, sampling

## 2. Extended Abstract

Apples of the varieties ‘Elshof’, ‘Holsteiner Cox’ and ‘Ingrid Marie’ were picked at maturity in October 2007. Apples were picked from top, bottom, eastern and western part of three trees of each variety. Nine apples from each zone (a total of 108 apples) were analysed individually for sugar, acid and aroma compounds.
GC-MS could tentatively identify a total of 59 aroma compounds in the samples and principal component analysis could completely separate the three cultivars based on aroma pattern. There was, however, only a weak effect of position on the tree when data from all varieties were analysed simultaneously. Therefore, further analyses were carried out on the cultivars separately. A discriminant PLS analysis revealed distinct differences according to (within tree) position for ‘Elshof’ apples (see Figure 1). Placed by themselves in the score plot, were apples from the top and bottom parts of the trees, while apples from east and west side were mixed. From the loading plot (not shown) it could be seen that ‘Elshof’ apples from top and bottom, among others had high levels of the compounds listed in Table 1. When the same type of data analysis was applied to the other two varieties similar, but less clear pictures were seen.

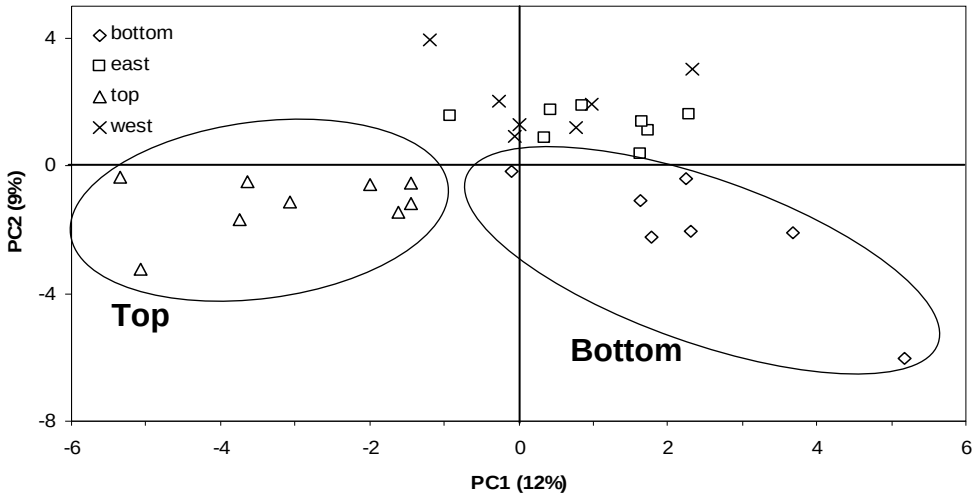


Figure 1: Score plot from discriminant Partial Least Squares regression analysis of data from 'Elshof' apples, predicting position on the tree from aroma data.

Table 1: Variables exhibiting significant differences due to position in 'Elshof' (values in a row with different letters are significantly different ($p<0.05$). Values for aroma compounds are GC-MS areas relative to internal standard area.

| | | Top | Bottom | East | West |
|---|---|---|---|---|---|
| | Sugar (%) | 13.4a | 12.3b | 13.3a | 12.9ab |
| | Acid (g/liter) | 6.8b | 7.5a | 7.1ab | 7.0b |
| High in top | 2-Methylbutyl acetate | 2400a | 1900b | 1600bc | 1300c |
| | 2-Methylpropyl acetate | 380a | 260b | 300b | 300b |
| | 2-Phenylethyl acetate | 2.7a | 1.9b | 1.9b | 2.5a |
| | 2-Pentyl propanoate | 0.98a | 0.27b | 0.81a | 0.69a |
| | 2,4-Hexadienal | 10.0a | 8.2a | 5.1b | 4.1b |
| High in bottom | Hexanal | 900b | 1300a | 1100ab | 900b |
| | 2-Butanol | 3.5b | 5.6a | 4.1b | 3.9b |
| | 2-Hexenoic acid | 0.03b | 0.38a | 0.18ab | 0.23ab |
| Other compounds | Ethyl butanoate | 1.1b | 2.8a | 1.4b | 0.5b |
| | Decanal | 1.3b | 3.5ab | 1.4b | 4.1a |
| | Butyl butanoate | 22a | 21a | 26a | 2b |

Several of the compounds listed in Table 1 have been reported as character impact odorants of apple cultivars identical to or closely related to the ones in this study (Fuhrmann & Grosch, 2000; Dixon & Hewett, 2000). It is therefore concluded that when apples are sampled for categorisation and characterisation of new or existing cultivars, it is extremely important to sample from all parts of the trees or clearly define from which position the fruits are sampled. Data also indicates the importance of good light exposure on fruits to ensure high quality.

## References

Fuhrmann, E. and Grosch, W., (2002) *Nahrung/Food*, 46 (3), 187-193.

Dixon, J. and Hewett, E.W., (2000) New Zealand Journal of Crop and Horticultural Science, 28, 155-173.

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# Session S-5E: White BioTech & Related Processes (Food-2a)

| Abstract Number | Paper Title & Authors | Included |
|---|---|---|
| 56 | Extraction of antioxidants from grape seed meal by aqueous ethanol solution<br>A Patankar | No |
| 2242 | Development of a starter culture for cocoa fermentation in West Africa<br>K T Debrah, D S Nielsen, M Jakobsen, S S Dedeh | Yes |
| 3522 | Enzyme Extraction of Phenolics from Skins Grape Skins (Vitis vinifera L.) and apples (Malus domestica) in relation to the Chemical Compositional<br>A. A Arnous, B A S Meyer | Yes |
| 3815 | Regulation of pyrG gene expression in Lactococcus lactis is controlled by the amount of CTP through an attenuator mechanism involving reiterative transcription also known as stuttering<br>J, Martinussen,K Hammer | Yes |
| 3924 | High gravity brewing- study of the affect of ethanol tolerance and nitrogen supplementation<br>M Piddocke, L Olsson, T L Soerensen, R Festersen | Yes |

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# Development of a starter culture for cocoa fermentation in West Africa

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## 1. Summary

Export of cocoa plays a significant economic role in West African countries like Cote d'Ivoire, Ghana and Nigeria - the major cocoa producing region in the World. Fresh cocoa beans have to go through fermentation and drying steps to obtain the desired characteristic "cocoa flavour"(1). In practice, cocoa beans are made to ferment spontaneously by the actions of contaminating microorganisms (1). This work was done to develop a starter that could be used to control the fermentation process to improve the quality of cocoa. Twenty-four yeast, ten acetic acid bacteria (AAB) and ten lactic acid bacteria (LAB) strains from stocks of isolates obtained from fermenting cocoa samples from Ghana were screened based on their contributions to the biochemical changes which take place during cocoa fermentation, to select some for formulating starter cultures. Six of the yeast strains which produced high amounts of ethanol and also showed pectin-degradation activity and three each of the acetic acid and lactic acid bacteria were selected and used in cocoa fermentation studies. Based on performance, three of the yeasts, the three selected AAB and one LAB were finally used to formulate two inocula ready for field fermentation trials. The studies generally confirmed the feasibility of using starter cultures to enhance the rate of fermentation of cocoa. Data obtained in both the initial screening work and the fermentation studies suggested that at least a combination of yeast and AAB would be required to appropriately ferment cocoa. The colour development however seemed to be influenced more by the LAB. pH and organic acid concentration of cocoa fermented with different single or mixed-strain cultures significantly varied to affect the quality of the fermented cocoa.

Keywords: Starter culture development, cocoa fermentation, artificial cocoa pulp medium, cocoa fermentation starter culture

## 2. Extended Abstract

**Aim**: To systematically screen and select a number of yeasts, acetic acid bacteria (AAB) and lactic acid bacteria (LAB) with potentials for the development of a starter culture for cocoa fermentation.

**Methods and Results**: Twenty-four yeast strains, 10 LAB and 10 AAB, all previously characterized(2), were singly-cultured in sterilized artificial cocoa pulp and tested for assimilation of citric acid, glucose, fructose and sucrose and the production of ethanol, lactic acid and acetic acid, the principal metabolites involved in the biochemical changes during cocoa fermentation. The yeasts were also tested qualitatively for pectinolytic activities. Six high alcohol producing yeasts strains with pectinolytic activities were identified and selected. Three AAB and three LAB strains which seemed to grow well and produced the metabolites of interest were also identified and selected. The selected isolates were used for further investigations using the artificial medium in mixed inoculations, and then as starter cultures in cocoa fermentation trials to determine their potential contributions to the cocoa fermentation.

**Conclusion**: Three yeast strains, *Saccharomyces cerevisiae* strain 1891, *Hanseniaspora guilliermondii* strain 16-6, and *Pichia membranifaciens* strain 4-8, three AAB strains (*Acetobacter tropicalis* strain A 250, *A. malorum* strain A123, *Gluconobacter oxydans* strain 292) and one LAB strains (*Lactobacillus fermentum* strain L18) were selected and combined into a starter culture which was used to produce cocoa beans comparable in quality to samples of cocoa beans traditionally produced in Ghana.

**Significance and Impact of the study**: The possibility of using a starter culture to enhance the fermentation rate of cocoa is confirmed and also potential isolates for the formulation of a cocoa fermentation starter culture were identified

**Acknowledgement**
This research was supported by a *Marie Curie International Fellowship* within the 6th European Framework Programme. We are very grateful to the Commission of the European Research Directorate for the fellowship for this project, MIFI-CT-2005-008143.

**References**

1. Thompson, S.S., Miller, K.B. and Lopez A.S., (2001). Cocoa and Coffee In: *Food Microbiology: Fundamentals and Frontiers* (Doyle, M.P. Beauchat, L.R. Montville, T.J., Eds). 2nd ed. Pp. 721-733. ASM Pres, Washington, D.C., USA

2. Jespersen L., Nielsen D.S., Hønholt S. And Jakobsen M. (2005). Occurrence and diversity of yeasts involved in fermentation of West African cocoa beans. *FEMS Yeast Research* 5, 441-453.

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# Enzyme extraction of phenolics from skins grape skins (*Vitis vinifera* L.) and apples (*Malus domestica*) in relation to the chemical compositional

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## 1. Summary

In this work we aimed to conduct a deep and detailed exploration of enzyme-assisted extraction of phenolics from berry and grape skins. The exploration will provide a ground base for understanding how the phenolics are bound to fruit skin cell wall polycarbohydrates-lignin matrix.

Keywords: Enzyme extraction, phenolics, skin cell wall, *Visit vinifera* L., *Malus domestica*, HPAEC-PAD / HPLC-DAD

## 2. Extended Abstract

Hypotheses: a)Whether it is possible to extract and retain selectively the most potent antioxidants by novel physical and enzymatic treatments of the berry and grape skin.
b) Whether it is possible to modify enzymatically the phenolics to optimize their health potential

Practically there is sparse knowledge about the phenolics detailed locations, and how they are bound into the fruit skin polysaccharides matrix. Knowledge about skins composition is needed. As first attempt we used grapes and apples skins as a model to understand the relation between skin cell walls polysaccharides and phenolics compounds. Such detailed compositional knowledge is a key issue to upgrade the wine making press residues or valorise the fruit skin for phenolics phytochemicals production as functional food additive. Polysaccharides composition of fruits skins cell walls are usually determined by measuring the monosaccharides released after hydrolysis with acid, alkaline, or enzyme. Chemical hydrolysis is simple, standardised, and an easily repeatable technique. By acid chemical hydrolysis, chromatographic data are simplified and interferences from undesired substances could be minimized. Cell wall polysaccharide composition (CWPC) data produced by acid chemical hydrolysis were helpful during extend enzyme assisted-extraction. Building CWPC reference helped comparing different enzymes under different conditions acting on different CWPC fruits materials.

For extended enzyme assisted-extraction selected commercial plant cell wall macerating enzymes were tested. The enzymes were Pectinex® BE Colour, Vinozym® FCE G (both enzymes are employed in the wine and juice industry), Celluclast® 1.5 L FG, mix of Celluclast® 1.5 L FG + Pectinex® BE Colour (1:1), and mix of Celluclast® 1.5 L FG + Vinozym® FCE G (1:1). The enzymes were added to pulverized, lyophilized grape skin samples -of defined particle size - of Cabernet Sauvignon and Merlot grapes in a full factorial design template. In the factorial design the type of enzyme preparation, enzyme loading, reaction temperature, and time were systematically varied. The extents of polysaccharides hydrolysis were evaluated by assessing levels of total water soluble carbohydrates and by monosaccharide quantification by HPAEC-PAD analysis. The extents of carbohydrate hydrolysis were then compared with the phenolics release pattern as measured by colorimetric quantification of total phenolics, and phenolics profiles by HPLC-DAD.

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# Regulation of *pyrG* gene expression in *Lactococcus lactis* is controlled by the amount of CTP through an attenuator mechanism involving reiterative transcription also known as stuttering

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## 1. Summary

As many metabolic processes involve nucleotides, a constant supply of these compounds is required for optimal growth. In order to fulfil this demand, the pathways in nucleotide metabolism are subject to regulation at both genetic and enzymatic levels. CTP is formed in a single reaction in which UTP is aminated. The reaction is catalyzed by CTP synthase encoded by *pyrG*[1]. The objective of this project is to determine the impact of regulation at the genetic level in the adjustment of the CTP pool size. By using metabolic control analysis we were able to show, that the CTP synthase has full control of the CTP pool size; an increase in *pyrG* expression leads to an increase in CTP pool size, whereas the CTP pool size decreases during reduced *pyrG* expression[2]. The expression of *pyrG* is regulated by the CTP pool size[3]. Therefore, the main conclusion is that the regulation of *pyrG* expression has the key role in CTP homeostasis at least in exponential growing cultures. The regulation of *pyrG* expression is found to be regulated at the transcriptional level by attenuation. During high CTP levels a terminator structure immediately in front of the structural gene is preferentially formed, whereas low CTP levels leads to formation of an anti-terminator. Evidence is presented that suggest that the intracellular CTP level is sensed by the speed of the RNA polymerase and this determines whether additional G-residues are incorporated at the very start of the transcript. The number of G-residues determines which structure preferentially is formed. *In vitro* data confirmed the model including the observation that no additional factor is required.

Keywords: Nucleotide metabolism, *pyrG*, gene expression, attenuation, Lactic acid bacteria

### References

(1.) Kilstrup, M.; Hammer, K.; Ruhdal, J. P.; Martinussen, J. *FEMS Microbiol.Rev.* **2005**.

(2.) Jorgensen, C. M.; Hammer, K.; Jensen, P. R.; Martinussen, J. *Eur.J.Biochem.* **2004**, *271*, 2438-2445.

(3.) Jorgensen, C. M.; Hammer, K.; Martinussen, J. *J.Bacteriol.* **2003**, *185*, 6562-6574.

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# High gravity brewing- study of the effect of ethanol tolerance and nitrogen supplementation

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## 1. Summary

In the process of high gravity beer fermentation, the yeast is exposed to a number of stressful conditions: high osmotic pressure, high specific gravity, low temperature and high ethanol concentrations at the end of the fermentations. In this study, we investigated the two major limiting factors of the yeast performance in high gravity brewing- ethanol toxicity and limited nitrogen availability. Two lager beer yeast strains- the ethanol tolerant AJL 3126, and the less ethanol tolerant strain AJL 2252 have been characterised in series of batch cultivations, at average gravity of 14°Plato as well as at high gravity at 21°Plato. When comparing the two strains, both for normal and high gravity fermentations, AJL3126 showed higher specific growth rates and higher final ethanol concentration. From all fermentations, samples from early exponential and stationary phases were collected for extensive metabolome analysis.
For studying the effect of addition on different nitrogen sources, the addition of urea, ammonium sulphate and enzymatically generated nitrogen source on the fermentation performance at 21°Plato of the strain Weihenstephan 34/70 were examined. In all cases of nitrogen supplemented fermentations, the yeast strain showed higher specific growth rate and higher ethanol production rate compared to the control non-supplemented fermentations. In addition, nitrogen supplemented fermentations showed improved flavour and aroma profiles of the final beer, as for example in terms of reduced amounts of acetaldehyde, ethyl acetate and isoamyl acetate.

Keywords: high gravity brewing, ethanol tolerance, metabolome, nitrogen supplementation;

## 2. Extended Abstract

High gravity brewing is currently one of the most popular brewing techniques. Whereas normal gravity beer fermentation is performed in the range of 12-15ºPlato, high-gravity fermentation involves production of beer wort of up to 18ºPlato and even higher. We characterised, in series of batch cultivations, at average gravity of 14°Plato as well as at high gravity- 21°Plato, two yeast lager beer strains: the ethanol tolerant AJL 3126, and the less ethanol tolerant strain AJL 2252. The wort at 21° Plato

was prepared with the addition of highly fermentable maltose or glucose syrups to the wort with average gravity. The physiological characterisation included growth characterisation as well as characterisation of the carbohydrate profiles by HPLC. When comparing the two strains, both for normal and high gravity fermentations, AJL3126 showed higher specific growth rates and a higher ethanol yield. The final ethanol concentration was up to 54 g/L, for the fermentations at 14° Plato and up to 86 g/L for the fermentations at 21° Plato. For both strains maximum cell concentrations were observed after 120 hours for the fermentations at 14°Plato and after 150 hours for the fermentations at 21° Plato. For the fermentations at 21° Plato, the fermentations ended with some residual amounts of maltose and maltotriose. Samples for metabolome analysis from early exponential and stationary phases from all fermentations have been collected. The metabolome analysis from the samples collected from the stationary phases of the fermentations showed distinct separation between the two strains as well as between the glucose and maltose supplemented fermentations for the less ethanol tolerant strain.

For the effect on addition of different nitrogen sources, we examined the effect on the addition of urea, ammonium sulphate and enzymatically generated nitrogen sources on the fermentation performance at 21°Plato of the popular lager beer yeast strain Weihenstephan 34/70. In all cases of nitrogen supplemented fermentations, the yeast strain showed slightly higher specific growth rates and higher ethanol productivity compared to the control non-supplemented fermentations. Both of the fermentations with enzyme additions increased the amount of the higher alcohols 3-methyl-1-butanol and 2-methyl-propanol in the final beer. Nitrogen supplemented fermentations showed improved flavour and aroma profiles of the final beer, as for example in terms of reduced amounts of acetaldehyde, ethyl acetate and isoamyl acetate. The addition of enzymes was shown to decrease concentrations of the ester ethyl acetate. In beer produced from wort with enzyme additions or with urea supplementation none, or only very small amounts of acetaldehyde was detected.

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# Session S-5Ee: White BioTech & Related Processes (Food-2b) – 5 min oral presentation plus poster presentation

| Abstract Number | Paper Title & Authors | Included |
|---|---|---|
| 651 | Influence of exopolysaccharide producing starter cultures and incubation temperatures on the physical and rheological properties of low fat set type yogurt<br><br>H Abbasi, M R Ehsani, S M A E Mousavi, Z E D Jomeh, M Vaziri | No |
| 3671 | Date syrup alternative source for lysine production<br><br>H Ahmadloo, F Naeimpoor | Yes |
| 4051 | Effects Of Axial Compression On Dry Active Saccharomyces Cerevisiae Structure<br>C T Sampsonis, F Fayolle, A Perronnet, A Arhaliass, J Legrand, E Auclair | Yes |
| 4101 | Rapid, extensive extraction of polyphenols from red grapes<br>J S Jensen, B Blachez, M Egebo, A S Meyer | Yes |
| 4103 | Utilization of natural food wastes as new adsorbent for precious metal ions<br>M Goto | No |

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# Date syrup alternative source for lysine production

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## 1. Summary

Lysine is an essential, economically important amino acid used as food and feed supplement. However, economical production of lysine depends on the availability of local resources. In *Middle East* countries, date is an important agricultural product which has the potential for being a nutritious source for lysine production.
In this work, date syrup was selected as an alternative complex sugar source for lysine production. *Brevibacterium flavum* and *Corynebacterium glutamicum* were used to examine the effect of variation in culture composition and operating conditions on growth and lysine formation. we have 5.42 g/l lysine production at the maximum level with *Corynebacterium Glutamicum* and 4.35 g/l lysine production with *Brevibacterium flavum*.

Keywords: Lysine, Corynebacterium glutamicum, Brevibacterium flavum, Date syrup

## 2. Extended Abstract

***Microorganism:*** Our microorganisms were taken from Persian Type Culture Collection. *Corynebacterium glutamicum*, PTCC 1603 and *Brevibacterium fluvum*, PTCC1532 these are used widely for industrial production of lysine [2].
***Cultures preparation & incubation*** Culture making is divided into 3 important steps, include: solid fermentation, inoculum producing and making main complex culture.

***Measurement methods:*** In this paper measurements are divided into 2 parts: biomass, and lysine analysis. Spectrophotometer UV/V is SP8001 (MeterTech.) is utilized in all cases for biomass determination OD of all samples in 660 nm are recorded. Then decolourization method applied and Chaynard (1992) Lysine detector is employed. After preparation of all samples, OD of them is recorded in 512 nm [4]. *Winrobust* software (accomplishing *Taguchi* method and analyzing results) for all optimization steps is employed**.**

**Results and Discussion:** At first Growth and lysine production by two bacteria species fermentation were studied in five different media with 5%,10%,18% date syrup, 1% glucose +5% date syrup, 1% ammonium sulphate + 5% date syrup. Results were analysed (comprising growth and lysine production) and the best media were selected (5% date syrup+1% glucose+1%amonium sulphate for *Corynebacterium*

and10% date syrup+1% glucose+1%amonium sulphate for *Brevibacterium*) to continue the next steps. Afterwards, optimization of culture medium by taguchi method was performed. Nitrogen, Potassium, Magnesium and Iron sources were studided in this step. Finally the best proposal mediums were cultured for 6 days and results were comprise with previous similar studies.

**Table1**. *Corynebacterium &Brevibacterium* results

| *Bacteria* | *Corynebacterium glutamicum* | *Brevibacterium fluvum* |
|---|---|---|
| *Parameters* | *Value* | *Value* |
| *Fermentation Duration* | *144 hr* | *144 hr* |
| *Temperature* | *32˚c* | *32˚c* |
| *Medium Volume* | *100 cc liquid in 250cc Erlenmeyer* | *100 cc liquid in 250cc Erlenmeyer* |
| *pH* | *7.5* | *7.5* |
| *Inoculm Size* | 10% | 10% |
| *Date Syrup* | 5% | 10% |
| *Ammonium Sulfate* | 4% | 2.5% |
| *Potassium di hydrogen phosphate* | 0.1% | 0.05% |
| *Magnesium sulfate* | *50mgr /100cc* | *50mgr /100cc* |
| *Iron Sulfate* | *0.2%* | *0.2%* |

**Table2**. *Comparison final fermentation by previous papers*

| ***Year-paper*** | ***Complex Medium*** | ***Lysine Production (gr/lit)*** | ***Bacteria*** |
|---|---|---|---|
| *2007-Our Study* | *Date Syrup* | *4.35 & 5.42* | *Brevibac.& Corynebac.* |
| *1983-Smackel* | *Hydrolyzed Paper* | *4.4-6.3* | *Brevibac.* |
| *1983-Pencholva* | *Fruits juice* | *2-2.4* | *Corynebac.* |
| *1997-Orchenco* | *Cane Liquor* | *3.4-4* | *Corynebac. & Brevibac.* |
| *1992-Pham* | *molasses* | *6.9* | *Corynebac.* |

## References

[1] Seibold G.,. Auchter M, Berens S. and Eikmann B. J, "*Utilization Of Soluble Starch by A Recombinant Corynebacterium glutamicum: Growth And Lysine Production* ", *Journal Of Biotechnology*, Vol. 105, pp-114-128, (2005)

[2] Haleemshah A., Hameed A., Ahmad S.and Majid khan G., " *Optimization Of Culture Condition For Lysine Fermentation By Corynebacterium glutamicum* ", *Journal Of Biological Science*, Vol. 2, No. 3, pp-151-156, (2002)

[3] Hermann T., " *Industrial Production Of Amino acids By Coryneform Bacteria* ", *Journal Of Biotechnology*, Vol. 104, No. 1-3, pp-155-172, (2003)

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# Effects of axial compression on dry active Saccharomyces cerevisiae structure

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## 1. Summary

In this work, an experimental strain of dry live yeast Saccharomyces cerevisiae agglomerated in spheres with diameters between 1000 and 2000µm was considered. The mechanical resistance of these spheres was studied with uni-axial compression tests (from 0 to 4000N) by using a texturometer. Effects of mechanical constraints on the structure of yeast spheres were described using binocular and electron scanning microscopy.

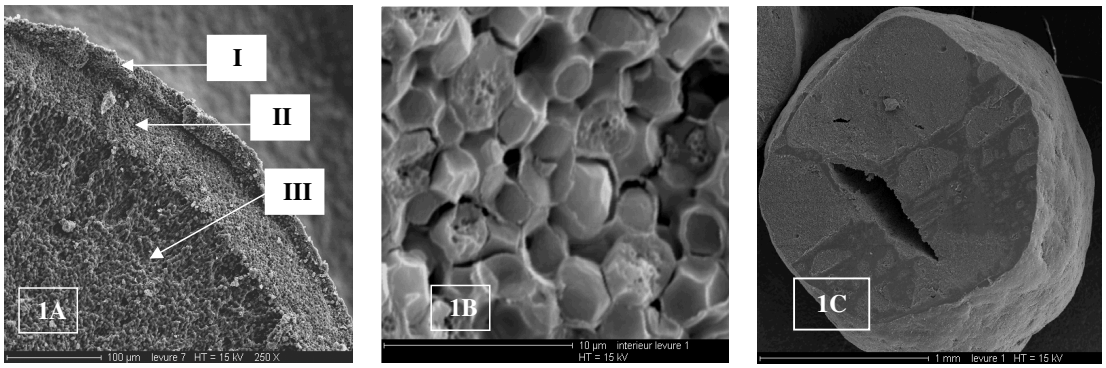
It was shown that the mechanical resistance of the spheres depended on their diameter, bulk density and moisture level. Indeed, a clear fracture of the spheres with a diameter higher than 1600µm and a low bulk density (749g/l in average) was observed at 1000N. In contrast, no clear fracture of the spheres with a lower diameter was seen with compression forces from 0 to 4000N specially with low moisture level spheres. The observation of the structure of the different spheres using scanning electron microscopy showed an air-filled area in the largest spheres (diameter higher than 1600µm). This empty space was responsible for the collapse of their structure. A decrease in spheres diameter (<1600µm) related to an increase in bulk density (>749g/l) and a low moisture level (<6,0%) could improve the mechanical resistance of such yeast spheres.

Keywords: yeast, texturometry, scanning electron microscopy

## 2. Extended Abstract

Live yeast is used extensively as probiotic agents in the ruminants feed industry (Newbold, 1996). However, as mechanical constraints are currently used in the feed manufacturing process, it was interesting to study the mechanical resistance of an experimental agglomerated *Saccharomyces cerevisiae* by an original combination of the texturometry method and electron scanning microscopy. After description of the yeast initial structure, the mechanical resistance of spheres to axial pressure was studied in relation to their physical properties (sphere diameter, bulk density and moisture level).

The experimental agglomerated yeast cells were spherical in form, with a smooth shiny surface. Seen in section (Figure 1A), the spheres presented three different structures (I, II and III) but formed with the same network of dehydrated yeast cells. Each cell was surrounded by a wall rigidified by the drying process (Figure 1B). The three different orientations of the structures were probably due to the speed of water migration during the drying phase (evaporation phenomenon).




**Figures 1:** initial agglomerated Saccharomyces cerevisiae structure seen by electron scanning microscopy

However some structural particularities differentiated large (fraction 1 : 1600-2000 µm) and small spheres (fraction 2 : 1000-1600 µm). Indeed, an air-filled area characterized spheres in fraction 1 (Figure 1C), and a full internal structure spheres in fraction 2. This structural difference explains why the behavior of spheres to axial compression was defined by different physico-chemical properties in relation to their diameter : bulk density and dry matter level for spheres in fractions 1 and 2 respectively.
Thus, the presence of an air-filled area caused the collapse at 1000N of the structure of large spheres with low bulk density (<749g/l). While moisture level was a more discriminating factor to predict the behavior of small spheres to axial compression. Moisture level was directly related to spheres elastic properties.

A decrease in spheres diameter (<1600µm) related to an increase in bulk density (>749g/l) and a low moisture level (<6,0%) could improve the mechanical resistance of such yeast spheres.

## References

Newbold, C.J., (1996) *Annual Zootechnology*, 45, 329-335.

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# Rapid extraction of polyphenols from red grapes

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## 1. Summary

Assessment of the phenolic content of red grapes is an important prerequisite for understanding how grape phenols impact wine quality. In the search for a rapid and robust extraction method we have investigated the influence of selected factors on extraction efficiency of phenols from eight different red wine grape cultivars. The effects of solvent contact time, extraction temperature, solvent levels of ethanol and hydrochloric acid on the extraction of total phenols and anthocyanins were investigated in statistically designed factorial experiments. Both extraction temperature and solvent levels of ethanol and hydrochloric acid were found to have highly significant effects on the extraction of both total phenols and anthocyanins. From these findings a rapid extraction protocol was defined, with high extraction degress of total phenols and anthocyanins.

Keywords: Solvent extraction, red grapes, anthocyanins, total phenols

## 2. Extended Abstract

Red wine color is mainly dependent on the content and composition of the phenolic substances present in the wine, including notably the total level of anthocyanins and polymeric pigments (Bakker et al. 1986, Mazza et al. 1999, Sacchi et al. 2005). The color forms an important part of the quality perception of red wine due to the influence of the color on the visual interpretation (Somers and Evans 1974), but phenolic compounds also affect the aroma and mouthfeel properties of red wines (Preys et al. 2006). A main hypothesis in our ongoing work on grape and wine phenols is that the phenolics present in the grapes have a significant influence on the quality of the finished wine and that it may be possible to predict the wine quality from quantification of the phenolics in the grapes.

A prerequisite for obtaining a proper evaluation of the phenolics present in grapes is the definition of a robust extraction method for the grape phenols. In order to do so a first requirement is to understand the influence of different parameters on the extraction efficiency and yields. This was investigated for selected parameters on grape homogenates of different grape cultivars in full factorial designs (Jensen et al. 2007). The results were bench-marked versus the "total" content as measured by a modified, 1 hour "total" extraction protocol and the data were expressed as relative extraction degrees. The extraction temperature, aqueous solvent levels of ethanol and

hydrochloric acid were each found to exert a highly significant impact on the extraction efficiency of total phenolics and anthocyanins (Table 1).

**Table 1** Effect tests and estimated model parameters for the mean extraction degree (%) of total phenols and anthocyanins for 3x3x2 full factorial design with three centerpoints. The factor levels were A) ethanol % v/v of 0, 25 and 50, B) temperature of 20, 40 and 60 ºC and C) hydrochloric acid concencentration of 0 and 0.1 M.

| Term | Total phenols (model fit: $R^2 = 0.99$ ) | | | Anthocyanins (model fit: $R^2 = 0.96$ ) | | |
|---|---|---|---|---|---|---|
| | Prob > F [a] | β estimate [b] | Std Error | Prob > F [a] | β estimate [b] | Std Error |
| Intercept | <.0001 | 66.49 | 0.36 | <.0001 | 83.40 | 0.76 |
| EtOH | <.0001 | 18.75 | 0.48 | <.0001 | 12.80 | 1.00 |
| HCl | <.0001 | 6.69 | 0.39 | <.0001 | 8.00 | 0.82 |
| Temp | <.0001 | 6.63 | 0.48 | <.0001 | 5.48 | 1.00 |
| EtOH*HCl | 0.446 | -0.37 | 0.48 | <.0001 | -5.54 | 1.00 |
| EtOH*Temp | 0.425 | 0.48 | 0.58 | 0.032 | -2.92 | 1.22 |
| HCl*Temp | 0.367 | 0.45 | 0.48 | 0.093 | -1.80 | 1.00 |

[a] Prob > F describes the probability that a term does not have a significant effect.
[b] The β estimates of the linear model using mean centered factor levels scaled between -1 and +1.

The data were used to design an optimal extraction protocol which encompassed the following extraction conditions: 50 % v/v acidified aqueous ethanol (0.1 M HCl) and 1:1 solvent:grape v/w ratio at 40 ºC, followed by sample neutralization and workup for analysis. By this method it was possible to extract an average of 82.1 % total phenols and 91.5 % of the anthocyanins from the grapes with only five minutes of solvent contact time. The protocol thus allowed high extraction of grape phenolics with acceptable relative standard deviations of 3.8 % for total phenols and 6.0 % for anthocyanins across eight different cultivars.

## References

Bakker, J., Preston, N.W., and Timberlake, C.F. 1986. The determination of anthocyanins in aging red wines - comparison of HPLC and spectral methods. Am. J. Enol. Vitic. 37:121-126.

Jensen, J.S., Blachez, B., Egebo, M., and Meyer, A.S. 2007. Rapid Extraction of Polyphenols from Red Grapes. Am. J. Enol. Vitic., in press.

Mazza, G., Fukumoto, L., Delaquis, P., Girard, B., and Ewert, B. 1999. Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Columbia. J. Agric. Food Chem. 47:4009-4017.

Preys, S., Mazerolles, G., Courcoux, P., Samson, A., Fischer, U., Hanafi, A., Bertrand, D., and Cheynier, V. 2006. Relationship between polyphenolic composition and some sensory properties in red wines using multiway analyses. Anal. Chim. Acta 563:126-136.

Sacchi, K.L., Bisson, L.F., and Adams, D.O. 2005. A review of the effect of winemaking techniques on phenolic extraction in red wines. Am. J. Enol. Vitic. 56:197-206.

Somers, T.C. and Evans, M.E. 1974. Wine Quality - Correlations with Color Density and Anthocyanin Equilibria in A Group of Young Red Wines. J. Sci. Food Agric. 25:1369-1379.

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# Session S-5F: Flexible Production, PAT & Modelling (Food-3a)

| Abstract Number | Paper Title & Authors | Included |
|---|---|---|
| 1172 | Contribution to the modelling of chocolate tempering process<br>F Debaste, Y Kegelaers, H B Hamor, V Halloin | Yes |
| 1501 | Characteristic of a Dairy Production Line under Uncertainty<br>H Cheng, A Friis | Yes |
| 1641 | Modeling and simulation of vegetable oil processes<br>A Martinho, H A Matos, R Gani, B Sarup, W Youngreen | Yes |
| 1643 | System Analysis and Automated Control of Fruit Ripening Processes<br>V C Hass, S Toemmers, K Kuehn, O Mierig | Yes |
| 2362 | Effect of the formulation on the continuous manufacturing of foamed products<br>I Narchi, C Vial, G Djelveh | Yes |
| 3904 | Modelling of physical and chemical processes in the small intestine<br>A Tharakan, P Rayment, I T Norton, P J Fryer | Yes |
| 3918 | Dynamic optimization of baking operations using refinement method<br>H D C Esveld, R M Boom, G Straten, A J B Boxtel | Yes |
| 3999 | A tool for productive and environmentally efficient food production management<br>K Östergren, J Berlin, B Johansson, B Sundström, J Stahre, A-M Tillman | Yes |

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# Contribution to the modelling of chocolate tempering process

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## 1. Summary

The tempering of chocolate, i.e. the process of crystallization of the desired morph; is a key step in its manufacturing by professional chocolatiers. In this work, a model of a tempering process based on seeding with solid chocolate grains is developed to enhance understanding and control of the system. The model aims to predict temperature field during melting and crystallization of the product. Therefore a mechanical stirrer is designed to simulate the manual mixing. Resulting flow field is modeled using CFD. Based on simulation results, the heat transfer problem is simplified using an effective thermal conductivity. The parameter value is fitted on experimental results. The heat conduction equation obtained is solved using Comsol Multiphysics. Melting of chocolate particles is added to the model using a sink term having the form of a kinetic reaction whose parameters are identified from an adiabatic melting experiment. The resulting model gives an accurate prediction of the cooling rate and the temperature field within the melted chocolate seeded with small solid grains.

Keywords: Chocolate tempering, Heat transfer, Computational fluid dynamics

## 2. Extended Abstract

Tempering process refers to a controlled melting and cooling of chocolate in order to achieve at the end the correct crystalline structure of the constituent cocoa butter (form V of the six different polymorphic forms). Well tempered chocolate is shiny, even-colored, crisp, and smooth tasting, while badly tempered chocolate is chalky and grainy, within the form of an unattractive, dull brown mass streaked with grey.
The trick to tempering is to control the temperature of the melted chocolate very precisely: first cooling it and then reheating slightly or adding crystallization nuclei such as solid chocolate.

In this study, a mathematical model is developed in order to get a better analysis and control of the tempering process.

The system considered here is the one used by professional pastry chefs and chocolatiers for dipping, molding and decorating. A batch of chocolate is first molten in a bowl placed in a temperature controlled water bath. The molten batch is then cooled at ambient temperature, while gently mixed by hand. At a given temperature, the liquid chocolate is seeded with a given amount of small solid pieces (for instance crystals of solid chocolate) to ensure the crystallization under the best form (V).

The modeling work aims to predict the evolution with time of the temperature field inside the chocolate during the cooling and crystallization phase.

Firstly, the mixing of melted chocolate is studied without taking care of any heat transport. A mechanical stirrer is designed to simulate the manual mixing in a controlled manner. Even if it can be anticipated that melted chocolate exhibits non-Newtonian behavior because of the importance of solid particles (of sugar, cocoa and milk powder) dispersed in the continuous fat melted phase, a laminar Newtonian flow is considered. A CFD simulation is performed using Fluent software, whose results display a mainly axial secondary flow.

Secondly, on the basis of this flow analysis, the transient heat transfer problem is simplified by neglecting the convective terms in the heat balance equation but using an effective thermal conductivity parameter to take into account enhancement of heat transport by the mixing process. This one is therefore modeled as a thermal diffusion process between neighboring fluid layers.
The resulting transient axisymetric heat conduction equation complemented with adequate boundary conditions is solved using Comsol Multiphysics. The value of the effective conductivity is fitted against experimental results, more precisely the temperature measured in the center of the bowl. A value of 10,7 W/m K is obtained, which is one order of magnitude higher than the thermal conductivity of molten chocolate.
A very good agreement is observed for the whole temperature field. Indeed, predicted transient temperatures compare very well to experimental data obtained with thermocouples set at 6 different locations within the melted chocolate.

Thirdly, a sink term is added to the thermal balance equation to take into account the additional cooling arising from the latent heat of melting of the solid pieces used as crystallization seeds. This term is written under the form of a kinetic reaction whose parameters are identified from an adiabatic melting experiment.

The resulting model gives finally an accurate prediction of the cooling rate and the temperature field within a mass of melted chocolate let at ambient temperature and seeded with small solid grains. On-going works focus on the development of a shrinking core model to better describe the step of seeds melting. The nucleation process will then be studied in order to complete the model. Such models should be useful to identify better criteria for good tempering conditions.

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# Characteristics of a dairy process under uncertainty

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## 1. Summary

In this work, the characteristics of a dairy production process under diverse product uncertainties are investigated through a process simulation. The flexibility analysis method of Grossmann and his co-workers (Swaney and Grossmann, 1985) is applied through a process simulation tool, PRO/II. A new set of physical property models for diary products were developed and built into PRO/II system. Milk products viscosity and process system pressure drop were employed as the process characteristic parameters to determine a process operation window. The flexibility of the operation window vertexes was evaluated with a minimization of the process pasteurization and cooling temperatures through vertex enumeration method. The quantitative analysis of the dairy process established a framework in developing of different flexible units, such as integrated milk and milk-based product productions, multi-task process unit, etc.

Keywords: flexibility, operability, milk production, simulation

## 2. Extended Abstract

To respond the needs of ever-changing food market, the development of a flexible processing strategy is very important for existing production units which were designed from the “one size fits all” concept. With the dramatically changed products, a set of optimal process operation methods are also needed for a food process in order to shift over production rapidly with minimal product loss. A quantitative understanding of the characteristics of a food process under diverse uncertainties is a foundation to develop the flexible and optimal production strategies.
Process system engineering theory and method have successfully been developed and applied in chemical engineering field for decades. Many reliable process simulation tools are available for chemical process engineering applications. In principle, the food process development can also adapt these process simulation tools if the physical property data (or models) of food are accessible. In this work, a process simulation tool, PRO/II, was selected to investigate the characteristics of a milk production process under different uncertain factors. A set of physical property models for liquid diary products were developed.

In a food production process, the main uncertain factors are the ever-changed product recipes (composition). Consequently, the process operation parameters and methods should be adjusted with the changed product recipes. In a dairy process, the change of product recipes reflects the physical property variations for those milk-based products. The processing method changes result in processing parameters and steps update. Among the changed physical properties for the milk-based liquid products, the fluid viscosity and enthalpy are the most important properties. In this work, the milk fluid viscosity and system pressure drop were selected to construct a process operation window. The operation window represents the process operability for the different milk based products. For the product-orientated production dealing with frequently changed products, the process operability should first be considered. After that, the process flexibility can be evaluated.

The method proposed by Grossmann and his coworkers (Swaney and Grossmann, 1985) was used to evaluate the process flexibility. If we assume the dairy process is flexible within its operation window, we only need to evaluate the singular points at the operation window vertexes. A minimization of the process pasteurization and cooling temperatures through vertex enumeration method was applied using PRO/II.

This work has demonstrated that the well-developed process system engineering theory and method in chemical engineering area can be employed in food process engineering applications. However, the development of food material physical property calculation models is very crucial in the applications.

Through a process simulation tool, such as PRO/II, the established process simulation framework can be adapted to any milk based liquid food production flowsheet because the developed milk physical property models are suitable for a wide composition and temperature ranges. From the quantitative description of the process operability and flexibility, we can explore various operation strategies for a dairy process.

**References**

Swaney, R. and Grossmann, I.E., (1985) *AIChE J*. 31 (4), 621-630.

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# Modeling and simulation of vegetable oils process

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## 1. Summary

The main target of this work is to provide a correct model of a vegetable oil process production namely soybean oil compounds. This involves: the creation of a new database with vegetable oil compounds with the estimation of their properties; the development of the models and their validation by comparison between simulations extracted data and real industrial data. Furthermore, the simulation models developed can also be used as a source data for process optimization.

Keywords: modeling, simulation, validation, recovery optimization, solvent-based extraction, vegetable oil

## 2. Extended Abstract

The goal of this work is modelling, simulation and optimization of a vegetable oil production process.

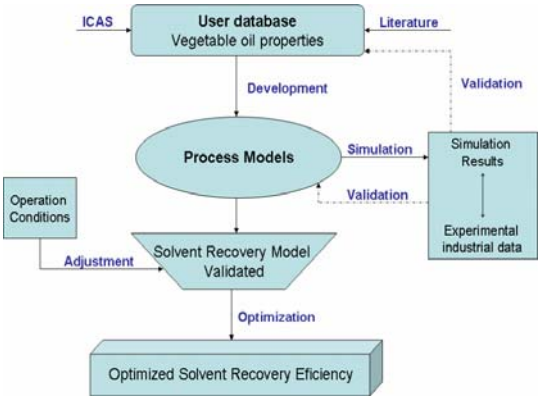


**Figure 1**- Methodology

A solvent-based extraction process for the production of vegetable oil from soybean seeds has been studied with special emphasis on the solvent recovery section of the process. The complete process is composed by: preparation, extraction, desolventization, oil and solvent recovery and the oil refining.

This study was dedicated to the solvent recovery section since, this part of the process has a great influence on how the plant can operate in an economical, environmental responsibility and general safety way. Thus, the solvent recovery section includes four parts: the evaporation of the hexane to concentrate the miscela (also part of the oil recovery), a condensation system, a mineral oil system and a water-solvent separation. The main compounds representing the vegetable oil (namely, soybean oil) usually consist of free fatty acids, triglycerides, tocopherols and sterols. The ICAS-ProPred software, added with literature data, has been used to generate the pure compound data and insert them into the database of a commercial simulator (ProII) (Figure 1). The next step has been the simulation of the process to validate the process models by matching the steady state data (mass balance) with industrial data (Figure 2). Two models were built: a double scrubber (deodorizer) which is used to remove the undesirable flavours and odours from the host triglycerides of the oil, making part of the oil refining section; and the solvent recovery, where all the hexane is recovered in a great extend as possible. The first was developed in ICAS software and the second in Pro II. The validation of these models by comparing the simulation results with the real industrial data, transforming them in a very useful tool for simulations/ prediction in other operational conditions. Moreover, the good choice of the generated compounds to represent the oil allows the prediction of the results for some eventual scenarios of improvements of the process. The oil behaviour is an important parameter in analysis, since the major compounds properties (with exception for the free fatty acids) were estimated in ICAS-ProPred. The final step has been to apply the validated process model to optimize the solvent recovery efficiency by adjusting operational variables.

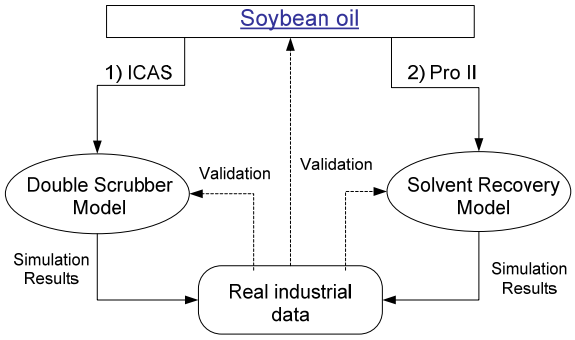


**Figure 2-** The models validation

For double scrubber model, global stream flows, present a standard deviation inferior to 0,7%, and for solvent recovery, the major difference obtained, is 17 ppm in oil composition of an air out stream. The solvent recovery efficiency is mainly affected by evaporation and condensation conditions zone.

## References

Hui, Y.H., *Bailey's Industrial Oil and Fat Products*, John Wiley & Sons, 5th edition, Vol.4

Book of Abstracts
European Congress of Chemical Engineering (ECCE-6)
Copenhagen, 16-20 September 2007

# System Analysis and Automated Control of Fruit Ripening Processes

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## 1. Summary

Rising competition on the consumer market forces the food industry to improve production processes. To fulfill the increasing demands and to generate a cheap and homogeneous product, a model-based automation strategy for the banana ripening process is being developed. The automated process control comprises an assurance and improvement off product quality. Energy and personal costs can be reduced with the help of a uniform way of controlling the process.

Keywords: banana ripening, optimized process, model-based control, automation strategy

## 2. Extended Abstract

To date, the ripening process of bananas has not been thoroughly analyzed or understood. The knowledge regarding this process is basically of qualitative nature. Process control in ripening chambers is based on the longstanding experience of a few people. In 40 % of the cases manual corrections on the process are still necessary.

Reasons for this can be found in the complexity and variability of the banana ripening process. There is a variety in the initial state of ripeness of the input bananas due to different pre-treatment. In addition, the course of the industrial banana ripening process is varying due to biological variables like enzyme activity. The ripening time may change due to logistic prerequisites.

The approach in the work presented here was to gather process understanding in a quantitative system analysis and the state of the art in an extensive technology analysis. The industrial ripening process is reproduced in a pilot plant, an experimental banana ripening box. Experiments in this box are used to search for a good indicator for the state of ripening. Plant parameters and significant process values must be assigned. Based on on-line measured values the actual state of the process will be estimated. Furthermore an applicable automation strategy must be worked out. Therefore, high-quality and highly efficient control loops are designed.

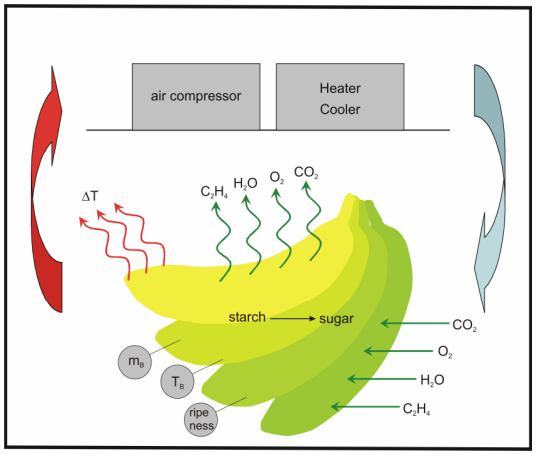


**Figure 1 :** structure and functioning of the industrial banana ripening and its significant parameters

A mathematical model is developed on the basis of the biochemistry of ripening bananas and on the plant environment. It describes the dynamics of the banana ripening process in the form of a system of coupled differential equations. The model will be used for the estimation of important state variables which then will be used to control the process. The model allows rapid simulation of different scenarios and thus to find solutions for varying courses of processes. Such a model based process control scheme should lead to moderate and efficient production techniques.

The varying parameters in the banana ripening process require a high performance process control strategy that flexibly adapts to the variable and imprecisely described process. Adaptive process control with the OLFO-method has been successfully applied in biotechnology [*Luttmann,* 1985; *Witte*, 1996 and *Frahm et al*., 2003] and shall be applied, transferred and further developed to the banana ripening process, as a process in the food industry. It is expected that this model based adaptive and optimizing automation strategy could be transferred to other fruit ripening processes.

This innovative automation strategy should enable an advanced process control of the banana ripening process that flexibly adapts to changing conditions and process values.

## References

Luttmann, R., Munack, A., Thoma, M., (1985) "Mathematical Modelling, Parameter Identification and Adaptive Control of Single –Cell Protein Processes in Tower Bioreactors", *Advances in Biochemical Engineering/Biotechnology*, 32, 95-205.

Witte, V.C., *Mathematische Modellierung und adaptive Prozesssteuerung der Kultivierung von Cyathus striatus*, Dissertation, VDI-Verlag, Düsseldorf (1996).

Frahm, B., Hass, V. C., Lane, P., Munack, A., Märkl, H., Pörtner, R., (2003) "Fed-batch Kultivierung tierischer Zellen - eine Herausforderung zur adaptiven, modellbasierten Steuerung", *Chemie Ingenieur Technik*, 4, 457-460.

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# Effect of the formulation on the continuous manufacturing of foamed products

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## 1. Summary

The aim of this work is to study the effect of formulation on the properties of the continuous phase, the gas/liquid interfacial properties and the continuous manufacturing of foamed products under laminar flow conditions. The continuous phase was a Newtonian glucose syrup (1 Pa.s) in which three polysaccharides were added at 0.1% (w/w): namely, xanthan, LM pectin and guar. Gas/liquid interfacial properties were modified by adding 2 % (w/w) of two kinds of proteins: sodium caseinate and whey proteins. The combined effects of polysaccharides and proteins were investigated by foaming experiments using a mixture of both types in glucose syrup solutions. Rheological and interfacial properties were measured by using a stress-controlled rheometer and a static tensiometer. Foaming was studied in the narrow annular gap unit of Djelveh and Gros (1995). Overrun and bubble size distributions were measured in foams at the exit of the column. Results showed that formulations containing 0.1 % xanthan or guar without proteins did not allow gas incorporation because of the blow-by phenomenon, while the formulation containing 0.1 % of pectin allowed the incorporation up to 100 % of the gas phase. In regards to results on proteins, the formulation containing 2 % Na-caseinate without polysaccharide allowed the incorporation of only 72 % of the gas phase. Conversely, experiments on formulations with 2 % of whey proteins alone gave abundant foam with an average bubble diameter of about 20 µm ± 2. About formulations containing 2% of whey proteins with polysaccharides, the formulation containing pectin gave abundant and very stable foams. The mixture of whey proteins and guar allowed the incorporation up to 100 % gas, while the formulation with 0.1 % xanthan exhibited a maximum gas incorporation of 72%.

Keywords: food foam, foaming process, foam formulation, polysaccharides, proteins

## 2. Extended Abstract

Foaming is a unit operation that consists in dispersing and stabilizing a gas phase in the form of bubbles into a continuous solid or semi-solid matrix, in order to confer a porous structure. It is frequently used in the food industry. Most common examples of foamed food are “sweet foams” (dairy desserts…), “acid foams” (textured cheese, fruit fools…) and “salted foams” (fish foams, pâtés...). Although the raw material

used in foaming are often emulsions exhibiting a non-Newtonian behaviour, the aim of this work is to investigate formulations without fat to better understand the gas incorporation in a continuous foaming process in laminar flow conditions.
The continuous phase is a Newtonian glucose syrup (1 Pa.s) in which three polysaccharides can be added at 0.1% (w/w): xanthan, LM pectin and guar. The effect on gas/liquid interfaces is investigated by adding 2% (w/w) of two kinds of proteins: sodium caseinate and whey proteins. The combined effect of polysaccharides and proteins is studied by using formulations containing a mixture of both components. Rheological and interfacial properties of the continuous phase are measured using a stress-controlled rheometer and a static tensiometer. Foaming is studied with a gas to liquid flow ratio of 10/30 and a rotation speed between 400 and 1600 rpm. At the exit of the column, foams are characterized by their overrun and their bubble size distribution using image analysis. The average size is defined using the Sauter diameter ($d_{32}$).
Results showed that the formulations containing 0.1% xanthan or guar without proteins did not allow gas incorporation because of the blow-by phenomenon. Conversely, the formulation containing 0.1% of pectin allowed the total incorporation up to 100% of the gas phase for all process conditions. The bubble diameter in these foams was about 50µm ± 10. According to the results on proteins, the formulation containing 2% Na-caseinate without polysaccharides allowed the incorporation of only 72% of the gas phase at 400 rpm. This value decreased to 60% when the rotation speed increased and, at 1600 rpm, blow-by appeared. Conversely, experiments on the formulation containing 2% of whey proteins confirmed their high foaming ability: for all operating conditions, abundant foam was obtained with an average bubble diameter about 20 µm ± 2. About formulations containing 2% whey proteins with polysaccharides, the formulation containing pectin gave abundant and very stable foams. The mixture of whey proteins and guar allowed the incorporation up to 100 % of the gas phase for all rotation speeds. Bubbles were very small, about 17 µm ± 3. Conversely, the formulation with 0.1% xanthan exhibited a maximum incorporation of the gas phase of about 72 % at 800 rpm, and then blow-by appeared.
Finally, experimental data demonstrates that pectin presents specific interactions with the glucose syrup that favour gas dispersion and stabilisation. In the presence of proteins, their interfacial properties stabilize gas/liquid interfaces. However, only the specific interactions described above between pectin and glucose syrup, coupled to the known interactions between pectin and whey proteins, can explain the remarkable foamability and stabilization ability of pectin/whey proteins mixtures. As a conclusion, LM pectin seems an interesting additive for food processors involved in foamed products based on glucose syrup, such as biscuits fillings.

**Reference**

Djelveh, G. and Gros, J.B., (1995) *Journal of Food Engineering*, 26, 45-56.

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# Modelling of physical and chemical processes in the small intestine.

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## Summary

The chemical and physical processing of food in the small intestine is an important step for the digestion and absorption of food. This work is an investigation into the mixing mechanisms induced by physiological intestinal contractions. By mimicking intestinal flow profiles it is possible to carry out sensitivity analysis on the system to determine the effect of changing food formulation parameters and aid the design of novel foods. The presentation covers how the impact that certain biopolymers have on the rates of nutrient absorption, can be investigated and understood using engineering models that have been developed as part of this work.

Keywords: transport phenomena, small intestine, *in-vitro* model, food formulation

## Extended Abstract

The objective of the work was to develop models of the small intestine with which food formulations could be investigated. So far the key developments that have been made are:

The development of a diffusion cell model that allows for the study into the way that different formulations affect molecular diffusion properties. An overhead rheometer has been added to this that allows for apparent shear rates to be determined during the mixing of formulations, while measuring the diffusion rate across a membrane.

A bench top model representing a section of the small intestine has been developed that can reproduce the segmentation motility action found in the small intestine. The small intestinal model (SIM) consists of an inner tube made from a membrane through which the molecule of interest can diffuse, and an outer tube that is impermeable to water. Both tubes are flexible and allow for deformation to take place. As the molecules of interest diffuse through the inner tube membrane into the fluid contained in the outer tube they are detected by analytical instrumentation.

The segmentation motility action of the small intestine is an annular contraction that moves inwards, radial to the centre. This action is responsible for the mixing of the intestinal contents and surface renewal. The action is reproduced for experimental

purposes by the inflation and deflation of a rubber cuff that is wrapped around the whole tube.

A computational fluid dynamics (CFD) model using the Fluent software has been developed. The segmentation action of the small intestine is modelled using a deforming mesh.

The diffusion cell has been used to investigate the effect of guar gum on diffusion and convection of small nutrient molecules. The results showed that for a 1% guar solution there was a reduction of 68% in the overall mass transfer coefficient of riboflavin compared to without guar. It has been shown that this reduction in the overall mass transfer coefficient is not due to binding of the molecule to the biopolymer or the fouling of the membrane. Experiments using the SIM have shown the effect of the segmentation motion on the nutrient mass transfer coefficient across the membrane for solutions containing different amounts of guar gum.

So far it has been shown that the reduction in postprandial hyperglycaemia as a result of presence of guar gum can be attributed in part to the reduction in diffusion coefficient seen in static diffusion cell experiments. It has also been shown using the SIM that convection influences the concentration on the inner surface of the membrane. The influence that segmentation has on the overall mass transfer coefficient is reduced as the viscosity is increased.

Further work will be investigating the influence of mixing on enzyme-substrate interactions and how the guar gum affects this under physiological process conditions. Also work on the mixing effectiveness of the segmentation action will be undertaken using flow visualisation techniques.

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# Dynamic optimization of baking operations using refinement method

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## 1. Summary

Baking is the major process in bakery production where product transformations are initiated by heating and which results in final product quality as crispness, brownness, crumb and water content. The baking process is described by a set of non-linear differential and algebraic equations [2]. For quality driven process design, optimal heating strategies are determined from input trajectories as a function of baking time. Control vector parameterization (CVP) is an effective method for the calculation of the input trajectories. However, for accurate optimization with a large number of parameters of the control vector computation time is large. Stepwise refinement of the CVP is proposed as an alternative to reduce the computation time.

*Keywords*: Baking, refinement, optimization, operation strategy

## 2. Extended Abstract

For the baking application, single run optimization with 45 control vector parameters requires about 18400 seconds to approach the optimal trajectory (Fig 1a). The computation time is related to the number of optimized input parameters, but the

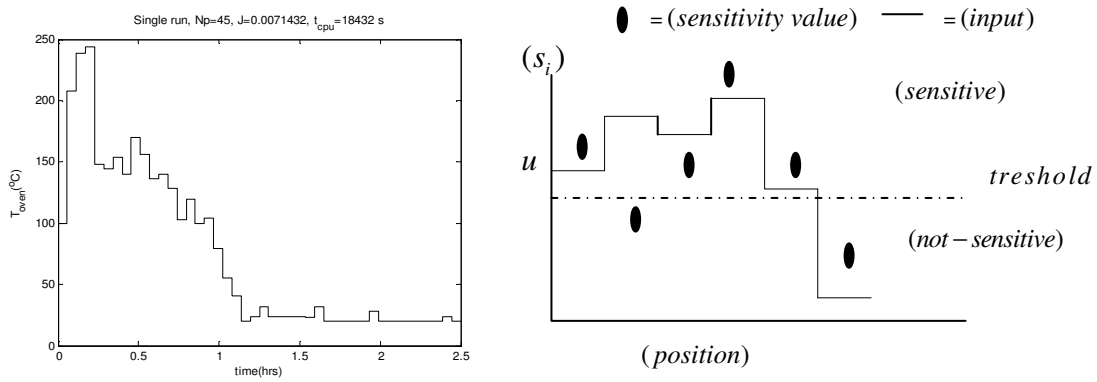


**Figure 1.** (a) Single run of control vector parameterization for 45 numbers of parameters, (b) the principle of refinement based on sensitivity value

objective function is not sensitive for changes of all parameters. A part of the parameters hardly contribute to the improvement of the objective function but require extra function evaluations and increase the computation time. Therefore, a refinement method is applied by adapting only the sensitive input parameters, while the others are kept constant (Fig 1b). To do so, a threshold value of sensitivity is used. Optimization calculations have been done for a piece-wise constant control vector, which is started with only six parameters. Figure 2 shows optimization progress for four succeeding levels of refinement. Table 1 gives the objective function values and computational time for the single run optimization of figure 1, for a refinement at all CVP-points [1] and the proposed refinement based on the threshold value. The proposed method is about 4 and 2 times faster than single run optimization and the refinement method on all CVP-points, respectively. Moreover the objective function value is also more beneficial.

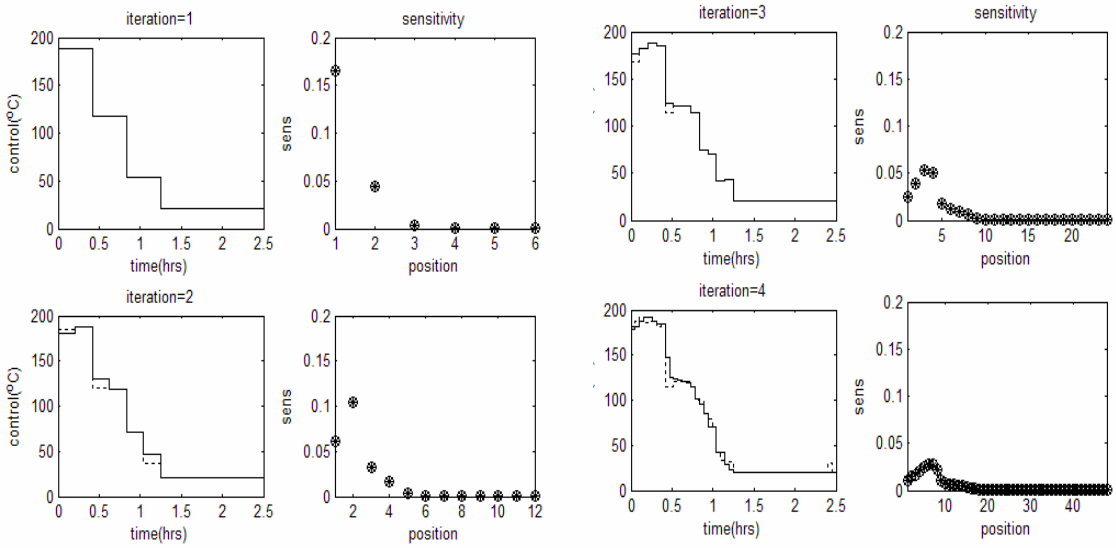


**Figure 2.** Baking temperature trajectory refinement for 4 iterations; refinement on all CVP-points (--) and refinement with threshold value (-).

**Table 1.** Computational time and performance index for three different methods

| Iteration | Refinement on all CVP-points | | | Refinement with threshold value | | | Single run | |
|---|---|---|---|---|---|---|---|---|
| | $N_u$ | J | $t_{cpu}$ (s) | $N_u$ | J | $t_{cpu}$ (s) | $N_u$ | J |
| 1 | 6 | 0.00718145 | 382.2 | 6 | 0.00718145 | 382.2 | 45 | 0.0071432 |
| 2 | 12 | 0.00697551 | 869.3 | 6 | 0.00697542 | 537.9 | | |
| 3 | 24 | 0.00692146 | 1718.9 | 12 | 0.00692135 | 1078.5 | | |
| 4 | 48 | 0.00686000 | 4941.5 | 24 | 0.00684551 | 2245.2 | | |
| *Total $t_{cpu}$* | | | *7911.9* | | | *4243.8* | | *18432* |

*Nu= Number of input parameters, J=performance index (calculated from square error from the setting value), $t_{cpu}$= computation time(s)*

## References

[1].García, M.S.G, Balsa-Canto, E., Alonso, A. A. and Banga, J.R. 2006. Computing optimal operating policies for the food industry, *Journal of Food Engineering, 74(1), 13-23*

[2].Hadiyanto, Asselman, A., van Straten, G., Boom, R.M., Esveld, D.C. & van Boxtel, A.J.B., 2007, Quality Prediction of bakery products in the initial phase of process design, *Innovative Food Science and Emerging Technologies,8(2),285-298*

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# A tool for productive and environmentally efficient food production management

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## 1. Summary

A tool for improving production efficiency based on the combination of discrete event simulation and environmental system analysis has been developed. The tool has been used to investigate how environmental impact and lead times are linked to production frequency in selected food production lines.

Keywords: food production, environmental system analysis, discrete event simulation, waste minimisation, productivity improvement

## 2. Extended Abstract

New production management concepts are required in the food industry for a number of reasons, including increasing diversity of products, low inventories, and demands on short lead times and high delivery accuracy. By producing on demand from the customer (pull flow) inventories can be minimised and costs reduced. In most cases, a pull flow will lead to an *increased frequency* in the production of specific products, which in turn requires flexible and stable production lines. On the other hand, most losses of raw material in food production occur when switching between products and at start-up and shut-down of a production line, which in many cases favours a *decreased production frequency.* Loss of raw material and product not only increases cost, but also environmental impact due to the large environmental impact of the agricultural step. This contradiction clearly shows that in order for production to be both efficient and environmentally friendly, production efficiency and environmental impact must be considered simultaneously. A tool for improving production efficiency based on the combination of discrete event simulation (e.g. Banks *et al*., 1995) and environmental life cycle assessment (LCA) (e.g. Baumann and Tillman, 2004) has been developed. The tool can be used to investigate how cost, revenue and environmental impact are linked to lead time, production planning, inventory and delivery accuracy in selected food production lines.

Based on the simulations, hot spots can be identified and improvements suggested, as exemplified below. Table 1 shows the influence of production frequency on product losses and overall production time for aseptic packaging of liquid foods. The absolute amount of product lost is determined by the number of product changes and the size of the equipment. Thus, the most efficient solution in this case is to increase the production frequency together with optimisation of batch size with respect to the equipment used.

.

| Normalised production frequency (N) | Relative change in product losses | Relative change in total production time |
|---|---|---|
| 2 | 0.3 | 1.08 |
| 1 | 1.0 | 1 |
| 0.5 | 2.5 | 0.98 |
| 0.25 | 5.4 | 1.01 |
| 0.125 | 11.2 | 1.38 |

**Table 1.** The influence of production frequency on product losses and total production time. The simulations were performed in AutoMod and are based on an annual production (~ 44 million packages/year) divided into 114 article numbers (Karlsson and Persson, 2007). The change in production frequency was implemented by changing the batch size. The same change in batch size was implemented in all production batches at each value of production frequency. N =1 corresponds to today's production system.

### *2.1. Conclusions*

Improvements can be identified by combining environmental analysis and process analysis, avoiding sub-optimisation. The most important benefit of a combined model is that all results are based on the very same set of inventory data describing the process conditions. Experience also shows that a combined working procedure is more time efficient than parallel application of discrete event simulation and environmental life cycle assessment.

## References

Banks, J., Carson, J. S. and Nelson, B. L., *Discrete-event System Simulation*, 2nd ed. Upper Saddle River, New Jersey (1995)

Baumann, H. and Tillman, A., *The Hitch Hiker's Guide to LCA,* Studentlitteratur, Lund, Sweden (2004)

Karlsson, J. and Persson, D., *Flow simulation of food industry production*, Master's Dissertation, Chalmers University of Technology, Sweden (2007)

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# Session S-5Ff: Flexible Production, PAT & Modelling (Food-3b) – 5 min oral presentation plus poster presentation

| Abstract Number | Paper Title & Authors | Included |
|---|---|---|
| 1057 | Innovated Food Design Visualized in the Nonlinear Analysis of Multi-functional Water Species Based on the Specified Physicochemical Parameters<br>Y Konishi, M Kobayashi | Yes |
| 1280 | Development Of Operational Strategies For Freeze Drying Process: Lemon Juice Application<br>E A Boss, R M Filho | Yes |
| 2981 | Application Of Multivariate Statistical Analysis For The Quality Control Of Food Products<br>A C Palou, S Simal, M Frau, V S Eim, A Femenia, C Rosselló | Yes |
| 3911 | Modelling of linseed oil expression curves. Impact of variety and maturity on the identified parameters of the model<br>R Savoire, J L Lanoisellé, E Vorobiev | Yes |
| 4060 | Designing and analyzing virtual cuts in 3D models of pig bodies by mapping cuts from a statistical atlas<br>M F Hansen, M V Christensen, S G Erbou | Yes |
| 4098 | A rethinking of large scale meal production<br>E H Engelund, P P Jacobsen, A Friis | Yes |

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# Innovated Food Design Visualized in the Nonlinear Analysis of Multi-functional Water Species Based on the Specified Physicochemical Parameters

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## 1. Summary

An innovated food design was challengingly studied in the course of the nonlinear analysis of multi-functional water species by using a drying process of Japanese squid at 30~50°C. Optimal design of the drying process requested was effectively visualized by using six physicochemical parameters as a function of the three multi-functional water species.[1,2)] The three water species were clearly distinguished as species-$A_1$ (mobile water species), species-$A_2$ (relatively restricted-water species) and species-$A_2$' (unfrozen-water species), all of which were exactly characterized by six physicochemical parameters. The six parameters were individually evaluated as effective diffusivity (*De*), activation energy of *De* ($E_D$), peak temperature of the temperature-programed-desorption profile ($T_P$), correlation time ($\tau_C$) derived by a proton NMR technique, bacterial growth coefficient (*m*), and color function ($L^*$, $a^*$, $b^*$), characterizing the three water species expressed by a non-linear function of the moisture content ($W_0$) of the squid as species-$A_1$ given at $W_0$ >120%-d.b. (designated $W_{0I}$), species-$A_2$ given at $W_0$ <120%-d.b. (designated $W_{0II}$), and species-$A_2$' given at -15>*T*(sample temperature)>-30°C.

Keywords: Three water species, bacterial growth coefficient, effective moisture diffusivity (*De*), activation energy of *De*, correlation time of water molecule

## 2. Extended Abstract

On *De*, as seen in Fig.1, it was evaluated as $1.8\times10^{-6}$ for the water species-$A_1$ and $0.6\sim1.7\times10^{-6}$ $m^2/h$ for -$A_2$, on $E_D$, 17 for -$A_1$ and 25~35 kJ/mol for -$A_2$, on $T_P$, 110 for -$A_1$ and 117~150°C for -$A_2$, on $\tau_C$, $4.8\times10^{-9}$ for -$A_1$ and $1.0\times10^{-8}\sim1.2\times10^{-7}$ sec for -$A_2$, on *m*, 0.35~0.55 for -$A_1$ and 0 $h^{-1}$ for -$A_2$, and on the values of $L^*$, $a^*$, $b^*$, it was recognized as milk white for -$A_1$ and dark brown for -$A_2$. On the water species-$A_2$', an unusual histerisis behavior for the value of $\tau_C$ was exactly appeared indicating two different locus's depending on the temperature decrease from 30 to 10°C (at which an

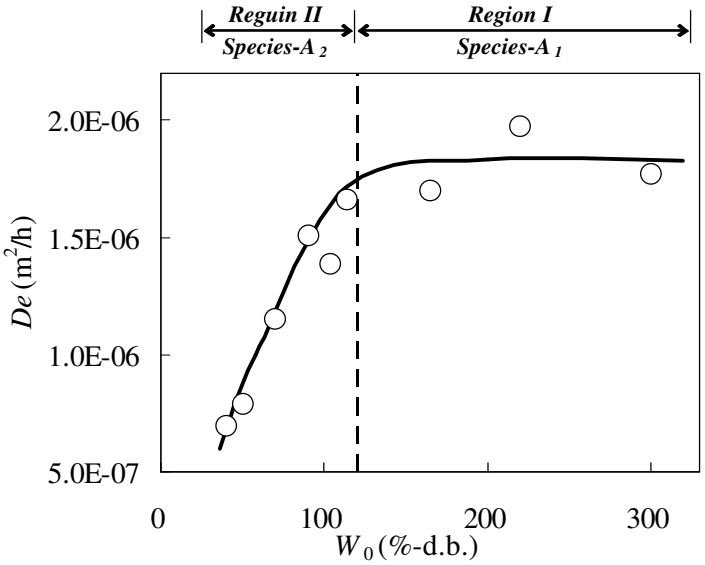


**Fig.1** Discrimination of species-$A_1$ and -$A_2$ recognized by *De*-$W_0$ dependency

activation energy of water molecule rotation, $E\tau_C$, was evaluated to be 3 kJ/mol for the water species-$A_1$ and -$A_2$) or the temperature increase from -25 to 0°C (at which $E\tau_C$ was 40 kJ/mol for the water species-$A_2$').

All values of the six parameters can individually be chosen depending on the food quality requested as milk white colored at $W_{0I}$ or dark brown colored at $W_{0II}$. The populations of species-$A_1$, -$A_2$, and -$A_2$' can easily be regulated by controlling $W_0$, $T_D$, and the relative humidity of supplied drying air (RH) as an operating parameter. The growth rates of staphylococcus and micrococcus for the squid sample were completely limited, as shown in Fig.2, by the species-$A_2$ or by an RH lower than 30% (designated as critical relative humidity, $RH_{30}$) at less than $10^3$ CFU/g without depending on $W_0$. The $RH_{30}$ was usefully employed as an optimal operation in the drying process.

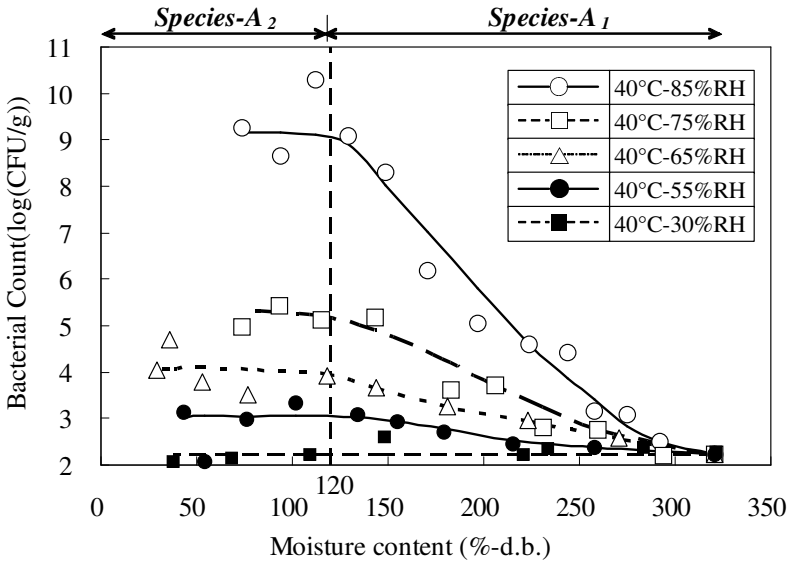


**Fig.2** Bacterial count as a function of moisture content and the relative humidity.

## References

(1)Konishi, Y. and Kobayashi, M., (2003). *Journal of Food Engineering* 59, 277-283.

(2)Konishi,Y., Miura,K. and Kobayashi, M., (2003). AIDIC Conference Series, Vol.6, 183-190.

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# Development of Operational Strategies for Freeze Drying Process: Lemon Juice Application

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## 1. Summary

The economy has became competitive, with impact on the industries of fruit juices which must advance in the sense of minimizing production costs and, at the same time, generating products that obey each time more rigorous patterns of quality.
In order to obtain a product with the market requirements as well as with high productivity and lower costs process improvements are necessary.
Bearing this in mind, in this work a parametric sensitivity analysis with experimental data and a suitable statistical procedure is presented.

Keywords: Lemon juice, Freeze-drying, Parametric Sensitivity Analysis, Factorial Design.

## 2. Description

The stability, storage, and distribution of certified reference materials which consist of liquid or frozen fruit juices are rather problematic (Ooghe et. al., 1998). A possible alternative solution for this problem is the storage and the transport in powder form. In the conventional dehydration high temperature leads to the product loss a flavour component and several of its components due to volatility. Freeze-drying process can be seen as an excellent option for solution of these problems, since this process works at low temperatures (Boss et. al., 2004). Improvements are necessary in order to obtain a product with the market requirements as well as with high productivity and lower cost.

The interesting factors to analyze are the structural rigidity, which facilitates rapid and almost complete rehydration at a later time, little loss of flavor, and minimization of degradative reaction, which normally occur in ordinary drying processes, such as protein denaturation, non-enzymatic browning and enzymatic reactions.
The experiments were carried out in freeze-dryer TELSTAR, model Cryodos-80. This equipment is composed of stainless steel cylinder chamber with eight manifolds for bottle connections. Inside this stainless chamber is a coil connected with a condenser.

Above the cylinder chamber there is a cover built with transparent material. Lemon juice was dried and each sample was retreated to verify the amount of removed water and product appearance (capacity to form powder) for each screening design trial.
Due to the relatively large number of variables to be analyzed it was necessary to submit the lemon juice freeze-drying process to an initial screening design. The variables analyzed are: thickness of the layer, frozen form, fragmentation and three different additives. The screening design was realized for eight hour of drying. It was necessary to choose a fixed drying time, since not all the samples were dried up the desired stage.
Once the relevant variables were selected by Plakett-Burman method, experiments were planned consisting of $2^3$ trial plus three central point, with removed water as response. Analyzing the screening design, it was possible to reduce the experimental design for the following variables: thickness of the layer (L), frozen form (FF) and acidic structural polysaccharides (ASP). The fragmentation variable will be included in the complete factorial design because the samples will be more fragmented than in screening design. It was only feasible to obtain the final product in the powder form with the use of the addictive acidic structural polysaccharides (ASP) and due to this the variable acidic structural polysaccharides (ASP) will not be participated as factor in the planning. All the samples will be prepared with acidic structural polysaccharides (ASP).
The variable that most influenced the process was the thickness of the layer. The best option for this variable is to maintain it at their lowest value.
In the complete factorial design executed for 1 to 16 hours of processing, it was possible to conclude that the experiments with low thickness of layer remove more water in shorter time. This is the variable that has larger effect in this process.

**References**

Boss, E. A., Toledo, E. C. V., Maciel Filho, R., (2004). Freeze-drying Process: real Time Model and Optimization. Chemical Engineering and Processing. v. 43, p. 1475-1485.

Ooghe, W.; Kramer, G.N.; Schimmel, H.; Pauwels, J., (1998) Comparison of some additives used in the preparation of freeze dried lemon juice candidate reference materials. *Fresenius Journal of Analytical Chemistry*. New York, v. 360, n. 3-4, p. 445-448.

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# Application of multivariate statistical analysis for the quality control of food products

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## 1. Summary

A proposal for the quality control of food products by using multivariate statistical analysis is described. Multivariate statistical methods comprising principal component analysis (PCA), cluster analysis of variables (CAV) and stepwise discriminant analysis (SDA) were applied to estimate the usefulness of various chemical, physical and sensory determinations for the differentiation and classification of different types of Majorcan cheese. PCA allowed the reduction of fifteen variables to three independent components accounting for the 85.6% of the explained variation. The first principal component (PC) was representative of the ripening stage. CA confirmed the correlation between the studied variables obtained by PCA. Using SDA it was determined which variables best classified the cheese samples according to their ripening stage. Finally, the classification functions allowed the classification of 93.5% of the cheese samples.

Keywords: quality control, principal component analysis, cluster analysis, discriminant analysis, Majorcan cheese.

## 2. Extended Abstract

Majorcan cheese is a non-cooked pressed type of cheese, salted in brine, produced on the island of Majorca (Spain) from cow's milk. There are no regulations about the cheese made in Majorca, neither about its characteristics nor the production process. The aim of this work was to set up a useful tool for the quality control of Majorcan cheese based on the differentiation and classification of three types of Majorcan cheeses: half-ripened, ripened and old-ripened. For this purpose PCA, CAV and SDA were used on the chemical, physical and sensory determined parameters.

The chemical composition [dry matter (DM) and non-protein nitrogen (NPN)] and the physical parameters [water activity ($a_w$), yellowness colour index (Z) and textural parameters (hardness, cohesiveness and springiness)] were measured. Sensory evaluation of the intensity of odour (IO) and aroma (IA), elasticity, firmness, friability, impression of humidity (IH), olfactory and gustative sensations (aftertaste) and persistence were carried out by 11 trained judges. PCA was applied in order to

establish the relationship between the different chemical, physical and sensory variables and to detect the most important factors of variability. PCA reduced fifteen variables into three PCs which accounted for 85.6% of the total data variance. As it can be observed in Fig. 1, DM, NPN, hardness, IO, IA, firmness, friability, aftertaste and persistence (variables which values usually increase with the ripening time) grouped in the positive side of the horizontal axis; whereas $a_w$, Z, springiness, cohesiveness, elasticity and IH (variables that decrease with the ripening time) were grouped in the negative side [1-3]. From these results, it could be concluded that the first PC could be considered as representative of the ripening stage. CAV was carried out to evaluate which variables behave similarly. As it can be observed in Fig. 2, the most correlated variables showed the smallest distances and grouped into two clusters, in agreement with the results obtained by PCA. SDA was used in order to determine which variables best distinguish among the three different groups and to propose linear functions capable of classifying new cases, not used in the computations. Using classification functions obtained by SDA, 93.5% of cheese samples were correctly classified according to their ripening stage.

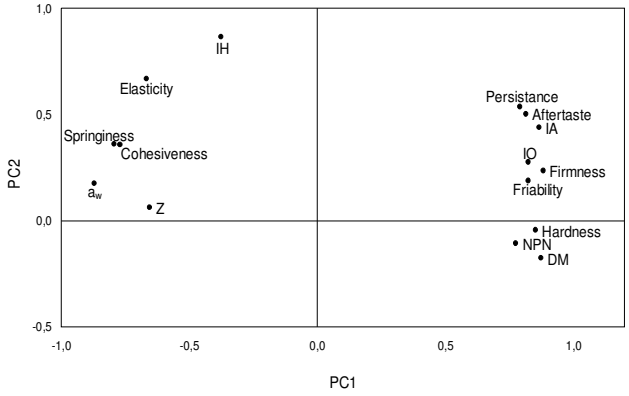


**Figure 1:** PCA. Representation of chemical, physical and sensory variables as a function of both the first (PC1) and the second (PC2) principal components

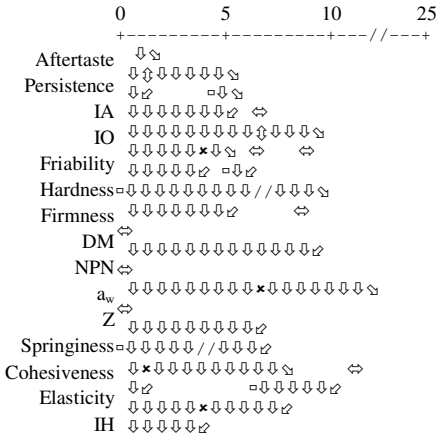


**Figure 2:** CAV. Chemical, physical and sensory variables against the distances between them

Overall, multivariate statistical methods comprising PCA, CAV and SDA have shown to be effective in order to select the optimal parameters for the quality control of Majorcan cheeses. Further, cheeses could also be correctly classified according to their ripening stage.

## References

[1]Frau, M., Simal, S., Femenia, A., Rosselló, C. and Sanjuán, E. (2003). *Alimentación, Equipos y Tecnología*, 181, 74-78.
[2]Hort, J. and Le Grys, G. (2001). *International Dairy Journal*, 11, 475-481.
[3]Poveda, J.M., García, A., Martín-Alvarez, P.J. and Cabezas, L. (2004). *Food Chemistry*, 84, 29-33.

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# Modelling of linseed oil expression curves

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## 1. Summary

Oilseed selection is actually performed on the basis of agricultural factors (biomass yield, plant disease resistance). However, the improvement of the oil expression ability is not taking into account. The objective of this work was to develop a new laboratory method adapted to the oil expression for small quantities of seeds. Linseed *(Linum usitatissimum L.)* obtained at the first stage of varietal screening was studied. A pressing chamber of 20.6 $cm^3$ volume allowed the expression of 3 to 10 g of oilseeds with a uniaxial stress. The chamber was attached to a food texture analyzer. During experiments the piston displacement versus the applied pressure was recorded. The expression curves obtained from one hour expression operation were modelled with a four Kelvin-Voigt elements viscoeleastic model previously developed for oilseed extraction. For the constant pressure phase of the expression (uniaxial compression creep test), this model allows the determination of oilseed mechanical intrinsic characteristics relative to different deformations of microscopic and macroscopic cake volumes (called intracellular, extracellular and extraparticular volumes). From the 4 compressibility modulii, a global compressibility modulus G, which represents the required stress for oil expression, is calculated. Those parameters are then related to the oil expression ability characteristics, mainly the oil yield. This method has been tested on seven linseed varieties harvested at different dates before maturity. By this way, an inverse correlation between global compressibility modulus and extracted oil mass is highlighted. Finally, an indicator of expression ability is proposed using a linear combination of studied variables.

Keywords: Linseed, expression, oil, modelling, Kelvin-Voigt viscoelastic model.

## 2. Extended Abstract

Flaxseed is a plant cultivated since the beginning of agriculture and its oil has mainly industrial applications in ink, varnishes and other coating applications due to its drying ability. In some countries, it is also an edible oil. Industrially, linseed oil is obtained by flaking, cooking and either continuous expression or expression followed by solvent extraction. The expression of oil from oilseeds has been studied mainly on laboratory scale. The impact of pre treatment on oil yield was studied, as well as the impact of oilseed composition, principally water content (Dedio and Dorrell, 1977), more recent studies evaluate the expression on pilot screw presses (Zheng *et al.*, 2003). In all these studies, expression is evaluated in term of process optimisation.
Our objective here is to use oil expression characteristics to select optimal new varieties for process.

Linseed (Linum usitatissimum L.) from seven commercial or under development varieties were studied. The crushed seeds conditioned to the same humidity were expressed for one hour at 50°C under constant uniaxial pressure (10MPa). The test cell was fixed on a TA.HDi

texturometer (Stable MicroSystems, Surrey, UK) and the applied force and piston displacement were record in function of expression time. The obtained curves were modelled with a four Kelvin-Voigt elements viscoeleastic model previously developed for oilseed extraction (Lanoisellé et *al.*, 1996) where $G_i$ are the compressibility modulus, $\upsilon_i$ the inverse of the characteristic time of each phase and $h_\infty$ the displacement corresponding to the theoretically maximum quantity of oil extractible during an infinite time pressing.

$$h = \frac{h_\infty}{\sum_{i=1_0,1,..,n} \frac{1}{G_i}} \left[ \sum_{i=1_0,1,..,n} \frac{1}{G_i} (1 - e^{-\upsilon_i t}) \right] \quad \text{(eq.1)}$$

Each exponential correspond to the consolidation of a different space, $1_0$ to the primary extra-particular volume consolidation, 1 to the creep consolidation of the extra-particular volume consolidation and 2 and 3 to the extra-cellular and intra-cellular volume consolidation respectively. $h_\infty$ was first determined using a linearized form of eq.1. Other parameters are determined using TableCurve 2D software (AISN Software, Jandel Scientific,USA), the same weight is allowed to each consolidation step (the same number of points is used in each step). A global compressibility modulus was calculated as the sum of the inverse of $G_i$, it represents the required stress for oil expression.

The Figure 1 presents the modelled and experimental curve and the error between the model and the experiment.

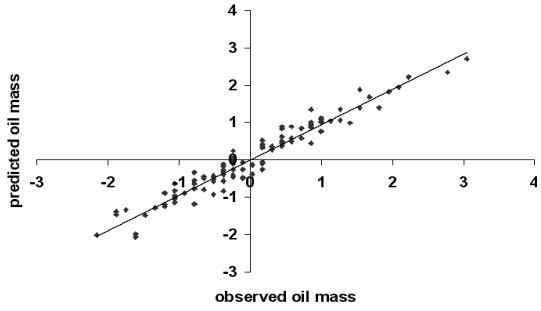


**Figure 2:** predicted values of oil mass in function of observed oil mass for regression with model parameters

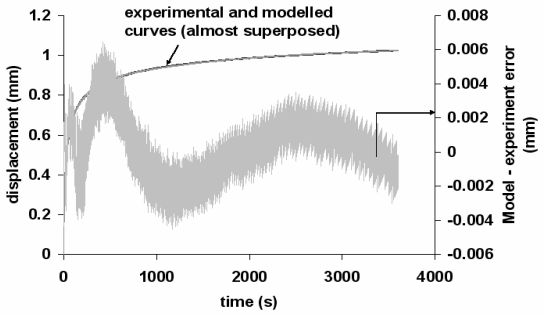


**Figure 1:** experimental and modelled curves (almost superposed) and mode- experiment error

A statistical analysis was performed on standardized variables of 130 experiments in order to reveal the adequacy between the model and the experiments. The explanatory parameters are the compressibility modulii $G_i$ and G, the characteristics times of each consolidation and $h_\infty$ and the explicate parameter is the extracted oil mass. The linear combination of the explanatory parameters permits to explain 93.6 % of the variability in oil mass. The figure 2 shows the predicted values of oil mass in function of the observed one.

The experimental data are well described by the considered model. Further experiment for integrated the biochemical composition of seeds are needed to explain the 6% of variability which are not represented by the model. A correlation between laboratory experiments on micro-scale and pilot experiments on screw-press are envisaged.

## References

Dedio, W. and Dorrell, D.G., (1977), *Journal of the American Oil Chemists' society*, 54(8), 313-315.

Lanoisellé, J.-L., Vorobyov, E.I, Bouvier, J.-M. and Piar G., (1996), *AIChE journal*, 42(7), 2057-2068.

Zheng, Y.-L., Wiesenborn, D.P., Tostenson, K. and Kangas, N., (2003), *Journal of the American Oil Chemists' society*, 80(10), 1039-1045.

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# Designing and analyzing virtual cuts in 3D models of pig bodies by mapping cuts from a statistical atlas

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## 1. Summary

The presentation will give a detailed description into the construction of statistical atlases including the process of image registration of CT scans of pig carcasses. A statistical atlas can be a valuable and important tool in a wide range of application such as virtual cutting, design of slaughter robots and for general extraction of statistical information from the population of pig. Furthermore, a number of virtual cutting examples will be given, and it will be demonstrated how a statistical atlas together with CT scans of pig carcasses can be used to extract meat quality parameters with performing any invasive procedure.

Keywords: Computed Tomography, statistical atlas, meat quality and virtual cutting.

## 2. Extended Abstract

The development and introduction of new products within the slaughter industry is a slow and expensive process as it is difficult to analyze the products wrt. earning potential and quality. The difficulty primarily stems from the unfortunate fact that it until recently was impossible access the quality of a product or combination of products until the cutting had been carried out. With Computed tomography (CT) it is possible to construct 3D models of the interior of pig bodies without performing an invasive procedure. A CT scanner generates a 3D volume of the interior of an object by moving an x-ray source in a spiral motion around the object while sensors continuously record the attenuation of the intensity of the x-ray beam. Given a population representative database of 3D pig models, it becomes possible to compare the earning potential and the overall quality of product combinations, as well as to determine which types of pigs are suitable for a given product range. In order for such an analysis to be carried out successfully it is essential that identical cuts can be located in the 3D models and that the crucial quality parameters can be extracted consistently across the models. These demands necessitate the establishment of an almost complete physical correspondence between the models in the database. This can be accomplished by registration of the 3D pig models to an atlas. An atlas could simply be a selected 3D pig model where anatomical points and regions have been

identified by an expert. Cuts or parts of cuts can be mapped from an atlas to the 3D pig models and vice versa. Substantial challenges remain in converting the vaguely defined cuts from product catalogues, which requires human interpretation and prior knowledge, to concise mathematical models which can be applied to an atlas.

In addition to mapping cuts, an atlas can also provide important and valuable statistical information about the population of pigs, which makes an atlas a widely applicable tool for a variety of research and development projects, e.g. the development of slaughter and cutting robots.

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# A rethinking of large scale meal production

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## 1. Summary

The study pertains to improvement of the quality of meals produced in large scale kitchens. Focus is on systematic shortening of production time and a customized application of convenience products when striving for improved product quality. As more and more meals are produced (and consumed) outside home and the production is concentrated at fewer production sites of increasing size. This is turning the preparation of meals into more traditional production-like scenarios and brings forward a need for process optimization according to these new conditions. Traditionally, upgrade of large scale meal production has been done by integrating new technologies into the existing systems leaving a need for a systematic rethinking of production flow unaddressed. Tools for rethinking of entire processes are offered in the production and management literature, but these theories seldom connects to optimization of meal production. In this work data obtained during case studies of several large-scale kitchens are analyzed using known production theories (lean management, agile production, mass customization). The outcome of this is base for a suggestion of methodologies for redesign of large scale meal production where quality and production planning is addressed concurrently.

Keywords: Systems, optimization, production

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# Session S-5G: Process & Product Innovation (Food-4a)

| Abstract Number | Paper Title & Authors | Included |
|---|---|---|
| 96 | PGSS - The innovative production of fluid-filled microcapsules<br>T Wendt | Yes |
| 125 | A short review of capacitive dielectric treatment of foodstuffs<br>F Marra | Yes |
| 512 | Removal Of Dark Compounds From Fruit Juices By Membrane Separation<br>M E Carrín, M B Buglione, J E Lozano | Yes |
| 2227 | Tools for complementary product development<br>J R Olsen, A Friis | Yes |
| 4034 | Low-fat frying with infrared heating<br>L Ahrné | No |
| 4055 | New innovative fats and oils with an healthy fatty acid profile<br>N D Clercq, I Foubert, K Dewettinck | Yes |

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# PGSS - The innovative production of fluid-filled microcapsules

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## 1. Summary

The PGSS (Particles from Gas Saturated Solutions) process is suitable for the micronisation of several substances and various polymers. With a couple of investigations it was shown that fine powders with different morphologies can be manufactured. Aim of this investigation was the production of powdery food products using the PGSS process. In the context of this work 165 experiments with the model systems palm fat/water, castor fat/water and PEG 6000/rapeseed oil were used to determine the interdependencies between process parameters of the PGSS process and particle and powder properties on the other hand. The aim was to investigate important influences on product specific characteristics. Afterwards, the knowledge obtained with the model systems was applied for the production of fluid-filled micro powderous composites for the food industry. Here, special attention was put on the influence of the process parameters on particle size and bulk density. The evaluation of the three model systems showed that the PGSS process is suitable for the production of powderous composites with variable characteristics. The particles can be adjusted by the process parameters in bulk density, particle size distribution and morphology.

## 2. Extended Abstract

Product life cycles in industry shorten every year. Manufacturers constantly expect new products. This innovation pressure is dealt with on the one hand by the development of new formulations, on the other hand new processes are called for, which permit the production of products that are presently not accessible, yet.

In the last years micro encapsulation has increasingly been used. The objective here is not only the protection of the products – microcapsules open new possibilities for the controlling of the release of active substances. In the food industry the control of

release characteristics in the range between 10 °C (50 °F) to 100 °C (212 °F) is of special interest and represents a special challenge for process engineering. This challenge can be met by using a high pressure technology called PGSS (Particles from Gas Saturated Solutions). The possibilities for the production of fluid-filled particles of this process will be shown in this presentation.

The PGSS process permits a careful processing in an inert gas atmosphere at low temperatures, moderate pressures and is suitable to produce powders and composites of solids, very viscous melts and even liquid substances. With this technique powders with different particle morphology and –size distribution are obtained. At the same time compressed $CO_2$ has a strong germ-killing effect.

For the generation of fluid-filled particles a shell material (e.g. chocolate, palm or castor fat) is provided in a melted condition. The core material (e.g. liquid extracts or flavors) and a supercritical fluid (mostly $CO_2$) are admixed into a mixing system. Inside the mixing system the core material is dispersed in the continuous liquefied shell material. This dispersion is expanded through a nozzle into a spray tower to ambient pressure, forming fine droplets. Simultaneously, the Joule-Thomson phenomenon of the expanding gas causes the solidification of the droplets. Finally, fine powders are obtained, which consist of a dispersed liquid phase encapsulated by the shell material.

No organic solvents, emulsifying agents or other additives are necessary for the production of these microcapsules. Thus, the PGSS process offers a special preservation of the products and is environmentally friendly. The production of composites has been demonstrated in the last years with many different systems. Dry and free flowing powders with a dispersed content of up to 60 wt.-% liquid (e.g. rum, honey, soy sauce, etc.) were achieved. In the presentation not only the encapsulation technique will be shown, but also the influence of different quantities of liquid on the powder parameters like the morphology, the bulk density and the particle size distribution.

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# A short review of capacitive dielectric treatment of foodstuffs

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## 1. Summary

Recently the literature has shown an increased interest in dielectric heating at radio-wave frequencies (reported often as RF heating) for heat processing foods. Reported benefits include even and more rapid heating, increased quality of final product and higher energy efficiency. Most of recent papers on this subject deal with solid-like foodstuffs, predominantly meat based products, vegetables and fruits. For meat products and vegetables the process objective is the cooking and sterilization or the pasteurization of the product. For fruits (in-shell or not) radio-frequency heating is applied basically for insect and pest control. In this paper, a short overview of advances of research about capacitive dielectric heating is presented. Improvements in process understanding, factors influencing heat transfer as well as modeling considerations are also discussed.

Keywords: dielectric heating, heat transfer, radio-frequency processing

## 2. Extended Abstract

Capacitive dielectric heating is a technique using electromagnetic energy to heat a lossy medium sandwiched between two parallel electrodes. The electrodes are connected to generator working in the frequency field of radio-waves (between 1 MHz and 300 MHz). It is generally recognized as an even and fast heating method. A comprehensive discussion about the basic principles of capacitive dielectric heating is reported by Piyasena *et al*. (2003). This review deals with the advances of capacitive dielectric heating in food processing, in the last 9 years, when more than 40 papers about radio-frequency heating appeared on international journals (please note that only a selection of these papers have been presented in the reference list). In recent works, considered foodstuffs included meat (McKenna *et al*., 2006), fruits (Birla *et al*., 2005), vegetables (Zhong *et al*., 2004), liquids (Awuah *et al*., 2005), eggs (Luechapattanaporn *et al*., 2005), fish and ready meals. The recent literature offered important contributions to the understanding of RF heating and covers some of the lack emphasized in the past. Analysis of dielectric properties of foodstuffs (as function of composition and temperature) has improved (Brunton *et al*., 2006); effects of RF heating in terms of physical, chemical and sensory properties have been

investigated (Tang *et al*., 2005) and mathematical modelling of RF heating was compared with experimental results (Marra *et al*., 2007). In brief, this analysis of literature leads to the following conclusions:

- there is an increasing interest for RF heating of food products;
- among selected frequency values (13.56, 27.12 and 40.68 MHz) for ISM applications, 27.12 MHz is the most used value for capacitive dielectric heating;
- sterilization and cooking of meat based products and pest control in fruits are the most suitable applications. The technique is promising also for ready meals;
- shape and orientation of food product influence the temperature distribution and can lead to poor heating of some areas within products;
- electrode distance and gap between food product and electrodes are key parameters often not considered or optimized by researchers;
- to prevent post process contamination and loss of components of composition during heating encasing of food product prior the RF treatment is sometimes necessary, especially for meat based products;
- in cased products arching can be a serious limitation to RF heating but water recirculation around the treated product during heat processing has been reported to minimise this effect;
- a simplified mathematical approach, based on a quasi-static assumption of electric field distribution, allowed modelling to be used for better understanding heat transfer during RF heating and for setting optimal process conditions;
- more work is needed in terms of sensory analysis and panel test of ready meals processed by capacitive dielectric heaters.

**References**

Awuah, G.B., Ramaswamy, H.S., Economides A. and Mallikarjuanan, K. (2005) *Innovative Food Science & Emerging Technologies*, 6 (4), 396-402

Birla, S.L., Wang, S., Tang, J., Fellman, J.K., Mattinson, D.S. and Lurie, S. (2005) *Postharvest Biology and Technology*, 38 (1), 66-79

Brunton, N.P., Lyng, J.G., Zhang, L. and Jacquier, J.C. (2006) *Meat Science*, 72 (2), 236-244

Luechapattanporn, K., Wang, Y., Wang, J., Tang, J., Hallberg, L.M. and Dunne, C.P. (2005) *Journal of Food Science*, 70 (4), E288-294

Marra, F., Lyng, J., Romano, V. and McKenna, B. (2007) *Journal of Food Engineering*, 29, 1661-1676

McKenna, B.M., Lyng, J., Brunton N.and Shirsat N. (2006) *Journal of Food Engineering*, 77 (2), 215-229

Pyasena, P., Dussault, C., Koutchma, T., Ramaswamy, H.S. and Awuah, G.B. (2003) *Critical Reviews in Food Science and Nutrition*, 43 (6), 587-606

Tang, X., Cronin, D.A. and Brunton, N.P. (2005) *Food Chemistry*, 93 (1), 1-7

Zhong, Q., Sandeep, K.P. and Swartzel, K.R. (2004) *Innovative Food Science & Emerging Technologies*, 5 (4),475-483

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# Removal of dark compounds from fruit juices by membrane separation

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## 1. Summary

The objective of this work is to carry out de-coloration of browned apple and grape juice by physical separation of melanoidins with combined use of ultrafiltration (UF) and nanofiltration (NF) membranes. Proposed actions are focused to reduce de-coloration agents added to juice in according with current trends in food processing. Ultra and nanofiltration de-coloration of browned juices were performed with a cross-flow membrane filtration unit, with membrane cut-off in the range 150-450 Da (thin-film composite based in polyamide) and 2-30 kDa (cellulose acetate or polyvinylidene fluoride). The following parameters were analyzed in the obtained permeates and compared with the non-browned clarified juice sample: Soluble sugars; pH; absorbance at 420 nm; soluble solids concentration as °Brix; and tristimulus color parameters, L, a, b and ΔE. Browned apple juice color was reduced to a level close to a fresh clarified juice after filtration with NF membranes (Cut-off < 2,000 Da). Although changes in pH of permeates were not observed, reduction in °Brix occurred when UF changed to NF. After membrane treatment of highly browned apple juice with NF membranes (< 2,000 Da) permeates with color characteristics closer to a no-browned juice were obtained. Contrarily, color recovering by NF resulted unviable for highly colored grape juices and traditional active carbon discoloration should be used. Moreover, from a practical point of view, soluble solids concentration reduction after NF must be carefully taking into account, since additional concentration step may be required.

Keywords: fruit juice, membrane, discoloration

## 2. Extended Abstract

Apple fruit (Granny Smith) and grape fruit (Merlot) were sorted, washed and crushed. Juice was produced by pressing in a hydraulic press, followed by screening, steam heating to 95°C, clarification by ultrafiltration (50 kDa hollow fiber) and concentration under vacuum at 60 °C to 72 °Brix. These concentrated juices were stored at relatively high temperatures to obtain a considerable browning level. Ultra and nanofiltration de-coloration of browned juices were performed with a cross-flow membrane filtration unit Sepa® CF Membrane Cell (Osmonics; Minnetonka; Mn; USA). The cell body accommodates any 19 cm x 14 cm flat sheet membrane for a full

155 cm2 of effective membrane area, with cut-off in the range 150-450 Da (thin-film composite based in polyamide) and 2-30 kDa (cellulose acetate or polyvinylidene fluoride). A total of 3 UF membranes (4, 20 and 30 kDa) and 3 NF membranes ( 0.3, 0.45 and 2 kDa) were used. Restored browned juice was treated at 30 °C in the UF/NF equipment, in batch mode. UF experiences were done consecutively in diminishing order of membrane molecular weight cut-off (MWCO), using permeate obtained in previous experience like feed to the next separation. On the other hand, NF experiences were done using as feed the same ultrafiltered juice batch. All experiences were done at 0.05 L/min of retentate flow with a transmembrane pressure in the range 41 to 90 kPa.

The following parameters were analyzed in the obtained permeates and compared with the non-browned clarified juice sample: Soluble sugars were quantified using a VARIAN VISTA 5500 (Varian, Assoc. Inc., Palo Alto, CA, USA) liquid chromatograph equipped with a differential refractometer VARIAN SERIES RI-3 and a Aminex® HPX-87C (Bio Rad; USA) column; pH (DigipHase Cole-Parmer pHmeter); absorbance at 420 nm (Perkin-Elmer Lambda3 UV/VIS spectrophotometer); soluble solids concentration as °Brix (Reichert Abbe Mark II digital refractometer); and tristimulus color parameters, L, a, b and ΔE in a HunterLab UltraScan XE colorimeter (Hunter Assoc. Laboratory; VA, USA).

Browned apple juice color (ΔE=61.43) was reduced to a value of ΔE=42.71 with the UF treatments and to the less value of 15.23 with the 0.3kDa NF membrane. Sample luminosity (L) reduced from 94.86 (without browning juice) to 46.89 with browning compounds, was increased to a value of 93.01 when NF treatment was applied. So, a level close to a fresh clarified juice after filtration with NF membranes could be obtained. Although changes in pH of permeates were not observed (pH value around 3.6), reduction in °Brix occurred when filtration process was conducted specially when ultrafiltration changed to nanofiltration. The reduction of °Brix value were from 9.4 to 8.9 in the UF range, and from 8.9 to 5.7 in the NF range. Sucrose concentration changed when 50 kDa UF membrane was used, principally. On the other hand, fructose concentration showed a significant diminution when filtration changed from UF to NF. After membrane treatment of highly browned apple juice with nano-filtration membranes (< 2,000 Da) permeates with color characteristics closer to a no-browned juice were obtained.

Contrarily, browned grape juice reached values of ΔE=27 and L=77.3 and they could be modified by membrane processing to 17 and 87.2, respectively. So, color recovering by NF resulted unviable for highly colored grape juices and traditional active carbon discoloration should be used. Moreover, from a practical point of view, soluble solids concentration reduction after NF must be carefully taking into account, since additional concentration step may be required.

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# Tools for complementary product development

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## Summary

Food producers can potentially benefit from collaboration on product development, e.g. in relation to meal solutions, by combining complementary resources. Potential benefits are flexibility, customization, increased and more consistent quality, knowledge sharing, decreased development costs etc. However, barriers to collaboration also exist, including low levels of trust, uncertainty, lack of commitment and common goals as well as opportunism. This dilemma is discussed in the light of research in the Danish food industry. Furthermore, tools for overcoming some of the obstacles of collaboration on product development, while also harvesting the benefits, are presented. These include tools for relationship strategy planning in relation to product development, modularisation of meal solutions, evaluation of collaboration and partners as well as improving quality through product development.

Keywords: Product development, food producers, collaboration, strategy, modularisation, quality, meal solutions

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# New innovative fats and oils with a healthy fatty acid profile

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## 1. Summary

Today, cardiovascular diseases (CVD) are death cause number one in the Western society. Another prosperity disease, type 2 diabetes is reaching epidemic proportions. Its prevalence is also enforced by the fact that more and more people are becoming obese. The major cause of these diseases is the too fat rich diet of humans and moreover it contains too much “unhealthy” fat, like trans and saturated fatty acids. One of the strategies to make the fat healthier is by changing the fatty acid profile. The efficacy of such a public health dietary strategy requires a multifaceted approach, which includes a well-informed and motivated consumer and the availability of affordable, acceptable food products with enhanced fatty acid profile. The latter can be obtained by mixing or enzymatic interesterification with healthy oils like rapeseed, sunflower, hazelnut, walnut, rice bran and flaxseed oil. The changing of the fatty acid profile may have major implications on the physicochemical properties. In this research the underlying mechanism of these properties is revealed so that changes can be made in an adequate way. Techniques like NMR, DSC and HPLC-ELSD are used in this research.

Keywords: healthy oils, fatty acid profile, blending, physicochemical properties

## 2. Extended Abstract

In this study palm oil, its low melting fraction palmolein and its high melting fraction palmstearin where blended with three oils with a healthy fatty acid profile. For this purpose hazelnut oil (88% C18:1; 10% C18:2); rice bran oil (42% C18:1; 33% C18:2) and walnut oil (20% C18:1; 49% C18:2; 12% C18:3) were selected. Blending was preferred because it has the advantage that no chemical processes are involved consistent with the consumer trend toward edible oil devoid of any chemical treatment and with natural characteristics and natural flavour.
Nine different binary blends were prepared and within each blend the two components were mixed at composition intervals of 10%. All the samples were analyzed for their SFC after being crystallized and tempered at 26 °C by NMR. The aim of the study was also to determine the melting characteristics of the different blends. The different blends were evaluated for their use in value-added products like spreads and margarines.

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# Session S-5Gg: Process & Product Innovation (Food-4b) – 5 min oral presentation plus poster presentation

| Abstract Number | Paper Title & Authors | Included |
|---|---|---|
| 228 | Cryogenic freezing of food materials as preparation for crushing<br>K Eugeney | No |
| 1960 | Innovation - where is the room for improvement?<br>C Nellemann | No |
| 2129 | Effect of ozonation on table grapes preservation in cold storage<br>D Kontoulis, K Katsieris, P Vlachos, M Kornaros | Yes |
| 2289 | Effect of Ultrasound as Abiotic Elicitor on the Production of Trans-Resveratrol in Vitis vinifera L.<br>E Erte A Güvenç B Kunter N Keskin Ü Mehmetoğlu | Yes |
| 3860 | Enzymatic structural modification of antioxidants for omega-3 oil protection<br>M B Let, LSchultz,H Zhang, Z Guo, C Jacobsen, X Xu | Yes |
| 4141 | Influence of surface structure on the cleanability of surfaces<br>U Bobe, K Sommer, U Beck, G Reiners, B Winzer, W Peukert | Yes |

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# Effect of ozone exposure on decay development of table grapes in cold storage

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## 1. Summary

The aim of this study was to investigate the effect of gaseous ozone exposure on table grapes contaminated with the phytopathogenic fungus *Botrytis cinerea* under cold storage conditions. The efficacy of four ozone treatments was evaluated against the fruit decay caused by *B. cinerea*. The application of 15ppm ozone for 1h per day was proved as the optimum process, based on the achieved elimination of disease incidence, the observed weight loss and the economic feasibility, compared to all other tested ozone treatments. This type of treatment resulted to a 6 week preservation time of table grapes.

Keywords: table grapes, *B. cinerea*, gray mould, preservation, ozone

## 2. Extended Abstract

### 2.1 Introduction

Since the declaration of GRAS status (Generally Recognized As Safe) for ozone in the United States in 1997 (US-FDA, 1997), the interest in developing ozone applications in the food industry has been greatly increased.

### 2.2 Materials and Methods

Artificially inoculated table grapes (Sultanina variety) with *B. cinerea* via two different ways, namely immersion of grapes into a suspension and direct injection of a suspension (containing fungus spores) on a single grape, were stored for 6 weeks, at 2-3$^{o}$C and 85-90% RH, under different ozone dosages. The following ozonation treatments were tested during this study: a) low ozonation (15ppm of ozone for 1h on a daily basis), b) high ozonation (2500ppm of ozone for 1h on a daily basis), c) periodical ozonation (2500ppm of ozone for 1h on a three day basis) and d) high initial ozonation (2500ppm of ozone for 1h only at the initial day). The decay frequency, the appearance of external disease, the number of infected grapes, the weight loss as well as a variety of quality parameters such as the sugars and proteins content were checked on a three day basis.

## 2.3 Results and discussion

According to the obtained results, it was concluded that the application of either the low or the high ozonation treatment, restricted the incidence of the gray mould caused by *B. cinerea* on table grapes, during 6 weeks of storage at 2-3$^{o}$C (Table 1). The latter finding was valid for both types of artificial contamination applied on table grapes. In contrast, the high initial and the periodical ozonation treatments were proved to be insufficient for preventing the decay development since the decay frequency reached 100% in all cases (Table 1). However, the exposure of table grapes to ozone atmosphere, resulted to significant weight losses, compared to the respective values achieved at control experiments, with an exception in the case of the high initial ozonation (Table 1), indicating that ozone affects the physiological responses of the table grapes (Palou et al., 2002). In addition, the measurements regarding the quality parameters of table grapes during their storage time (data not shown) revealed that the ozonation did not significantly affect their levels in table grapes. The lower weight loss along with the lower operational cost accomplished via the low ozonation treatment, when compared with the high ozonation one, proved that the former would be the optimum choice.

Table 1. Influence of ozone treatments on decay frequency and weight loss of artificially contaminated table grapes with *B. cinerea*, after 6 weeks of cold storage

| | Cluster | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | | Immersion without ozone | | Immersion with ozone | | Injection without ozone | | Injection with ozone | |
| Treatment | DF (%) | WL (%) | DF (%) | WL (%) | DF (%) | WL (%) | DF (%) | WL (%) | DF (%) | WL (%) |
| Low ozonation | 4.2 | 4.6 | 57.7 | 4.4 | 2.2 | 10.0 | 3.9 | 4.7 | 3.6 | 6.4 |
| High ozonation | 30.4 | 2.9 | 100 | 3.2 | 2.9 | 23.4 | 55.6 | 1.9 | 22.2 | 21.5 |
| Periodical ozonation | 2.1 | 4.9 | 89.8 | 6.8 | 93.3 | 21.4 | 100 | 10.1 | 100 | 16.1 |
| High initial ozonation | 2.1 | 4.9 | 89.8 | 6.8 | 49.4 | 7.4 | 100 | 10.1 | 100 | 6.2 |

DF=Decay Frequency, WL=Weight Loss

## 2.4 Conclusions

The exposure to 15 ppm ozone for 1h on a daily basis combined with cold storage, resulted to a 6 week preservation of table grapes, against the decay caused by the Botrytis blight.

### References

Palou, L., Crisosto, C. H., Smilanick, J. L., Adaskaveg, J. E., Zoffoli, J. P., (2002) *Postharvest Biology and Technology*, 24, 39–48.

U.S. Food and Drug Administration - EPRI (Electrical Power Research Institute) 'expert panel', *Substances generally recognised as safe, proposed rule*, Federal register 62 (74): 18937-18964 (1997).

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# Effect of ultrasound as abiotic elicitor on the production of *trans*-resveratrol in *vitis vinifera* L.

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## 1. Summary

In this study, the effect of ultrasound as an abiotic elicitor on the production efficiency of *trans*-resveratrol in *Vitis vinifera* L.(Kalecik Karasi (KK) - fresh and frozen - cultivar) was investigated. The *trans*-resveratrol concentration increased 20 fold by ultrasonic irradiation in pulse mode during 1 h at the end of incubation time of 24 h for irradiated fresh KK at 20 kHz according to nonirradiated sample. The maximum *trans*-resveratrol concentration (3.63 mg/g skin) was reached at these conditions.

Keywords: *trans*-resveratrol; ultrasound; *Vitis vinifera* L.; antioxidant; abiotic elicitor

## 2. Extended Abstract

Phytoalexins are organic metabolites that are produced by plants in response to biotic elicitor (e.g. fungal infection) or abiotic elicitor such as UV light, heavy metal ions or ultrasound. Resveratrol (trans-3,5,4'-trihydroxystillbene) is one of the most important phytoalexins. It is synthesized particularly in the skins and seeds of grape berries and only trace amounts are present in the fruit flesh. It exists in two isomeric forms, *cis*- and *trans*-; the *trans*-isomer is present in the skins of *Vitis vinifera* L., but the *cis*-isomer has not been reported. A strong antioxidant property of *trans*-resveratrol is emphasized in medicine and pharmacy literature. Recently, it is carcinopreventive activity has been proved.

In this study, to investigate of the effect of ultrasound as an abiotic elicitor on the production efficiency of *trans*-resveratrol in *Vitis vinifera* L. (Kalecik Karasi clone 15 (KK) -frozen and fresh- cultivar) was aimed. Ultrasound was given into the system by the application ultrasonic irradiation in pulse and continuous mode at 20 or 30 kHz. A cylindrical glass reactor with jacket (100 mL) was used. *Trans*-resveratrol was analyzed by HPLC. The parameters were chosen as freezing (-20 $^{o}$C), ultrasound application types (in continuous mode (C-US) or in pulse mode: 0.5 s on/ 0.5 s off (P/05-US) or 0.1 s on/ 0.9 s off (P/01-US)), ultrasound application time (10 min or 1 h) and incubation times (0, 24 h, 48 h and 72 h) and frequency (20 kHz and 30 kHz).

The *trans*-resveratrol concentrations were similar for fresh (0.18 mg/g skin) or frozen (0.17 mg/g skin) samples before they were irradiated as shown in Figure1. The *trans*-

resveratrol concentration increased 20 fold by ultrasonic irradiation in pulse mode during 1 h at the end of incubation time of 24 h for irradiated fresh KK at 20 kHz according to nonirradiated sample. Similarly, the *trans*-resveratrol concentration increased 8 fold by ultrasonic irradiation in pulse mode during 1 h at the end of incubation time of 48 h for irradiated frozen KK at 20 kHz according to nonirradiated sample. Generally, the application of ultrasound in pulse mode was more effective than that of in continuous mode. After the samples were irradiated at 20 kHz, the highest *trans*-resveratrol concentration was obtained by ultrasonic irradiation in pulse mode (P/01-US) for both fresh (3.63 mg/g skin) and frozen (1.33 mg/g skin) samples. Similarly, after the samples were irradiated at 30 kHz, the highest *trans*-resveratrol concentration was obtained by ultrasonic irradiation in pulse mode (P/05-US) for both fresh (2.92 mg/g skin) and frozen (1.09 mg/g skin) samples. The high *trans*-resveratrol concentrations were reached at the incubation times of 24 h (3.63 mg/g skin and 2.92 mg/g skin) and 48 h (1.33 mg/g skin and 1.09 mg/g skin) for fresh and frozen samples, respectively, for ultrasonic irradiation at both 20 kHz and 30 kHz. It may be said that the more *trans*-resveratrol could be produced using fresh grape skin according to frozen grape skin by ultrasonic irradiation. Although it was known that freezing may act as an abiotic elicitor, it was not observed positive effect of freezing on the *trans*-resveratrol synthesis in this study. As a result, it could be said that ultrasound is a suitable abiotic elicitor for *trans*-resveratrol synthesis in grape skin. This positive effect of ultrasonic irradiation could be enhanced by using together another abiotic elicitor such as ultraviolet irradiation.

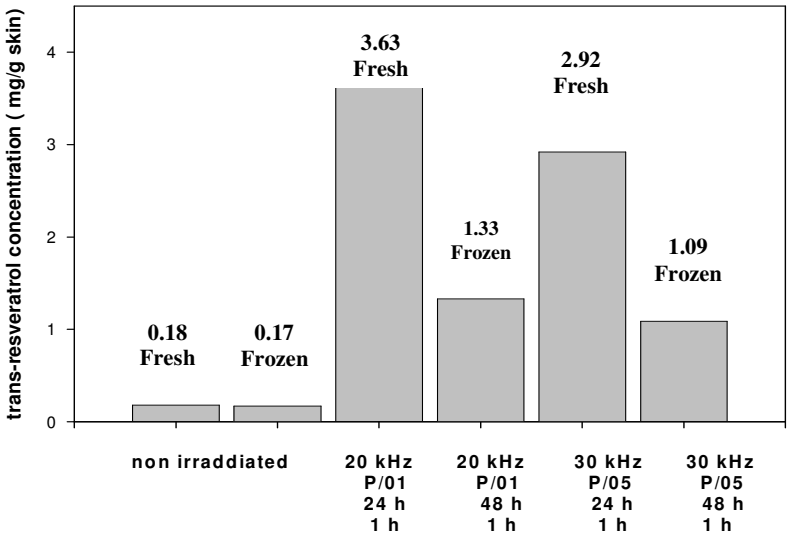


**Figure 1.** The effect of ultrasound on the *trans*-resveratrol synthesis in grape skin.

## Acknowledgements

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# Enzymatic structural modification of antioxidants for omega-3 oil protection

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## 1. Summary

The main barrier against a successful launch of foods containing oils rich in the long chain polyunsaturated fatty acids (omega-3 PUFA) is the high susceptibility to lipid oxidation. The complex nature of real food systems further complicates antioxidative stabilisation and studies have shown that many of the commercial free radical scavenging antioxidants are not efficient in preventing lipid oxidation in fish oil enriched foods. This poster presents the modification of antioxidants using enzymatic synthesis targeted for better antioxidative properties in fish oil enriched foods. Investigations of the enzymatic esterification of ascorbic acid with conjugated linoleic acid (CLA) is presented. In t-butanol only Novozymes 435® turned out to be a useful enzyme preparation for the production of ascorbylCLA-ester. Response surface modelling showed that the optimum reaction conditions in this system were determined to be 4 hours at 50°C and a molar ratio of R(CLA/ascorbic acid) = 4. Furthermore, the product formation was enhanced by increasing the ascorbic acid concentration above saturation limits (10 g/L). In addition, the esterification reaction was transferred to an ionic liquid system, in order to reduce the amount of organic solvents used. It was evident that only methyltrioctylammoniumtriflouroacetate could provide a proper reaction environment for production of ascorbylCLA-ester when using Novozymes 435®.

Keywords: enzymatic synthesis, antioxidants, omega-3, lipase, ionic liquids

## 2. Extended Abstract

An increasing amount of evidence compiled over the last 30 years, supports the nutritional benefits of dietary omega-3 PUFA, which are present in high amounts in marine oil triglycerides. Due to the high degree of unsaturation, omega-3 PUFAs are highly susceptible to oxidative deterioration which will lead to the formation of unpleasant fishy off-flavours and reactive free radicals and aldehydes. Oxidative deterioration seems to be particularly prominent in emulsions and complex food systems, and the particular mechanisms of oxidation can differ significantly between different food emulsion systems. Moreover, the efficacy of antioxidants seems to be

influenced by their localisation in the food system, which is dependent on the polarity of the antioxidant and on the emulsifier used. Therefore, there is a need to develop new antioxidants based on natural sources but with improved physical properties, which are designed to be located where they are needed and to have the right antioxidative properties required in the particular food system (e.g. free radical scavenging or metal chelating properties). This poster presents investigations of the enzymatic synthesis of ascorbyl esters in both organic solvent medium and in an ionic liquid system.

The esterification of ascorbic acid with CLA in t-butanol was only efficient using the immobilised enzyme preparation Novozyme 435® (55°C), and the maximum concentration of product was obtained already after 4 hours. Application of Lipozyme RM® gave just small amounts of the desired product, whereas Lipozyme TL®, AK20 and PS30 were not efficient at all. To determine the efficiency of the enzymes, the samples were analysed by HPLC, and the formation of esterified product was semi-quantitatively determined as peak area of the ester compound (UV-detection).

High yields was obtained in both t-butanol and acetone, but the esterification reaction, as mediated by Novozymes 435®, did not take place in 1-butanol, hexane, and only to a limited extent in 2-methyl-2-butanol. The better performance of the more hydrophobic solvents was likely to be due to increased solubility of ascorbic acid in these solvents. Accordingly, further experiments showed that increasing ascorbic acid concentrations above saturation limits increased product concentration significantly.

Response surface modelling indicated that neither temperature changes in the interval 40 – 60°C nor reaction times between 2 and 6 hours influenced the formation of ascorbylCLA-ester significantly. The influence of the molar ratio between acyl donor and ascorbic acid were investigated in the interval between R(CLA/ascorbic acid) = 1 to 10. A four-fold increase was observed when increasing the ratio from R = 1 to R = 5, but only minor increases were observed between R = 5 and R = 10. The optimum ratio were therefore determined to R = 5. Further studies showed that the estereification followed first order reaction kinetics.

In order to minimize the use of organic solvents, the esterification reaction was transferred to an ionic liquid system. A screening of five different ionic liquids showed that only methyltrioctylammoniumtriflouroacetate could provide a proper reaction environment and result in useful levels of ascorbylCLA-ester. Similarly to esterification in the solvent system, an increase in ascorbic acid concentration above its saturation limits increased ester formation, and reaction rate increased significantly as temperature increased from 50 to 70°C. On the other hand, removal of water by applying vacuum during reaction did not increase product formation further.

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# Influence of surface structure on the cleanability of surfaces

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## 1. Summary

The paper discusses the influences of surface parameters on the detachment of adhered particles. Surface energy, roughness and roughness structure have been the topometric and topographic parameters of interest.
The effects of variations of the cleaning fluid on particle detachment became also analysed.
As one of the most critical soilings exists as soon as particles, cells or microorganisms "cooperate" during adhering and attaching to a surface it is extremely important for the cleaning process to investigate the influence of the surface structure on the detachment of foulings and biofilms.

Keywords: topometry, surface, roughness, cleaning, adhesion

## 2. Extended Abstract

In former research experiments had been done to investigate the effect of a surface topography / topometry on its cleanability [1,2]. These experiments had been carried out with single particles (spherical) and single living organisms (spherical and elliptical) and showed no dependence of the cleaning result on the surface roughness. This fact leads to the theory that there is a huge variety of roughness structures on a realistic surface which are dominating the adhesion force rather than the size of the roughness. Figure 1 shows that the van der Waal force differs in orders of magnitude for different roughness structures (e.g. factor $> 10^6$ for technical roughnesses $Ra > 0{,}1\ \mu m$).
Next to the cleaning of single particles the effect of crosslinked contaminations like colloids and surface foulings on th cleaning efficiency will be presented. The influence of different structures of surface topography will be shown and if there is an structure-dependent effect on the cleaning similar to the Lotus-Effect® (which doesn't work in the immerged system).

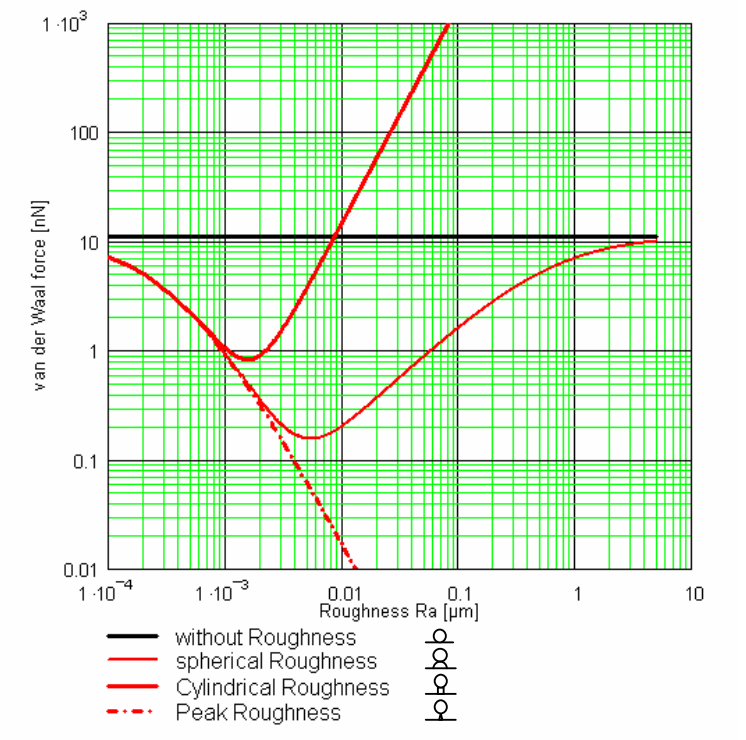


Figure 1: van der Waal force between a particle (Ø = 1 µm) and a surface with different roughness structures

## References

[1] Bobe U, Sommer K., Beck U., Reiners G., *AiF-Project 13586 Final Report* (2005)

[2] Beck U., Bobe U., Gamer N., Reiners G., Sommer K., (2005) *CIT*, Vol. 77, No. 12, 1942-1946

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# Session S-5H: Hygiene, Hygienic Design & Unit Operations (Food-5a)

| Abstract Number | Paper Title & Authors | Included |
|---|---|---|
| 2263 | Effects of ultra-high pressure homogenization on microbial and physico-chemical shelf-life of milk<br>J Pereda, V Ferragut, J M Quevedo, B Guamis, A J Trujillo | Yes |
| 2768 | Food sterilisation under high pressure-Fundamentals,new insights and challenges<br>A Mathys, S Toepfl, V Heinz, D Knorr | Yes |
| 3332 | Pathogen inactivation by pulsed electric fields - a survey of processing conditions, equipment design and potential applications for heat sensitive products<br>S Toepfl and V Heinz | Yes |
| 3900 | Importance of CIP velocity<br>B B B Jensen | Yes |
| 3977 | The effect of thermosonication and pulsed electric fields on the inactivation of Listeria innocua in low fat milk<br>M W Ribeiro | Yes |
| 4120 | Modelling for food safety in simulated cheeses<br>E Noriega, A Laca, M Díaz | Yes |
| 4132 | Process Integration For The Fractionation Of Milk Proteins<br>M P Mier, R Ibáñez, I Ortiz | Yes |

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# Effects of ultra-high pressure homogenization on microbial and physico-chemical shelf-life of milk

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## 1. Summary

The effect of ultra-high pressure homogenization (UHPH) on microbial and physico-chemical shelf-life of milk during storage at 4ºC was studied and compared to a conventional heat preservation technology used in industry. High pressure treatments applied were 200 and 300 MPa (single stage) with milk inlet temperatures of 30 and 40ºC. UHPH-treated milks were compared to high-pasteurized milk (PA; 90ºC for 15 s). The microbiological quality and physico-chemical parameters (viscosity, color, pH, acidity, and residual peroxidase and phosphatase activities) were assessed in milks. Microbial results of treated-milks during storage at 4ºC showed that UHPH treatment produced milk with a microbial shelf-life between 14 and 18 days, similar to that achieved for PA milk. UHPH treatments reduced the L* value of treated-milks and induced a reduction in viscosity values of milks treated at 200 MPa compared to PA milks, however these differences would not be appreciated by consumers. Hence, alternative methods such as UHPH may give new opportunities to develop fluid milk with an equivalent shelf-life to that of PA milk in terms of microbial and physico-chemical characteristics.

Keywords: ultra-high pressure homogenization, milk shelf-life, microbial inactivation, physico-chemical characteristics.

## 2. Extended Abstract

### Introduction

Ultra high-pressure homogenization is a new food preservation treatment that is being developed as an alternative to heat-treatments. To date, physico-chemical and microbiological changes generated by UHPH in milk have been studied after treatment but not during storage (Hayes et al., 2005; Pereda et al., 2006). Therefore, the aim was to compare the effects of heat and UHPH treatments on the microbial and physico-chemical shelf-life of milk during storage at 4ºC.

## Material and Methods

Fresh raw bovine milk was collected from a local farm (Can Badó, Barcelona, Spain) and was standardised at 3.5 ± 0.2 % of fat. Milk was processed using a Stansted High Pressure homogenizer. Treatments applied were 200 and 300 MPa with inlet temperatures (Ti) = 40ºC and 30ºC. UHPH-treated milks were compared to high-pasteurized milk (PA; 90ºC-15 s). Microbiological quality was studied by enumerating total counts, psychrotropic bacteria, lactococci, lactobacilli, enterococci, coliforms, spores and *Pseudomonas* spp. Physico-chemical parameters assessed were viscosity, color, pH, acidity and residual lactoperoxidase and alkaline phosphatase activities.

## Results

After treatment, UHPH was as efficient in reducing psychrotrophic, lactococci and total bacteria as was PA treatment, reaching important reductions (~3.5 log cfu/ml). Coliforms, lactobacilli and enterococci were completely eliminated.

Small but significant differences were detected in the pH value of milk treated at 200 MPa at 30ºC (~ 6.72) in comparison to raw milk and to the other treated milks (~6.74). For each day of sampling, the pH of milks treated at 200 MPa at 30ºC was always below the values of the other milks. After 18 days of storage a decrease in pH was observed except for pasteurized and 300 MPa at 30ºC milks, which continued with a constant pH until the end of storage.

UHPH reduced the L* value of treated-milks and it induced a reduction in viscosity values of milks treated at 200 MPa compared to PA milks, however these differences would not be appreciated by consumers.

Related to enzymes, heat treatment performed at 90ºC for 15 s inactivated completely the lactoperoxidase. Temperature achieved during UHPH treatments at 300 MPa also produced total enzyme inactivation. However, at 200 MPa at 30 and 40ºC was observed a residual activity of 35% and 1%, respectively. Both, heat and UHPH treatments reached a complete inactivation of Alkalinephosphatase.

## Conclusions

UHPH treatment, besides achieving a reduction in microbial counts, generated changes in physico-chemical properties such as color, viscosity, pH and acidity. Nevertheless, differences in instrumental color and viscosity measurements between UHPH-treated milks and PA milk were not visually and sensorially obvious.

Therefore, UHPH technology could be a good alternative to be used as one step homogenization/pasteurization to produce commercial milk with a microbial and physico-chemical shelf-life equal to that of high-pasteurized milk.

## References

Hayes, M.G., Fox, P. F. and Kelly, A. L., (2005) Journal of Dairy Research, 72, 25-33.

Pereda, J., Ferragut, V, Guamis, B. and Trujillo, A., (2006). Milchwissenschaft. 61, 245-248.

Book of Abstracts
European Congress of Chemical Engineering (ECCE-6)
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# Food sterilisation under high pressure- Fundamentals, new insights and challenges

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Consumers all over the world demand high quality and convenient products with natural flavor and taste, and greatly appreciate the fresh appearance of minimally processed food. Sterilization under pressure has been shown to be valuable for global production of high quality products. However, the mechanism of inactivation of bacterial spores by heat and pressure is still a topic of discussion. Obviously, the change of the dissociation equilibrium under pressure and temperature plays a dominant role in inactivation of microorganisms. Heat and pressure inactivation of G*eobacillus. stearothermophilus* spores at different initial pH-values in ACES and phosphate buffer confirmed this view. Thermal inactivation in ACES buffer at 122°C resulted in higher logarithmic reductions. Contrary, after pressure treatment at 900 MPa with 80°C phosphate buffer showed higher inactivation. These results indicated the different dissociation equilibrium shifts in buffer systems by heat and pressure. Due to preparation, storage and handling of highly concentrated spore suspensions, the clumping and the formation of aggregates can hardly be avoided. After particle analysis it could be shown that the lag phase often found in thermal spore inactivation can sufficiently be described by first-order inactivation kinetics when the agglomeration size is considered. The physiological response of *B. licheniformis* spores to pressure and thermal inactivation was investigated using multiparameter flow cytometry. For pressure treated spores, four distinct sub-populations were detected. For these sub-populations, we suggest a three step model of inactivation involving a germination step following hydrolysis of the spore cortex, an unknown step, and finally an inactivation step with physical compromise of the spore's inner membrane. With an improved understanding of the spore inactivation mechanism it will be possible to assess the benefits and sustainability of emerging technologies e.g. pressure assisted thermal sterilization with regards microbiological safety and stability.

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# Pathogen inactivation by pulsed electric fields - a survey of processing conditions, equipment design and potential applications for heat sensitive products.

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## 1. Summary

The feasibility of Pulsed Electric Field (PEF) application to inactivate pathogens in heat sensitive products was investigated. By variation of processing parameters (electric field strength, specific energy input and treatment temperature optimum treatment conditions for a gentle and energy efficient increase of shelf life have been identified for treatment of raw milk, nutrient or vitamin solutions. A combination of PEF and mild heat provided a potential to increase PEF efficacy and to reduce electrical energy consumption significantly. To separate thermal and PEF effects kinetic studies on thermal sensitivity and inactivation during a combined process have been performed. A technical scale pilot plant was realized to show the techniques applicability and to evaluate costs of investment and operation.

Keywords: Pulsed Electric Field, Non-thermal, Minimal Processing, Energy Efficiency

## 2. Extended Abstract

### 2.1 Introduction

As an alternative to thermal preservation a non-thermal, minimal processing by pulsed electric fields can be utilized for microbial decontamination of heat sensitive products. The effect of processing parameters on inactivation of different pathogenic microorganisms by pulsed electric field application was investigated.

### 2.2 Experimental design

A 3 kW pulse modulator to supply rectangular pulses with a voltage up to 25 kV was developed at DIL. The liquid media was exposed to electric field pulses in a co-linear treatment chamber with an inner diameter of 4 mm and an electrode gap of 4 mm at a flow rate of 10 l/h. Inactivation of *L. monocytogenes, S. aureus, Campylobachter jejuni, Enterobacter sakazakii* and *Salmonella sp*. in milk and other liquid media was investigated. The impact of electric field strength (10–40 kV/cm), initial temperature (20–55°C) and specific energy input (10–200 kJ/kg) on treatment efficacy has been evaluated. Pulse repetition rate was adapted to the desired processing parameters and the liquid media flow rate in a range of 10 to 90 Hz. Using glass capillaries the thermal inactivation in a temperature range of 40 to 90°C was investigated to allow a separation of PEF and thermal inactivation effects.

## 2.3 Results and discussion

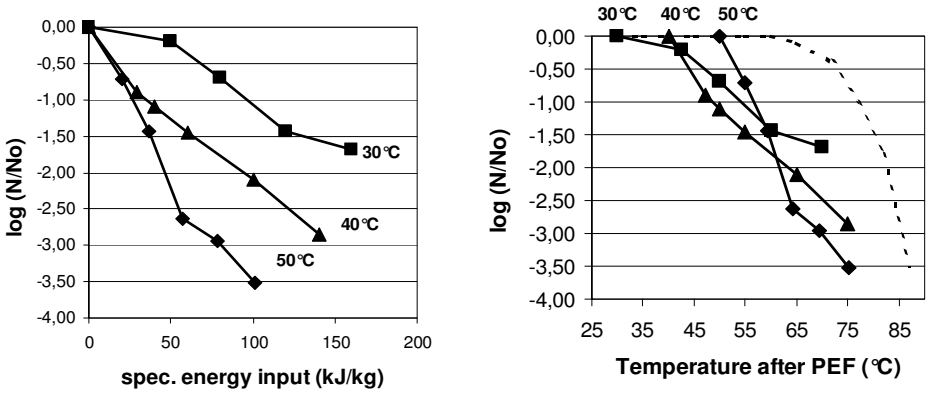


**Figure 1:** (a) Inactivation of *E. sakazakii* in milk by pulsed electric fields with a strength of 25 kV/cm and different initial treatment temperatures. (b) Inactivation of *E. sakazakii* by PEF related to maximum treatment temperature achieved.

Exemplarily the inactivation of *E. sakazakii* in milk at different treatment temperatures is shown in Figure 1(a). An inactivation of up to 5.5 log cycles was obtained at a field strength of 25 kV/cm. Due to dissipation of electrical energy the media temperature will increase during treatment, in Figure 1 (b) the inactivation is shown with relation to the maximum temperature achieved. At an initial temperature of 50°C and an energy input of 100 kJ/kg a maximum temperature of 75°C was obtained, after a residence time of 3 s the media was cooled in a heat exchanger. Applying a thermal treatment of 75°C for 3 s a 0.4 log inactivation was detected (dashed line), indicating the minor impact of thermal effects on microbial inactivation. Total extra costs of an optimized PEF treatment have shown to be in a range of 1 Euro-cent per litre. Similar results have been found for other pathogenic microorganisms (data not shown). A model has been developed for selection of optimum processing parameters dependent and microbial strain and product properties. Exceeding a maximum temperature of 85°C by increasing initial temperature or energy input the importance of thermal effects on total inactivation is increasing.

## 2.4. Conclusions

Utilizing synergetic effects of mild heat and PEF application optimum processing parameters can be selected with regard to maximum allowable product temperature or a minimization of electrical energy use. PEF application allowed a reduction of maximum temperature in a range of 10 to 20°C in comparison to a thermal treatment By selection of suitable processing conditions the technique will allow preservation of heat sensitive, premium foodstuff, nutrient media or protein solutions while minimizing thermal product deterioration. Based on the results of this study a technical scale pilot plant with a capacity of 200 l/h was realized, an industrial scale prototype is under development at present.

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# Importance of CIP velocity

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## 1. Summary

The flow of detergent is an important factor in the cleaning of closed processing equipment. Typically, cleaning is performed at constant flow rates throughout the system and the cleaning time decided based on the criteria; the area most difficult to clean must be cleaned at the end of the cleaning. This presentation focuses on practical and numerical experiments on cleaning of an upstand with different flow rates (and pulsation) and flow in a spehrical shaped valve house. It was shown that the pulsation only has minor effect on removal of the soil used, but more interestingly, that the areas always difficult to clean is located in the exact same positions independent on flow rate and pulsations. Steady state and transient computational fluid dynamics simulations are used to generate information about the flow patterns, so these phenomena can be explained. The reason for the idea of changing the flow rate is to change the size of recirculation zones and the position of stagnation points to improve cleaning effects.

Keywords: CIP, pulsating, velocity, cleaning, closed equipment

## 2. Extended Abstract

Dead-ends and shadow zones are problem areas that should be avoided in food processing equipment. During cleaning detergent may be stagnant in these zones. The stagnant nature of the detergent means that low velocity and consequently low wall shear stress is present on the surface, making cleaning difficult and ineffective. To worsen it fluid exchange from the bulk flow to these regions are often low which increases the problem with cleaning.

The reason for changing the flow rate during cleaning has to be found in the nature of the flow in and around the zones difficult to clean (dead ends and shadow zones). However, they do not give the full flow field, which is always 3D in pipes. The flow is characterised by recirculation zones and subsequent stagnation points. In the case of laminar flow in the pipes the length of a recirculation zone, typically located after a pipe expansion depends on the Reynolds number and the expansion ratio. For a relatively low Reynolds number the length increases up to a certain point after which it starts decreasing to the length the recirculation zones has at fully developed turbulent flow. At a fully developed turbulent flow only the velocity magnitude in the

recirculation zone changes a little with changing bulk velocity. The stagnation point is located at the end of the recirculation zone and moves accordingly.

For a dead-end geometry, a recirculation zone or a number of recirculation zones are always generated in the dead-end itself. The size, number and orientation of the recirculation zone depends on the length-diameter ratio (length and diameter of the dead-end – L/D). One recirculation zone rotating around a horizontal axis perpendicular to the axial direction of the main pipe will be seen until L/D is larger than 3-4. Longer upstands give additional recirculation zones. For very low L/D (at least < 0.2) we have shown, using CFD, that the "normal" recirculation zone in e.g. a L/D = 1 is no longer present in the dead-end. Instead, two recirculation zones are formed rotating around a vertical axis perpendicular to the axial direction of the main pipe. Stagnation points on the surfaces will appear where the axis of rotation intersects with the pipe walls.

The interesting observation about the dead-end is how the recirculation zone acts as a function of flow rate in the main pipe. Grasshof (1980) showed that increasing the velocity in the main pipe also increased the exchange of liquid from the upstand. For this study it is however, more interesting to ask the questions; is it possible to change the size of the recirculation zone by changing the flow rate and will the stagnation point change location?

In this presentation it is shown that changing the flow rate during cleaning does not have any effect on the location of the areas where flow conditions are unfavourable to cleaning. Increasing the flow rate increases velocity generally, but only very small increases are seen in areas already exposed to very low velocity (and thereby wall shear stress). Increasing the flow rate might reduce the problem areas, but problem areas will always be located where they have always been, as long as there is turbulent flow. Furthermore, it is shown how CFD can be applied as a tool for obtaining extra information that can be used to gain more knowledge on how to establish flow during CIP and also provide information on how to change future design of equipment with respect to cleanability. Also, CFD could be a valuable tool as a supplement to EHEDG testing, as CFD together with EHEDG cleaning results can be used to identify why certain areas in an EHEDG test are difficult to clean from a fluid mechanics perspective.

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# The effect of thermosonication and pulsed electric fields on the inactivation of *Listeria innocua* in low fat milk

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## 1. Summary

In this study the effect of two non-thermal technologies, thermosonication (TS) and pulsed electric fields (PEF) were examined individually, and in combination, for their ability to inactivate *Listeria innocua* inoculated in pre-sterilised low fat milk. TS was applied by either (a) allowing the milk to heat from 25°C at the point of entry into the sonication unit to 55°C at the point of exit (TS1) or (b) pre-heating the milk to 55°C prior to TS and maintaining this temperature during sonication (TS2). During PEF treatment milk, which had been pre-cooled to 5°C, was exposed to 30 and 40 kV $cm^{-1}$ for a total treatment time of 50 μs.
A maximum inactivation of 0.6 log cycles was observed for TS1 which involved processing the milk for 80 s at the highest power output (85 W $cm^{-2}$), while similar conditions for TS2 achieved a 3.3 log cycle reduction. Stand alone PEF treatment at 30 and 40 kV $cm^{-1}$ produced maximum reductions of 1.1 and 3.3 log cycles of *L. innocua*, respectively. Sequential treatment (TS1+ PEF at 40kV $cm^{-1}$) yielded a 6.8 log cycle reduction suggesting a synergy between the methods. By contrast, the combination of TS2 and PEF was additive with 4.5 and 6.3 log cycle reductions, respectively, being observed at 30 and 40 kV $cm^{-1}$. No significant difference ($P \geq 0.05$) was detected comparing the results obtained by TS1 or TS2 combined with PEF to the control high-temperature short-time (HTST) treatment at 72°C which caused a reduction of 7.0 log cycles.
*Keywords*: thermosonication, pulsed electric fields, *Listeria innocua,* milk

## 2. Extended Abstract

*2.1. Introduction:* Beverage processing by non-thermal technologies can achieve microbial inactivation levels comparable to conventional pasteurisation methods, while potentially retaining better quality in the products. TS and PEF are examples of such novel technologies, with the former largely believed to inactivate microorganisms by ultrasonic cavitation in conjunction with mild heating (*Piyasena et al. 2003*), while it is generally accepted that PEF inactivates microorganisms by breakdown of their cell membranes (*Dimitrov 1984*). An enhanced bactericidal effect could be achieved using a combination of TS and PEF at reduced treatment intensities (*Ross et al. 2003*). The aim of the present study was to achieve a reduction of *L. innocua* in low fat milk using a combination of TS and PEF equivalent to conventional pasteurisation.

*2.2. Methods:* Pre-sterilised low fat milk was inoculated with *L. innocua* prior to processing to achieve an initial population of approximately $10^8$ CFU $ml^{-1}$. TS was conducted at a power output of 85 W $cm^{-2}$. Milk was either heated from 25 to 55°C during the residence time (80 s) in the ultrasonic cell (TS1) or pre-heated to 55°C prior to sonication and held at this temperature during treatment using liquid refrigerant (TS2). The PEF treatment consisted of square-wave pulses (1µs) at 15 Hz and electric field strengths used were either 30 or 40 kV $cm^{-1}$ for a total treatment time of 50 µs. PEF was applied alone or in combination with TS1 or TS2. Conventional pasteurisation (72°C for 26s) was used as a control. All data were analysed by one-way ANOVA with a significance level of 5%.

*2.3. Results:* The reduction of *L. innocua* (Fig. 1.) was higher ($P<0.05$) when milk was treated using TS2 rather than TS1 (3.3 vs. 0.6 log cycles). Similarly, exposing milk to PEF at 30 kV $cm^{-1}$ led to a smaller reduction (1.1 log cycles) than PEF at 40 kV $cm^{-1}$ (3.3 log cycles). The combined treatments at 30 kV $cm^{-1}$ produced a microbial reduction of 3.8 and 4.5 log cycles for TS1+PEF and TS2+PEF respectively, while combined treatments at 40 kV $cm^{-1}$ resulted in significantly higher reductions of *L. innocua* (6.8 and 6.3 log cycles for TS1+PEF and TS2+PEF, respectively ($P<0.05$)).

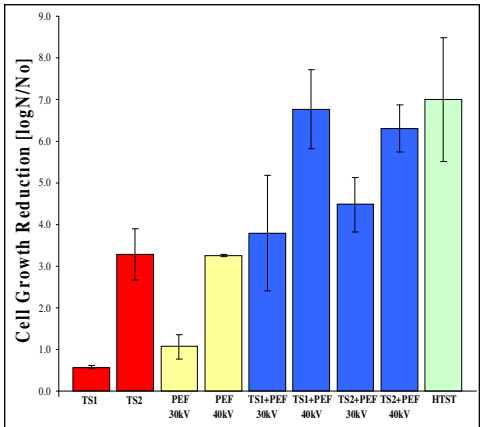

Fig. 1. Reduction of *Listeria innocua* achieved by thermosonication (TS), pulsed electric fields (PEF), combined TS and PEF, and high-temperature short-time pasteurisation (HTST) in UHT milk.

The reduction observed with the combined approach at higher field strength caused similar inactivation ($P\geq0.05$) to that obtained by conventional processing of low fat milk (7.0 log cycles).

*2.4. Conclusion:* Low fat milk samples treated individually with TS, PEF or combined TS and PEF at 30kV $cm^{-1}$ exhibited less effective reduction of *L. innocua* than a combination of TS and PEF at higher electric field strengths. A treatment synergy between TS1 and PEF was observed when combining both at 40kV $cm^{-1}$ which led to a *Listeria* reduction of 6.8 log cycles compared to 6.3 log cycles achieved with TS2 and PEF. Pre-heating the milk before TS (TS2) was proved unnecessary as the heat generated during TS followed by PEF (TS1) was sufficient to reduce the bacterial count to a similar level to conventional pasteurisation. Based on these results a combined non-thermal approach could therefore represent a valid alternative to conventional pasteurisation of milk. However, further research on the quality parameters of milk after combined TS and PEF processing will be required to prove its potential for milk processing.

*Acknowledgement:* This research has been part financed under the Food Institutional Research Measure, which is administrated by the Department of Agriculture and Food, Ireland.

## References

Dimitrov, D. S., (1984) *Journal of Membrane Biology*, 78, 53-60.

Piyasena, P., Mohareb, E. and McKellar, R. C., (2003) *International Journal of Food Microbiology*, 87, 207-216.

Ross, A. I. V., Griffiths, M. W., Mittal, G. S. and Deeth, H. C., (2003) *International Journal of Food Microbiology*, 89, 125-138.

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# Modelling for food safety in simulated cheeses

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## 1. Summary

Food safety in soft cheese production is a problem of main interest. In particular, to anticipate microbial behaviour in soft cheeses, which suffer the colonization of several pathogens, such as *Listeria monocytogenes*. In this work, a new model that combines diffusion and reaction terms has been developed to predict the evolution of cells and substrates within structured foods. To test the feasibility of the model, microbial evolution with time and position was determined in two model cheeses with a similar composition and structure to soft cheeses. This model enables to obtain the distribution of nutrients and products in the food, being possible to simulate different ways of contamination, and it providing interesting results.

Keywords: food safety, Listeria, growth modelling, model foods

## 2. Extended Abstract

Soft cheeses are risk products in food safety since their properties are favourable enough to support pathogens´ growth, such as *L. monocytogenes*. Thus, complete mathematical models are required to predict the safest operational conditions. Most available models have been developed from experiments in liquid phase, but the absence of diffusional limitations in these broths leads to not entirely reliable predictions of cell growth in cheeses, where physical structure has been reported as a significant parameter. Therefore, models describing microbial growth evolution not only with time but also with position within the food would be desirable. This implies that diffusional processes must be taken into account. In this work, mass balances for involved solutes have been set out by considering cheese as a homogeneous structure with cylindrical geometry, and oxygen as the limiting substrate of cell growth. A system of differential equations that includes diffusion and reaction processes was obtained:

-Biomass balance

$$\frac{\partial C_x}{\partial t} = D_x \frac{\partial^2 C_x}{\partial z^2} + r_x$$

$$r_x = K\, C_x\, (1 - \tau\, C_x)$$

-Substrate balance

$$\frac{\partial C_s}{\partial t} = D_s \frac{\partial^2 C_s}{\partial z^2} + r_s$$

$$r_s = -\,(Y\, r_x + m\, C_x)$$

- Initial conditions

$$t = 0,\ C_s = C_{s\,sat};\ C_x = C_{xo}$$

- Boundary conditions

$$z = L \text{ (surface)},\ C_s = C_{s\,sat}$$

$$z = 0 \text{ (bottom)},\ \frac{\partial C_x}{\partial z} = 0,\ \frac{\partial C_s}{\partial z} = 0$$

Prediction of biomass and substrate evolution with this type of model requires kinetics of free microorganisms under different aeration conditions. So, *L. innocua* growth was studied in two culture broths with a similar composition to soft cheeses, but with different lactic acid content in order to simulate low and high acid cheeses, and the effect of oxygen concentration on cell growth was also evaluated in both media.

To test the goodness of the prediction, real cheeses with heterogeneous characteristics make difficult to collect representative data, so that, microbial evolution with time and position was determined in two structured model cheeses with the same composition as the mentioned cheese broths and provided with a specific structure given by addition of κ-carrageenan. Finally, model predictions were compared with experimental results, showing a good agreement for lactic acid concentration lower than 3 g/L. However microbial behaviour for concentration higher than 3 g/L was completely opposite to that expected from broth experiments.

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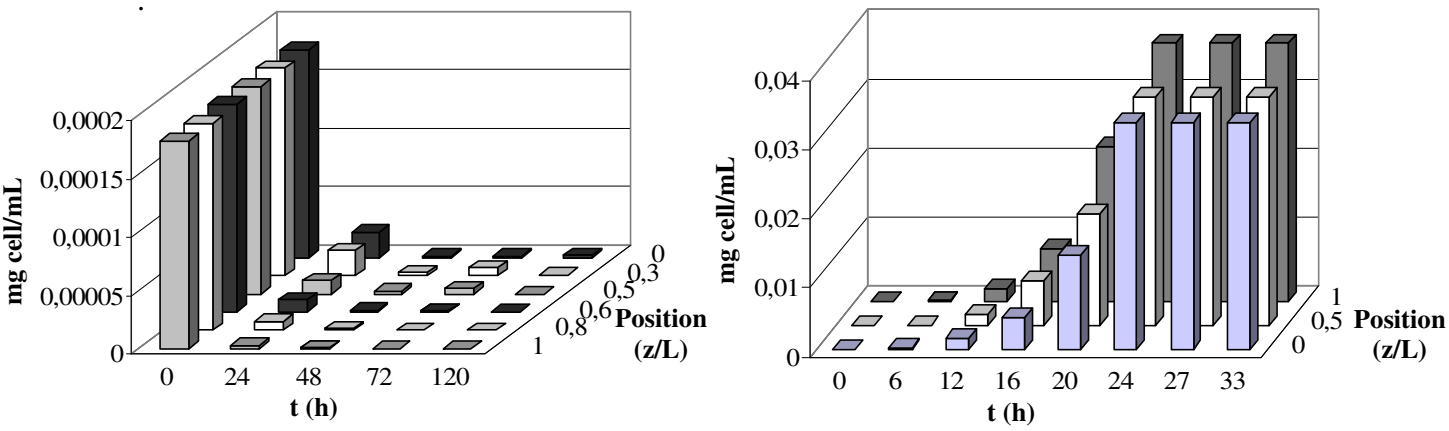


**Figure 1.** *L. innocua* growth in the structured model cheeses: 10.6 g/L lactic (left) and 3 g/L lactic (right). z/L = 0 (bottom); z/L = 0.5 (middle); z/L = 1 (surface).

## References

McMeekin, T.A., Olley, J., Ratkowsky, D.A., Ross, T., (2002). Predictive microbiology: towards the interface and beyond. *International Journal of Food Microbiology,* 73, 395-407.

Wilson, P.D.G., Brocklehurst, T.F., Arino, S., Thuault, D., Jakobsen, M., Lange, M., Farkas, J., Wimpenny, J., Van Impe, J.F., (2002). Modelling microbial growth in structured foods: towards a unified approach. *International Journal of Food Microbiology*, 73, 275-289.

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# Process integration for the fractionation of milk proteins

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## 1. Summary

The food and the pharmaceutical industries are of prime importance to the economy of the European Union (Gateway to the European Union). For these sectors, the substitution of traditional processes by means of more sustainable processes is essential to continue in the market and represent a real challenge. To accomplish this goal, process integration may be an attractive tool.

Thus, the aim of this study was to evaluate the performance of the integration of several membrane processes in order to separate casein and whey proteins from milk in the framework of sustainable development. Different operational conditions had been tested to check the technical viability of the process.

Keywords: protein separation, process integration, green engineering, membrane technology

## 2. Extended Abstract

Milk is one of the most important raw materials employed in the food industry. Milk proteins including casein and the so-called whey proteins have high nutritional values and also important functional properties. Due to these properties, milk proteins have become of great interest for the food industry and for pharmaceutical industry which are of prime importance to the economy of the European Union (Gateway to the European Union).

To date, milk proteins are isolated by fermentation processes, chemical precipitation processes, column chromatography or electrophoresis. These conventional processes need dangerous reagents, which are hazardous to transport and handle and also produce high quantity of effluents (Bazinet et al. 2001). However, new advanced separation processes are being developed. These processes are called to substitute conventional ones in the near future due to its environmental and also economical advantages.

The aim of this study was to evaluate the performance of the integration two membrane processes, Electrodialysis with Bipolar Membranes (EDBM) and High Performance Tangential Flow Filtration (HPTFF), in order to separate casein and whey proteins from milk. Different operational conditions had been tested to check the technical viability of the process.

**Table 1**. Powdered milk average composition

| | **Average values per 100 g** |
|---|---|
| **Protein** | 34 g |
| **Carbohydrates** | 52 g |
| **Fat** | Max. 1 g |
| **Energy** | 1499 kJ / 353 kcal |

Reconstituted milk from powder (composition shown in table 1) was employed to prepare the feed solution. Four different concentrations between 2.5% and 10% in weight were tested.

In a first step, the separation of casein and whey from milk has been performed by means of EDBM technology as an alternative to traditional chemical precipitation. Experiments were carried out working in a laboratory-scale plant purchased from Elektrolyse Project, Netherlands. A combination of commercial cationic, anionic and bipolar membranes were employed as described in P. Mier et al. 2007a. Experiments were carried out in batch mode and stopped when the pH of the milk solution decreased to 4.6. After centrifugation of the milk solution, lyophilization was employed to dry obtained casein while whey was recovered to feed the HPTFF system. Viability results were satisfactory and are compiled in P. Mier et al. 2007b.

In a second step different whey proteins of interest were separated by means of HPTFF technology. Experiments were carried out employing a laboratory-scale plant purchased from Millipore. A commercial BIOMAX membrane and a positively charged prototype membrane of 100kDa from Millipore were tested. Experiments were carried out in batch mode. Several feed pH values were tested in order to establish its influence in the process. As a result, the following set-up (figure 1) is proposed for process integration in order to separate casein and whey proteins from the point of view of green process development.

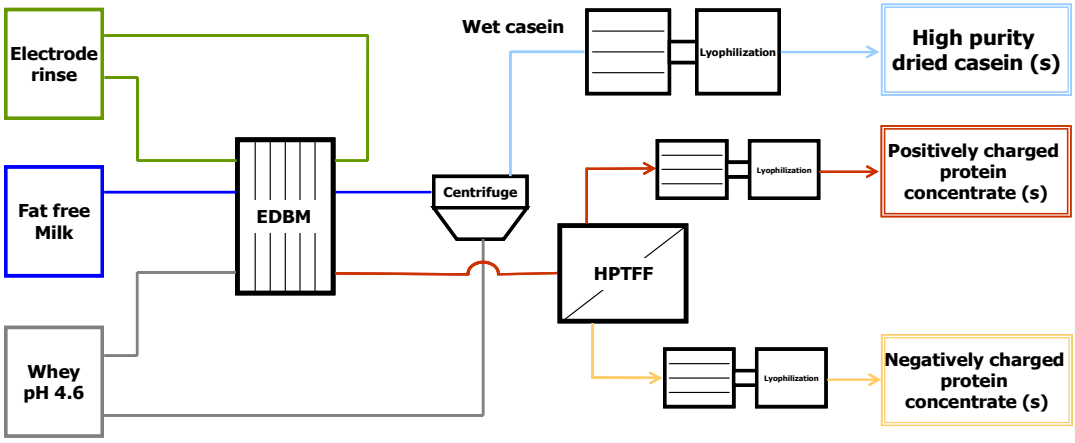


**Figure 1.** Proposed flowsheet for the separation of milk proteins

## References

Gateway to the European Union http://europa.eu.int
Bazinet, L.; Ippersiel, D.; Gendron, C.; Mahdavi, B.; Amiot, J.; Lamarche, F. (2001) Journal of Dairy Research 68, 237-250
Mier, P.; Ibáñez, R.; Ortiz, I. (2007a) Récents Progrès en Génie des Procédés 94
Mier, P.; Ibáñez, R. ; Ortiz, I. (2007b) Sent to Biochemical Engineering Journal

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# Session S-5Hh: Hygiene, Hygienic Design & Unit Operations (Food-5b) – 5 min oral presentation plus poster presentation

| Abstract Number | Paper Title & Authors | Included |
|---|---|---|
| 3095 | Yield Optimization In Carrot Juice Production<br>A Fariña, I Rodrigues, M Henriques, R Saraiva | Yes |
| 3954 | Pulsed light as a novel decontamination technology: factors affecting microbial inactivation<br>A Lasagabaster, I M Marañón | Yes |
| 3964 | Heat treatment improvement of dairy products via new continuous Ohmic heating technology by fluid jet<br>S Ghnimi, N F Malaspina, M Dresch, G Delaplace, J F Maingonnat | Yes |
| 4057 | Energy efficient microwave hot-air drying of porous materials<br>E Holtz, L Ahrné, T H Karlsson, A Rasmuson | Yes |

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# Yield optimization in carrot juice production

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## 1. Summary

The consumption of fruit juices has recently increased due to its incorporation in the consumers diet, partly displacing regular fruit and soda beverages. The vitaminic and antioxidant characteristics of carrot juice led to its introduction in fruit juice formulations. The main problems in carrot juice utilization are the low production yield and its chemical and microbiological stability.
The objective of this work was the optimization of the yield in carrot juice production. The factors tested were the branching time (60, 90, 120, 150 s) and the addiction of enzymes (pectinase, alpha-amilase and celulase) during the grinding process. We also evaluated the influence that the thermal processes had in pH, ºBrix, acidity and colour of the produced juice. Microbiological analyses for the commercial sterility evaluation of the bottled juice were also performed.

Keywords: carrot juice, yield, blanching, enzymatic treatment, sterilization

## 2. Extended Abstract

Carrots possess a significant number of health benefits, such as the prevention of cancer, diabetes and even heart diseases. Juicing adds to these benefits, because it removes the fiber, making the important nutrients and phytochemicals found in carrots more easily absorbed by the body. Carrot juices are a rich source of the antioxidants beta-carotene, alpha-carotene, phytochemicals and glutamine, calcium, potassium and vitamins (Anderson and Clydescale, 1980).
In carrot juice production the blanching has been the operation that affects more significantly the final product quality. The alteration in structure walls during this process leads to carotenoids and vitamins degradation. Borowska et al. (2006) suggests that the optimization in blanching time, size and shape of carrots pieces could contributes to improve final products nutritional value. The use of enzymes is pointed out as increasing the production yield, and decreasing both the energetic consumption and the waste production (Helbig and Laperche, 2001).

*Results*

The carrot *Danucus carota* L. was the raw material used in the juice production, belonging to the family *Apiácia Umbilifera*. The parameters evaluated in juice were

the production yield, the ºBrix, the pH, the acidity, the colour and microbiological stability. The use of enzymes increase the global production yield (Figure 1), particularly pectinase, but the increase in blanching time does not benefit this parameter. The increase in soluble solids content and acidity is more effective also with pectinase, and in this case the chemical stability could be achieved more easily. Enzymes don't influence colour juice. The microbiological stability was achieved with autoclave sterilization, but other techniques could be employed to avoid the thermal degradation in juice quality.

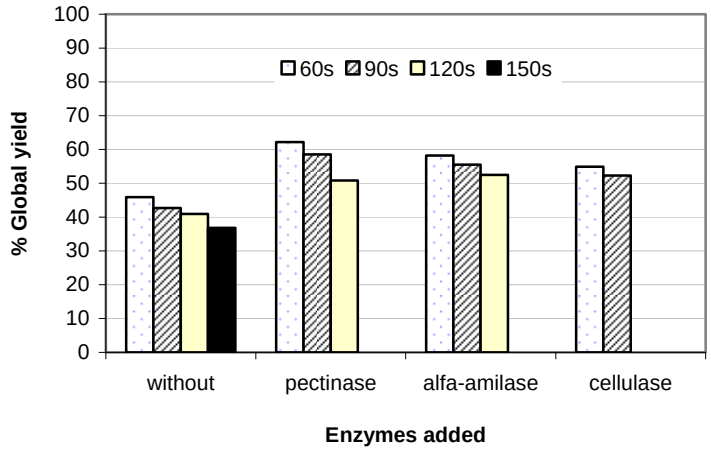


**Figure 1.** Global process yield with the use of different enzymes, for the blanching times of 60s, 90s, 120s and 150s.

## 3. Conclusions

The increase in the blanching time prejudices the yield of the global process, mainly by decreasing the yield in the pressing operation. The use of enzymes favours the production yield. The pectinase was the more efficient because it allows an increase in yield of 20 %. The use of pectinase improves the soluble solids content (12 ºBrix) and the acidity (0,055 % (w/w)). The colour is not affected by the blanching time. Only the use of enzymes leads to a small alteration in this parameter.The comparison between the juice produced and commercial juices shows that the acidity is lower and the pH consequent higher. This could be mainly because commercial juices incorporate products for chemical stabilization, for example lemon juice or citric acid. The microbiological stability was achieved with autoclave sterilization.

### References

Anderson, E. and Clydescale, F. (1980) *Journal of Food Science*, 45,1533-1537.
Borowska, E.J., Zadernowski, R.; Szajdek, A.; Zadernowski (2006) *Fruit Processing*, 1,22-27.
Helbig, J. and Laperche, S., (2001) *Fruit Processing*, 2,324-347.

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Copenhagen, 16-20 September 2007

# Pulsed light as a novel decontamination technology: factors affecting microbial inactivation

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## 1. Summary

Pulsed light technology has been shown to be a quick and effective non-thermal decontamination process which could induce more than 7 Log CFU reduction in *Listeria innocua* viability at relatively low doses (0.7 $J.cm^{-2}$) and short treatment time (325 μs). Antimicrobial efficacy increased with the pulse energy, the number of pulses and the light dose. Although additional studies are needed, pulsed light appears as an economical and promising non-thermal decontamination technology which could be used to improve the microbial safety and quality of fresh or minimally processed food products.

Keywords: Pulsed light, non-thermal process, decontamination, microbial inactivation, minimally processed food.

## 2. Extended Abstract

Consumers have a growing preference for fresh-like, minimally processed and convenience food products, as well as for safe and high quality foodstuffs. The need to assure the microbial safety of food products without compromising their sensory and nutritional quality has led to development of suitable non-thermal preservation technologies. Pulsed light is a novel non-thermal process which consists of high power pulses of a broadband light emission with a considerable amount of light in the short-wave UV spectrum (Dunn *et al.* 1995, Wekhof 2000).

The aim of this work was to study the impact of some pulsed light processing factors on microbial inactivation effectiveness. The most effective conditions to inactivate *L. innocua*, a non-pathogenic surrogate for *L. monocytogenes*, were identified. This strain was inoculated in KPBS and on agar surface to simulate contaminated liquid and solid (surface) foodstuffs.

Results showed that a single light pulse induced more than 7 Log CFU reduction at relatively low doses (0.7 $J.cm^{-2}$) and short treatment time (1 pulse: 325 μs). No

significant rise in temperature was observed for all tested treatments. As shown in figure 1, microbial inactivation increased with the pulse energy (J) and the number of pulses in both liquid and solid systems. Furthermore, the shorter the distance of the sample from the lamp the better the efficacy of the process. *L. innocua* reduction in both liquid and solid samples increased with the light dose received by the sample, i.e. the higher the microbial exposure to the light the better the efficacy of the pulsed light process (figure 2).

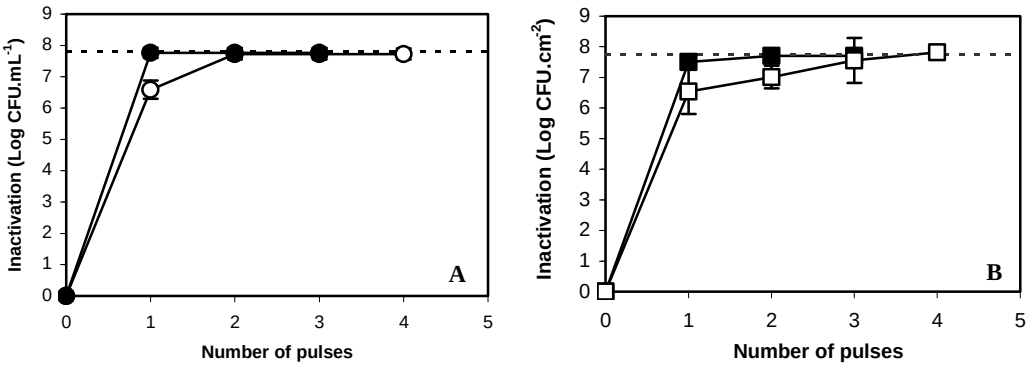


Figure 1: Impact of pulse energy and the number of pulses on *L. innocua* inactivation in (A) liquid and (B) solid samples. ○,□: 300 J; ●,■: 600 J. Dashed line represents the maximum detectable inactivation level.

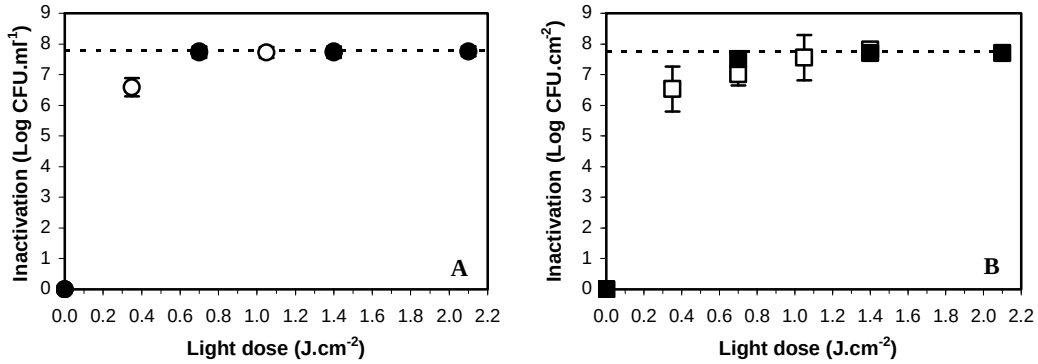


Figure 2: Impact of light dose on *L. innocua* inactivation in liquid (A) and solid (B) samples. ○,□: 0.35 J.cm$^{-2}$ per pulse; ●,■: 0.7 J.cm$^{-2}$ per pulse. Dashed line represents the maximum detectable inactivation level.

Although additional studies are needed to point out the suitability of this process to improve the microbial safety and quality of fresh or minimally processed food products, pulsed light appears as an economical and promising non-thermal decontamination technology for this kind of products.

## References

Dunn, J., Ott, C. and Clark, W., (1995) *Food Technology*, 49, 95-98.

Wekhof, A., (2000) *Journal of Pharmaceutical Science and Technology*, 54, 264-276.

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# Heat treatment improvement of dairy products via new continuous Ohmic heating technology by fluid jet

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## 1. Summary

Ohmic heating is well known to minimize the fouling phenomenon compared to indirect heat transfer technologies (plate heat exchangers, tube heat exchangers). Although the technique appears both simple and advantageous, several difficulties are encountered in its application. Our developed design is based on the elimination of the product–wall interface. It consists in applying an alternative electrical current directly in the falling jet between two stainless steel electrodes. The aim of this experimental study was to evaluate thermal performances of this new technology using a whey protein solution designed to mimic dairy products behaviours. A thermal sensor, based on the hot wire technique, was used in order to monitor fouling/cleaning phenomena in the Ohmic heater. The experimental investigation highlights the potential application of this innovated technology in the heat treatment of dairy products.

Keywords: Ohmic heating, dairy fluid, fouling, fluid jet, hot wire method

## 2. Extended Abstract

Figure 1 presents the schematic diagram of the designed Ohmic heating cell. The flow domain consists of a cylindrical glass tube connected to the electrodes; which are tightly held in position using rubber rings and four iron bars with nuts and bolts. The inlet, a round jet of small cross-section through which liquid leaves from the nozzle tip, is connected to the phase. The outlet, a conical receptacle through which the liquid is taken out, is connected to the mass. Electrolysis is prevented by the use of high frequency alternating voltage. It had a frequency up to 30 kHz with switching voltage up to 3800 V. The model fluid used in the fouling experiments was an aqueous solution of β-lactoglobulin (1% *w/w*; Armor protein, France) and chloride sodium (0.5% *w/w*) in water. The scanning electron microscopy observation (Figure 3) reveals that the addition of 0.5% NaCl to the protein solution doesn't change the microstructure of the deposit (a spongious and soft texture). The flowrate was fixed at 200l/h and the inlet and outlet temperatures were fixed respectively at 60°C and 90°C.

Taking account the totally different way of heating in this geometry, deposit thickness were determined directly at the outlet of Ohmic cell using a thermal sensor based on the hot wire technique (Fillaudeau et *al*, 2004) as shown in Figure 3. By weighting the Ohmic cell before and after fouling runs (Figure 2), the deposit thickness inside the electrode was indirectly detected. We demonstrate that whey protein fouling doesn't impact the thermal and electrical performances of the Ohmic cell. In fact the essential of the heating occurs in the fluid jet (free surface). In the heating zone, the detected deposit was negligible. This technology can be a means of energy savings compared to traditional processes and offers potential applications in dairy industry.

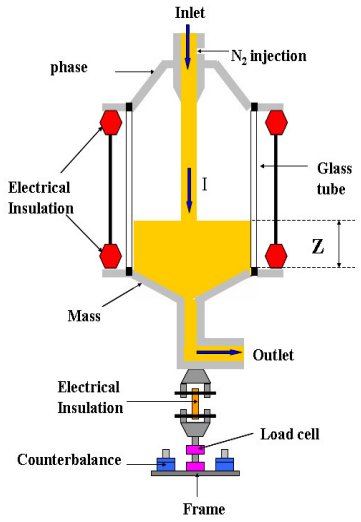


**Figure 1:** Schematic diagram of the Ohmic heating cell by fluid jet.

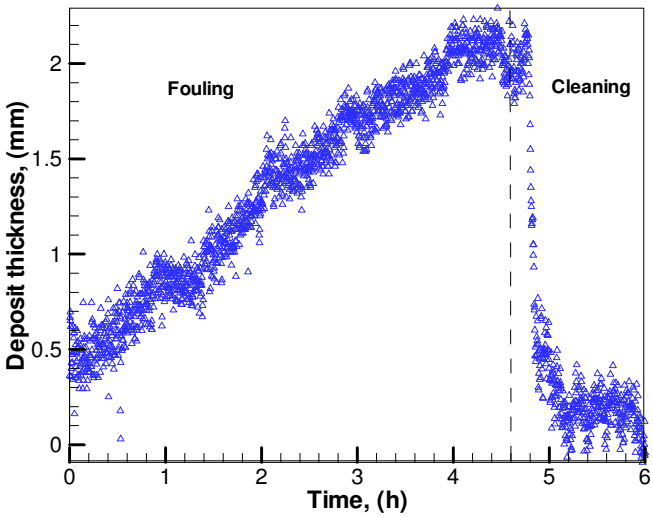


**Figure 2:** On-line fouling/cleaning monitoring by hot wire method at the outlet of the Ohmic heater.

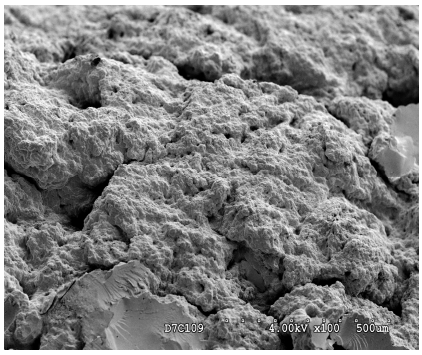


**Figure 3:** SEM observations of deposit generated by Ohmic heating of protein solutions.

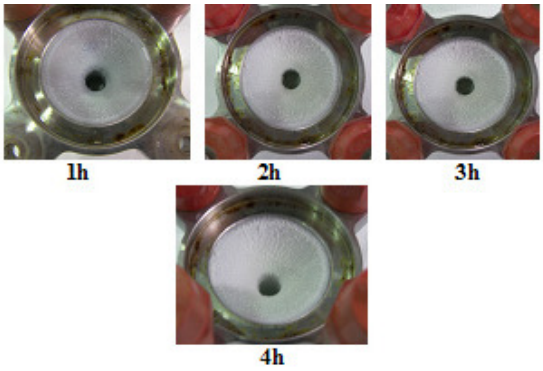


**Figure 4:** Photographs of the electrode (mass) after 1-, 2-, 3- and 4-h fouling experiments.

## References

Fillaudeau, L., Cardenas, R., Korolzuck, J., Lejaye, J., Cozic, F. and Debreyne, P., (2004) *International conference engineering and food, ICEF-9*

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# Energy efficient microwave-convective drying of porous materials

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## 1. Summary

Combined microwave convective drying has the potential of yielding dried foodstuffs of better quality and with shorter processing time than convective drying alone. As a result, questions related to processing and production feasibility of the combined process arise. One such question is whether the combined process is energy efficient. In the work presented here microwave convective drying has been studied in a small scale equipment specially designed for the study of drying kinetics and energy efficiency. The effect of processing parameters, such as microwave power, air velocity, air temperature and air humidity on energy efficiency has been studied.

Keywords: energy efficiency, combined process, drying kinetics, product temperature

## 2. Extended Abstract

New drying processes, including combined methods, have been developed to increase dehydration rates and simultaneously improve the quality of dehydrated products. Microwave assisted air-drying is a promising technique for plant foods, due to the shorter drying time and better quality of the resulting products. These advantages are mostly due to the fast, instantaneous, volumetric heating caused by microwave energy, while air flow can contribute to a more homogeneous heating and effective removal of evaporated moisture. In order to develop an energy efficient process, air and microwave energy needs to be balanced taking into account the dominating heat and mass transfer mechanisms, which change with the decrease in water content during drying.

In order to clarify the heat and mass transfer mechanisms involved in microwave convective drying, the effect of process parameters on drying kinetics is studied in a small-scale drying equipment, specially developed, for combined microwave-convective drying. A porous material (aerated concrete) was chosen as a model product to avoid deformation and chemical changes of the sample during drying.

The equipment was designed to create a well defined drying situation, in order to simplify the description of the heat and mass transfer mechanisms. The equipment consists of a magnetron with power supply and a waveguide. Air can flow across the waveguide through slots in the waveguide walls. During drying a rectangular shaped sample is placed inside the waveguide. The microwave power can be maintained at low constant power, without pulsing. Microwave power, air velocity, air temperature and air humidity can be varied and are recorded during the course of drying. The sample is weighed at intervals to calculate moisture content, and sample temperature is monitored by infrared pictures of samples that have been split in two, or by using optic temperature fibres.

The model material, aerated concrete, is characterized by measuring dielectric data as a function of moisture content and by sorption isotherms.

The process parameters studied are microwave power, air temperature, air velocity and air humidity. These parameters significantly affect the energy efficiency of the combined drying which is evaluated as the total specific energy consumption for drying and includes microwave energy as well as energy consumed to produce hot air for convection. Microwave power is the parameter with the greatest influence on drying time and consequently on energy efficiency, as short drying time reduces energy consumption. However, for heat sensitive products such as foods the microwave power output has to be chosen to avoid detrimental quality changes due to high temperatures. Product quality is, therefore, a limiting factor for short drying time.

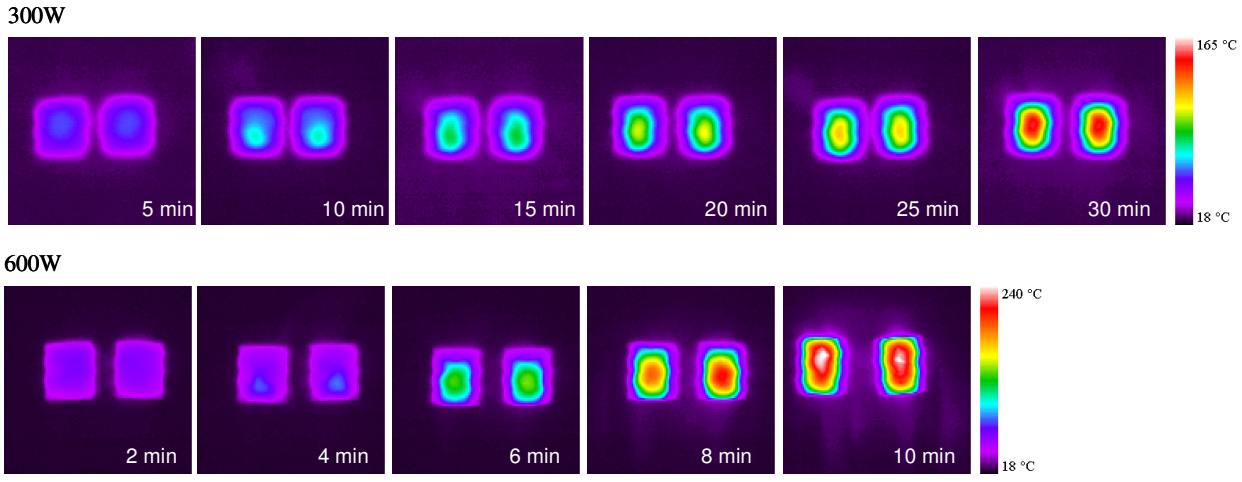


**Figure 1:** Infrared representation of product temperature during microwave convective drying at 300 and 600 W respectively. The sample has been split in two and the cut surfaces are displayed.

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# Session S-5I: Modern Analysis: Chemical & Multivariate Analysis (Food-6a)

| Abstract Number | Paper Title & Authors | Included |
|---|---|---|
| 3025 | Determination of the Fat Content of Beef using Microwave Methods<br>S K Ng, P Ainsworth, A Plunkett, A Gibson, G Parkinson, A Haigh, G Jacobs | Yes |
| 3867 | Rapid determination of alginate monomer composition using spectroscopy and chemometrics<br>T, Salomonsen, H M Jensen, F H Larsen, S B Engelsen | Yes |
| 3909 | Tools for easing understanding of complex data<br>A J Lawaetz | No |
| 4002 | In-line ultrasound Doppler based rheometry for processing applications<br>J Wiklund | No |
| 4035 | Quantification of active lactic acid bacteria by flow cytometry<br>J Worm, R Stavnsbjerg, R M Schoth, K F Hansen | Yes |
| 4041 | Rapid Pattern Recognition, An Alternative Food Safety Solutions Or A Scientific Artefact?<br>M Bassompierre | Yes |
| 4056 | Quantifying Biological Variation<br>S G Erbou, M V Christensen, R Larsen, E V Olsen, B K Ersbøll | Yes |
| 4062 | Gourmet potatoes: How to evaluate this?<br>A K Thybo, S Kreutzmann, M Bassompierre | Yes |

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# Determination of the fat content of beef using microwave methods

S. K. Ng,[a] P. Ainsworth,[a] A. Plunkett,[a] A. Gibson,[b] G. Parkinson,[b] A. Haigh,[b] G. Jacobs[b]

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## 1. Summary

Experiments on beef samples with varying fat content have been undertaken to establish the feasibility of using microwave methods to determine the fat content of beef. Two methods are used to determine the relative complex permittivity. The first method utilized a waveguide cell which incorporates specially designed Perspex windows and the second method employs a 1.6 mm microwave coaxial probe. Lean meat and beef dripping were used as the basic constituents to construct the measurement samples. Six samples were produced in 10% steps up to 50% of added fat. Both ingredients with specific ratios were well mixed in a food processor to ensure sample homogeneity. The results obtained show a strong, almost linear relationship between both the real and imaginary parts of permittivity and the fat content of the meat.

Keywords: Coaxial probe, fat content, meat, permittivity, transmission line.

## 2. Extended Abstract

### Introduction

Meat industry faces a persistent need for new methods of meat quality evaluation. The expectation of consumers for improved meat quality continually rises, which induces the necessity of more stringent quality control at every level of meat processing. Although accurate chemical analysis is available, the development of a continuous real-time sensor that can discriminate the fat content of meat will be of great interest. In this research, experiments on beef with varying fat content have been undertaken to establish the feasibility of using microwave methods to determine the fat content of beef rapidly.

### Microwave measurement

The first measurement technique has been implemented in waveguide 10 cell (WG10) operating at 2.5-3.5 GHz in the dominant mode ($TE_{10}$). The relative complex permittivity is derived from the transmission coefficient using the Musil-Zacek equations [1]. The second method utilized a microwave coaxial probe (Agilent 85070E, 'Performance' probe). The relative complex permittivity is determined by the refection coefficient at the probe-sample interface which is measured as a function of frequency using a Vector Network Analyser [2]. During measurements, care is taken to eliminate air bubbles from the probe and Perspex windows surfaces in both methods.

## Results

The results obtained from the microwave waveguide cell and microwave coaxial probe measurement are presented in Figs. 1 and 2. Fig 1 illustrates the real and imaginary part of the permittivity at 3 GHz against % of fat measured using the waveguide cell. This plot clearly shows the linear relationship between permittivity and fat content. The real and imaginary part of the permittivity decreases as the % of fat increases. Fig 2 shows the results obtained using the microwave coaxial probe at 10 GHz. The real and imaginary part of the permittivity decreases as the % of fat added increases. The imaginary part gives better measurement resolution compared to the real part in discriminating the same range of meat to fat ratios in both methods. Comparatively, the waveguide cell methods yield a more linear result.

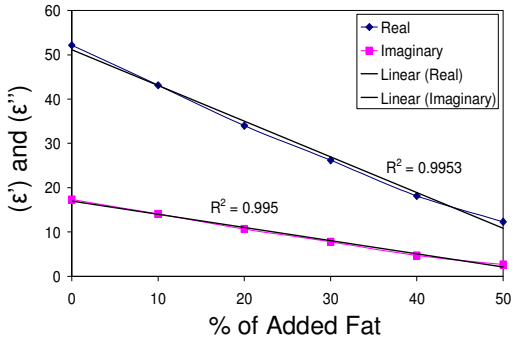


**Fig.1:** Complex permittivity of beef samples as a function of fat content at 3 GHz using waveguide cell method

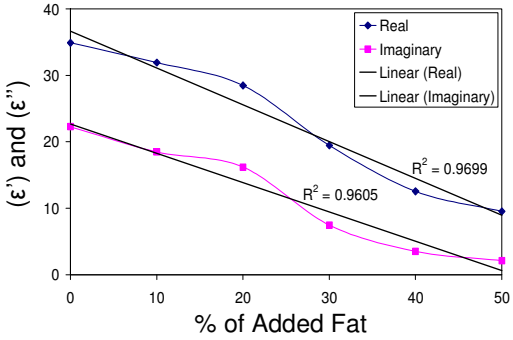


**Fig.2:** Complex permittivity of beef samples as a function of fat content at 10 GHz using coaxial probe method

## Conclusion

The complex permittivity of beef samples with varying fat content over a fixed frequency range was evaluated using a microwave waveguide cell and dielectric probe technique. Plots of permittivity (real & imaginary) against fat content show good potential for the microwave methods to be accurately calibrated as a fast and reliable measurement technique for determining the fat content of beef products.

## References

[1] A. D. Haigh, F. Thompson, A. A. P. Gibson, G. M. Campbell, and C. Fang, "Complex permittivity of liquid and granular material using waveguide cells," *Subsurface Sensing Technologies and Applications*, vol. 2(4), pp. 425-434, 2001.

[2] D. V. Blackham and R. D. Pollard, "An improved technique for permittivity measurements using a coaxial probe," *IEEE Transactions on Instrumentation and Measurement*, vol. 46, pp. 1093, 1997.

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# Rapid determination of alginate monomer composition using spectroscopy and chemometrics

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## 1. Summary

The potential of using near infrared (NIR), infrared (IR), Raman and $^{13}C$ CP MAS NMR spectroscopy for rapid and non-destructive analysis of alginates has been investigated.

Keywords: alginate, spectroscopy, chemometrics

## 2. Extended Abstract

Alginates are extracted from brown seaweed (*Phaeophyceae*) and used as thickeners, stabilisers and gelling ingredients in the food and pharmaceutical industry. Chemically, they are a family of binary copolymers of (1-4) linked β-D-mannuronic acid (M) and its C-5 epimer α-L-guluronic acid (G). Their functionality in different applications is closely related to the M/G ratio and the distribution of M and G along the chain, of which especially the former can easily be determined by solution-state $^{1}H$ nuclear magnetic resonance (NMR) (Grasdalen *et al.*, 1979 & 1983). However, at the concentrations required for NMR analysis, alginate solutions are too viscous to give well-resolved spectra. To reduce the viscosity, it is necessary to partially degrade the alginate chain by a mild acid hydrolysis prior to NMR analysis. This hydrolysis is relatively time-consuming and labour intensive and may possibly alter the information about the M and G distribution of the original alginate polysaccharide. Thus, it will be preferable to develop an alternative rapid method for characterization of the alginate structure.

In this work, Fourier transformed infrared (FT-IR) spectroscopy, near infrared (NIR) spectroscopy and Raman spectroscopy have been tested as alternative rapid methods for prediction of the M/G ratio measuring directly on the alginate powders. Using the multivariate regression method partial-least-squares (PLS) regression, the M/G ratio of alginate can be predicted from the FT-IR spectra with a prediction error comparable to the error from the NMR reference method. Due to the diamond ATR (Attenuated Total Reflectance) sample preparation of the FT-IR method, which can measure the alginate powder directly and non-invasively, the measurement time was reduced considerably. The prediction error is comparable to the error from the NMR

reference method. Figure 1 shows the measured versus predicted plot of the calibration set (79 samples) and the test set (20 samples). It can be concluded that the model is very robust since the M/G ratio of the test set samples, which have not been used in the model development, is predicted with the same accuracy as the calibration samples (Salomonsen *et al.,* 2007).

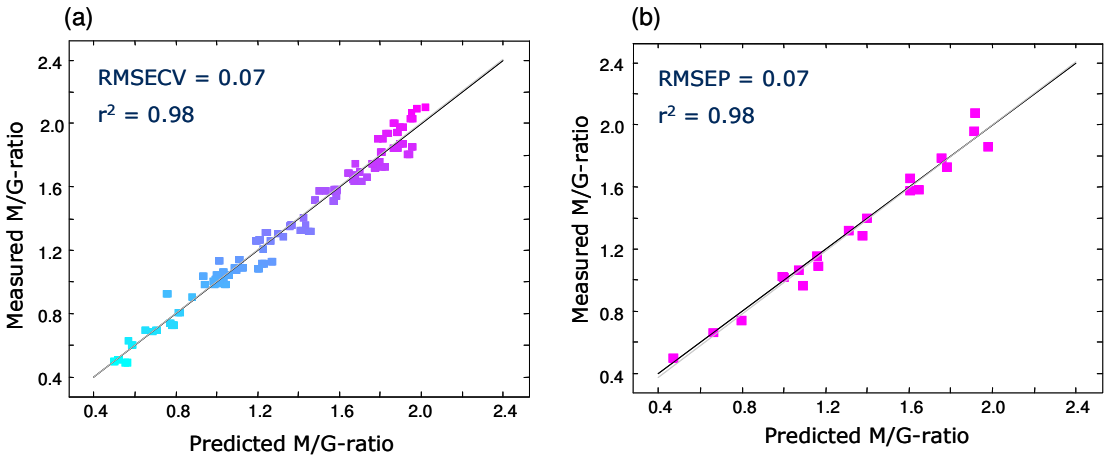


**Figure 1**. Measured versus predicted plot of the (a) calibration samples and (b) test samples using two PLS components. RMSECV: root mean square error of cross validation. RMSEP: root mean square error of prediction.

Another issue of concern was the inherent destructive nature of the hydrolysis procedure, which was necessary to obtain well resolved NMR spectra introducing a source of error in the form of degraded, terminal carbohydrates during the hydrolysis. For this reason, the potential of solid state $^{13}C$ cross-polarisation magic angle spinning (CP MAS) NMR to determine the composition of the intact alginate was investigated. In addition, these results may also allow for determination of the distribution of the M and G units along the chain and thereby obtain information not provided by IR, NIR and Raman spectroscopy.

## References

Grasdalen, H., Larsen, B. and Smidsrød, O. (1979). NMR study of the composition and sequence of uronate residues in alginates. *Carbohydrate Research,* 68, 23-31.

Grasdalen, H. (1983). High-field $^{1}H$ spectroscopy of alginate: sequential structure and linkage conformations. *Carbohydrate Research,* 118, 255-260.

Salomonsen, T., Jensen, H.M. and Engelsen, S.B. (2007). Rapid determination of alginate monomer composition using spectroscopy and chemometrics (in preparation).

Quantification of active lactic acid bacteria by flow cytometry
Proceedings of European Congress of Chemical Engineering (ECCE-6)
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# Quantification of active lactic acid bacteria by flow cytometry

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## Abstract

Chr. Hansen produces lactic acid bacteria (LAB) for the food industry world wide. The bacteria are provided as frozen or freeze-dried biomass and when applied their performance i usually dependent on their active metabolism, eg. in the dairy industry where LAB metabolise lactose of milk into lactic acid and other by-products. Therefore, a major focus in process development and quality control is to provide a biomass of required size and quality. The discrimination between size and quality reflect the simple relationship that the cells not only need to be present. They also have to perform correctly by the customer and continue to do so according to the specified limits.

During the last couple of years, single-cell analysis of bacteria by flow cytometry becomes more and more a standard microbiological tool. At Chr. Hansen, flow cytometry is used in research and development to characterize and enumerate bacteria in different applications. Especially for enumeration of bacteria, a very precise and reproducible assay has been setup. The system is almost completely automated by use of robotic sample preparation and autosampling, and even with capacity for high-through-put performance.

This presentation address the analytical potential and limitation for the quantification of LAB by flow cytometry especially relative to the traditional cultivation technique. Special focus is directed to the combined application of robotics and flow cytometry providing more, better and faster results than possible with related techniques.

Keywords: lactic acid bacteria, analysis, flow cytometry

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# Rapid pattern recognition, an alternative food safety solution or a scientific artefact?

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*Summary*

The general cause of high levels of persistent lipophylic organic pollutants (PLOP's), such as dioxin, in human tissue is primarily caused by bioaccumulation through the food chain in the lipid phase. Food, especially fish products, is the main cause of human exposure. Accordingly, reliable, time- and cost-effective PLOPs screening methods are required in foods and feeds for effective reduction of this intake through the nutritional pathways.
We have investigated methods for dioxin screening, employing chemometrics and using fatty acid pattern recognition to a large number of samples: above 400 fishmeal and fish samples.
Our work illustrated strong covariances between fatty acid profile and PLOPs probably due to eating habits, geographics and the related local pollutants. These covariances, when employed for developing dioxin and PCB screening methods based on Partial Least Square regressions yielded Root Means Square Error of Prediction between 5 and 10% of the considered range and of approximately twice the reference methods repeatability. These results should be considered in contrast to accepted screening methods such as Ah receptor based methods that are time consuming and very costly.

Keywords: fish, dioxin, pollutant, fatty acids, screening

## 1. Extended Abstract

Very promising relationship was found within fatty acid profiles and dioxin teq (Bassompierre et al., 2004, 2007) in a reduced set of 75 fish meal samples. Therefore, our focus was orientated towards this relationship for wild and farmed fish, and fish meal. 500 samples were analysed for dioxins and fatty acids. The investigated relationship between fatty acid patterns and dioxin /PCB teq resulted in valid chemometrics model for screening of dioxin in fish meal and of dioxin and PCB in fish. The developed predictive Partial Least Square regression models, relying on fatty acid analyses by GC FID after direct sample methylation and automatic peak integration returned an uncertainty of approximately twice the repeatability of the reference method and below 10% of the investigated range.
However, the tremendous requirement of dioxin analyses has resulted in a positive trend: accredited commercial laboratory have reduced both price and time delay (Figure 1). Whereas, the advantages of screening methods based upon Ah receptor might be reduced to the important sample throughput capacity they offer, dioxin screening by fatty acid pattern recognition remains as one of the few potential for fish based companies desiring to enhance their internal control: the in house cost and time being related to fatty acid analyses, i.e., ~5 € and an hour pr sample.

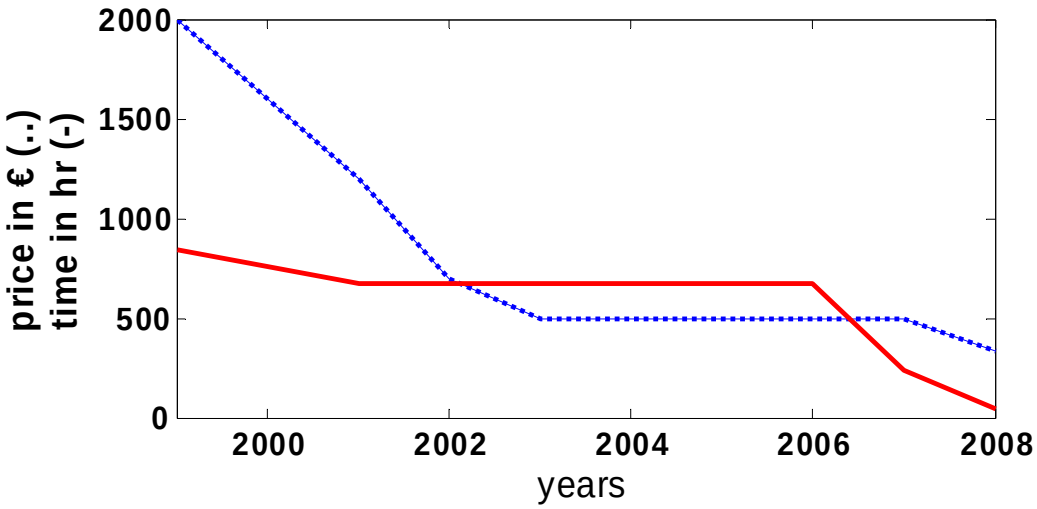


Figure 1. Price and delay time trends
for dioxin/PCB reference analyses

For a fish meal manufacturer, the commercial and contractual budget will remain related to the reference analyses, however dioxin screening by fatty acid pattern recognition should enable internal control and thereby facilitate stock and cleansing management.

**References**

Bassompierre, M., Munck, L. Bro, R. and Engelsen, S.B., (2004) *the analyst*, 129, 553-558.
Bassompierre, M., Tomasi, G., Munck, L. Bro, R. and Engelsen, S.B., (2007) *Chemosphere*, 67, S28–S35.

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# Quantifying Biological Variation

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## 1. Summary

A method for obtaining a compact 3D statistical shape model of porcine bone structures from 2D CT scans is presented. The model encapsulates the biological variation of the bone structures and its parameters and can be used for interpretation and prediction purposes.

Keywords: Image analysis, computed tomography, 3D statistical shape model, biological variation

## 2. Extended Abstract

Statistical shape models (Cootes et al., 1995) are often used to encapsulate complex biological variation in medical applications. They can be used for obtaining a better understanding of a shape, as prior knowledge in automated segmentation schemes or as discriminating features in classification tasks. In order to do statistics on shapes corresponding points are required, which are not feasible to acquire manually in 3D.

In this work dense 3D surfaces of bones are estimated from anisotropic voxels using Radial Basis Functions (Turk et al., 1999). The data consists of 2D CT scans of 40 porcine carcasses separated along the medial plane. Each scan has a slice thickness of 10mm with a spacing of 10mm between each scan. Voxel dimensions are [x, y, z] = [0.88, 0.88, 10]mm. The full length of the carcasses is scanned resulting in approximately 130 scans per carcass, but only 30 scans per carcass are used in this application, covering the parts around the region of the pelvic bone. Using an extended iterative closest point algorithm (Besl et al., 1992) a rigid registration is obtained, c.f. figure 1, and a compact statistical shape model is built. Figure 2 shows the mean shape. The model quantifies the biological variation of the bone structures and by applying varimax rotation (Kaiser, 1958) the modes are localized and their interpretation is simpler.
Stepwise regression is used for detecting significant model parameters and a leave-one-out cross validation scheme is applied for measuring how well the model generalizes when used for predicting the weight of the bones and the meat in the hind part of the carcass. The true weights are obtained by manual dissection. It is

emphasized that the bone in the model is only part of the complete bone structure in the hind part of the carcass. 7 significant parameters of the model can predict the weight of the bones with a standard deviation of the errors relative to the true observations of 4.6%. When predicting the weight of the meat the standard deviation of the relative errors is 5.4%. Some of the uncertainty is related to the manual dissection, but how much is difficult to determine.

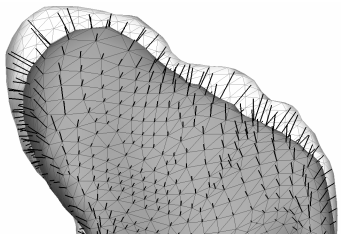

**Figure 1 -** Registration of end parts of two bones

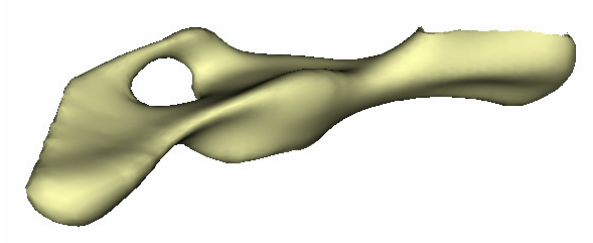

**Figure 2 -** Mean shape of part of the hip bone.

## References

Besl, P. J., Mckay, N. D., (1992) A method for registration of 3-D shapes, *IEEE Transactions on Pattern Analysis and Machine Intelligence*, 14(2), 239-256.

Cootes, T. F., Taylor, C. J., Cooper, D. H., Graham, J., (1995) Active shape models - their training and application, *Computer Vision and Image Understanding*, 61(1), 38-59.

Kaiser, H. F., (1958) The varimax criterion for analytic rotation in factor analysis. *Psychometrika*, 23, 187-200.

Turk, G., O'Brien, J. F., (1999) Shape transformation using variational implicit functions. *Computer Graphics*, 33, 335-342.

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# Gourmet potatoes: How to evaluate this?

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## 1. Summary

Eleven potato varieties were screened for applicability for 4 common applications: Cooked, baked, oven slices and mashed potatoes. These data were related to actual tailor-made sensory quality profiles and chemical composition which gained an insight in the chemical and sensory attributes being of relevance for describing applicability of potatoes. This information is meant to be available for the consumers in a future quality labeling. A Videometer was used to screen the raw potatoes and these data were also correlated to some of the quality characteristics. The combination of a quality description and a rapid quality sorting equipment can form the basis for a quality labeling system in the future.

Keywords: potatoes, sensory quality, gastronomy, dry matter, prediction

## 2. Extended Abstract

Like many other food products potatoes follow the trends in the food market now-a-days by evolving from a commodity product to a gourmet side dish – a new luxury product. A substantial knowledge on potato raw material quality in relation to sensory quality and applicability is needed.
Potatoes have a high gastronomic diversity as they can be used in a variety of different applications as cooked, baked or mashed potatoes etc. Here a large variation in texture plays an important role. Marketing of potatoes due to these applications is not well established in DK as it is in many other countries.
The aim of this presentation is to establish the link between gastronomic diversity assessment and quality assessment by sensory analysis and chemical characterisation. In the future this knowledge will be significant for quality labelling of potatoes.
Eleven potato varieties were processed into 4 potato products to study the applicability (suitability) and evaluated by three cooks for applicability on a five point hedonic scale. All products were subsequently profiled by a trained sensory panel for 4-5 sensory attributes by a tailor-made sensory profile for each product. The eleven potato varieties were screened by a Videometer and then characterised by chemical and physical analysis such as dry matter content, starch quality and texture.

A large diversification of the applicability was found between varieties (Fig. 1). Varieties found to be optimal for water cooking were very little suited for mashed potatoes. Potatoes applicable for oven dishes were classified into a third group.

*Which sensory attributes determines the optimal application?*
The correlation between the sensory attributes and optimal application was very different for the various products. E.g. for oven products brown appearance was highly correlated to optimal applicability, for cooked potatoes a high potato flavour was an important sensory attribute and for mashed potatoes low graininess and high fluffiness and high potato flavour were correlated to applicability.

*Can chemical composition predict the optimal application?*
The dry matter content is known to be highly correlated to texture attributes (Thybo 1998), which this study also documented. Moreover the size of raw starch grains and other structural components were able to give more specific information about the sensory quality and optimal applicability.

*Can sensory quality and applicability of potatoes be predicted by Videometry?*
The surface of raw potatoes with peel was measured by a Videometer in order to study the possible predictions of sensory quality and applicability. Very high predictions were obtained for many of the chemical components (dry matter content r=0.99, amylose r=0.96, starch grain size r=0.98) and sensory quality parameters (oven mealiness r=0.89, graininess in mashed potatoes r=0.99). The results give some preliminary indications about the perspectives in using Videometry as an on-line sorting system for sorting and labelling potatoes with different qualities.

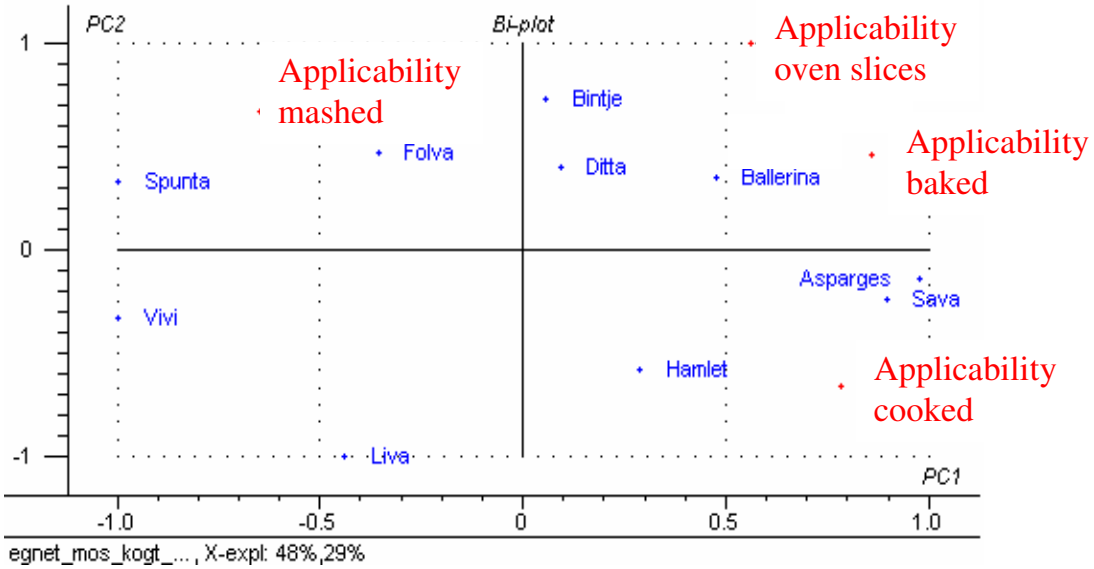


**Fig. 1.** A Principal Component Analysis (PCA) plot of the applicability scores for 11 potato varieties

## References

Thybo, A.K. 1999. *Sensory and instrumental characterisation of cooked potato texture*. Ph.D thesis, The Royal Veterinary and Agricultural University, Copenhagen

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# Session S-5Ii: Modern Analysis: Chemical & Multivariate Analysis (Food-6b) – 5 min oral presentation plus poster presentation

| Abstract Number | Paper Title & Authors | Included |
|---|---|---|
| 1415 | Application Of Correlation Between Biot And Dincer Numbers For Determining Moisture Transfer During The Air Drying Of Coroba Slices<br>O Corzo, N Bracho, A Pereira, A Vásquez | Yes |
| 2467 | Use of Artificial Neural Network Models for Prediction of Drying rates of Agricultural Products<br>K Movagharnejad, M Nikzad | Yes |
| 4047 | Spectroscopic and sensory characterization of low- and nonfat cream cheeses – relationships and method differences<br>C M Andersen | No |
| 4059 | Accelerated shelf-life evaluation of ready-to-eat foods<br>M Nuin | No |
| 4100 | Evaluation of glass transition of frozen foods. A benchmark of selected methods using conventional DSC and modulated DSC. Application to selected food samples<br>A L Bail | No |

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# Application of correlation between Biot and Dincer numbers for determining moisture transfer during the air drying of coroba slices

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## 1. Summary

Application of the correlation between Biot (Bi) and Dincet (Di) numbers for determining the mass transfer parameters during the air drying of coroba slices was investigated. The coroba slices were put in drying tray and dried in cabinet air dryer operated at air velocity of 0.82, 1.00 or 1.18 m/s and temperature 71, 82 or 93 ºC with drying from the top surface of the drying slices. Changes in weight of slices were monitored at 3 min intervals for drying time to equilibrium. Linear regression was used to fit experimental dimensionless moisture content data to Dincer and Dost model. The high coefficients of determination ($R^2 > 0.97$) indicated the goodness of fit of experimental data to Dincer and Dost model.

Keywords: Bi-Di correlation, moisture transfer, air drying, coroba slices

## 2. Introduction

Fick's law of diffusion, has been used to describe the moisture diffusion process for food products by many researchers. However, simplified models still remains popular in obtaining values for diffusion coefficient. Simple diffusive models are often accurate enough for engineering purposes as it was confirmed by new correlations for solids drying. The aim of this work was to test the simple models based on unique correlation between Biot and Dincer numbers in order to describe mango drying kinetics at different maturity stage, drying temperature and air velocity.

## 3. Materials and methods

The fruit of coroba palm was peeled and the pulp cut into slices ($1.86 \times 10^{-3}$ m). Moisture content was determined. The coroba slices were put in drying tray and dried in cabinet air dryer operated at air velocity of 0.82, 1.00 or 1.18 m/s and temperature 71, 82 or 93 ºC. Changes in weight of slices were monitored at 3 min intervals for drying time to equilibrium. The moisture content data at the different drying conditions were converted to the dimensionless moisture content. Linear regression was used to fit experimental dimensionless moisture content data to Dincer and Dost model:

$$MR = \frac{X_t - X_e}{X_0 - X_e} = G \exp(S\,t) \quad (1)$$

where $G$ is the lag factor, $S$ is the drying coefficient and $t$ is the drying time.
The moisture diffusion coefficient relation ($D$) developed by Dincer and Dost for slab samples is given as:

$$D = \frac{S\,L^2}{\mu^2} \quad (2)$$

where $L$ is the half thickness of the slice and $\mu$ is a root of solution to the moisture diffusion coefficient given in a simplified form for slab geometry:

$$\mu = -419.24\,G^4 + 2013.8\,G^3 - 3615.8\,G^2 + 2880.3\,G - 858.94 \quad (3)$$

Moisture transfer coefficient ($k$) of coroba slices can be evaluated using the Bi-Di correlation proposed by Dincer and Hussain (2002):

$$Bi = \frac{k\,L}{D} = 24.848\,D_i^{-\frac{3}{8}} = 24.848\left(\frac{v}{S\,L}\right) \quad (4)$$

where *Bi* is the Biot number for moisture transfer, *Di* is the Dincer number which is a dimensionless number and *v is* the velocity of the drying air.

## 4. Results and discussion

The high coefficients of determination ($R^2 > 0.97$), and no pattern evident with the residuals across the range of coefficients indicated the goodness of fit of experimental data to Dincer and Dot model. Values of G and S ranged from 1.022 to 1.294 1.249 and from 2.892 x 10-4 to 4.189 x 10-4 s-1, respectively. The Bi numbers were in the range 0.100-0.114, indicating that during coroba slices drying an internal and external resistance to the mass transfer existed. Using values of S and Bi, the moisture diffusion (D) and moisture transfer (k) coefficients were calculated. The values of D and k ranged from 2.68 x 10-12 to 4.53 x 10-12 m2/s and from 0.903 x10-4 to 1.570 x10-4 m/s, respectively.
The predicted dimensionless moisture content profiles were calculated as function of Biot and Fourier numbers ($F_o$):

$$MR = \exp\left(\frac{0.2533\,B_i}{1.3 + B_i}\right)\exp(-\mu\,F_o) \quad (5)$$

The mean relative error between experimental and predicted profiles ranging from 8.72 to15.02% showed that the Bi-Di correlation could be applied for determining the mass transfer parameters during air drying of coroba slices at studied conditions.

## References

Dincer, I. (1996). *International Journal of Energy Research*, 20(5), 419–422.

Dincer, I. and Dost, S. (1995). *Drying Technology*, 13(1&2), 425–435.

Dincer, I. and Hussain, M. M. (2004). *International Journal of Heat and Mass Transfer*, 47, 635–658.

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# Use of artificial neural networks models for prediction of drying rates of agricultural products

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## 1. Summary

Empirical mathematical correlations are usually used to describe the drying behaviour of natural materials. These empirical equations are not able to describe a range of drying conditions and their use is limited to single specific experimental conditions. In this study we are going to show that this problem may be avoided by using artificial neural network (ANN) models. These models are able to describe the drying behavior of different natural materials (tomato, potato, tangerine, garlic , pomegranate and orange). The experiments show that in each case ANN models predict the drying behavior of agricultural products in a range of experimental conditions with great accuracy.

Keywords: artificial neural network, drying rate, drying, empirical correlation

## 2. Extended Abstract

Drying is defined as a process of moisture removal due to simultaneous heat and mass transfer (Gogus, 1994). This complicated process depends on different factors such as air temperature and velocity, relative humidity of air, air flow rate, physical nature and initial moisture content of the drying material, exposed area and pressure (Akpinar et al., 2003). Knowledge of drying behavior is important in the design, simulation and optimization of drying process (Senadeera et al., 2003).In order to show the ability of ANN models for prediction of the drying behavior of natural products, standard drying tests were conducted for six different agricultural products.

**2.1 Materials and methods:** Drying was performed in a pilot plant tray dryer (UOP 8 Tray Dryer, Armfield, UK). The unit consists of a floor standing tunnel, in one end of which is mounted an axial flow fan. Downstream of the fan a bank of electrically heated elements heats the air flowing to the drying chamber. Sample weight was continuously determined and displayed by a balance. The air flow velocity was measured at the end of the tunnel by a digital anemometer. Agricultural products were obtained from local market in suitable seasons. Standard drying experiments were conducted for each agricultural product in four different experimental conditions. The drying experiments were continued for near 12 hours and the absolute moisture content of the samples was calculated at regular times.

**2.2 Mathematical Modelling:** Four different empirical correlations were used in this study for fitting of experimental data. The regression analysis was performed using

the Table Curve 2D, Systat software. Mean square error ( $MSE$ ) and Mean relative percent error ( $P$ ) were the statistical criterions for selection of best empirical correlations. The calculations showed that different correlations would be the best model in different cases and the fitted constants vary considerably from an agricultural product to another and from one experimental condition to the next.

**2.3 Neural Network Architecture and results:** The type of network used in this work is the multi-layer perceptron network. Multi-layer perceptron networks are one of the most popular and successful neural network architectures, which are suited to a wide range of applications such as prediction and process modelling. Experimental data from this study were used to train and test ANN models for prediction of moisture ratios during the drying process. Using MATLAB Neural Network Toolbox, a feed-forward ANN model was designed using back-propagation training algorithm. The number of neurons in input and output layers depends on independent and dependent variables, respectively. The activation function was logarithmic sigmoid. Plotting the model output against the desired response and also evaluating Mean relative percent error ( $P$ ) assesses the performance of the network. Values of $MSE$ and $P$ of ANN models have been listed in table 1. It is clear from the table 1 that the ANN model is able to predict the drying behaviour of various materials in different conditions.

## 2.4 Conclusions

This study shows that different empirical correlations may be fitted to a drying system in different conditions, while a simple ANN model is able to describe the whole of experimental conditions more accurately. The ANN model is also able to be retrained and the range of experimental conditions may be expanded by addition of new sets of experiments. So it can be concluded that ANN model may be considered as an alternative for description of drying behaviour of agricultural products.

## References

*Akpinar, E.K., Bicer, Y., Yildiz, C., 2003. Thin layer drying of red pepper. Journal of Food Engineering 59, 99-104.*

*Gögüs, F., 1994, The effect of movement of solutes on millard reaction during drying, Ph.D. Thesis, Leeds University, Leeds.*

*Senadeera, W., Bhandari, B.R., Young, G., Wijesinghe, B., 2003. Influence of shapes of selected vegetable materials on drying kinetics during fluidized bed drying, 58, 277-283.*

**Table 1**

| Agricultural Product | Tangerine | Orange | Garlic | Potato | Tomato | Pomegranate |
|---|---|---|---|---|---|---|
| P (%) | 2.70 | 3.65 | 2.08 | 3.34 | 1.18 | 2.76 |

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# Session S5-P-1: Innovations in Food Technology – Poster Session I

| Abstract Number | Paper Title & Authors | Included |
|---|---|---|
| 354 | Color (gray values) estimation during roasting coffee<br>J A Hernández, B Heyd, C Irles, G Trystram | Yes |
| 1319 | Application of discrete modelling approach to yeast drying<br>F Debaste, V Halloin | Yes |
| 1325 | The ecological impact of the sugar sector – Aspects of the change of a key industrial sector in Europe<br>M Narodoslawsky | No |
| 1497 | Objective function analysis for tropical fruits and coffee drying: Energy vs. Quality aspects<br>C A Cardona, C E Orrego, V R Nicoletti, P J Amaral, G I Giraldo | Yes |
| 1499 | Energy and Exergy analysis of tropical fruits dehydration using different dryers<br>C A Cardona, C E Orrego, J Tellis, A L Gabas, O D Hernández | Yes |
| 2629 | Modelling of an Immobilized Glucose Isomerase Packed Bed Bioreactor<br>R Khalilpour, R Roostaazad, J Razavi | Yes |
| 3366 | Antioxidant Components Preservation In Vegetables Under Microwaves Processing<br>A A Barba, A Calabretti, M Amore, A L Piccinelli, L Rastrelli | Yes |
| 3811 | The orotate transporter oroP from Lactococcus lactis can be used both as a very efficient, food-grade selection and counter-selection marker for strain construction in many different organisms<br>E Defoor, J Martinussen | Yes |
| 3813 | Analysis of plasmid pDBORO – A plasmid encoding an orotate transporter from Lactococcus lactis subsp. lactis biovar. diacetylactis strain DB0410<br>E Defoor, J Martinussen | Yes |
| 3915 | "Green" Alternatives to Conventional Organic Solvent: A Closer Look at the Screening of Room Temperature Ionic Liquids (RTILs) for the Biosynthesis of Lipophilic Flavonoid Esters<br>B M Lue, Z Guo, X Xu | Yes |
| 4004 | Quality of paprika powder during heating by infrared radiation<br>N Staack, E Borch, D Knorr, L Ahrné | Yes |
| 4008 | Oxidative stability of mayonnaise based salads enriched with fish oil<br>A-D M Sørensen, N S Nielsen, C Jacobsen | Yes |
| 4016 | Peptidase activities in different strains of Lactobacillus | Yes |

| | | |
|---|---|---|
| | helveticus<br>M P Jensen, F K Vogensen, Y Ardö | |
| 4038 | Regulation of early gene expression in the temperate lactococcal phage TP901-1<br>M Pedersen, L L Leggio, J G Grossmann, S Larsen, K Hammer | Yes |
| 4042 | The dependence of sucrose, glucose syrup, furcellaran and citric acid concentrations on the properties and self-life stability of jelly<br>A Salumets | No |
| 4064 | The effect of concentration, temperature, pH, and ions on the rheological properties of xanthan gum, tara gum and their mixtures<br>K Vaher, K Laos, A Sõrmus | Yes |
| 4066 | Crystallization of the supersaturated sucrose solutions in the presence of fructose, glucose, corn syrup and lactose<br>E Kirs | No |
| 4106 | Connection between sensory quality and consumer's preference of salmon<br>D G Petersen | No |
| 4108 | Quartz crystal microbalance with dissipation analysis of interactions between specific milk molecules and instestinal brush border membranes of importance for mammalian health<br>S B Nielsen, D E Otzen, J Stagsted | Yes |
| 4114 | Direct-Affinity Reverse Extraction (DARE) screening for bioactive food-derived peptides<br>A L W Jørgensen, H R J Madsen, J Stagsted | Yes |
| 4116 | Eating quality of wheat bread with potato fibres<br>U Kidmose | No |
| 4117 | Shelf-life prediction of convenient foods by accelerated storage studies and sensory analyses<br>M Nuin , B Alfaro, C Abaroa | Yes |
| 4119 | Texture modification in acid milk gels produced at low temperature<br>H A Ashish, S Pyett, B Bergenståhl | Yes |
| 4121 | Rheological Measurements Of Yolk To Characterise Origin And Processing Properties Of Eggs<br>A Laca, B Paredes, M Díaz | Yes |
| 4122 | Casefindings From Interviews With Danish Food Sector Executives On Their Responsibilit In Counteracting Overweight And Obesity<br>M S Jørgensen, B Mikkelsen | Yes |
| 4123 | Mechanisms of Selenium-dependent antioxidant properties in milk<br>M R Clausen, J Stagsted | Yes |
| 4127 | Molecular gastronomy – cooking of meat<br>L Mortensen | No |
| 4128 | Perspectives, Knowledge And Consumption Frequency Of University Students For Functional And Light Foods<br>A Nazan, C M Ali | No |
| 4129 | Nutritional Habits And Food Consumption Frequency Of Obese And Non Obese Women | No |

| | | |
|---|---|---|
| | A Nazan, S Hasipek | |
| 4130 | Evaluation of lipid and protein oxidation during processing and storage of fatty fish mince<br>S Eymard, C P Baron, C Jacobsen | Yes |
| 4148 | The Effects Of Packaging Materials And Filling Methods On Some Characteristics Of Herby Cheese (otlu Peynİr)<br>Z Tarakci | No |
| 4149 | The Effect Of Adjunct Cultures On Some Chemical And Biochemical Properties Of White-brined Cheese<br>Z Tarakci, Y Tuncturk | No |
| 4150 | Optimization of drying parameters for sweet pepper (Capsicum annum. L)<br>J P Pandey | No |
| 4151 | Partition coefficient of ions (Na+, K+, Mg++, Ca++, Cl-) in solid/liquid system<br>C Mouawad, E Tehrany,S Desobry | No |

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# Color (gray values) estimation during coffee roasting

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## 1. Summary

In order to guarantee the quality of roasted coffee, it is important to measure and control a large number of important variables during the process. Image analysis allows on-line measurement of essential values such as the color and swell of the beans. However this technique of image analysis is difficult to employee in the industries of coffee roasting. For this reason, it is necessary to developer a technique to estimate on-line color. Consequently, this work proposes a mathematical model based in artificial neural network for the color (gray values) estimation during coffee roasting. The mathematical model considers as input variables the stay time and bean temperature. A feedforward network with one hidden layer was used to predict the gray values. The best fitting training data set was obtained with three neurons in the hidden layer, which made possible to predict gray values with accuracy at least as good as that of the experimental error, over the whole experimental range. On the validation data set, simulations and experimental data test were in good agreement (R2>0.987). The developed model can be used for a reliable on-line state estimation and control of coffee roasting.

Keywords: coffee roasting, color (gray values), quality and neural networks.

## 2. Extended Abstract

Coffee roasting is a very important unitary operation in order to develop specific organoleptic properties (flavors, aromas and color), underlying the quality of coffee to guarantee a good cup of coffee. Nevertheless, this process is very complex since the quantity of heat transferred to the bean is key. During coffee roasting, there are moisture loss and chemical reactions (oxidation, reduction, hydrolysis, polymerization, decarboxilation and many other chemical changes), as well as important changes (color, volume, mass, form, bean pop, pH, density and volatile components), and in addition $CO_2$ is generated.
Several parameters can be used as indicators to determine the roasting degree (ex. aroma, flavor, color, beans temperature, pH, composition chemical, bean pop, mass loss, gas composition and volume). Nevertheless, in the industry it is very difficult to

on-line estimate these parameters, normally the roaster master take up an essential position. He determines the operation conditions by measuring obtained out-line from organoleptic properties (color, aroma and flavor), as well as physic measures (air temperature and stay time of process). Now well, this work proposes a strategy for estimating the color of the bean in real time. Colombian green coffee beans (*Arabica*) were roasted using hot air flow as heating medium. The experiments were carried out at constant air velocity of 4 m/s and different air temperatures (190, 200, 210, …300°C). Figure 1 shows experimental data of the bean color (gray values) obtained in real time. These data were obtained from a camera video CCD Sony® (XC-711P) with 50 mm. The behavior of color is very interesting during the process: the first 20 *s* the color remains constant after the gray values diminishes lightly, and when the moisture is abundant on the bean surface, the gray values augment up to overcoming the initial color, for finally to descend up to reaching the optimum color fixed by the master roasted. A model is proposed to determine the gray values from the bean temperature and stay time as input parameters in the neural model. For the model, the Levenberg-Marquardt learning algorithm, the hyperbolic tangent sigmoid transfer-function and the linear transfer-function were used. Three neurons in the hidden layer are possible to predict gray with accuracy at least as good as that of the experimental error, over the whole experimental range. The developed model can be used for a reliable on-line state estimation and control of coffee roasting.

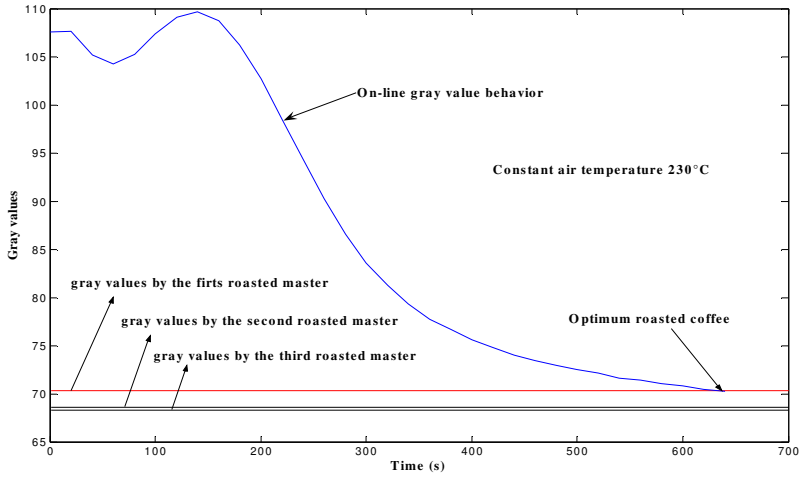


**Fig. 1.** On-line gray values of roasted coffee and three different optimum gray values

## References

Hernández, J. A., Heyd, B. Irles, C., Valdovinos, B. and Trystram, G., (2007) *Journal of food engineering*, 78,1141-1148.

Herández Pérez, J. A., *Étude de la torrféaction: modélisation et détermination du degré de toreffaction du café en temps réel,* PhD these of the ENSIA, France (2002).

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# Application of discrete modelling approach to yeast drying.

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## 1. Summary

Yeast drying is widely used to facilitate its transport and conservation. This work deals with application of pore network model to baker's yeast drying in fluidized bed. Classical equations at the reactor scale are coupled with the pore network for the grain. This model takes into account diffusion in the gas phase, transport by liquid film in partially saturated region and pressure gradient effects in the liquid phase. Porous structure to be applied in the model is obtained using environmental scanning electron microscope. Results are compared to experimental results obtained on a laboratory pilot fluidized bed and to a simplified receding core, continuous model.

Keywords: yeast drying, fluidised bed, discrete model, pore network model.

## 2. Extended Abstract

Drying of yeast is of major importance for food industry. Without it, transport and storage of yeast couldn't be easily achieved [1]. Unfortunately, the process has some important drawbacks: it is highly energy consuming and the quality of the product, including viability, can be altered. Study and modelling of yeast drying is then useful to limit energy loss and to minimize product alteration. Classical models of yeast drying are based on desorption isotherms: experimental curves of equilibrium solid moisture content as a function of air moisture content. Such models have several limitations. Firstly, the production of experimental desorption isotherms is a difficult and expensive process. Moreover, the isotherm depends on solid geometry, so if yeast grains are modified, in theory the isotherm measurements have to be repeated. Secondly, models are very sensitive to isotherms. So, a precise model would require a very precise isotherm, which cannot always be obtained experimentally. Thirdly, although isotherms are based on equilibrium, they are often used to model the kinetic of the drying; some kinetics effect cannot be highlighted by such a method.

Most alternative models developed to integrate the effect of solid structure on drying are based on a continuum approach, where the porous system is modelled by a fictive continuous medium [2]. In this paper, a discrete modelling of yeast grain is used. In the discrete approach, the medium is modelled directly at the pore scale by

considering a network of pores linked together by throats. Simplified local transport equations for each throat and pore are then solved. Such models naturally take into account phenomena that classical models can't easily handled as the fractal form of the drying front. It represents a powerful complementary tool to continuous approach [3]. The model used in our simulations includes most features of existing fundamentals pore network models for evaporation: diffusive vapour transport, capillary and viscous effect in the liquid phase and film flow in the partially saturated area [4]. Network parameters of the models are deduced from the analysis of the solid structure observed with an environmental scanning electron microscope.

The model is validated by comparison with experiments of drying of a small quantity of yeast in a thermogravimetry analyser. The practical case of fluidized bed drying is then studied. The model couples the pore network model for yeast grains to classical transport equations for the whole reactor. The results are compared to experimental results on a laboratory pilot plant and to a simple receding front continuous model. This comparison allows highlighting strengths, weaknesses and complementarities of both approaches.

**References**

[1] D. Bayrock and W.M Ingledew. *Fluidized bed drying of baker's yeast: moisture levels, drying rates, and viability changes during drying*. Food research International, 30(6):407–415, 1997.
[2] S. Whitaker. *A theory of drying in porous media*. Advances in heat transfer, 13 :119–203, 1977.
[3] M. Prat. *Discrete models of liquid-vapour phase change phenomena in porous media.* Revue générale de Thermique, 37 :954–961, 1998.
[4] A.G. Yiotis, A.G. Boudouvis, A.K. Stubos, I.N. Tsimpanogiannis, and Y.C. Yortsos. *Effect of liquid films on the drying of porous media*. AIChE Journal, 50(11) :2721–2737, nov 2004.

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# Objective function analysis for tropical fruits and coffee drying: Energy vs. Quality aspects

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## 1. Summary

Convective drying, vacuum drying, spray drying and freeze–drying are widely used in industry for food processing. Usually freeze drying is considered as a high energy process not suitable as a first option for large scale productions. On the other hand Freeze-drying is a suitable technique to preserve taste, color, flavor, nutritional and antioxidant content of fresh fruits and vegetable extracts. Other advantages are the high solubility and rehydration ability of the dried product. For all other convective heat driven processes used for drying this type of materials the situation is usually inverse. In this way to compare these technologies energy analysis is not the unique criterion to be considered. Quality, production volumes and market analysis must be also included.

For that purpose, simulations for each dryer were performed calculating the heat requirements of each process and outlet conditions according to technical and economic parameters (based on retail prices) required in the market. Then an objective function was developed including the cited criteria allowing the analysis of advantages or disadvantages of these technologies using fruit and coffee industry as examples.

The obtained methodology and results allow the choice of appropriated technologies for different biomaterials drying based on quantitative analysis.

Keywords: Objective Function, freeze drying, convective drying, juice, extract

## 2. Extended Abstract

In food processing drying has been a widely used operation to reduce moisture in products reducing transportation and storage costs, and also improves its shelf-life. There exists a great variety of options for drying, convective drying, vacuum drying, spray drying and freeze–drying are some of the examples.

Conventional air-drying is the most frequently used dehydration operation in food and chemical industry. In this case, the drying kinetics is greatly affected by air temperature and material characteristic dimension, while all other process factors exert practically negligible influence. Convective drying is usually seen as the first choice in drying operation because it is a well established method of drying. It uses flowing equipment for air to carry out moisture of samples and is energy consuming because usually air is preheated. Vacuum drying systems does not involve air-flowing equipment but drying is carried out by using vacuum systems that can be highly energy-consumers and expensive.

Spray drying is a technique used to convert either solutions or slurries into powder particles. There is a wide variety of spray dryer configurations suited to a particular type of powder or feed consistency. Spray drying involves a previous atomization of feed into a spray. There, the feed is put in contact with air resulting in moisture evaporation countercurrently or cocurrently. Freeze drying is usually considered the last alternative in choosing a drying method because of his technical complexity and high cost, and is based on a separation of liquid water from a wet solid product or from a solution or dispersion of given concentration in the form of a solid phase, ice, and its subsequent removal by vacuum sublimation and consists of mainly four operations: freezing, vacuum, sublimation and condensing.

An integrated objective function for the process to compare four types of drying technologies for coffee and other fruits in terms of capital, energy, quality and environmental impact costs was analyzed. For that purpose, simulations for each dryer were performed calculating the heat requirements of each process and outlet conditions according to technical and economic parameters (based on retail prices) required in the market. Additionally the WAR (Cardona et al. 2004) algorithm was used in the parametric form with the resulted values obtained for each simulation.

The obtained methodology and results allow the choice of appropriated technologies for different biomaterials drying based on quantitative analysis. The application of the generated strategy of comparison can be used in software form suitable for optimization of the selected drying technology. As a general result the feasibility of the process can be evaluated in a simple way before the invests are made.

**References**

Cardona, C. A., V. F. Marulanda and D. Young (2004), Analysis of the environmental impact of butylacetate process through the WAR algorithm, *Chemical Engineering Science*, **59**, 5839-5845.

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# Energy and Exergy analysis of tropical fruits dehydration using different dryers

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## 1. Summary

Drying is an important operation for food industry, which is based on a simultaneous heat and mass transfer of moisture to an air stream. Although, there are many different kinds of dryers, it's difficult to analyze the conditions of the operation for selecting one or another. In this work, the first law of thermodynamics represented in energy consumption is applied to the process of drying tropical fruits (juices) and vegetables (extracts). These results are subsequently analyzed using exergy balances for different technologies. Convective drying, vacuum drying, spray drying and freeze–drying were analyzed. For that purpose, a simulation using different models for each dryer was performed calculating the heat requirements of each process and outlet conditions according to parameters given in the literature. Selection of the most appropriated models was based on accuracy and calculation time. After that, an economic comparison is made. Application of the second law results in an exergy balance for every part of the process. This exergy balance yields an estimate of the quantity of exergy loses, that are useful as a thermodynamic comparison of the processes. This mathematical tool gives an assessment between the processes and also permits an analysis of the most efficient and least efficient part of the drying equipment. Useful quantitative results agreeing with experimental data were obtained, allowing the choice of most convenient alternative for different fruits drying.

Keywords: Drying Process, Exergy, juice, extract

## 2. Extended Abstract

In food processing and other industries, drying processes are used to eliminate moisture of samples to save money in transportation costs and during storage. There exist different processes to use as a dryer convective drying, vacuum drying, spray drying, and freeze – drying are some. Convective drying is usually seen as the first choice in drying operation selection because it is a well known method of drying. It uses flowing equipment for air to carry out moisture of samples. Simulations showed that the energy consumption in this case with natural gas as a fuel is not so expensive

as people think, however the quality of the product and the level of water removed in some fruits made it not so suitable for dehydration. Vacuum drying systems does not involve air-flowing equipment but drying is carried out by using vacuum systems that can be highly energy-consumers. Here simulations give us an interesting result, the optimal point between the vacuum and the quality of the drying. Spray drying is a technique used to convert either solutions or slurries into powder particles. There is a wide variety of spray dryer configurations suited to a particular type of powder or feed consistency. In this case, calculations for different fruits drying yields a high energy demand dependence on the characteristics and use of the product. Freeze drying is usually considered the last alternative in choosing a drying method because of his technical complexity and high cost, and is based on a separation of liquid water from a wet solid product or from a solution or dispersion. Optimization of this type of drying technology from the point of view of energy consumption is more limited to the freeze level to be achieved. However, the capital and energy costs for fruits as for example the tree tomato (*Cyphomandra betacea)* have no any importance due to the fact that the quality of the powder is the best.

All the simulations made for these drying technologies had as a critical point the efficiency of these systems for producing the required energy for heating or freezing. This is quite difficult for understanding in the simple framework of energy meaning. To solve this problem, in this work the exergy analysis was applied. Exergy is a measure of how an energy form (work, flow, internal, radiative and other sources of energy) is useful or its quality or its potential to cause(Syahrul, Hamdullahpur et al. 2002). Recently it has been used to understand the efficiency and environmental impacts of process, and among them drying systems (Midilli and Kucuk 2003; Gunhan, Demir et al. 2005; Kavak Akpinar, Midilli et al. 2005; Akpinar, Midilli et al. 2006). Exergy balances based on the second law of thermodynamics and the previous simulation of drying processes to specify temperature, moisture, and enthalpy conditions allowed calculating the exergy losses in each part of the process. This was helpful to decide which process is better to use and how each part affects the whole performance. In this work these balances have been applied to processes for drying coffee, apple juice, orange juice and other fruits for the cited dryers. Different results were obtained and summarized in tables and figures.

**References**

Akpinar, E. K., A. Midilli and Y. Bicer (2006), *Journal of Food Engineering*,72, 320-331.

Gunhan, T., V. Demir, E. Hancioglu and A. Hepbasli (2005), *Energy Conversion and Management*,46, 1667-1679.

Kavak Akpinar, E., A. Midilli and Y. Bicer (2005), *Energy Conversion and Management*,46, 2530-2552.

Midilli, A. and H. Kucuk (2003), *Energy*,28, 539-556.

Ratti, C. (2001), *Journal of Food Engineering*,49, 311 - 319.

Syahrul, S., F. Hamdullahpur and I. Dincer (2002), *Exergy, An International Journal*,2, 87-98.

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# Modeling of an immobilized glucose isomerase packed bed bioreactor

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## 1. Summary

In this study, the performance of immobilized glucose isomerase enzyme (in cylindrical form) for isomerization of glucose to fructose was mathematically modeled.

Keywords: Fructose, Glucose Isomerase, Packed Bed, Bioreactor, Immobilized Enzyme

## 2. Extended Abstract

In this study, the performance of immobilized glucose isomerase enzyme (in cylindrical form) for isomerization of glucose to fructose was mathematically modeled. A modified Michaelis-Menten type relation (reversible kinetics) was used to describe the enzyme kinetics. The kinetics parameters are in table 1:

| **Parameter** | **Quantity** |
|---|---|
| $V_{mf}$ | 8.768e-4 kg/m$^3$.kg catalyst |
| $K_{mf}$ | 126.112 kg/m$^3$ |
| $K_{mr}$ | 81.072 kg/m$^3$ |
| $K_{eq}$ (at 60°C) | 1 |

**Table 1:** Kinetic parameters of isomerization reaction

Mass transfer inside the biocatalyst particle and through the bed column was analyzed simultaneously. Using measured data, physicochemical properties including diffusivity, viscosity and density of sugar solutions were correlated with its concentrations and were used to provide precision in solving the set of model equations. Model equations were solved using the Runge-Kutta and Gauss-Seidel algorithms and Finite Difference numerical method in Matlab environment.
Model output was used to demonstrate the effect of parameters such as velocity and bulk substrate concentration on the concentration profile within the biocatalyst particle, effectiveness factor and bulk substrate concentration along the bed.
Model predictions were further validated against experimental data collected from a lab scale isomerization bioreactor. Measurements of the overall bioreactor conversion at various substrate concentrations were shown to lie within 5% of the values as put

forth by the model (Figure 1). Using experimental data, a criterion was proposed to maximize the isomerization conversion from the immobilized bioreactor.

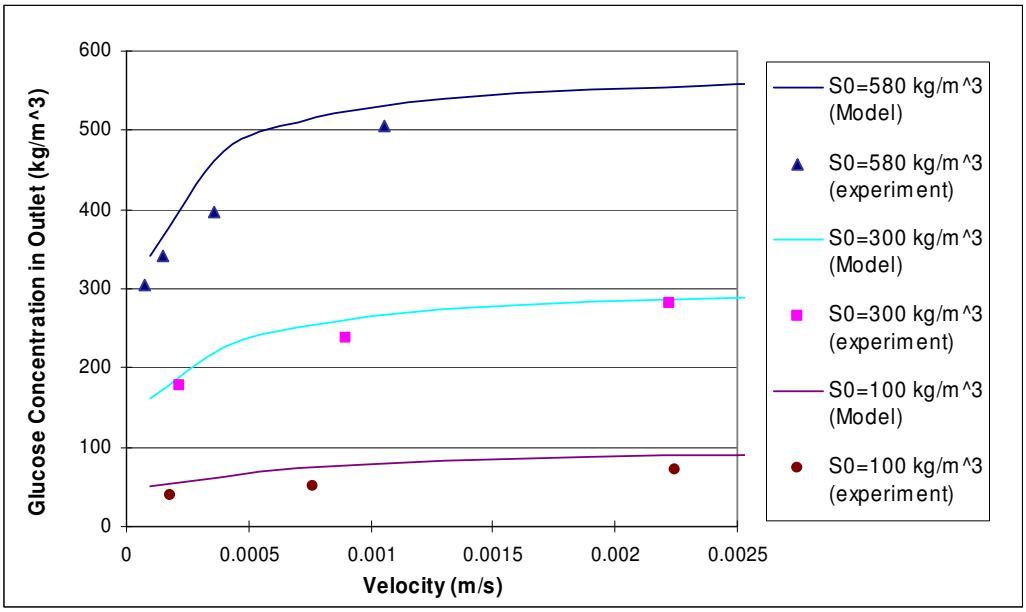


**Figure 1:** Comparison of model and experiment results

## References

Bales, V., Rajniak, P., 1986. Mathematical simulation of fixed bed reactor using immobilized enzyme. *Chem. Papers* 40(3), pp. 329-338.

Beck, M., Kisser, T., Perrier, M. and Bauer, W., 1986, Modeling glucose/fructose isomerization with immobilized glucose isomerase in fixed and fluidized-bed reactors. *Canadian Journal of Chemical Engineering* **64**, p. 553.

M. Dadvar, M. Sohrabi and M. Sahimi, 2001, Pore network model of deactivation of immobilized glucose isomerase in packed-bed reactors. I. Two-dimensional simulation at the particle level. *Chemical Engineering Science* **56** (2001), p. 2803.

Vasic-Racki, D.V., Pavlovic, N., Cizmek, S., Drazic, M. and Husadzic, B., 1991. Development of reactor model for glucose isomerization catalyzed by whole-cell immobilized glucose isomerase. *Bioprocess Engineering* **7**, p. 183.

Ozdural, A.R., Tanyolac, D., Boyaci, I.H., Mutlu, M. Webb, C., 2003. Determination of apparent kinetic parameters for competitive product inhibition in packed-bed immobilized enzyme reactors. *Biochemical Engineering* 14, pp.27–36.

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# Antioxidant components preservation in vegetables under microwaves processing

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## 1. Summary

Fruits and vegetables are considerable sources of natural antioxidant components useful in the human diet for their health benefits. These antioxidants (carotenoids, vitamins, flavonoids, other phenolic compounds etc.) offer protection against injurious free radicals and have been strongly associated with reduced risk of chronic diseases, such as cardiovascular disease, cancer, diabetes. To consume or to increase their shelf-life, fruits and vegetables undergo to specific treatments, which can consist in minimal handling or deep transformations. In particular, vegetables require treatments necessary to obtain edible foods in terms of digestibility, palatability, and softening features. Depending on the methods which are used to process the vegetables physical and/or chemical alterations are possible using low or high temperatures. Being the cooking operation a process that involves simultaneous heat and mass transfer, the role of convective (boiling and steaming methods) and radiative (microwaves method) heat transport phenomena are related to the final quality of the processed vegetable. The intent is to emphasize if dielectric heating is suitable for foods processing in terms of nutritional factor preservation and energetic sustainability since, actually, packaged pre-cooked and cooked foods have an enormous market success as microwaveable preparations (soups, salads, etc.). The work has been focused on the antioxidant components (carotenoids and ascorbic acid) preservation in pumpkin cooking treatments.

Keywords: vegetable, thermal treatments, microwave, antioxidants.

## 2. Extended Abstract

### *2.1. Material and methods*

*Vegetable*. As vegetal matrix pumpkin (*Cucurbita maxima*) is used supplied from a local greengrocery. No preliminary treatments (i.e. blanching step) are performed on the fresh product before cooking processes.
*Antioxidant components analysis*. The carotenoid (α-carotene, all-trans/cis β-carotene, lycopene and lutein) and the ascorbic acid contents have been analysed by the high performance liquid chromatography with diod array (HPLC-DAD). All extraction procedures are repeated three times and results are given as mean ± SD.
*Cooking methods*. The fresh pumpkin is cut in slices (30 mm in diameter and 6 mm in thickness) and then cooked with different methods to have samples well-done but firm to the bite. All cooking procedures (boiling, steaming and microwaving – at 2.45 GHz and 1000 W in power - with or without water) are repeated five times; pumpkin

samples are monitored in temperature and weighed in order to analyse temperature profiles and mass changes.

*2.2. Results and discussion*

In Fig.1 are reported the cooking times and the water losses of pumpkin samples processed with different methods. Microwaves methods show short treatment times but the higher water losses. All the samples attain 100°C, but the ones steam-cooked. These latter do not overcome 90°C. In Fig.2 and Fig.3 are reported the contents of α-carotene, all trans β-carotene, cis-β carotene and lutein and lycopene, respectively. The cis β-carotene content is assayed to estimate the effects of the cooking on the isomerization of all trans β -carotene, being the cis isomer less bioavailable. All the cooking methods lead to a significant release of α, all trans-β carotene and its cis-isomer (carotenoids total content increases during the thermal treatments varying from 350 mg/g - fresh vegetable- until to 900 mg/g -boiled product-); in lycopene and lutein contents, except in boiled samples, no significant changes or losses have been detected. As a matter of fact cooking leads to a softening of the plant tissue so that the carotenoids can be extracted much easier. It is assumed that an increased extractability may be associated with an improved bioavailability.

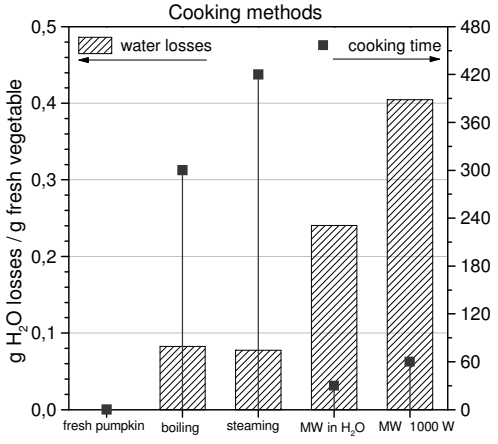


**Fig. 1** Cooking times and the water losses of pumpkin samples processed with different methods.

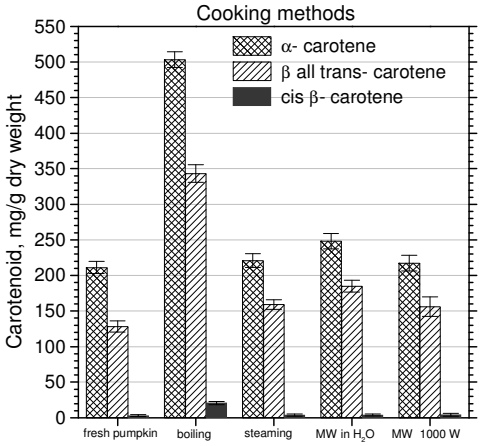


**Fig. 2** α-carotene, all trans β-carotene and cis-β carotene contents in fresh and cooked pumpkin samples.

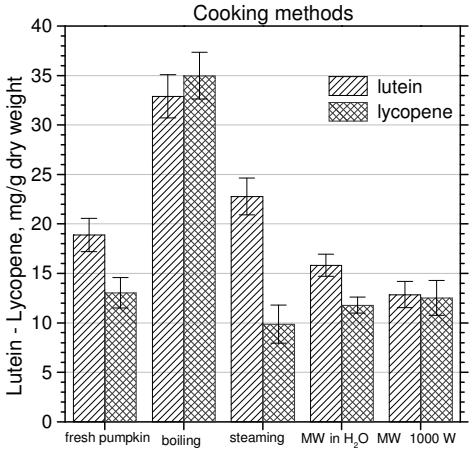


**Fig. 3** Lutein and lycopene contents in fresh and cooked pumpkin samples.

Ascorbic acid is a vitamin very sensitive to technological procedures. Its loss from pumpkin has been evaluated to evidence the degradation induced by thermal treatments. Analyses have shown a content of 9,56 mg/100g in fresh products and very low residual contents in all cooked samples: 1,72 mg/100g in boiled samples; 1,97 mg/100g in steam-cooked samples and less then of 0,10 mg/100g in microwave-cooked samples.

Microwaves treatments have been shown to cause heavy losses. However the cooking conditions used in this work are probably extreme and are likely to be experienced.

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# The orotate transporter *oroP* from *Lactococcus lactis* can be used both as a very efficient, food-grade selection and counter-selection marker for strain construction in many different organisms

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## 1. Summary

A new lactococcal plasmid, pDBORO, was isolated from the *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* strain DB0410 responsible for the sensitivity of DB0410 towards the pyrimidine-analog 5´-fluoroorotate. The plasmid pDBORO complete nucleotide sequence has been determined. The open reading frame *oroP* on pDBORO necessary for the uptake of orotate was identified. A number of industrial important strains like *Lactococcus lactis*, *Bacillus subtilus*, and *Bacillus licheniformis* have been shown to be unable to metabolize orotate. If the *oroP* gene was introduced into these strains they acquired the ability to utilize orotate. If the strains had a pyrimidine requirement, the *oroP* gene could function as a selectable marker when growing in the presence of orotate as sole pyrimidine source. In an otherwise resistant strain, *oroP* was shown to sensitize the strain towards the analog 5-Fluoroorotate. It was shown that strains who have lost the *oroP* gene could easily be selected in the presence of 5-Fluoroorotate, thus being an efficient counter-selection marker[1].

Keywords: Genetic engineering, Nucleotide metabolism, Selection, Counter-selection

### References

(1.) Martinussen, J. and Defoor, E. M. C. Patent WO2005078106-A1.

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# Analysis of plasmid pDBORO – A plasmid encoding an orotate transporter from *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* strain DB0410

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## 1. Summary

A new lactococcal plasmid, pDBORO, was isolated from the *Lactococcus lactis* ssp. *lactis* biovar *diacetylactis* strain DB0410 responsible for the sensitivity of DB0410 towards the pyrimidine-analog 5´-fluoroorotate. The plasmid pDBORO amounts to 16404 bp and its complete nucleotide sequence has been determined. Fifteen open reading frames (ORFs) were encountered of which three insertion-sequence (IS) elements identified as two IS946 and one IS982. Two ORFs are incomplete due to the insertion of an IS element in their carboxy terminal end. Homologs for four ORFs were found on the IL1403 sequence: the *copB* gene, coding for a copper-potassium transporting ATPaseB and the *ysbA*, *ysbB* and *ysbC* genes. The structural organization of the pDBORO replication region is highly similar to other theta-replicating plasmids both at the cis- (*repA*) and trans-acting (*repB*) sequences. By plasmid deletion analysis and molecular cloning, we show that the open reading frame *ysbC* on pDBORO is responsible for the utilization of orotate as the sole pyrimidine source. The gene was accordingly renamed to *oroP*[1]. Surprisingly, *oroP* homologs are found on the *Lactococcus lactis* IL1403 and MG1363 chromosomes despite the fact that they are resistant towards fluoroorotate.[2]

Keywords: Nucleotide metabolism, Lactic acid bacteria, plasmid, orotate

### References

(1.) Martinussen, J. and Defoor, E. M. C. Patent WO2005078106-A1.

(2.) Kilstrup, M.; Hammer, K.; Ruhdal, J. P.; Martinussen, J. *FEMS Microbiol Rev.* **2005**, *29*, 555-590.

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# "Green" Alternatives to Conventional Organic Solvent: A Closer Look at the Screening of Room Temperature Ionic Liquids (RTILs) for the Biosynthesis of Lipophilic Flavonoid Esters

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## 1. Summary

Room temperature ionic liquids (RTILs) are gaining popularity as a media for biocatalysis reactions. In this study, esterification reactions of flavonoids (rutin, esculin) with long chain fatty acids (palmitic acid, oleic acid) were investigated in a wide range of novel RTILs. Based on the results, one can see that ionic liquid (IL) structure has a large influence on the reaction system. Screening results showed that many ILs were not compatible with the system and the lipase (Novozym 435) used as no or very little activity was detected. However, several promising candidates were identified in this system for further investigations.

Keywords: room temperature ionic liquids (RTILs), flavonoids, screening, lipase, biocatalysis

## 2. Extended Abstract

Room temperature ionic liquids (RTILs) are often referred to as solvents of the future and are regarded as promising replacements for traditional organic solvents due largely to their lack of vapour pressure. Besides not releasing organic volatiles into the air (hence a "green" technology), they are regarded as possible substitutes in enzyme biocatalysis for many other reasons, including their high thermal stability, wide liquid range and their potential for solubility modifications (i.e. through appropriate cation & anion selection).

In this system, flavonoids were esterified with long chain fatty acids in order to increase the hydrophobic character of the compounds since these esters have much potential in food systems and in human nutrition (i.e. as antioxidants). In general, differences in solubilities of flavonoids and fatty acids make the development of an efficient enzymatic system for the acylation of flavonoids in organic solvent media problematic. Butanol, pentanol & acetone remain among the more efficient conventional solvents used to date for these reactions. In this work, a range of ionic liquids (ILs) were screened for use in the biosynthesis of fatty acid esters of the flavonoids rutin and esculin.

The ILs investigated were composed of a range of cation moieties such as imidazolium, pyridinium and tetraammonium while anion moieties included structures such as $Cl^-$, $BF_4^-$, $PF_6^-$, trifluoroacetate, alkylsulfates and sulfonates. ILs were screened in order to determine which structures maintained maximal lipase activity in the reaction system of interest.

Overall, it was apparent that not all ILs were appropriate for this type of reaction system. Biosynthesis of long chain fatty acid esters of the flavonoid rutin were found to be much slower than esculin, due to the bulky nature of the rutin substrate. However, based on this screening, it was possible to elucidate further as to the type of structure compatible with the maintenance of enzymatic activity. Anion structure seemed to have a more pronounced effect on enzymatic activity, with $BF_4^-$, $PF_6^-$ and $Tf_2N$ among the most promising candidates for this system. Lastly, experimental results could also be compared with initial predictions made using the COSMO-RS (Conductor-like Screening Model for Real Solvents) quantum chemical model.

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# Product and microbial quality of paprika powder during heating by infrared radiation

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## 1. Summary

In this work, infrared radiation (IR) was explored as a technique for decontamination of paprika powder. Wetted paprika powder was exposed to infrared (IR) heat treatments with constant heat flux. The effect of IR on paprika was evaluated in terms of temperature distribution, colour, water activity, and reduction of bacterial spores. Changes of colour and water activity were observed on the surface. However, the overall colour and water activity were not significant affected. At $a_w$ of 0.96, total reduction of *Bacillus cereus* spores was observed in the inside of the powder, but the overall sample showed only a reduction of 1.5-2 $\log_{10}$ colony forming units (CFU)/g, due to high viable counts on the surface.

Keywords: Infrared radiation; Water activity; Paprika powder; Colour; *Bacillus cereus* spores

## 2. Extended Abstract

Despite their contribution to taste, colour and odour in foods, spices are also known to be a large source of food spoilage, due to their high microbial contamination. One of the most frequently found spore-forming bacteria concerning spices is *Bacillus cereus*. However, the decontamination of dried powders is difficult, and the difficulty correlates with the presence of a spore-forming microflora adapted to low water content as well as the high thermal resistance of microorganisms in dry systems.
In this work, infrared radiation (IR) was explored as a technique for decontamination of paprika powder. Infrared is a part of the electromagnetic optical spectrum (wavelength 0.76-1000 μm), that causes heating on surfaces. The advantage of IR is the fast heating, as energy is transferred by radiation from the heater to the food without heating the surrounding air.
Paprika powder having water activities ($a_w$) of 0.5, 0.8 or 0.96 was exposed to infrared heat treatments with constant heat flux of medium IR wavelength at 5 $kW/m^2$. The effect of IR on paprika was evaluated in terms of temperature distribution, colour, water activity, and reduction of bacterial spores. Temperature

was measured in four different locations (on surface, and in depths of 1, 3 and 8mm) throughout a 10 mm powder bed. Thermal inactivation in paprika powder was studied for the natural background flora and for inoculated spores of *Bacillus cereus*.

The temperature distribution in paprika powder during the infrared treatment is affected by the water activity (Fig. 1). Lower water activity showed higher surface temperature and higher temperature difference between the surface and the inside of the powder. Water activity of 0.96 showed longer heating time, but a more uniform temperature distribution. Results of changes in colour and water activity during IR treatment are expressed in figure 2 and 3, respectively. Due to the higher surface temperature, changes of colour and water activity were observed on the surface. However, the overall colour and water activity were not significant affected.

Decontamination of *Bacillus cereus* (results are not shown) was not very effective for infrared heating at lower water activities of 0.5 and 0.8 (around 1 $\log_{10}$ CFU/g) due to fast water lost in the surface. At $a_w$ of 0.96, total reduction of *Bacillus cereus* spores was observed inside the powder (detection limit at 1 $\log_{10}$ CFU/g), but the overall sample showed only a reduction of 1.5-2 $\log_{10}$ CFU/g, due to high viable counts on the surface. Wetting of powder is therefore necessary to achieve significant microbial reduction.

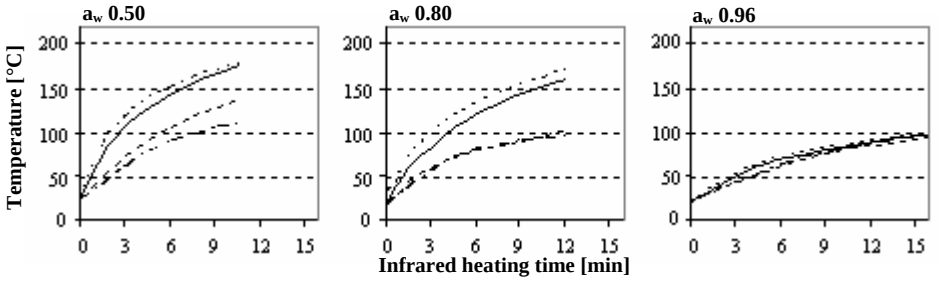


Figure 1: Temperature of paprika powder measured on the surface (····) and at depths of 1 (——), 3 (---), and 8 mm (·-·-·-) at water activity levels of 0.5, 0.8, and 0.96.

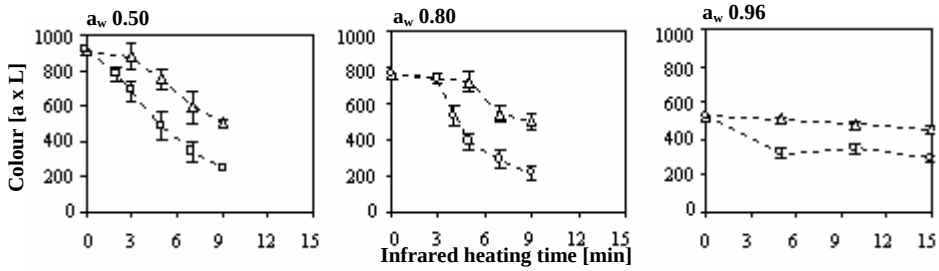


Figure 2: Surface (Δ) and overall (○) colour measurements (a x L) of paprika powder at $a_w$ 0.50, 0.80, and 0.96.

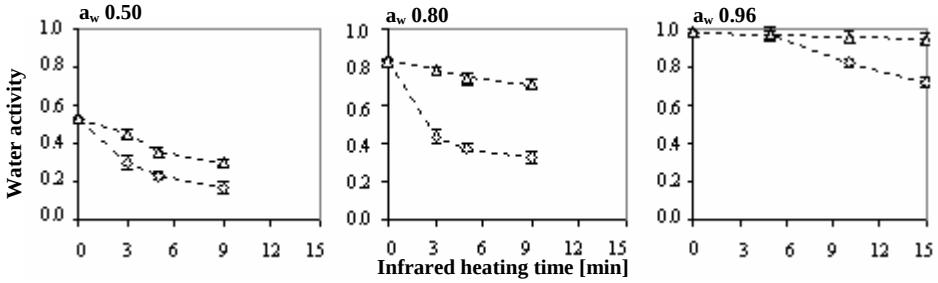


Figure 3: Effect of infrared heating on surface (□) and overall (Δ) water activity in paprika powder at $a_w$ 0.5, 0.8, and 0.96.

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# Oxidative stability of mayonnaise based salads enriched with fish oil

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## 1. Summary

The marine omega-3 polyunsaturated fatty acids (PUFA) have received increasing interest during the last decades. This is due to several health effects associated with the intake of omega-3 PUFA. Hence, several efforts have been made to incorporate marine oils into various food products e.g. milk, yoghurt and mayonnaise. Food products containing omega-3 PUFA are highly susceptible to oxidation, which causes undesirable flavors and loss of those omega-3 PUFA that are beneficial.

Most food products consist of a complex matrix e.g. mayonnaise based salads, and several factors in these food systems might influence the oxidative stability. Thus, a better understanding of the effects of the different ingredients on oxidation of omega-3 PUFA as well as the effect of possible interactions between the ingredients is essential when omega-3 enriched foods are produced.

The study aimed at evaluating the oxidative stability of the lipids in fish oil enriched mayonnaise based salads during storage. The influence of the different ingredients and their interactions on lipid oxidation in the products was evaluated. Furthermore, the antioxidative effect of different spices (thyme, oregano and rosemary) in the mayonnaise based salads was assessed. The samples were stored at 2 °C in closed plastic boxes in the dark up to 8 weeks. Lipid oxidation progress in the different salads was assessed by determination of the peroxide concentrations (peroxide value, IDF) and the concentration of volatile oxidation products (dynamic headspace GC-MS). Moreover, sensory analysis was performed on a few selected mayonnaise salads to support the chemical analyses.

The oxidative stability of the different mayonnaise salads enriched with fish oil, interactions of different ingredients and effect of spices on the lipid oxidation in these salads will be described in more details on the poster.

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# Peptidase activities in different strains of *Lactobacillus helveticus*.

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## 1. Summary

*Lactobacillus helveticus* is very peptidolytic and has in recent years been used as an adjunct culture in different kind of cheeses to accelerate ripening. Therefore, we wished to examine the peptidolytic activity of different Lactobacillus helveticus strains. Cell free extracts of different Lb. helveticus strains was incubated with specific synthetic substrates. The results revealed various aminopeptidases with different specificities and level of activity depending on the strain.

Keywords: *Lactobacillus helveticus*, Peptidase activities, Strain dependence

## 2. Extended Abstract

### *2.1. Background*

*Lactobacillus helveticus* is a fastidious lactic acid bacterium that is auxotrophic for several amino acids. In order to grow in milk *Lb. helveticus* is dependent on its proteolytic/peptidolytic system for the release of essential amino acids. The proteolytic and the peptidolytic activities as well as amino acid catabolism is important for cheese flavour development.
The objective of this study was to examine the peptidolytic activity of different strains of *Lb. helveticus*.

### *2.2. Materials & methods*

Seven different strains of *Lb. helveticus* were selected on the basis of their rep-PCR profiles. The seven different strains were *Lb. helveticus* CNRZ32, *Lb. helveticus* CNRZ303, *Lb. helveticus* LHC2, *Lb. helveticus* ATCC15009 and *Lb. helveticus* L100 and two thermophilic bacteria isolated from the cheeses Arla Kadett and Cougar Gold respectively.
The bacterial strains were grown in MRS, harvested and then cell free extracts (CFE) were prepared using a French Press. The peptidolytic activity of the CFE was measured using different synthetic substrates - *para*-nitroanilides (pNA).

*2.3. Results*

The highest activity expressed by all strains was observed towards Gly-Pro-pNA, Lys-pNA and Leu-pNA. Moderate activity was observed against Bz-Phe-Val-Arg-pNA, Pro-pNA and Gly-pNA while only very low activity was observed against the substrates Phe-pNA and Glu-pNA. Additionally, significant strain variations were observed with *Lb. helveticus* CNRZ303 showing highest peptidolytic activity.

*2.4. Conclusion*

The *Lb. helveticus* strains all possessed general aminopeptidases, peptidases with proline activity and endopeptidases with a strain dependent activity level.

*2.5. Acknowledgement*

This study was kindly supported by the Danish Dairy Research Foundation and the Innovation Law

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# Regulation of early gene expression in the temperate lactococcal phage TP901-1

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## 1. Summary

Bacteriophages are viruses that infect bacteria and use the host to generate new phage progeny. Infection of starter cultures by bacteriophages poses a major problem in the dairy industry and influences the quality of the finished products. After infection of a sensitive host by a temperate phage such as the lactococcal phage TP901-1, the phage must choose either a lytic or a lysogenic life cycle. A genetic switch controlling transcription of different genes regulates the choice between these two life cycles. The genetic switch of the temperate phage TP901-1 consists of two divergently oriented promoters $P_L$ (lytic) and $P_R$ (lysogenic) being responsible for transcription of the early gene clusters in the TP901-1 genome. The initiation of transcription from both $P_L$ and $P_R$ is regulated by the TP901-1 repressor, CI, in consort with the modulator of repression, MOR. It has been suggested that during early lytic infection MOR functions as an anti-repressor that interacts with CI thereby preventing binding of CI to the operator sites. We have shown that *mor* encodes a protein and that this protein is required for having the choice between the two different life cycles. In order to get further insight into the mechanism of action of the CI repressor we constructed specific mutations and C-terminal deletions in *CI* and studied the effects in vivo. Combined with protein studies of the purified mutated or truncated forms of the CI protein the N-terminal part was determined to contain the DNA binding domain and the C-terminal part for being responsible for oligomerization. The overall structure of CI seems to be a flat disc-like structure according to small angle X-ray scattering.

Keywords: Genetic switch; repressor mutants; DNA binding domain; oligomerization domain; SAXS analysis

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# The effect of temperature on the properties of Xanthan's tara's and their blend's solutions

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## 1. Summary

Rheometer was successfully used to measure and characterize the effect of temperature on the rheological properties of investigated materials**.** During heating xanthan´s, tara´s and their blend´s solutions liquefy reversibly. It is possible to get stronger solutions after heating process. This work is undoubtedly very important for food industry and needs further investigation to gain complete knowledge about these food hydrocolloids and their properties.

Keywords: xanthan, tara, rheometer

## 2. Extended abstract

Xanthan gum is an extracellular polysaccharide secreted by the microorganism *Xanthomonas campestris* (Sworn, 2000). It exhibits a synergistic interaction with galactomannans. Tara gum is a galactomannan with the rheological properties that are similar to guar gum and locust bean gum. These hydrocolloids can be used as thickeners and stabilizers in food applications (dairy products, desserts) and in non-food applications (pharmaceuticals, cosmetics). These two food hydrocolloids have many good rheological properties; the most important would be their ability to produce highly viscous aqueous solutions at relatively low concentrations. New opportunities may exist in the unique rheology and performance improvements resulting from mixtures of xanthan and tara gum.
The purpose of the present work was to investigate the effect of temperature on the rheological properties of xanthan´s, tara´s and their blend´s solutions.

### Temperature ramp

In food industry food products often undergo heat treatment after the addition of hydrocolloids. Due to this it is very important to know how temperature influences their structure.

Temperature was raised from 5$^{o}$C to 90 $^{o}$C and then decreased form 90 $^{o}$C to 5 $^{o}$C. The effect of temperature on the storage modulus of xanthan´s and tara´s solutions in different concentrations is shown in Figure 1. Storage modulus (G`) relates to the elastic nature of

the material (Gunasekaran, Mehmet, 2000). Temperature rise causes a drop in storage modulus of xanthan´s and tara´s 2% solution but has bigger effect on tara´s solutions than xanthan´s. After cooling the storage moduluses increase again to the same value as at the beginning. From Fig. 3 it is seen that temperature has smaller effect on the xanthan and tara solutions with lower concentration compared to solutions with higher concentrations.

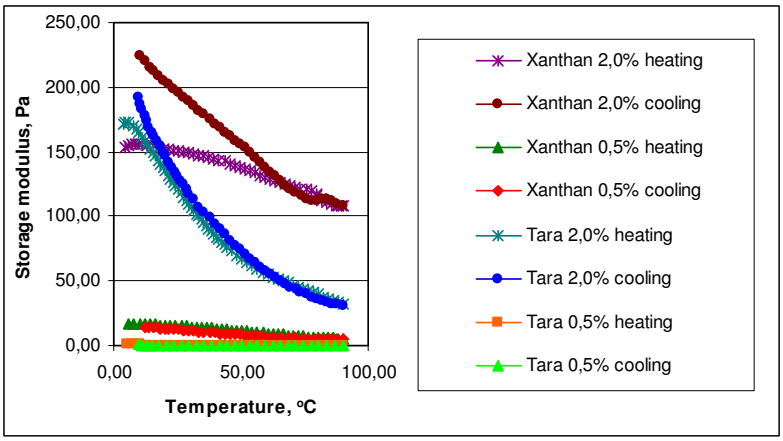


**Figure 1:** The effect of temperature on the storage modulus of xanthan´s and tara´s solutions in different concentrations

The effect of temperature on the storage modulus on xanthan´s, tara´s and their blend´s solutions is seen in Figure 2. At 5°C the storage modulus of xanthan´s and xanthan:tara blend´s solutions were similar but during heating the storage modulus on xanthan:tara blend decreased more strongly. After cooling the storage modulus of xanthan:tara blend got much bigger that it was at the beginning. It means that synergistic interaction of xanthan and tara blends appears after heating.

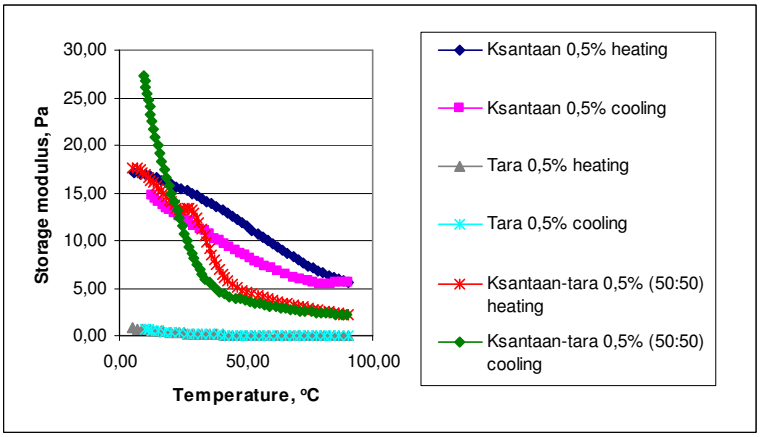


**Figure 2:** The effect of temperature on the storage modulus of xanthan´s, tara´s and their blend´s solutions

## References

1) Gunasekaran, S. and Mehmet, M (2000). Dynamic oscillatory shear testing of foods – selected applications – In: Trends in Food Science & Technology 11, pp. 115-127

2) Sworn, G. (2000). Xanthan gum – In: *Handbook of hydrocolloids* (ed. by: Phillips, G.O., Williams, P.A.) Woodhead Publishing, Cambridge, pp. 103-116

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# Quartz crystal microbalance with dissipation analysis of interactions between specific milk molecules and intestinal brush border membranes of importance for mammalian health

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## 1. Summary

Through an innovative use of the quartz crystal microbalance with dissipation technique (QCM-D), we wish to advance research on molecular interactions between specific food-derived components and the intestinal brush border membrane by continuously measuring the binding of components to brush border membrane vesicles (BBMV). By combining QCM-D with chromatographic separation of milk components, we hypothesize that novel bioactive proteins or peptides encrypted in milk proteins with potential beneficial effects on mammalian health can be identified. Preliminary results show that this approach is feasible.

Keywords: Bioactive milk components, Quartz crystal microbalance, Small intestinal brush border membrane, Mass spectroscopy

## 2. Extended Abstract

Dietary proteins may provide – in addition to their nutritional effects as a source of energy and essential amino acids - a wide range of biological and functional effects. Through actively communicating with the gastrointestinal (GI) tract, milk may target mammalian health beyond a supply of amino acids, carbohydrates and lipids. While some proteins, particularly caseins, contain an appropriate amino acid composition for growth and development of the developing newborn, a number of proteins and peptides encrypted in milk proteins have been shown to possess various biological effects: antimicrobial, disease resistance and to target certain nutrients and minerals to specific receptors[1-3]. The *in vivo* relevance of the identified interactions is, however, often questionable.

The QCM-D technique is based on the piezoelectric properties of quartz. An AC voltage applied over the gold electrodes of the quartz crystal induces oscillations at a fundamental frequency and overtones thereof which can be measured. An addition of a mechanical stress occurring when matter is deposited to the quartz crystal microbalance results in a change in the resonance frequency which can be related to a deposited mass by the sauerbrey equation[4]. Thus, it is hypothesized that a biosensor can be prepared by depositing BBMVs displaying characteristics similar to intact enterocytes[5;6] to the surface of the QCM crystal. Thereby, specific components in

foods interacting with BBMV, serving as a model of the small intestine, can be identified. We therefore hypothesize that specific components of milk interacting with the GI tract can be identified using the QCM-D technique combined with chromatographic and mass spectrometry methods.

In the present work, interactions between BBMV deposited on the gold surface of the quartz crystal and defatted colostrum are analyzed using the QCM-D technique as seen in **Figure 2**. In this way, specific components contained in defatted colostrum that bind to BBMV can be identified using a novel application of the QCM-D apparatus through separation of proteins and peptides by size exclusion chromatography coupled with the quartz crystal microbalance with dissipation apparatus (LC-QCM-D). Preliminary experiments show that this approach is feasible as seen by the correlation between eluting components and binding to brush border membrane vesicles, resulting in changes in the resonance frequencies of the quartz crystal, see **Figure 1.**

This experimental setup will used with a simulated small intestine digestion of milk/colostrum protein components using pepsin and pancreatic protease isolates to identify whether specific proteolytic fragments bind to BBMV. In addition, proteins and peptides that have been identified with our LC-QCMD setup will be characterized using mass spectrometry. With this setup, we hope to identify novel bioactive components with potential beneficial effects on human and animal health.

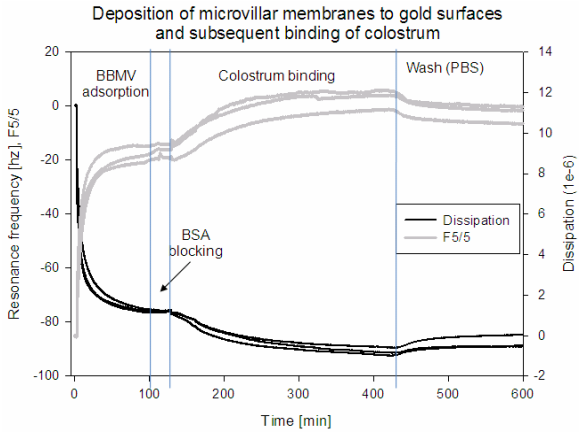


**Figure 2** Binding of colostrum to brush border membrane vesicles deposited on gold surface quartz crystals. Three replicate traces shown.

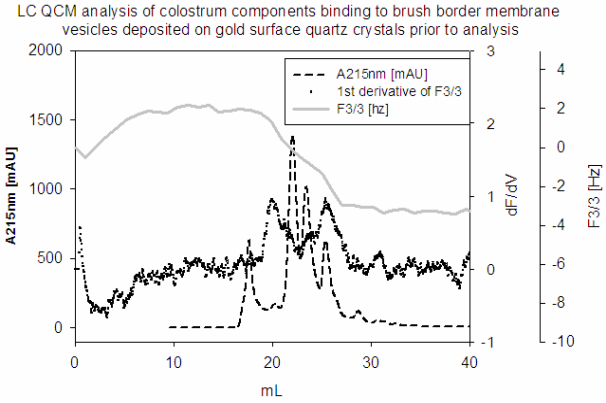


**Figure 1** LC-QCM analysis of colostrum components binding to brush border membrane vesicles deposited to crystals prior to binding experiment.

## Reference List


1. H. Kawakami and B. Lonnerdal, *Am J Physiol Gastrointest Liver Physiol* 261, G841-G846 (1991).
2. S. F. Gauthier and Y. Pouliot, *J.Dairy Sci.* 86, 78-87 (2003).
3. A. R. Jensen, J. Elnif, D. G. Burrin, P. T. Sangild, *J.Nutr.* 131, 3259-3265 (2001).
4. K. K. Kanazawa and J. G. Gordon II, *Analytical Chemistry* 57, 1770-1771 (1985).
5. M. Tosco, A. Faelli, C. Sironi, G. Gastaldi, M. N. Orsenigo, *Journal of Membrane Biology* 202, 85-95 (2004).
6. M. Knopfel, C. Smith, M. Solioz, *Biochemical and Biophysical Research Communications* 330, 645-652 (2005).

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# Direct-affinity reverse extraction (DARE) screening for bioactive food-derived peptides

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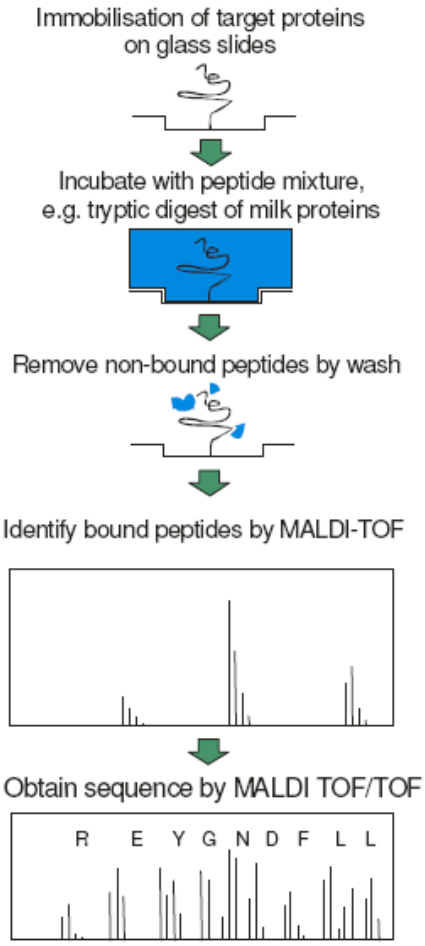
## 1. Summary

Foods of the future should provide not only appropriate nutrients and sufficient energy for human function but also contribute to the well-being and health of consumers. In order to rapidly and efficiently characterize novel foods for presence of potentially bioactive components, we propose to identify bioactive peptides by Direct Affinity Reverse Extraction (DARE), arguing that for a peptide to have a biological effect it must bind to a relevant target protein with high affinity. We have developed strategies for immobilising biotin- or polyhistidine-tagged biomolecules as targets for the DARE technology. Subsequent incubation of the target biomolecules with milk-derived peptides results in binding of putative bioactive peptides, and through selective washing we hope to identify peptides with high affinity for the target biomolecules. The peptides may then be identified directly through sequencing by MS/MS. We use biomolecules of importance for the innate immune response as targets, e.g. members of the family of Toll-like receptors, and the activity of the identified putative bioactive peptides will then be tested using a murine intestinal epithelial cell line transfected with the luciferase reporter gene under control of NF-κB. Activity of ligand-induced TLR signaling in the presence of these peptides can be simply measured as luciferase activity in cell lysates.

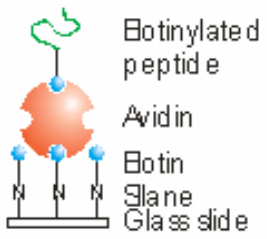



Keywords: bioactive peptides, mass spectrometry, nanoscale-purification, innate immunity, peptide-protein interactions

## 2. Extended Abstract

The DARE method utilizes purified target biomolecules to extract peptides with high binding affinity from complex food-derived hydrolysates. The method requires that i) immobilized targets do not disintegrate in the MALDI process as a result of laser-induced evaporation, ii) peptides bound to targets are released in the MALDI process either as a result of incubation with matrix or due to laser-induced evaporation, iii) immobilization of targets must not influence ability to bind ligands/peptides, iv) density of immobilized targets must be sufficiently high to allow detection of bound peptides.

Immobilization of target proteins on glass slides is a critical step. First, functional amino groups are generated on the glass using N-silane treatment that allows subsequent coupling chemistry, e.g. reaction with N-hydroxysuccinimide biotin. This generates covalently attached biotin sites that bind avidin with high affinity. Multiple binding sites for biotin on the avidin molecule then allow binding of different biotinylated target proteins. Another strategy is to introduce a nitrilotriacetic group (NTA) onto the N-silane functionalized glass slide that will bind Ni ions, generating high-affinity sites for commonly used hexahistidine-tagged recombinant proteins.




Principle for attaching target molecules to glass slides.

We initially used a model system with biotin-avidin slides to show that we can recover biotinylated peptides added to complex milk hydrolysates and that the biotinylated peptide is released from avidin under our conditions for MALDI analysis. Furthermore, the results showed that avidin does not fragment during the process and that the amount of recovered biotinylated peptide bound to the slides is sufficient for detection by MALDI-MS. Addition of internal standards will enable quantification of bound peptides.

Immobilized target proteins were then incubated with milk-derived peptides from a simulated gastrointestinal digest of milk with pepsin and Corolase ™. Low molecular weight peptides in the digests were recovered by reverse dialysis and incubated with immobilized innate immune receptors (TLR2 and -4, CD14) as well as their ligands. Both soluble TLR2 and CD14 have been detected in milk (LeBounder et al., 2003) and these potent regulators of the innate immune response or fragments thereof present in gastrointestinal digest of milk may be part of our immune response regulating the balance of pathogenic bacteria in our gut. We will present results from these experiments.

### References

LeBouder, E., Rey-Nores, J.E., Rushmere, N.K., Grigorov, M., Lawn, S.D., Affolter, M., Griffin, G.E., Ferrara, P., Schiffrin, E.J., Morgan, B.P. and Labeta, M.O. (2003) *Journal of Immunology*, 71, 6680-6689.

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# Shelf-life prediction of convenient foods by accelerated storage studies and sensory analysis

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## 1. Summary

Accelerated Shelf-Life Testing (ASLT) was used to predict the shelf life of four types of ready-to-eat vegetable foods packed in retortable plastic containers. For heat stable products, the end of shelf life is limited by changes in sensory properties that eventually, make the product unacceptable for the consumers. Product acceptability was established using sensory analysis conducted by 30-50 consumers with samples stored at different storage temperatures (4, 23, 30, 35, 40ºC) and with different storage times for each temperature. Consumer's acceptance or rejection data were analyzed by survival analysis and an Arrhenius model.

Keywords: Shelf life, Accelerated Shelf life Testing (ASLT), sensory, convenient food, prediction

## 2. Extended Abstract

Shelf-life of heat processed foods packed in plastic packaging is lower than the same product canned with an equivalent heat process. This is due to the different permeability of the packaging materials to external factors affecting product stability, primarily oxygen and light. Therefore, food companies that decide to adapt their canned products to the new packaging trends need to recalculate the shelf life of their products. For practical reasons, especially when the actual storage time is long (up to 5 years in heat-stable products), the industry resorts to accelerated test techniques to shorten the process of obtaining the experimental data.

In this study, Accelerated Shelf-Life Testing (ASLT) was used to predict the shelf life of four types of ready-to-eat vegetable foods packed in retortable plastic containers. For heat stable products, the end of shelf life is limited by changes in sensory properties that eventually, make the product unacceptable for the consumers. Therefore, product acceptability was established using sensory analysis conducted by 30-50 consumers who answered "yes" or "no" to whether they would consume samples stored at different storage temperatures (4, 23, 30, 35, 40ºC) and with

different storage times for each temperature. Additionally, they rated the overall acceptance using a 9-point hedonic scale, with 1= dislike extremely, 5= neither like nor dislike, and 9= like extremely. A staggered design was used, increasing the number of consumers as the sampling time approached the end of shelf life. End of shelf-life, at a certain temperature, was the time at which 50% of consumers found the product unacceptable. Consumer's acceptance or rejection data were analyzed by survival analysis and an Arrhenius model.

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# Texture modification in acid milk gels produced at low temperature

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## Extended Abstract

Milk is a complex biological system and it has some of the interesting properties which make it important in formulation of many delicious food products. One peculiar property of milk is that its protein's (Protein system) gets disturbed in presence of acid, heat or both. This property of milk proteins is desired in one product while it is not in another. The magnitude of such physico-chemical changes in protein system and thus in the final quality of product differs with different physical and chemical conditions and history of milk handling. Our aim in this research project was to examine changes induced by in situ acidification at temperature lower than 5°C in a model fat free milk system and to know how addition and different degree of complexation of whey proteins with colloidal system yields different types of acid milk gels.

In our study we tried to increase the protein content by addition of whey proteins to four different levels (0, 0.44, 0.88 and 1.32% by wt) corresponding to its natural level of 0.44%, followed by formation of four different protein systems based on literature reports. This includes; 1. Heating (90°C for 10 min) at pH 6.55 to produce coating of casein micelles by whey proteins. (1) 2. Heating (90°C for 10 min) at natural milk pH of 6.7 to produce Partial coating of casein micelles with whey proteins and partial formation of whey protein aggregates (1) 3. In situ formation of whey protein aggregates (>90%) by heating at (90°C for 10 min) at pH of 7.1 (2) 4. Addition of pre formed whey protein aggregates (heating of whey protein solution to 90°C for 10 min) in the heated milk system. (3)

The pH of gelation was monitored in all above systems and gelled systems were physically characterized by the textural analysis at uniform acidification time with same amount of acidulant added. Sensoric analysis was also carried out using trained panel. Findings of this study has revealed that pH and heat treatment along with addition of whey proteins to milk leads to early gelation and produces gels with more hardness (figure-1). In fact as a general rule we observed that increase in whey protein addition level by three times leads to increase in gel hardness by three times showing a clear contribution of whey protein in acid gelation of milk in these systems. However, in contrast to the aforementioned result we found some interesting observation in texture profile when whey protein aggregates were added to heated

milk at 0.44%. This system was having very different sensoric-texture profile then the remaining samples (Table-1, figure-2). Also in sensory testing gel made with 0.44% whey protein aggregates was found to be two times less clumpy than normal milk gel. Mixed protein system (as in Normal heated milk) with partial whey protein coating on casein and partial whey protein aggregates formation gives highest gel hardness, suggesting that the two gel systems may be incorporated in one another. This study shows new ways to modify texture of set yoghurt type products to meet different consumer demands without addition of non-milk ingredients.

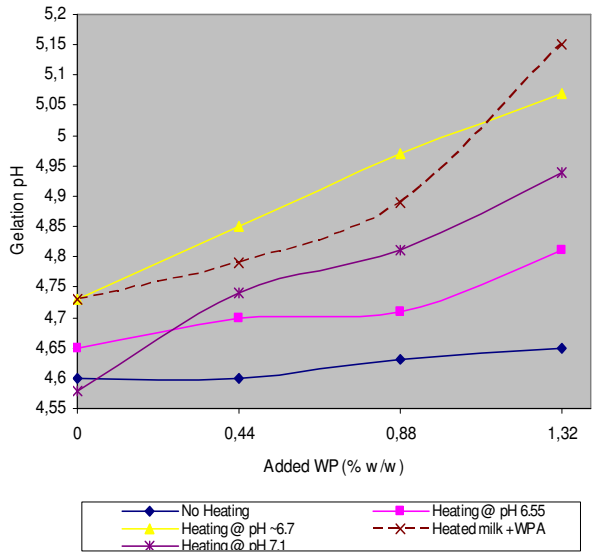


**Figure 1:** Effect of heating milk at altered pH, with & without WP addition in different forms on Gelation pH.

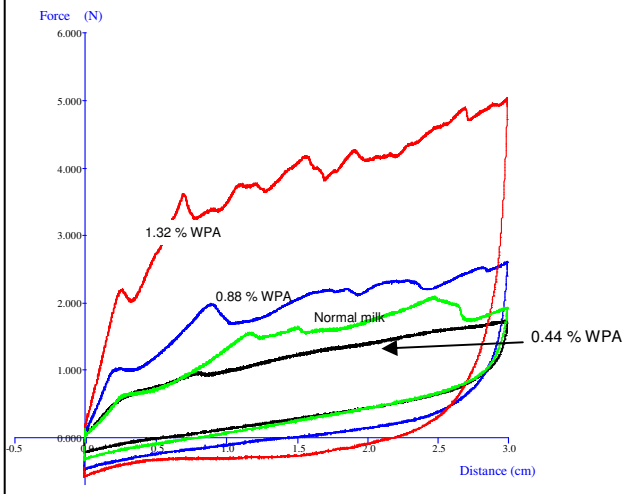


**Figure 2:** Effect of addition (increasing) of WPA in heated milk on gel texture.

| Analysis | Normal Milk gel | Gel of Milk heated at 6.55 pH with 0.44% WP | Gel of Milk heated at 6.7 pH with 0.44%WP | Gel of Milk added with 0.44% WPA | Deviation |
|---|---|---|---|---|---|
| Apparent Thickness | 5,07 | 4,13 | 5,72 | 4,84 | 0,0128 * |
| Mouth feel Thickness | 1,99 | 1,28 | 1,87 | 1,74 | 0,0249 * |
| Mouth feel Lumpiness | 1,42 | 0,89 | 2,27 | 0,69 | 0,0027 * |

*L.S.D. at 5% level of significance*
** Significant result*

**Table 1:** Sensoric evaluation results of selected acid milk gel systems.

## References

1. Vasbinder A. J., and Kruif C. G., (2003) *International Dairy Journal*, 13, 29-38
2. Anema S. G., and Li Y., (2003) *Journal of Agriculture and Food Chemistry*, 51, 1640
3. Schorsch C., Wilkins D. K., Jones M. G., and Norton I. T., (2003) *Journal of Dairy Research*, 68, 471-481

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# Rheological measurements of yolk to characterise origin and processing properties of eggs

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## 1. Summary

Egg proteins and lipoproteins are responsible of a wide range of interesting properties of many emulsions, foams, gels and other foods processed by industry. The modifications of functional properties of these proteins during food processing determine a complex behaviour that can result in structural changes of difficult prediction, and rheological measurements are a useful tool to study them. In this work, egg yolk samples coming from different breeds of laying hens, and from differently fed hens, have been analysed using steady state and dynamic tests in a rotational rheometer. The rheological tests were completed with analysis of pH, wet content and differential scanning calorimetry (DSC). The results gave valuable information about egg yolk functional and structural properties, which contributes to improve processes control and hence obtaining final products with better qualities.

Keywords: rheological properties, egg yolk, proteins, rheology.

## 2. Extended Abstract

In this work, three lines of investigation will be considered, although other several rheological applications are of interest in egg yolk characterizations.

Firstly, different hen breeds (Leghorn, Bantam and Asturian Regional hen) were characterized according to the values of their “n” (behaviour index) and “K” (consistency index) parameters. These parameters were calculated fitting to the Power Law equation the data obtained from steady state tests. As can be seen in table 1, the values of “n” and “K” are different enough to differentiate the breeds studied, so it could be possible to determinate egg yolk origin based on the value of these parameters.

| | | "n" (dimensionless) | "K" ($Pa.s^n$) |
|---|---|---|---|
| Leghorn | Average | 0.688 | 9.7 |
| | SD | 0.022 | 3.2 |
| Asturian Regional hen | Average | 0.677 | 12.6 |
| | SD | 0.015 | 3.6 |
| Bantam | Average | 0.668 | 7.8 |
| | SD | 0.029 | 3.2 |

**Table 1:** Values of "n" (dimensionless) and "K" ($Pa.s^n$) of egg yolks obtained from different hen breeds

Secondly, the evolution of egg yolk with the time was studied. The parameters "n" and "K" were calculated in the same way explained before and analyses of pH and wet content were developed in order to complete the rheological measurements. The results of the assays, shown in table 2, proved that, under controlled feeding conditions, certain modifications in egg yolk between 9 and 13 days of age can be observed. The study of these modifications would be very useful in the egg quality control.

| Egg age (days) | "n" (dimensionless) | "K" ($Pa.s^n$) | % Dry Extract | pH |
|---|---|---|---|---|
| 1 | 0.679 | 12.9 | 54.3 | 5.93 |
| 5 | 0.675 | 12.1 | 53.9 | 5.97 |
| 9 | 0.696 | 10.2 | 52.6 | 6.01 |
| 13 | 0.702 | 9.8 | 53.9 | 6.00 |
| 19 | 0.685 | 10.1 | 52.5 | 6.06 |
| 23 | 0.692 | 11.2 | 52.8 | 6.03 |
| 27 | 0.695 | 8.9 | 53.0 | 6.07 |
| 34 | 0.694 | 8.2 | 51.8 | 6.15 |

**Table 2:** Values of different yolk parameters obtained from eggs of different age coming from caged laying Leghorn hens

Thirdly, yolk coagulation kinetics were analysed using dynamic tests. These tests (specially "cure tests" whose results are reported in table 3) have shown that the way of feeding the laying hen is decisive on the yolk coagulation kinetics. This has been proved not only by rheological measurements, but also by DSC analyses.

| | G' (Pa) | G'' (Pa) |
|---|---|---|
| Control Diet | 225.7 | 188.2 |
| "Ecological" Diet | 307.4 | 207.6 |
| n-3 Fatty Acids Diet | 196.8 | 171.9 |

**Table 3:** Values of the parameters G' (storage modulus) (Pa) and G'' (loss modulus) (Pa) at the gelation point of egg yolks obtained from hens differently fed

## References

Cordobés, F., Partal, P. and Guerrero, A., (2004) *Rheol. Acta*, 43, 184-195.

Kiosseoglou, V., (2003) *Current Opinion in Colloids and Interface Science*, 8, 365-370.

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# Case-findings from Interviews with Danish Food Sector Executives on Their Responsibility in Counteracting Overweight and Obesity

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## 1. Summary

Traditional forms of representative democracy are being supplemented by "dual governance" and "self regulation" approaches in which the food sector is expected to engage in preventive measures. In order to explore the nature of this type of engagement in the Danish food sector, a study based on telephone interviews was conducted among Danish food sector stakeholders who had participated in the Danish Obesity Summit. The results show a tendency that the more the products is in focus as contributing to overweight and obesity, the more the food sector interviewees are in favour of giving the consumers the responsibility in avoiding obesity and the less active roles they see for business and government. On the other hand, companies that have taken initiatives see the roles of business and government as much more active. The findings suggest that there is a need for more research about how dual governance policy making and measures can be used to counteract unhealthy eating.

Keywords: healthy eating, self regulation, food industry, corporate nutritional responsibility, dual governance

## 2. Extended Abstract

The increasing incidence of overweight and obesity (International Obesity Task Force, 2004) has placed preventive strategies on the top of the public health nutrition agenda in many countries. Governmental authorities and institutions try to influence the behaviour of citizens towards healthier eating habits and lifestyles. But according to contemporary political science, modern "network" societies experience a development in which governments, NGOs and business interact and govern in new way to addresses important societal issues. "Policy communities" and "issue

networks" (Bogason & Zøllner, 2007) is used as terms to characterise these new policy making entities. In counteracting obesity and overweight the role of non-public actors such as industry and business organisations have increasingly been given attention and traditional representative forms of democracy seems to some extent to have been supplemented by "dual governance" (Lang, 2007), self regulation (Marsden et al, 1998) and partnerships (Mikkelsen & Trolle, 2004), where stakeholders in the food sector increasingly are engaging in private nutritional or diet related schemes. Although this kind of self-regulation is the dominant way of EU regulation in a number of adjacent areas, e.g. food safety and environmental standards, public health experts have mostly been sceptical about the role the food sector could play in promoting a healthy diet. In order to explore the nature of the engagement of food sector stakeholders in counteracting overweight and obesity, a study was conducted among Danish food sector stakeholders who had participated in the Danish Obesity Summit (www.fedetopmoede.dk). The aim was to analyse what kind of responsibility Danish food sector executives see as necessary in relation to overweight and obesity. The focus was on identification of types of ongoing initiatives and mapping of the stakeholders' views on necessary additional initiatives, including who should take such initiatives. The study was based on qualitative telephone interviews with 16 of the commercial participants among the 48 participants in the Danish Obesity Summit 2005. The results show differences in the perception of the necessary initiatives and in the practice of different stakeholders in relation to overweight and obesity. There is a tendency that the more the products, which the interview person is responsible for or representing, are in focus as contributing to overweight and obesity, the more is the person in favour of giving the consumers the responsibility in avoiding obesity and overweight and not on taking an active role as business. Furthermore, these stakeholders see the role of the government as primarily providing information and not taking more active roles like supporting product development or implementing different levels of tax on different types of food. On the other hand, companies that have taken initiatives like reducing the fat content in products or represent products that are not so much in focus as contributing to obesity, see the role of business and government as much more active. The findings show the need for more research about how dual governance policy making and measures can be used to counteract unhealthy eating.

**References**

Bogason, P., & Zøllner, M., Methods for Network Governance Research: an Introduction. In: *Methods in Democratic Network Governance*, 1-20, Houndmills, Basingstoke: Palgrave Macmillan (2007).

International Obesity Task Force (2004) http://www.iuns.org/features/obesity/obesity.htm

Lang, T., Food Wars, Keynote at the 10th congress of the Federation of European Nutrition Societies Congress. Paris 10-13 July (2007).

Marsden, T., Flynn, A , Harrison, M., (1998) *Agribusiness*, 13, 2 , 211 - 226

Mikkelsen, B.E., Trolle, E. (2004) *Scandinavian Journal of Nutrition*, 48, 2, 61-69

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# Mechanisms of selenium-dependent antioxidant properties in milk

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## 1. Summary

The effect of selenium supplementation of cows on the antioxidant properties of milk is investigated, with emphasis on radical quenching ability.

Keywords: Selenium, oxidation, milk, proteins

## 2. Abstract

Selenium is an essential trace element for humans and animals since it is necessary for a narrow range of critical enzymes and thus plays a significant role in the immune system.
Se is specifically incorporated as selenocysteine into glutathione peroxidase (a major antioxidant enzyme of cells) and other Se-enzymes. Selenomethionine (Se-Met), in contrast, is incorporated non-specifically into proteins in place of methionine.
Thus Se-Met may contribute distinct redox chemistry to proteins, since it is more easily oxidized compared to its sulfur analogue, and this may be important for the oxidative stability of milk.

Preliminary studies indicated that oxidation is altered in milk from selenium-supplemented cows. We have therefore initiated a supplementation experiment, where cows are supplemented with selenomethionine (as Se-yeast, Sel-Plex®), and our research focuses on describing the Se-dependent antioxidant mechanisms in milk.

It is well known that milk proteins exert antioxidant properties, and we want to investigate the effects of the non-specific incorporation of selenomethionine into proteins on their antioxidant properties. This requires fractionation of milk into individual components and therefore we are using size exclusion chromatography (SEC) in combination with different functional antioxidant assays.
Figure 1 shows that this approach allows us to evaluate the relative antioxidant capacity of the milk constituents, determined as the ability to quench $ABTS^{\cdot+}$.

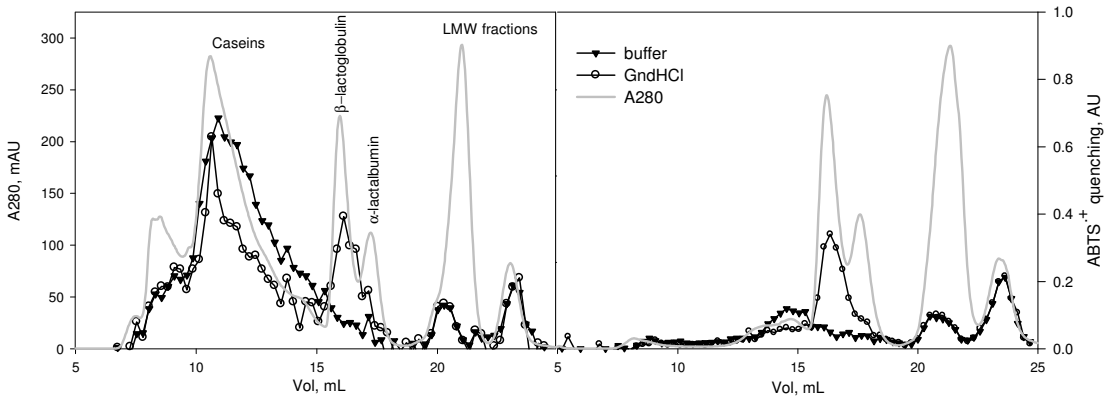


**Figure 1.** Size exclusion chromatography (Superdex 200 column, 10 mM trisHCl, pH 8) of skimmed milk and milk serum (prepared by ultracentrifugation, 100,000 g, 16 h) combined with evaluation of $ABTS^{\cdot+}$ quenching ability in buffer, ▼, or 6 M guanidinium hydrochloride (GndHCl), ○.

It is evident that the major antioxidant capacity of milk is associated with the casein fraction. In the serum fraction one antioxidant protein peak is observed in buffer, while the major serum proteins, β-lactoglobulin and α-lactalbumin, exert no antioxidant capacity. However upon denaturation with GndHCl the antioxidant capacity of β-lactoglobulin increases dramatically, with little or no effect on α-lactalbumin. This can be attributed to exposure of a buried cysteine within the β-lactoglobulin molecule.
Two low molecular weight fractions with radical quenching ability are observed. Both peaks are non-protein peaks, as determined by the Biorad assay, and as expected their quenching ability is not affected by GndHCl. The first peak is seen as a shoulder on the major A280 peak, and can therefore not be attributed to the same compound(s). In contrast the other radical quenching peak is clearly not different from the A280 peak.

So far we have not been able to detect significant differences between selenium and control milk. This may be due to instability of selenomethionine, which is easily oxidized. Our current focus is therefore on improvements of assay parameters in order to be able to detect the most labile compounds.

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# Evaluation of lipid and protein oxidation during processing and storage of fatty fish mince

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## 1. Summary

Fatty fish species have the potential to provide high nutritional value products. However during processing and storage lipid and protein oxidation occurs resulting in the degradation of nutritional and physico-chemical properties of the products. The objective of the study was to evaluate oxidative reactions that take place in both lipid and protein fractions of fish mince during processing and storage of fish products, but also to identify critical factors that can lead to deleterious quality loss during storage. Horse mackerel mince products were produced by mimicking the washing steps in the surimi production. Three products, characterised by their qualitative and quantitative differences in their lipid and protein fractions, were obtained and stored 96 hours at 5°C or 6 weeks at -10°C. Lipid and protein alterations were measured during storage.

Lipid and protein oxidation developed differently in the three products during processing and storage at 5°C or -10°C. Several factors such as, tocopherol level, iron content, concentration in lipid oxidation products, impact on the alteration of lipids and proteins during processing and storage. Lipid and protein oxidation seemed to develop simultaneously during processing and storage of fish mince but it is necessary to investigate to which extent these reactions are linked. By focusing on the development of oxidation in both the lipid and protein fractions, this study gave a new insight into oxidative reactions in fatty fish, especially in protein oxidation.

Keywords: lipid oxidation, protein oxidation, fatty fish, fish processing

## 2. Extended Abstract

The production of mince from fatty fish species has the potential to provide high nutritional value products with appreciable level of omega 3 polyunsaturated fatty acids. Unfortunately, omega 3 fatty acids are very sensitive to oxidation. Protein fraction is also sensitive to degradation during processing and storage of fish products. Compounds formed by alteration of the lipid and protein fractions have a deleterious effect on nutritional and physico-chemical properties of the products.

The objective of the study was to evaluate oxidative reactions that take place in both lipid and protein fractions of fish mince during processing and storage of fish products, but also to identify critical factors that can lead to deleterious quality loss during storage.
Horse mackerel mince- products were produced by mimicking the washing steps in the surimi production. Three products were obtained 1) horse mackerel mince, 2) mince with intermediate fat content and 3) mince with a low fat content. These products were characterised by their qualitative and quantitative differences in their lipid and protein fractions but also by their initial level of oxidation. In order to evaluate oxidative degradations during storage, the different products were stored for up to 96 hours at $+5^{o}$C and for up to 6 weeks at $-10^{o}$C. Development of primary and secondary lipid oxidation products were evaluated at regular intervals during storage. Protein modifications were determined by measuring protein solubility, carbonyl groups and free thiol groups content. In order to identify if specific proteins are oxidised during processing and storage, gel electrophoresis and western immuno-blotting against protein carbonyls groups were performed.

For both storage temperatures, levels in primary and secondary lipid oxidation compounds varied most in the first part of the storage period, 24h hours for 5°C and 2 weeks for -10°C, but remained stable thereafter. During the first part of the storage period, oxidation developed differently in the different minces while the three minces showed the same oxidation pattern during prolonged storage. The samples stored at 5°C were more heavily oxidized than samples stored at -10°C. A decrease in protein solubility was also observed in the first part of the storage period for all products and for both temperatures. Free thiol contents decreased during storage of the products indicating formation of disulfide bonds, which might be responsible for protein aggregation. Protein carbonyls groups developed during processing of the intermediate and low fat products to reach a maximum at T0. Thereafter, carbonyl content remained steady during storage at 5°C of these two products. In contrast, the mince presented low level of carbonyls at T0 but protein oxidation developed very rapidly during storage at 5°C and the carbonyl reached their maximum after 12 hours storage. Western immuno-blotting showed that high molecular weight proteins oxidized very rapidly during processing while low molecular weight proteins were not oxidized at T0. However, protein oxidation progressed during storage and low molecular weigth proteins were also oxidized in the different products after 96 hours of storage at 5°C.

Lipid and protein oxidation seemed to develop simultaneously during processing and storage of fish mince but it is necessary to investigate to which extent these reactions are linked.

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# Session S5-P-2: Innovations in Food Technology – Poster Session 2

| Abstract Number | Paper Title & Authors | Included |
|---|---|---|
| 329 | The isomerization kinetics of lactose to lactulose in the presence of sodium aluminate<br>A Hashemi , F Z Ashtiani | Yes |
| 597 | Homogenisation of dairy products at high fat content using the valve technology<br>A K Köhler, B F Aguilar, C A Hensel, D K Schubert, E H P Schuchmann | Yes |
| 647 | A New Process for Deproteinization of Chitin from Shrimp Head waste<br>M Mizani, B M Aminlari | Yes |
| 761 | Apple Juice Clarification: Identification Of Pectin Residues By Immunogold Labeling<br>V Sorrivas, D B Genovese, J E Lozano | Yes |
| 888 | Drying Of Alcohol Precipitated Pectin Gel With Low Pressure Superheated Alcohol Vapor<br>M J Urbicain, D B Genovese, J E Lozano | Yes |
| 1515 | Predicting The Moisture And Mass Loss Of Mamey Strips During Osmotic Dehydration<br>N Bracho,O Corzo, O Ramírez | Yes |
| 2530 | Effect of whey pretreatments on lactose recovery<br>Z Akbari, F Zokaee, T Ghomashchi | Yes |
| 2603 | Inactivation of microorganisms and pathogens by an alternating high pressure treatment<br>N Ebel, B Frey, J Brandmayer, A Schopper, M Herrmann, E Schlücker | Yes |
| 2933 | Investigation Of Solvent Extraction Kinetics Of Antioxidants From Grape Marc<br>G Spigno, L Tramelli, D M D Faveri | Yes |
| 2960 | A Novel Enzymatic Maceration Treatment To Enhance The Phenolic Content Of Apple Juice<br>M Pinelo, A S Meyer | No |
| 3102 | Optmization of invertase immobilization in Duolite A-568<br>L Marquez | No |
| 3576 | An experimental study of various whey proteins concentrates addition effects on some textural and physical properties of direct expanded maize extrudates<br>M Brncic, B Tripalo, S R Brncic, D Semenski, D Jezek, T Bosiljkov | No |

| | | |
|---|---|---|
| 3809 | Purine nucleosides and Cytidine uptake in Lactococcus lactis share a common transporter of the ABC type<br>J Martinussen | Yes |
| 3862 | Comparative fatty acid content of sunflower seeds of genotypes Romanian inbred lines using Artificial Neural Networks<br>D Bratfalean, D Bratfalean, MV Cristea, D F Irimie, P S Agachi | Yes |
| 3891 | Reduction of Campylobacter on Chicken Carcasses by SonoSteam® Treatment<br>D Hansen, B S Larsen | Yes |
| 3946 | Infusion pasteurization of skim milk: Effects of different time-temperature combinations<br>A B Hougaard, M Hammershøj, R Ipsena | Yes |
| 3948 | Analysis of PurR from Lactococcus lactis<br>C B Jendresen, M Kilstrup, J Martinussen | Yes |
| 3952 | Salting dynamics for anchovy (Engraulis anchoita) with salt replacers<br>R L Holgado, M A Zubiaga, I P Díez, M I Salaverri, I G Palacios | Yes |
| 3973 | Impact of type of microorganism and different intrinsic and extrinsic factors on the high-pressure carbon dioxide inactivation of microorganisms<br>L V Ginneken | No |
| 4032 | Drying of rice paddy using a microwave vacuum dryer<br>K Cheenkachorn, C Cheepsathit, E Pattala | Yes |
| 4046 | Production of Beverages and Curds made from Peas<br>G Zweytick, E Berghofer | Yes |
| 4067 | Classification of Penicillium fungi<br>L H Clemmensen, M E Hansen, B K Ersbøll | Yes |
| 4096 | Shrimp shells: A ressource<br>By Mr Jørgen Risum (Technical University of Denmark, Denmark) | No |
| 4102 | Wine Dilution Affects the Reliability of Tannin Analysis by Protein Precipitation<br>J S Jensen, H H M Werge, M Egebo, A S Meyer | Yes |
| 4105 | Investigation of lipid oxidation in freeze-dried membranes using Confocal Laser Scanning Microscopy<br>L Kurtmann | No |
| 4107 | Does extra-cellular DNA play a role in biofilm formation of Listeria monocytogenes EGDe?<br>M Harmsen<br>T Skov | Yes |
| 4145 | Textural Properties of Amylomaltase Modified Starches compared to Gelatine<br>M R Hansen (Copenhagen University, Denmark) | No |
| 4146 | Kinetics of microbial and biochemical change of chocolate during storage<br>J P Pandey | No |
| 4147 | Enzymatic synthesis of lipophilic flavonoid derivatives in organic solvents containing ionic liquids<br>Yifan Hu | Yes |

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# The isomerization kinetics of lactose to lactulose in the presence of sodium aluminate

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## 1. Summary

Lactulose (4-O-β-D-galactopyranosyl D-fructose) is a synthetic disaccharide which is synthesized by the isomerization of lactose, a widely available natural disaccharide.
In this research the isomerization kinetic of lactose to lactulose in presence of sodium aluminate as a catalyst has been investigated. The aim of this work is to introduce a suitable reaction model for isomerization of lactose to lactulose with sodium aluminate in order to find a beneficial tool to predict the trend of reaction in different conditions and to study the effect of important parameters such as pH and temperature on reaction. The experiments were carried out in three temperatures (50, 60 and 70$^{o}$ C) and two aluminate/lactose molar ratios (1 and 2). Samples were taken at different times. The contents of different sugars then were determined by using HPLC method.
The obtained data from HPLC used for finding the optimum reaction condition and calculating of rate constants. Comparing the experimental and model predicted data shows that the model is well fitted with experimental data and so could have been used in simulation and industrial purposes.

Keywords: Lactulose, Lactose, Isomerization, Kinetic

## 2. Extended Abstract

Lactulose (4-O-β-D-galactopyranosyl D-fructose) is a synthetic disaccharide synthesized by the isomerisation of lactose, a widely available natural disaccharide.
Many processes for preparing lactuolse by isomerization of lactuolse are known. One of the most important agents which could be used in this process is complexing reagents such as sodium borate and sodium aluminate.
In the first step of study the reaction model proposed for the isomerization of lactose to lactulose in presence of Sodium Aluminate. The proposed model is shown in Figure 1

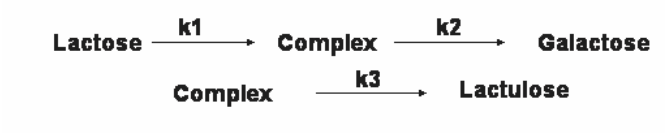


**Figure 1:** the reaction model

Solving the kinetic equation for this model leads to finding the equation for the concentration of lactose and lactulose as a function of rate constants ($k_1$, $k_2$ and $k_3$) and time. The experiments were carried out in three temperatures (50, 60 and 70$^o$ C) and two aluminate/lactose molar ratios (1 and 2).The final result shows that the model can well predict the reaction. The comparison of experimental data and model prediction for one of experiments is shown in figures 2 which CRE and CRT are the experimental and model predicted lactulose concentrations respectively. The CAE and CAT are also representing the experimental and model predicted data for lactulose.

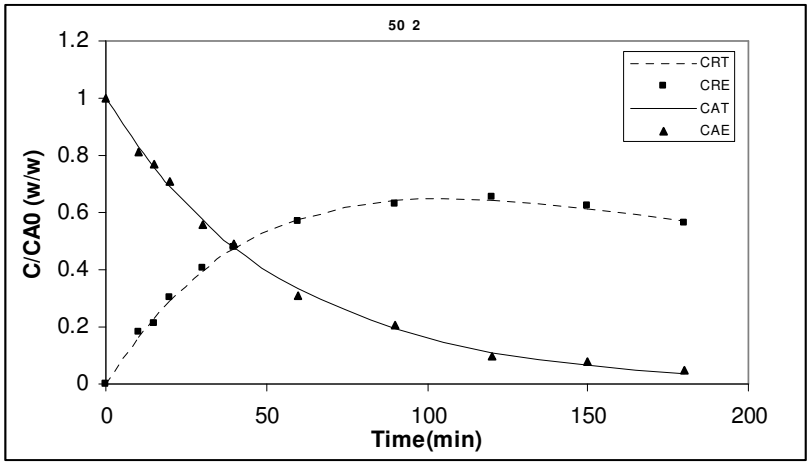


**Figure 2 :** Experimental and model predicted data for aluminate to lactose ratio =1 and T=50 $^o$C

## References

Zokaee, F., Kaghazchi, T., Zare, A., Soleimani, M.,2002, Isomerization of lactose to lactulose-study and comparison of three catalytic systems,*Process Biochemistry*,37:629-635

Nagasawa, T., Tomita, M., Tamura, Y., Obayashi, T. and Mizota, T.; Process for preparing a lactulose syrup, *U.S.Patent 3814174*, (1974).

De Haar, W.T. and Pluim, H.;Method of preparing lactulose, *European patent 0339749*,(1991).

Carobbi, R., Miletti, S. and Franci, V.; Process for preparing lactulose from lactose, in the form of a syrup or a crystalline product, *U.S.Patent4536221*, (1985).

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# Homogenisation of dairy products at high fat content using the valve technology

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## 1. Summary

High pressure homogenisation of milk and milk products is today state of the art. But it is limited by a fat concentration of 17 %. In this work we will present a mechanism to homogenise milk with fat contents up to 42 % and mix them to 3.5 % fat milk in one step. This mechanism will be explained by CFD-simulations and experiments. This new homogenisation process has the advantage that the energy input can be reduced by 60%.

Keywords: milk, high-pressure homogenisation, micro-structured system, partial homogenisation, dairy products

## 2. Extended Abstract

High pressure homogenisation is a unit operation widely used in the production of emulsions in the submicron range. In food industry it is used for the homogenisation of e.g. dairy products. An emulsion premix is pressurised to several hundred bars and then pumped through homogenising valves where droplets are disrupted (full stream homogenisation). Product properties such as colour, consistency, taste, and creaming stability can be improved by homogenisation [Kessler 1996, Schuchmann 2005]. Besides conventional flat valves (as used for dairy products) a simple orifice may also serve as homogenisation valve [Stang 1998]. Recently we investigated the homogenisation efficiency of orifice-type valves on several emulsion-based products.
In most homogenising devices droplets are not only deformed and disrupted by one single mechanism. Usually, different mechanisms affect the droplets simultaneously. In a simple orifice valve droplets are deformed in a laminar elongational flow partially superimposed by shear flow in front of the borehole, whereas behind the borehole forces in turbulent flow predominate. Furthermore, cavitation phenomena may occur.
The research was focused on the influence of parameters like the homogenisation pressure, temperature and the valve geometry on the particle size distribution of the product. Flow patterns within the homogenisation valves were determined. The influence of flow patterns on droplet deformation and disruption was investigated. Based on these results, impingement jet deflection valves were developed, and scale-

up rules were established [Aguilar 2006]. In emulsions containing slow adsorbing emulsifiers (as milk and cream), droplets formed in homogenisation tend to aggregate and coalesce. For these systems, a micro-structured system was developed in cooperation with the Forschungszentrum Karlsruhe [Schubert 1993]. This micro-structured system ('combi-valve') combines a homogenisation valve (of orifice type) with a T-shaped micro-mixer. The micro-mixer allows adding a second, emulsifier containing phase to the first one being just homogenised.
In the combi-valve only one component is high-pressure homogenised. In the case of dairy products, the first component stream is cream coming from the separator. The cream contains most of the fat globules, especially the big ones. The second component, skimmed milk, enters the valve at significantly reduced pressure. Skimmed milk contains high amounts of dairy proteins being able to adsorb at the new fat globule interfaces. Fat globule aggregation or coalescence - as it is usually found in homogenisation of cream at fat contents above 17 % - can thus be suppressed efficiently. Thus cream of a fat content up to 42 % can be efficiently homogenised. In the same time, the fat content is adjusted to the target product value. Hence, the volume to be high-pressure homogenised is significantly reduced without loosing product quality resulting in huge energy savings. In order to realise this, the second component stream has to be mixed to the high-pressure homogenised stream within milliseconds resulting in three effects: First, an intense and fast mixing is realised as the second stream enters in a region of high turbulent flow. Secondly, the intruding second component stream influences positively the homogenising effect taking place mainly after the homogenising valve by applying back-pressure and inducing additional kinetic turbulent energy [Aguilar 2004]. Thirdly, additional emulsifier molecules can be added via the second component stream directly into the zone of droplet break-up, enabling the improvement of the stabilisation of new droplets.

**References**

Aguilar, F. A.; Freudig, B.; Schuchmann, H. P.: *Herstellen von Emulsionen in Hochdruckhomogenisatoren mit modifizierten Lochblenden.* Chemie Ingenieur Technik Vol. 76 (4), **2004**, 396-399.

Aguilar, F. A.; Schuchmann, H. P.; Schubert, H.: *Scale-up verbesserter Düsen zum Hochdruckhomogenisieren und –emulgieren.* 3. Jahresbericht AiF-Projekt Nr. 13731 N **2006**.

Kessler, H. G.: *Lebensmittel- und Bioverfahrenstechnik - Molkereitechnik.* 4. Auflage, Verlag A. Kessler, München, **1996**.

Schubert, K. et al.: *Mechanische Mikrofertigung – Verfahren und Anwendungen*. 1.Statuskolloquium des Projektes Mikrosystemtechnik, KfK 5238, Karlsruhe,.**1993**.

Schuchmann, H. P.; Danner, T.: *Emulgieren: Mehr als nur Zerkleinern.* Chemie Ingenieur Technik Vol. 76 (4), **2005**, 364-375.

Stang, M.: *Zerkleinern und Stabilisieren von Tropfen beim mechanischen Emulgieren.* 527, vdi Fortschrittsberichte, VDI verlag, Düsseldorft, **1998**.

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# A new process for deproteinization of chitin from shrimp head waste

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## 1. Summary

Shrimp (*P.Semisulcatus*) head waste is an excellent source of valuable materials, containing on the dry basis of the offals about 6% chitin and 66% protein. Chitin is linked to proteins by glucosidic bonds and deproteinization process should be accomplished by using alkali digestion. It is known that severe alkali treatment results in degradation of chitin polymer chains and also reduces the quality of the protein extracts. Application of sodium sulfite, Alcalase (a commercial proteinase), Triton x-100 and combination of these reagents was investigated and the effect of each treatment on the quality of chitin was evaluated. The highest protein recovery (64%) was obtained from shrimp head waste when sodium sulfite (200mmol/l) and Alcalase (0.5%) were used and the remained chitin could be more purified by a mild alkali digestion (0.5% NaOH, 60℃, 0.5hr). The chitin produced by this two-stage process had a good quality, according to its degree of acetylation and content of residual protein.

Keywords: shrimp-head waste, chitin, alkali digestion, deproteinization, Alcalase

## 2. Extended Abstract

According to Figure1, chitin was produced by a two stage process. Eight different deproteinization process and the amount of protein recovery achieved by each process is summarized in Table1. The highest protein recovery was obtained by combination of sodium sulfite and Alcalase and the remained precipitate which was rich in chitin, was analyzed to determine its nitrogenous composition. The results showed that using chemical agents in combination with proteolytic enzyme could reduce proteinaceous fraction of precipitate, to ~3%, which is lower than the those reported by other investigators (Synowiecki, 2000).The precipitate obtained from the first stage (A+S) was treated by different alkaline process [60℃, 0.5hr, 1/10 (w/v)] with NaOH concentration varying from 0 to 2%. The results showed that increasing alkali concentration to above 0.5% did not significantly change the protein residue content and degree of acetylation (DA) in chitin. The final characteristics of purified chitin

produced under optimum condition were in the range for food grade commercial chitin (protein residue=0.870%, DA=77.670%) (Subasinghe, 1999).

**Table 1:** Protein recovery (%) obtained by using different deproteinization process[1,2]

| Treatment | Protein Recovery(%) |
|---|---|
| N | 37.0 |
| S | 48.7 |
| T | 38.5 |
| A | 45.7 |
| S+T | 53.5 |
| S+A | 64.3 |
| T+A | 39.0 |
| S+T+A | 63.2 |

[1]N= no treatment, T= Triton x-100(0.01g/kg), S= sodium sulfite (200mmol/l), A=Alcalase (12AU/Kg, i.e.0.5% v/w)
[2]The protein recovery is calculated as the percentage of the total protein content of shrimp head waste (65.8%, db)

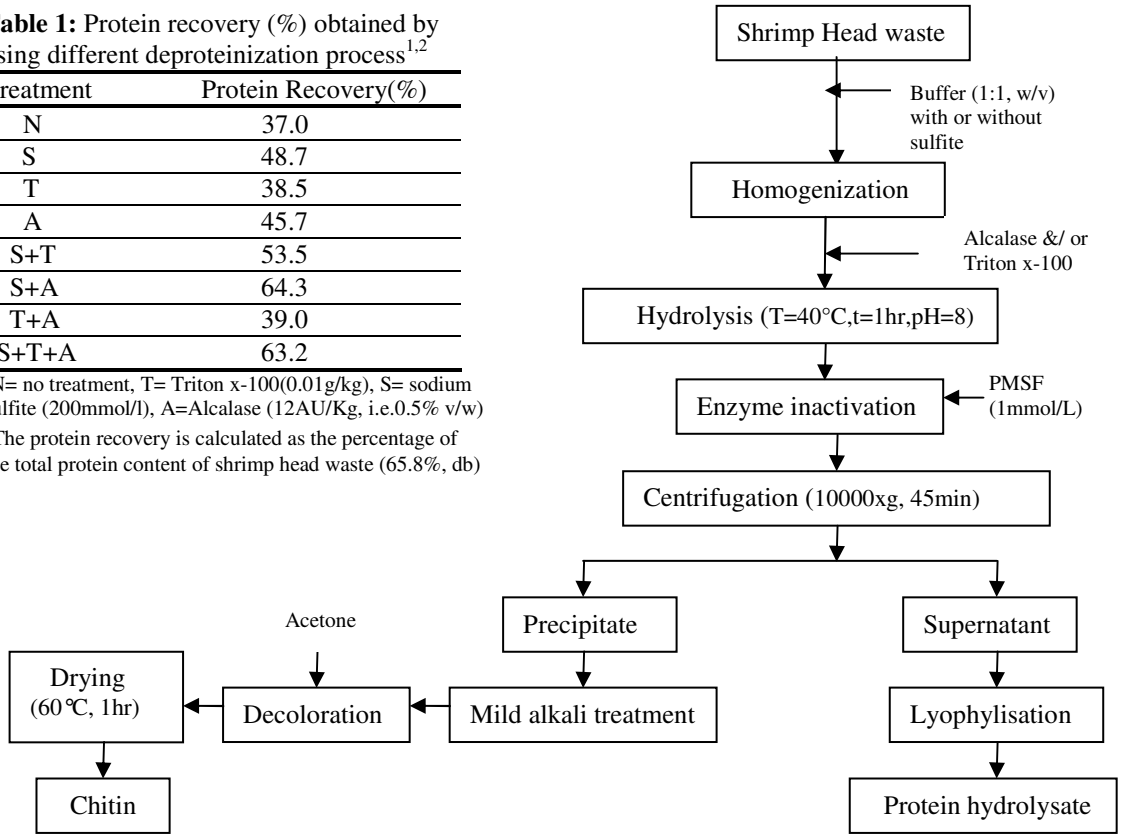


**Figure1:** The two- stage method for chitin production

## References

Subasinghe, S., (1999), *Chitin from Shellfish Waste-Health Benefits Over Shadowing Industrial Uses,* Infofish International, 3, 58-65.

Synowiecki, J. and Al-Khateeb, N.A.A.Q, (2000) *The Recovery of Protein Hydrolysate during Enzymatic Isolation of Chitin from Shrimp Cragon cragon Processing Discards*, Food Chemistry., 68,147-152.

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# Apple juice clarification: Identification of pectin residues by immunogold labeling

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## 1. Summary

To elucidate the enzymatic clarification mechanism of an apple juice, monoclonal antibodies (MAB) complexed to colloidal gold, were used to investigate the real surface structure of cloud apple juice particle. The application of these cytochemical tool shown that pectinase do not completely de-polymerize the pectin located on the particles, explaining why cloudy apple juice may remain as a stable colloid even at a Zeta potentials lower enough to assure flocculation.

**Keywords:** Apple juice, clarification, pectin, immunogold labeling

## 2. Extended Abstract

The conventional clarification process employed in industrial apple juice processing includes hydrolysis of pectin with specific enzymes (pectinases), flocculation of turbidity with clarifying agents, and filtration through plate and frame or vacuum filters. Turbidity of a cloudy apple juice (CAJ) is provided by particulate material that remains in suspension, a complex mixture of cellulose, hemicelluloses, pectins and proteins (Sorrivas et al., 2006). Particles (< 0.5μm) are retained in suspension due to mutual charge repulsion and the protective effect of pectin. Cloud particles have been modeled to consist of negatively charged pectin wrapped around a positively charged protein-carbohydrate core (Endo, 1965). This simplified model assumed that after the enzymatic breaking of pectin, positively charged proteins result partially exposed on the particle surface, promoting flocculation. However, the phenomenon of particle stabilization in cloudy juices was not completely elucidated yet (Genovese and Lozano, 2006). To better explain the apple juice clarification mechanism the participation of pectin after enzymatic treatment should be known.
Monoclonal antibodies (MABs) are excellent tools for localizing pectic components (Knox, 2005). In this work MABs specific to different pectin epitopes were used to investigate the real surface structure of CAJ particles. Five MABs (LM5, LM6, LM7, JIM5 and JIM7) kindly provided by Dr. Knox (Leeds, UK) were selected for pectin characterization. While antibodies LM5, LM6 and LM7 recognize galactan, arabinan and de-esterified polygalacturonase epitopes, respectively; antibodies JIM5 and JIM7 recognize a range of partially methyl-esterified pectin epitopes, respectively (Knox, 2005). Juice samples were incubated in grids with the *hybridoma* supernatants of the

MABs (JIM5, JIM7, LM5 and LM7) and incubated in a wet chamber with a goat anti-rat antibody (immunoglobulin G) complexed to colloidal gold (15nm). Grids were finally stained with uranyl acetate and lead citrate, dried, and observed with a JEOL 100CXII Transmission Electronic Microscope at 80kV.Observed particles obtained from an enzymatically treated CAJ showed galactan, de-esterified polygalacturonase, and un-esterified pectin epitopes on their surfaces. Fig. 1 shows, as an example, colloidal gold on an apple particle after treatment with LM7 antibody.

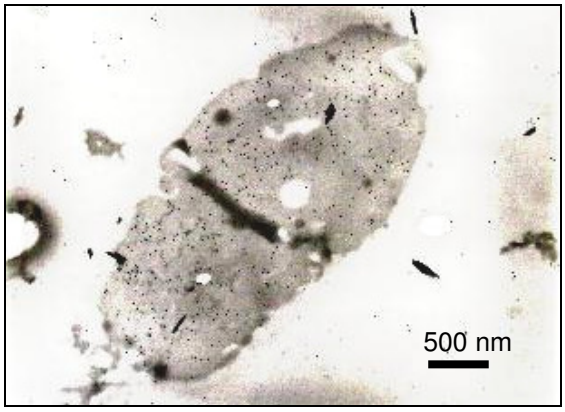


**Figure 1.** Immunogold-labeled sections of an enzymatically treated cloudy apple juice with LM7. Bar = 500 nm

Results appeared to contradict somewhat the generally accepted theory that pectinolytic treatments completely de-polymerize the apple pectin, promoting juice clarification by particle surface charge modification. As a conclusion, these cytochemical tools explained why cloudy apple juice remained as a stable colloid despite the enzymatic treatment, behavior attributed to the low effectiveness of pectinases to completely degrade pectin attached on particles.

## References

Endo A. (1965). Studies on the enzymatic clarification of apple juice. *Agriculture and Biological Chemistry*, **2**, 25-31

Genovese D.B. and Lozano J.E. (2006). Contribution of colloidal forces to the viscosity and stability of cloudy apple juice. *Food Hydrocolloids* 20:767–773

Knox P. (2005). List of Monoclonal Antibodies. http:// www.bmb.leeds.ac.uk/ staff/jpk /antibodies.htm

Sorrivas V., Genovese D.B. and Lozano J.E. (2006). Effect of pectinolytic and amylolytic enzymes on apple juice turbidity. *Journal of Food Processing and Preservation*, 30, 118-133.

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# Drying of alcohol precipitated pectin gel with low pressure superheated alcohol vapor

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Keywords: Drying, pectin, superheated vapors

## 1. Summary

Superheated ethanol vapor at moderate temperatures and pressures below the equilibrium ones was used to the recovery of pectins from the alcoholic pectin gel, obtained during the pectin extraction process of apple pomace, and compared with the conventional air drying technique. Results indicated that the proposed method renders a better product in a shorter time and offers a technology worth to be further explored.

## 2. Extended Abstract

The use of superheated steam at moderate temperatures and pressures below the equilibrium ones, as the heating agent in drying of foodstuffs instead of hot air, has been reported elsewhere by these authors among others, as a successful technique to obtain dry products of much better quality than those dried by the conventional hot air procedure (Elustondo et al., 2002a). In this work, this technique has been applied to the recovery of pectins extracted from apple pomace produced in juice concentrate manufacturing plants.

Pectins are precipitated from the water solution by successive washing with ethanol yielding a gel, saturated with a water-alcohol mixture, which requires to be dried before further processing. This drying is performed with the apparatus illustrated in Fig. 1 (Elustondo et al., 2002b) a closed chamber where the gel sample is brought into contact with an alcohol superheated vapors stream.

Vaporization of the retained alcohol is provoked by the heat transferred to the wet gel from the gas stream, at a prevailing pressure lower than the atmospheric one, such that the liquid phase boils at a relatively low temperature.

The vapors removed from the gel are mixed with the superheated vapor and recycled after being reheated again by a controlled electric resistance. An equivalent mass is simultaneously removed from the camera by a vacuum pump and condensed in a film condenser. In this particular case, the liquid obtained is almost pure ethanol.

Drying is produced in a thin boundary within the gel, as the evaporation takes place in the liquid-vapor interface, making the boundary to move inwards. The required latent vaporization heat is conducted through the dry matter, while the evaporated alcohol diffuses through it outwards.

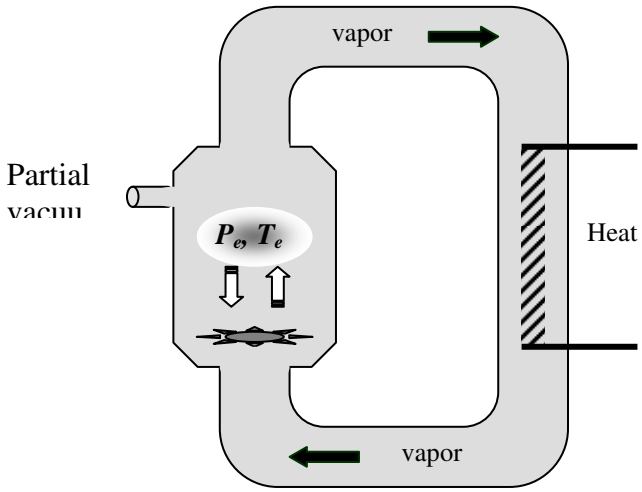


**Figure 1**: Sketch of the low pressure superheated vapor dryer.

A typical superheated alcohol temperature is in the order of 60ºC, while the wet zone remains at the temperature in equilibrium ($T_e$) with the prevailing pressure ($P_e$) (order of 40ºC), building a suitable temperatures difference for the heat transfer to take place with no risk of damaging the product.
Conventional drying was carried out on the same samples in a typical hot air drier at 60o C, and all samples were ground and screened, to obtain a dry powder with a particle size smaller than 250.
Comparative results indicated that air drying took 2 hours and the product measured 158 °USA-SAG and 85.50 ED% while those dried with superheated alcohol required only 1 hour to yield pectin with 165 °USA-SAG and 88.21 ED%.
It can be concluded that the proposed method renders a better product in a shorter time and offers a technology worth to be further explored.

## References

Elustondo D., Elustondo M. y Urbicain, M.J. (2002). Drying with Superheated Steam: Maximum drying rate as a lineal function of pressure, Chemical Engineering Journal, 86, 69-74

Elustondo, D.M Mujumdar A.S and Urbicain M. J. (2002) Optimum Operating Conditions in Drying Foodstuffs with Superheated Steam, Drying Technology 20(2), 38

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# Predicting the moisture and mass loss of mamey strips during osmotic dehydration.

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## 1. Summary

Application of the Peleg model was investigated for predicting moisture and mass loss by mamey strips during osmotic dehydration using osmotic solution at different concentrations (40-45-50 °Brix) and temperatures (40-45-50 °C). Mamey was cut into strips and the moisture content was determined by quadruplicate. Four groups with four mamey strips in each were introduced simultaneously into an osmotic solution of a given concentration and temperature. One group was removed at intervals of 1 h during 4 h. The moisture content and mass were determined. Data base was fitted to Peleg model using simple linear regression. The high regression coefficients ($R^2$ = 0.98) indicated the acceptability of Peleg model for predicting both moisture and mass loss. Models for equilibrium moisture content ($R^2$ = 83.4) and mass ($R^2$ = 78.4) as a function of brine concentration and temperature were found.

Keywords: moisture loss, Peleg model, mamey strips, osmotic dehydration

## 2. Introduction

During the osmotic dehydration, the solute and moisture concentrations, and consequently, the mass, change, and finally there will be an equilibrium state. Peleg model has been used to describe sorption processes in various foods and water desorption and sucrose absorption during osmotic dehydration of fruits. The objectives of this study were the determination of the applicability of Peleg equation in predicting moisture and mass loss of mamey strips during osmotic dehydration.

## 3. Materials and methods

3.1 Osmotic dehydration

The mamey fruits (*Mammea americana*) were peeled and cut into strips (20.1x15.0x 6.4 ± 0.2 $mm^3$). The moisture content was determined. Randomly groups with 4 strips in each were formed. Mass was determined for each strip. Four groups were introduced simultaneously into an osmotic solution of a given concentration and temperature. One group was removed at intervals of 1 h during 4 h. The moisture content and mass were determined. The temperature and concentration were 40, 45 and 350ºC and 40, 45 and 50 ºBrix, respectively. Experiments were carried out by duplicate.

3.2 Peleg model

Peleg (1988) proposed a two-parameter sorption

$$\frac{t}{X_w - X_{w0}} = K_1 - K_2\, t \qquad (1)$$

where $X_w$ is moisture content expressed as dry basis at time t, $X_{w0}$ is initial moisture content expressed as dry basis, $K_1$ is the Peleg rate constant, and $K_2$ is the Peleg capacity constant.

Similarly, for mass loss uptake the Peleg model can be also written as:

$$\frac{t}{M - M_0} = K_3 - K_4\, t \qquad (2)$$

where $M$ is mass at time t, $M_0$ is initial mass, $K_3$ is the Peleg rate constant, and $K_4$ is the Peleg capacity constant.

The Peleg capacity constant relates to equilibrium attainable mass. As t → ∞, the relations between equilibrium moisture content ($X_{we}$) and mass ($M_e$) and $K_2$ and $K_4$ are:

$$X_{we} = X_{w0} \pm \frac{1}{K_2} \qquad (3) \qquad\qquad X_{se} = X_{s0} + \frac{1}{K_4} \qquad (4)$$

## 4. Results and discussion

The coefficients of determination ($R^2$) ranged from 0.98 to 0.99 for both moisture and mass loss. The p-values for model, intercept and slope were slower than 0.0001. Such $R^2$ as p-values indicate a good fit to the experimental data.

The rate constants ranged from 4.64 to 14.73 h (kg/kg db)$^{-1}$ and from 107.79 to 178.46 h (kg/kg db)$^{-1}$ for moisture and mass loss, respectively. The effects of concentration and temperature on these rates did not show a pattern. The capacity constant ranged from 0.239 to 0.352 (kg/kg db)$^{-1}$ and from 1.69 to 4.28 (kg/kg db)$^{-1}$ for moisture and mass loss, respectively. At constant temperature the capacity constant for moisture loss increased ($p < 0.05$) with increasing concentration while that for mass loss decreased ($p < 0.05$).

The equilibrium moisture content ranged from 1.11 to 3.061 kg water/kg db and equilibrium mass ranged from 1.16 to 2.95 kg/kg db. Multiple linear regression (Table 5) fitted data of equilibrium moisture and mass as a function of brine concentration (C) and temperature (T). The models as fitted correspond to:

$$\ln X_{we} = 4{,}596 - 0{,}067\,(C) - 0{,}023\,(T)$$

$$\ln M_e = 4{,}312 - 0{,}035\,(C) - 0{,}050\,(T)$$

The models explained the 83.4 y 78.4 % of the variability in equilibrium moisture and mass.

**References**

Peleg, M. (1988). *Journal of Food Science* 53: 1216-1219.

Corzo, O. and Bracho, N. (2006). J*ournal of Food Engineering*, 75, 535-541.

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# Effect of whey pretreatments on lactose recovery

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## 1. Summary

Whey is a by-product of the dairy industry whose major components are lactose, proteins and mineral salts. Lactose recovery is an important task both in environmental and in food sciences. The recovery of lactose from whey is generally accomplished by the simple process of concentration and crystallization. Preparation various grades of lactose generally include methods for removing whey proteins and ash components prior to crystallization. Five pretreatments (acid-base, lime, Al2O3, Fecl3 and Alcl3 along with boiling), for removing whey protein and ash, are considered in this study. Whey after pretreatments was analyzed for lactose (HPLC), ash, protein (Kjeldhal Method) , Aluminium (Atomic absorbtion), Iron (Spectrophotometry) and Calsuim (Flame Photometry). Results show that best way for removal of protein is accomplished with adding HCl to change it's from pH 6 to 4, boiling 20 min, filtering protein aggregates. Also partial demineralization in whey solution for reducing ash is accomplished by adding NaOH to increase pH up 7.2 and heating solution for 20 min. After pretreatment, whey concentration was carried out in evaporator under vacuum at 62C. For lactose crystallization, a crystallizer which equipped with temperature controller and stirrer, is used. The stirring rate was fixed at 200 rpm in all experiments and temperature program is: reducing temperature from 62C to 15C during one day and remaining at 15C for two days. Results show that removal of protein along with partial demineralization would improve lactose purity up to 99% from whey.

Keywords: Whey, Protein Denaturation, Lactose, Crystallization, pretreatment.

## 2. Extended Abstract

According to the literature review about whey pretreatment, most important pretreatments which considered in this study are summarized in table 1.

| Type of pretreatment | Description |
|---|---|
| acidic-alkaline | Adding Hcl to change from pH 6 to 4 , boiling 20 min, filtering protein aggregation, adding NaOH to increase pH up 7.2, heating for 20 min. |
| Lime | Adding lime for changing to pH 6.2, boiling 10 min, filtration |
| Fecl3 | Adding 8 gr Fecl3 5% to 100 gr whey, boiling 10 min, filtration |
| Alcl3 | Adding 1 gr Alcl3 5% to 100 gr whey, boiling 10 min, filtration |
| Al2O3 | Adding 0.02 gr Al2O3 to 100 gr whey, boiling 10 min, filtration |

Table 1: Different pretreatments which were used in this study

| Type of ingredient | Initial amount | Amount of ingredient after acidic step | Amount of ingredient after alkaline step |
|---|---|---|---|
| Lactose | 4% | 4% | 4% |
| Cl | 1491ppm | 2130ppm | 2130ppm |
| Protein | 0.95% | 0.45% | 0.27% |
| Ash | 0.0559 | 0.0453 | 0.013 |
| Calcium | 400ppm | 370ppm | 162ppm |

Table 2: Whey composition after acidic and alkaline pretreatments

| Type of pretreatment | Final amount of protein |
|---|---|
| Whey without any treatment | 0.95% |
| Acidic/Alkaline | 0.27% |
| Lime | 0.41% |
| Fecl3 | 0.37% |
| Alcl3 | 0.35% |
| Al2O3 | 0.26% |

Table 3: Amount of residual protein in whey after each type of pretreatment

| Type of Pretreatment | Type of mineral | Initial amount | final amount after pretreatment |
|---|---|---|---|
| Acidic/Alkaline | Ca | 400ppm | 162ppm |
| Lime | Ca | 400ppm | 282ppm |
| Fecl3 | Fe | 352ppm | 32ppm |
| Alcl3 | Al | 68.68ppm | 6.8ppm |
| Al2O3 | Al | ---- | <4ppm |

Table 4: Amount of minerals in sample before and after each type of pretreatment

## References

Pederson, J., *Treatment of whey*, US patent: 4202902, (1980).
Vembu., *Separation of minerals from whey permeate*, US patent 5639501(1997).
Zadow, J.G., *Whey and lactose processing*, Elsevier science publishers LTD,(1992).
Harju , et al., *process of recovering lactose from whey*, US patent :4955363 ,( 1990).
Drabent,Z., Zoltowski, M., *Deproteinization of whey with iron salts in lactose manufacture*, dairy Sci. 29,930, (1966).

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# Inactivation of microorganisms and pathogens by an alternating high pressure treatment

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## 1. Summary

Keywords: Inactivation, high hydrostatic pressure, alternating pressure, microorganisms, pathogens

## 2. Extended Abstract

Static high pressure (sHP) technology has become a unique and powerful tool in life sciences. It is primarily applied in food and pharmaceutical processing. In contrast to other commonly used procedures, such as heat, radiation, magnetic or electrical fields, and ultrasound, the sHP treatment offers distinctive features in sterilising products reducing adverse effects on the quality of the products. The sHP treatment avoids Maillard browning and destruction of temperature sensitive vitamins and other biologically relevant molecules. An innovative technology for the inactivation of liquid foods and pharmaceuticals based on pressure change was developed. This technique, called dynamic high pressure (dHP), combines the advantages of the common static HP batch processes with a continuous process and thereby leads to reduced process costs.

In this work we present the viability analysis of Saccharomyces cerevisiae and Escherichia coli (K12) subjected to dHP treatment at varied pressure, duration, temperature, frequency of the pressure changes, and pressure ramping.
First results show a complete inactivation of both organisms after 5 min with pressure continuously alternating between 3 and 400 MPa. An interdependence of inactivation and number of pulses could be stated using single pressure pulses in 30 sec intervals. These basics offer new potentials towards a continuous process.

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# Investigation of solvent extraction kinetics of antioxidants from grape marc

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## 1. Summary

Extraction of phenolic compounds from red grape marc by a solvent extraction procedure (with ethanol, at 60°C up to 5h) was investigated. Aim of the research was to evaluate the effect of water addition to ethanol on recovery yield, the extraction kinetics at different stirring rate, and finally the eventual influence of extraction time on quality of extracted phenols in terms of composition (percentage of tannins on total phenols) and antioxidant power.

Keywords: antioxidants, grape, kinetics, marc, solvent extraction.

## 2. Extended Abstract

A previous research(1) had shown the feasibility of a one solvent-one-step extraction to recover phenolic compounds from grape marcs, a waste of wine-making production. The first optimisation experiments had led to solvent selection (ethanol), working temperature (60°C) and time (<5 h). In fact, ethanol, besides being a safe solvent in view of food application of the extracts, allowed for high recovery yields, while trend of phenols yield could be well described by a first order kinetics up to a 5h time. Since mixtures of alcohols and water have revealed to be more efficient than the corresponding mono-component solvent system(2), the influence of water addition on phenols yield was investigated. Furthermore, in order to optimise the process the extraction rate fort time <5h, and the influence of extraction length on extract quality (composition and antioxidant power) were also investigated.

### *2.1. Materials and methods*

Raw materials were pressed marcs by red grape. Marcs were oven dried at 60°C (final moisture 2-4%), milled (final size <=2mm), and extracted with a 4/1 (v/w) ratio solvent/sample. The liquid extract was separated by centrifugation (5350g for 5min), and freeze-dried. In the first phase of experimentation extractions at 60°C for 5h were carried out in a rotary shaker (80rpm) with absolute ethanol containing different volumes of water (from 10 to 60%). In the second phase yield and extract quality were monitored over time, varying the stirring rate (by using the rotary shaker or a magnetic stirrer). Total phenols content was determined by absorbance reading at 280nm, and expressed as gallic acid equivalents (GAE). Yield(%) was calculated as $g_{phenols}/100g_{dried\ marc\ (d.w.)}$; phenols content as $g_{phenols}/100g_{freeze\text{-}dried\ extract}$. Percentage of

tannins was estimated by the PVV method[3], and the antioxidant power (AOP) was assessed according to the ABTS assay[4]. All the trials were performed in triplicate.

*2.2. Results and discussion*

Increase of water content of ethanol was statistically influent (linear regression analysis using SPSS v.11.5, $P<0.05$) (Fig. 1). Tukey's post-hoc test confirmed that phenols yield was improved increasing the water percentage of ethanol from 10% to 30%, and, then it did not significantly change for water content between 30 and 60%. On the other hand the phenols content in the extracts increased for water content from 10 to 30%, and decreased for water content above 50% (Fig. 1(A)), in agreement with other works.[2,5]

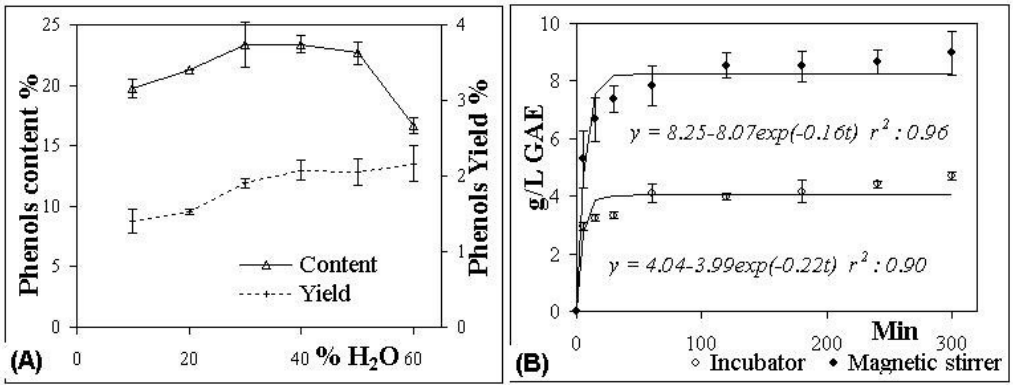


**Fig. 1:** (A) Influence of water content of ethanol on phenols yield and content. (B) Extraction kinetic curves at different stirring modalities.

Experimental data obtained by both the stirring modalities could be described accurately by the use of the characteristic function in the general case of a polydispersed anisotropic solid[6]: $C_l = A - B\exp(-Ht)$, where $C_l$ is the liquid phase concentration. The increase in stirring rate almost duplicated the phenol concentration (Fig. 1(B)). Percentage of tannins of the extracted phenols did not significantly changed with extraction time (67.9-72.8% range), such as the AOP which for all the samples showed the same trend as a function of GAE concentration.
Results could then suggest to extract phenols with a 40:60/water:ethanol mixture, for no more than 2h at 60°C and with a high mixing rate.

**References**

1. Spigno, G. and De Faveri, D.M., (2007) *Journal of Food Engineering,* 78,793-801.
2. Yilmaz, Y. and Toledo, R.T., (2006) *Journal of Food Composition and Analysis,* 19,41-44.
3. Makkar, H.P.S., Bluemmel, M., Borowy, N.K. and Becker, K., (1993) *Journal of the Science of Food and Agriculture,* 61,161-165.
4. Re, R., Pellegrini, N, Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C., (1999) *Free Radical Biology and Medicine*, 26(9/10),1231-1237.
5. Cacace, J.E. and Mazza, G., (2003) *Journal of Food Science,* 68(1),240-248.
6. Simeonov, E., Tsibranska, I. and Minchev, A., (1999) *Chemical Engineering Journal*, 73,255-259.

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# Purine nucleosides and Cytidine uptake in *Lactococcus lactis* share a common transporter of the ABC type

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## 1. Summary

In order to optimize the performance of the cheese starter *Lactococcus lactis*, knowledge of the central biochemical pathways is of crucial importance. Some of the most important biochemical pathways are those involved in nucleotide metabolism, since any growing organism needs nucleotides for the synthesis of DNA, RNA and several co-enzymes[1]. Moreover, nucleotides are acting as allosteric effectors for a large number of enzymatic reactions. Therefore, alteration of nucleotide pool sizes may have significant effects on a large number of biochemical pathways, leading to changes in cell physiology. In this work, the presence of two different high affinity nucleoside transporters is demonstrated: One system is responsible for uridine uptake and another system is responsible for the uptake of all purine nucleosides and cytidine[2]. Transposon induced mutations in the purine/cytidine transporter have been obtained, and the affected genes identified. The transporter was shown to be of the ABC type. The unusual properties of nucleoside transport are used to establish physiological conditions where nucleotide pools are altered by addition of nucleosides in different combinations. Inhibition of inosine uptake in a purine requiring strain by cytidine leads to reduction of internal purine nucleotide pools and a decreased growth rate. A similar effect is seen for inhibition of cytidine uptake in a pyrimidine requiring strain by purine nucleosides, except that pyrimidine pools were lowered.

Keywords: Lactic acid bacteria, Nucleotide metabolism, Transport, Nucleotide pool

### References

(1.) Kilstrup, M.; Hammer, K.; Ruhdal, J. P.; Martinussen, J. *FEMS Microbiol.Rev.* **2005**.

(2.) Martinussen, J.; Wadskov-Hansen, S. L.; Hammer, K. *J.Bacteriol.* **2003**, *185*, 1503-1508.

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# Comparative fatty acids content of sunflower seeds of Romanian inbred lines genotypes using Artificial Neural Networks

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## 1. Summary

Among species of oleaginous plants grown in Romania, a high-ranking positive place is held by the sunflower varieties. Sunflower seed is considered an important oilseed source crop due its high nutrition oil composition used in human or animal food and nonfood applications. Several genotypes of Romanian sunflower oil have been considered in the study of fatty acids profile content using laboratory analytical techniques. The methyl ester analysis has been made by gas-chromatography technique. Methodologies based on Artificial Neural Networks (ANNs) were observed to be the ideal tools in many problems involving property classification and prediction. A two-layer probabilistic ANN, using a radial basis layer and a competitive layer, has been used for classification. The first designed ANN has been used for classifying the category of polyunsaturated fatty acid based on acid profile content: C14:00, C15:00, C16:00, C16:01, C17:00, C18:00, C18:01, C18:02, C18:03, C20:00, C20:1 and C22:0. The three classes of polyunsaturated fatty acids are: group 1 of less than 40% polyunsaturated fatty acid, group 2 of 40%-50% polyunsaturated fatty acid, and group 3 of higher than 50% polyunsaturated fatty acid. The second designed ANN has been used for classifying the category of linoleic-acid/oleic-acid ratio in three groups: group 1 of linoleic-acid/oleic-acid ratio higher than 2, group 2 of linoleic-acid/oleic-acid ratio between 1-2 and group 3 of linoleic-acid/oleic-acid ratio less than 1. The classification results of the three groups reveal good accuracy of the trained ANNs for classifying in three classes the fatty content behavior, with no error.

Keywords: Sunflower genotypes, Fatty acids, polyunsaturated fatty acid, Oilseeds, ANN.

## 2. Methodology

A set of 15 inbred lines genotypes sunflower have been considered in the study and submitted to the following investigations: fats content, fatty acids content of fats and division of the samples according to the fatty acids content into two categories. The two considered categories are: the first category of polyunsaturated fatty acids and the second category of linoleic-acid/oleic-acid ratio. ANNs were used for separating the

samples into three classes, for each of the two categories[7]. The first classification of polyunsaturated fatty acids category was performed by the ANN for dividing the percentage of polyunsaturated fatty acids in three classes. The ANN was trained using a set of 15 input–output literature data (percentage content of: Miristic acid-C14:00, Pentadecanoic acid-C15:00, Palmitic acid-C16:00, Palmioleic acid-C16:01, Heptadecanoic-C17:00, Stearic acid-C18:00, Oleic acid-C18:01, Linoleic acid-C18:02, Linolenic C18:03, Arachidic acid-C20.0, Behenic acid-C22:00, Gadoleic acid-C20:1). A testing set 15 experimental data obtained from Romanian inbred lines was given to the ANN in order to test the ability of the trained ANN to perform the classification. The classification results, presented in Fig 1, show the three classes. The second classification of the linolenic-acid/oleic-acid ratio, was also based on training using a set of 15 input literature data. Testing was performed on the set of 15 experimental data obtained from Romanian inbred lines, in order to test the ability of the trained ANN for classifying Romanian inbred lines data, Fig 2. For both categories, the classification revealed a perfect fit with the classes obtained by the experimental results.

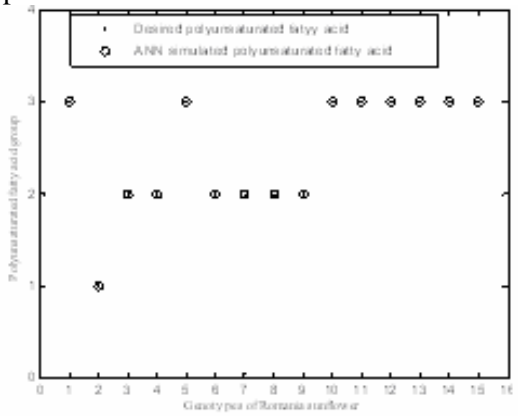


**Figure 1**: Classification results of polyunsaturated fatty acids content for the Romanian sunflower oil genotypes testing set of data

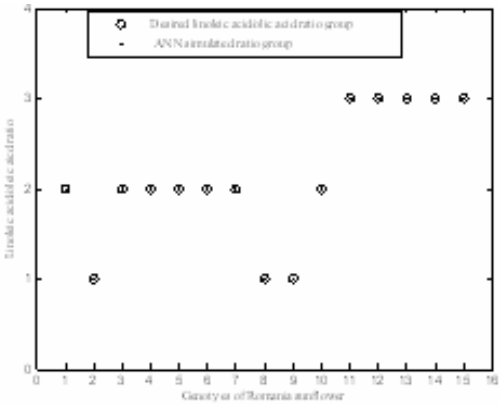


**Figure 2:** Classification results of linolec-acid/oleic-acid ratio for the Romanian sunflower genotypes testing set of data

## References

1. J.L. Luque-Garcya, M.D. Luque de Castro (2004) *Journal of Chromatography A, 1034 237–242*
2. Dorina Bratfaleana, Mircea Vasile Cristea, Paul Serban Agachi, Dan Florin Irimie, Ahmad Sarrafi, Michel Petitprez, (*2007) 17th European Symposium on Computer Aided Process Engineering – ESCAPE17*
3. Darinka Brodnjak-Voncina, Zdenka Cencic Kodbab, Marjana Novic (2005) *Chemometrics and Intelligent Laboratory Systems 75, 31– 43*
4. Mohammad Izadifar (2005) *Journal of Food Engineering 66 227–232*
5. M.V. Cristea, S. Varvara, L. Muresan and I.C. Popescu, (2003) *Indian Journal of Chemistry, 42A 764.*
6. Matlab, Neural Networks Toolbox, *MathWorks*, (2006)

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# Reduction of *Campylobacter* on chicken carcasses by SonoSteam® treatment

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## 1. Summary

*Campylobacter* infection is an increasing health problem and poultry is the most common source. SonoSteam® is a new technology for decontamination of surfaces by the use of steam and ultrasound combined, and the method was tested on freshly slaughtered chickens in an off-line pilot plant. Groups of 30 carcasses were included; one that was treated with SonoSteam® (sound-level of 160 dB and frequency of 20-25 kHz; steam at max. output of 100kg/h and temp. of 100° C) and another represented the references. All the carcasses were placed individually in sterile, airtight bags and analysed for the level of *Campylobacter* on the surface before and after SonoSteam® treatment. The results showed an average reduction of 2.51 $\log_{10}$ units (CFU/ml) and no visual changes of the chicken carcasses, which is as good as or better than currently known decontamination methods.

Keywords: Campylobacter; surface-decontamination, chicken, ultrasound, steam

## 2. Extended Abstract

### *2.1. Introduction*

Contaminated food or water as well as poor cooking and kitchen hygiene are the typical causes of human *Campylobacter* infections (DVFA 2006). It is an increasing health problem and since poultry is the most common source, intervention programmes have been initiated to help eliminate the occurrence of *Campylobacter* on fresh poultry products (Hansson 2005, Wieland 2006). Attempts to decontaminate the surface of fresh meat by various methods have so far not been sufficiently effective. SonoSteam® is a new technology for decontamination of surfaces by the use of steam and ultrasound combined, and the method was tested on freshly slaughtered chickens in an off-line pilot plant, which was designed for the present investigations.

### *2.2. Methods*

Groups of 30 carcasses were included; one that was treated with SonoSteam® (sound-level of 160 dB and frequency of 20-25 kHz; steam at max. output of 100kg/h and temp. of 100° C) and another represented the references. All the carcasses were

placed individually in sterile, airtight bags and analysed for the level of *Campylobacter* on the surface before and after SonoSteam® treatment.

*2.3. Results and conclusion*

The effect on the level of *Campylobacter* on the surface of chicken carcasses by treatment with a combination of steam and ultrasound (SonoSteam®) was investigated in this experiment. The two trials showed a significant decrease in the level of *Campylobacter* from 3.60 and 4.43 $\log_{10}$ units (CFU/ml) to 1.67 and 1.34 $\log_{10}$ units (CFU/ml), respectively.

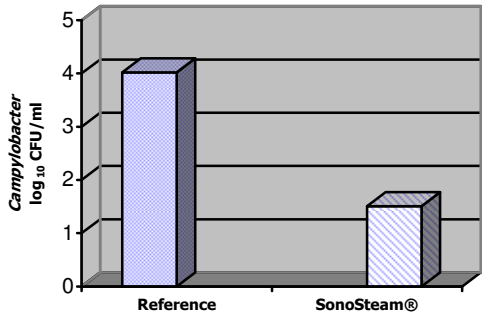


**Figure 1** Concentration of *Campylobacter* on chicken carcasses treated with a combination of steam and ultrasound (SonoSteam®) for maximum 10 seconds as well as untreated references.

The results showed an average reduction of 2.51 $\log_{10}$ units (CFU/ml) and no visual organoleptic changes of the chicken carcasses. This is as good as or better than currently known decontamination methods (Morgan 1996, Avens 2002). A SonoSteam® In-line system is being developed and will be tested in the near future.

## References

Avens, J. S., Albright, S. N., Morton, A. S., Prewitt, B. E., Kendall, P. A. and Sofos, J. N., (2002) *Food Control*, 13,445–450.

Danish Veterinary and Food Administration (DVFA), *Dansk særstatus og nye initiativer for Salmonella og Campylobacter i dansk og importeret kød og æg*, Bonzai Publisher, DK (2006).

Hansson, I., Ederoth, M., Andersson, L., Vagsholm, I. and Olsson Engvall, E., (2005) *Journal of Applied Microbiology*, 99,1149–1157.

Morgan, A. I., Radewonuk, E. R. and Scullen, O. J., (1996) *Journal of Food Science*, 61,1216–1218.

Wieland, B., Sandberg, M., Johannessen, G.S, Bohlin, J., Hofshagen, M. and Cudjoe, K.S., (2006) *Journal of Applied Microbiology*, 101,1027–1032.

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# Infusion pasteurization of skim milk: Effects of different time-temperature combinations.

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## 1. Summary

Infusion pasteurization technology was used in different time-temperature combinations for heat treatment of skim milk and compared to untreated skim milk and a standard pasteurization treatment. Aerobic count of microorganisms and activity of alkaline phosphatase showed that all infusion-pasteurized samples had received proper pasteurization. There were no difference in the size of casein micelles, but differences were seen in activity of the enzyme xanthine oxidase. The results indicate possible differences in properties of infusion-pasteurized skim milk compared to standard pasteurized skim milk.

Keywords: Skim milk, infusion pasteurization, aerobic count, enzymatic activity

## 2. Extended Abstract

Milk is normally pasteurized in order to obtain a product that is safe for the consumer and has an extended shelf life as compared to raw milk. The heat treatment does not only inactivate indigenous milk enzymes and unwanted microorganisms but also affects the chemical and physical attributes of the milk. Thereby the functional properties of the milk are changed and can be adversely influenced (Walstra et al., 1999). Therefore, it is of interest to develop and apply novel technologies for pasteurization of milk that result in less deterioration of the functional properties. One such technology is infusion pasteurization where very short heating and cooling times may ensure less damage to the natural attributes of milk along with the necessary inactivation rates of microorganisms and fulfil the regulatory criteria of enzymatic inactivation of alkaline phosphatase set to ensure adequate pasteurization.
Experiments were performed on skim milk that had only been heated to ~60°C in order to separate it from the milk fat at a commercial dairy. Infusion pasteurization was performed with three different holding times (0.1s, 0.2s and 0.7s), and for each holding time samples were drawn at five different temperatures (85°C, 90°C, 100°C, 110°C and 120°C). In the analyses the infusion-pasteurized samples were compared to the untreated skim milk as the control treatment and a standard pasteurization at 72°C for 15 seconds in a plate heat exchanger as the reference treatment.
Analysis of the total aerobic count at 30°C revealed that all of the time-temperature combinations used for the infusion pasteurization resulted in lower aerobic counts as compared to the standard pasteurization. There were no significant differences in the

aerobic counts between the infusion-pasteurized samples. Microscopy and testing of gram reaction for some characteristic colonies showed as expected that the microflora after heat treatment consisted of gram positive bacteria including spore formers. The aerobic counts were very low for all of the infusion-pasteurized samples, although tendencies of decreasing aerobic count with increasing intensity of heat treatment were seen. An explanation for the in general low level of aerobic counts, which made it impossible to distinguish the different infusion pasteurization treatments, may be that the raw milk was of very high quality and that the separation process had lowered the number of microorganisms present in the skim milk even further.

Analysis of the particle size (i.e. the size of the casein micelles) in the samples using a dynamic light scattering technique showed no significant differences among the infusion-pasteurized samples or in comparison with the untreated and reference samples. If the heat treatments cause denaturation of substantial amounts of whey protein these would be expected to react with κ-casein in the casein micelles and thus make the casein micelles increase in size (Anema & Li, 2003).

The results on enzymatic activity of alkaline phosphatase showed that all heat treated samples met the inactivation criteria for adequate pasteurization. The enzyme is relatively heat sensitive with a thermal resistance only slightly higher than those of non-spore forming pathogens that could be present in milk (Farkye & Imafidon, 1995). The activity level of alkaline phosphatase in the untreated milk was relatively low, which also indicates an effect of the heating to ~60°C during skim milk production. There were no significant differences between the reference and the infusion-pasteurized samples regarding the level of alkaline phosphatase inactivation.

Another important enzyme system in milk is the oxidation of hypoxanthine to uric acid via xanthine catalysed by xanthine oxidase. The enzyme is a part of the milk fat globule membrane and is released during handling of the milk. Xanthine oxidase is relatively heat stable and usually unaffected by standard pasteurization, which was confirmed in the present study. However, the infusion pasteurization temperature significantly ($P<0.001$) affected the xanthine oxidase activity regardless of holding time. Xanthine oxidase units were reduced to 69% and 58% at infusion temperatures of 85°C and 120°C, respectively, compared to the level in unheated milk.

In conclusion, the infusion technology ensures proper pasteurization of skim milk regarding microbiology and alkaline phosphatase activity criteria. Our results indicate that infusion-pasteurized skim milk could have properties differing from of those of milk pasteurized by use of a plate heat exchanger.

**References**

Anema, S.G. and Li, Y., (2003) *Journal of Dairy Research*, 70, 73-83

Farkye, N.Y. and Imafidon, G.I. (1995). Thermal denaturation of indigenous milk enzymes. In: *Heat-induced changes in milk*, 2nd edition. Ed. Fox, P.F., International Dairy Federation, Brussels, pp. 331-348.

Walstra, P., Geurts, T.J., Noomen, A., Jellema, A. and van Boekel, M.A.J.S., (1999). Heat Treatment, In*: Dairy Technology – Principles of Milk Properties and Processes*, Marcel Dekker Inc., New York, USA, pp. 189-239

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# Analysis of PurR from Lactococcus lactis

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## 1. Summary

Purine nucleotides constitute an essential part of every cell as building blocks of nucleic acids and cofactors, for energizing intermediates in numerous cellular processes, and as carrier of chemical energy between biological reactions. In the Gram-positive lactic acid bacterium *Lactococcus lactis*, purines can be salvaged from the environment or synthesized de novo. The biosynthetic genes are under transcriptional regulation by the regulatory protein PurR, which responds in a feed-forward manner to the starting molecule for salvage and biosynthesis, 5-phosphoribosyl-1-pyrophosphate (PRPP). Lactococcal PurR is homologous to the PurR protein in the Gram-positive model organism *Bacillus subtilis*. Although PurR works as a repressor in *B. subtilis*, the homolog in *Lactococcus lactis* was proved to act as an activator. Lactococcal PurR activates promoters with a conserved distance between the minus ten region and a "PurBox" motif with a consensus sequence of AWWWCCGAACWWT. Here we present results of expression of lactococcal PurR heterologously in *Escherichia coli* and purification. We examined the stoichiometry and DNA binding activity, and the effect of the inducer PRPP on DNA binding. While PRPP causes the *B. subtilis* repressor to relief DNA binding, the effect of PRPP on the lactococcal activator was found to be far smaller. Additionally, we used an *in vitro* footprint (DNase I protection) to confirm the PurBox motif as a true binding site of PurR. The *in vitro* results are discussed together with genetic evidence, and a combined model for PurR mediated regulation is presented.

Keywords: purine biosynthesis, PurR, DNA binding, protein purification, transcription regulation

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# Salting dynamics for anchovy (*Engraulis anchoita*) with salt replacers

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## 1. Summary

The salted anchovy (*Engraulis anchoita*) is a traditional heavy-salted food product in the Mediterranean countries with high nutritional value that contains approximately between 14 and 15 % of NaCl. However, changes in consumer life style motivate the tendency to reduce the amount of sodium chloride in food. The aim of the study was to compare and analyze the influence of salt replacers as a method to reduce the sodium content in salted anchovy. The texture and sensory properties and the penetration grade of sodium were studied during the ripening period. The results obtained showed that is it possible to develop a curing process with salts replacers for anchovy obtaining a reduction of 30-35% in the sodium content. From a sensorial and textural point of view, it is also possible to reduce the sodium content and obtain a product acceptable for consumers.

Keywords: anchovy, sodium content reduction, ripening, salt replacers.

## 2. Extended Abstract

The salted anchovy (*Engraulis anchoita*) is a traditional heavy-salted food product in the Mediterranean countries with high nutritional value that contains approximately between 14 and 15 % of NaCl. However, changes in consumer life style, who are concerned about the harmful effects that a high level of sodium chloride in their diet could have, motivate the tendency to reduce the amount of sodium in food. Different methods can be used for obtaining a reduction of sodium content in foods such as the use of salt substitutes in combination with masking agents, the use of flavour enhancers and the optimisation of the physical form of salt (Phelps and al, 2006).

The aim of the study was to compare and analyze the influence of salt replacers against NaCl during the curing period of anchovy in the textural and sensory properties and the penetration grade of sodium.
The salting experiments were carried out at 5ºC for 135 days, which is considered the time required in order to obtain commercial salted anchovy. Two different commercial salt replacers were used “Morton Salt” and “Costa Salt” against NaCl as control to study the penetration of Sodium in the decapitated and gutted anchovies

(*Engraulis anchoita*). Overpressure was applied during the salting process as in the traditional one.

The chloride content was determined using the Volhard method. A Perkin-elmer model 5100 atomic absorption spectrophotometer was used to determinate Sodium and Potassium content. Organoleptical analysis was realized by a selected and trained panel in fish products (Fisinger and al, 1982).

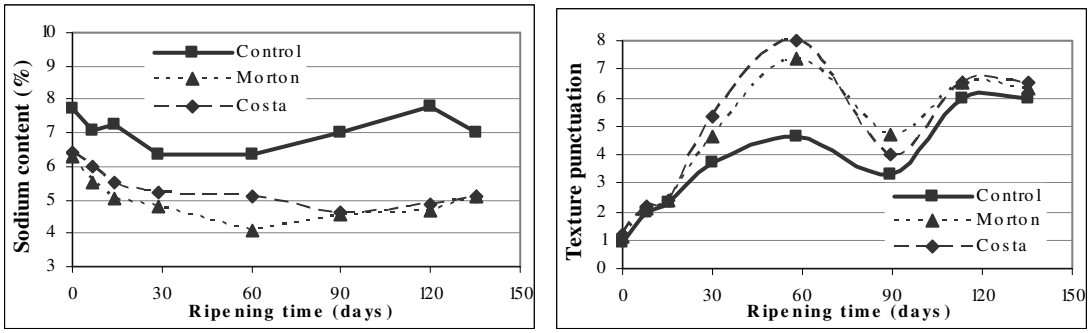


**Figure 1:** Sodium content (%) and Texture punctuation by an expert panel along ripening time of anchovies using Morton and Costa Salt as salt replacers.

Along the ripening process of anchovies with salt replacers, the penetration grade of sodium in them was lower in case of using Morton and Costa salt, being 30% less in sodium content at the end of the study. The main difference between salt replacers and the control was their high content in Potassium chloride.

In case of organoleptical parameters and more specifically with the texture parameter, at 60 days, it was observed an over ripened state of anchovies (8 points) when salts replacers were used (Fisinger and al, 1982). However, at the end of curing process, all of them showed the same textural properties and good panel acceptance.

These results indicate that it is possible to develop a ripening process with salts replacers for anchovy obtaining a reduction of 30-35% in the sodium content comparing with the traditional product. From a sensorial and textural point of view, it is also possible to reduce de sodium content and obtain a product acceptable for consumers.

**References**

Fisinger, B., Barassi, C.A., Lupin H.M. and Trucco, R.E. (1982) An Objective index for the evaluation of the ripening of salted anchovy. Journal of Food Technology, 17: 193-200.

Phelps, T., Angus, F., Clegg, S., Kilcast, D., Narain, C. and Ridder, C. (2006) Sensory issues in salt reduction. *Food Quality and Preference*, 17 (7-8), Pages 633-634.

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# Drying of paddy using a microwave vacuum dryer

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## 1. Summary

Drying of paddy is one of the important processes for the rice millers to produce good quality rice. A conventional drying process is done using hot air which results in time consuming and energy loss. In the present work, Pathumthani fragrant rice paddy was dried using a microwave-vacuum dryer. The drying experiments were carried out by varying two parameters affecting the drying performance including the microwave powers and the pulsation periods. The microwave powers varied from 170 to 500 watts while the pulsation periods varied from 30 to 120 seconds. Moisture contents in the paddy were observed for different drying conditions. The optimum operating condition was also investigated. The properties of milled rice before and after cooking were determined including percents of whole kernels, brokens, elongation, and gelatinization temperature. The results showed that the removal rate of moisture in paddy decreased as the microwave power increased. The higher microwave power resulted in an increased portion of brokens. The optimum conditions for decreasing the moisture contents of the paddy were found at the pressure of 40 kPa, the microwave power of were 245 Watt, and pulsation period of 30/60 seconds/seconds. The properties of rice from microwave vacuum dryer and the conventional process are insignificantly different.

Keywords: microwave, vacuum drying, rice paddy, drying

## 2. Extended Abstract

Rice is one of important crops of the world. Thailand is one of the biggest worldwide rice exporters with about 7 million tons of milled rice in 2006. After harvesting, paddy must be dried to prolong storage time and prevent mould growth. In this work, paddy was dried using a microwave-vacuum drying technique. The effects of microwave incident powers and pulsation period on percents of moisture, head rice, and rice brokens are shown in Table 1 and Figures 1-2.

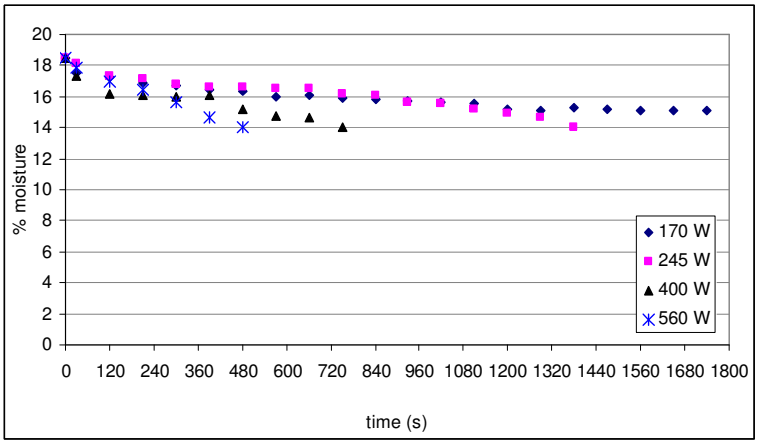


**Figure 1** Effects of microwave incident powers with a pulsation period of 30/60

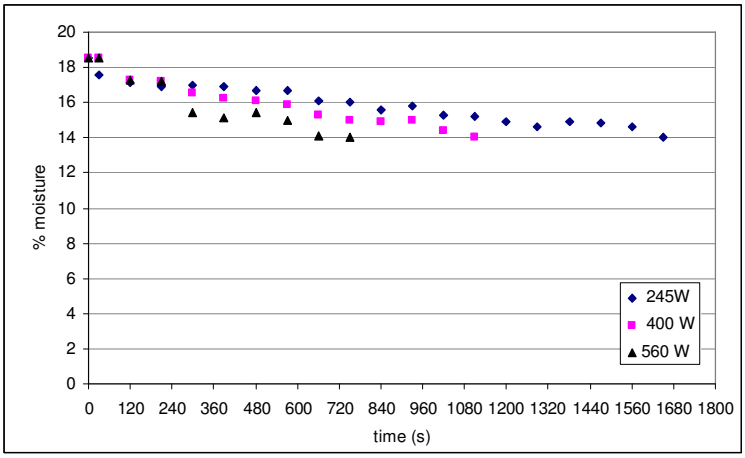


**Figure 2** Effects of microwave incident powers with a pulsation period of 30/120

**Table 1** Effects of microwave power and pulsation period on milled rice

| | Weight (g) | | | | | |
|---|---|---|---|---|---|---|
| | Pulsation period 30/60 | | | | Pulsation period 30/120 | |
| | 170W | 245W | 400W | 560W | 245W | 400W |
| Rice paddy | 300.0 | 300.0 | 300.0 | 300.0 | 292.5 | 300.0 |
| Milled rice | 196.6 | 192.5 | 192.5 | 193.8 | 184.5 | 194.0 |
| Head rice | 127.9 | 124.5 | 100.0 | 46.2 | 109.0 | 113.0 |
| Rice Broken | 69.3 | 68.0 | 92.5 | 147.6 | 75.5 | 81.0 |
| Head rice/rice Broken | 1.8 | 1.8 | 1.1 | 0.3 | 1.4 | 1.4 |
| % Broken | 35.1 | 35.3 | 48.1 | 76.2 | 40.9 | 41.8 |

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# Production of Beverages and Curds made from Peas

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## 1. Summary

Although legume seeds, such as peas, beans, and lupines, and products made from these materials offer several advantages for human nutrition, their consumption is decreasing in European countries. Legumes are a good source of dietary fibres, vitamins, minerals, and protein of high value and due to their high content of carbohydrates and protein they are suitable raw materials for the production of beverages and tofu-like curds. Applying a hot mash process coarsely crushed peas were suspended in water enriched with heat-stable amylases, heated up to 95 °C for 15 min. After cooling down to 65 °C the starch was saccharified by addition of amyloglucosidases. The solid particles were decanted and the resulting beverage was pasteurized at 95 °C. The influence of the water to pea ratio, the saccharification time, and the rotational speed difference during the decantation process on colour, sugar content, viscosity and sensory properties were investigated. The obtained beverage was then used as base for the production of a tofu-like curd. The influence of the concentration of the precipitation salt, the precipitation temperature, and the forming pressure on the responses texture, colour, and sensory properties were analysed.

Keywords: legumes, peas, beverages, curds

## 2. Extended Abstract

### *2.1. Production of Legume Based Beverages*

For the production of the milk-like beverage the peas were coarsly crushed and suspended in water of 75 °C, enriched with heat-stable alpha-amylases. The resulting suspension was heated up to 95 °C and kept at this temperature for 15 minutes. Then the mash was cooled down to 65 °C and ground in a cutter with a gap of 0.05 mm. The starch of the suspension was then saccharified by amyloglucosidase for one to three hours. Finally the solid particles were separated and the resulting tasty and sweetish beverage was pasteurized for five minutes at temperatures of more than 95 °C. For further optimization of the process the influences of the raw material to water ratio (1:10; 1:4), the saccharification time (15/65 min) and the rotational speed

difference during the decantation process (10/90 min-1) were investigated following a $2^3$-factorial statistical design. Due to the chlorophyll in the peas the colour of the produced beverage was light green. Highest saccharification, and thus, most palatable taste, was obtained at a pulses:water ratio of 1:10. After one hour of saccharification no increase in sweetness could be observed. The calculated results of the statistical design for the optimization of the produced drinks are shown in Table 1. In sensory tests especially the sweetish taste was judged as good and the green colour was assessed as interesting and innovative.

| | p-value dry matter | p-value protein | p-value starch | p-value glucose |
|---|---|---|---|---|
| **A:ratio: raw material to water** | 0,0011 | 0,0167 | 0,3406 | 0,0168 |
| **B:saccharification time** | 0,6029 | 0,9478 | 0,0793 | 0,0043 |
| **C:rotational speed difference** | 0,7546 | 0,7725 | 0,6246 | 0,4776 |
| **AB** | 0,9848 | 0,1456 | 0,2854 | 0,0109 |
| **AC** | 0,8976 | 0,8669 | 0,9744 | 0,5435 |
| **BC** | 0,9646 | 0,742 | 0,1331 | 0,7649 |
| **R-squared** | 94,60% | 82,72% | 74,97% | 94,66% |

Table 1: ANOVA for the responses: dry matter, protein, starch and glucose

### *2.2. Production of Curds*

The resulted beverage was also found to be suitable for the production of tofu-like curds. Pea milk as intermediate material for curd production requires a high protein and dry weight content, which was achieved at low water ratios. Curd made from peas had a very firm and rubber-like texture. Applying a $2^3$-factorial statistical design for curd optimization, varying the parameters precipitation salt (Nigari), precipitation temperature and forming pressure, all three parameters correlated positively, where 2.5 %(v/v) of Nigari, 95 °C and a forming pressure of 120 kg/m² were found as optimal conditions for the production of a curd with high dry matter and protein content, and thus, good texture. The calculated results of the statistical design for the optimization of the produced curds are shown in Table 2. Due to sensory and physical properties like texture and colour, curd made from peas was found as a very interesting and appealing alternative to already well known products.

| | p-value dry matter | p-value protein | p-value starch | p-value texture |
|---|---|---|---|---|
| **A:concentration of Nigari** | 0,2598 | 0,3984 | 0,8029 | 0,0666 |
| **B:temperature of precipitation** | 0,1067 | 0,2670 | 0,0610 | 0,0585 |
| **C:pressure** | 0,5955 | 0,6835 | 0,8850 | 0,2070 |
| **AB** | 0,3576 | 0,1566 | 0,2718 | 0,1110 |
| **AC** | 0,1635 | 0,1985 | 0,1173 | 0,2923 |
| **BC** | 0,9228 | 0,5679 | 0,3758 | 0,1555 |
| **R-squared** | 72,12% | 68,09% | 76,95% | 85,76% |

Table 2: ANOVA for the responses: dry matter, protein, starch and texture

## References

Bisby, F.A., Buckingham, J., and Harborne, J.B., *Phytochemical Dictionary of the Leguminosae*, Chapman & Hall, UK (1994).

Bremer, E., (1991) Process for the production of lupin milk containing protein, and process for the further treatment of this product and tofu-like foodstuff obtained thereby, EP044936.

Elmadfa, I., Freisling, H., and König, J., Österr. Ernährungsbericht 2003, Austria (2003).

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# Wine dilution affects the reliability of tannin analysis by protein precipitation

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## 1. Summary

Tannins in wine are a very diverse group of polyphenolic compounds that are defined by their ability to precipitate with proteins. A reported analytical method for tannin quantification relies on selective precipitation of tannins with bovine serum albumin. To test the reliability of tannin analysis by protein precipitation on wines having highly variable tannin levels we have measured the tannin content of various dilutions of five commercial red wines. The tannin contents of both very diluted and concentrated samples were systematically underestimated, which could be explained by a precipitation threshold and insufficient protein for precipitation, respectively. Based on these findings we have defined a valid range of the tannin response in the protein precipitation-tannin assay, which suffers minimally from these problems.

Keywords: wine, tannin analysis, protein precipitation

## 2. Extended Abstract

Tannins play an important role in the mouthfeel properties and colour stability of red wines and are therefore related with wine quality. Reliable quantitative analysis of wine tannins is challenged by the chemical diversity of tannins. The purpose of this study is to investigate the influence of dilution degree on the reliability of tannin analysis by protein precipitation. The method relies on that tannins can be separated by precipitation with bovine serum albumin and after resuspension measured by a colour reaction with ferric chloride (Hagerman and Butler 1978, Harbertson et al. 2003). Various dilutions of five commercial red wines produced from different grape varieties were analyzed by protein precipitation and the linear relationship between the inverse dilution factor and the measured tannin content was evaluated for each of the wines: Linear relationships were observed in parts of the dilution ranges and deviations from linearity happened at lower 1/(dilution factor) values for the wines having relatively high tannin contents. The slopes of the tannin response versus 1/(dilution factor) varied from 0.67 to 1.5 for the five different wines (Table 1). All wines caused negative intercepts, which supported the theory of a precipitation threshold (Hagerman and Butler 1978). At low tannin responses the intercept amounted to a large percentage of the response and hence caused too low tannin estimations. In addition, several dilutions with high tannin content deviated from the

linear relationship, probably due to insufficient protein being present for the precipitation step with high tannin levels.

**Table 1** Intercepts, slopes and correlation coefficients (r) from linear regressions in the linear range between the tannin response (in absorbance units) and the inverse dilution factor, by the method of least squares.

| | Wine 1 | Wine 2 | Wine 3 | Wine 4 | Wine 5 |
|---|---|---|---|---|---|
| Intercept | -0.033 | -0.020 | -0.025 | -0.010 | -0.018 |
| Slope | 0.67 | 1.17 | 1.52 | 0.97 | 0.83 |
| r | 0.999 | 0.999 | 0.997 | 0.999 | 1.0000 |

Considering the substantial impact of the intercept for highly diluted wines and the need for sufficient protein for the precipitation step, we defined a valid range of the tannin response, where the tannin precipitation suffered minimally from the described problems. By allowing a 5 % deviation of the measured to the maximum tannin content we recommend that the valid range of the tannin response lies between 0.3 – 0.75 units of absorbance at 510 nm (Figure 1).

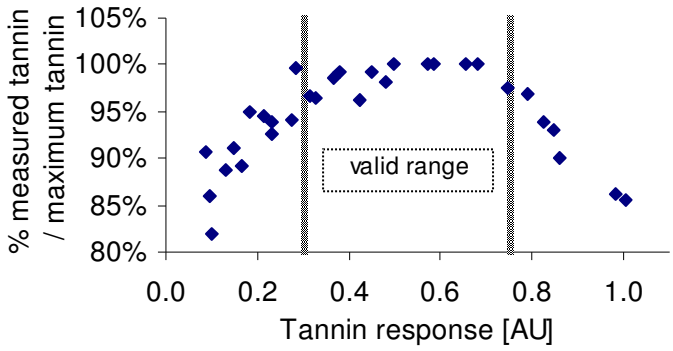


**Figure 1** The valid range of tannin response (absorbance at 510 nm, AU) defined from the relative proportion of the measured tannin content to the theoretical maximum tannin content (95-100%).

## References

Hagerman, A.E. and Butler, L.G. 1978. Protein Precipitation Method for Quantitative-Determination of Tannins. Journal of Agricultural and Food Chemistry 26:809-812.

Harbertson, J.F., Picciotto, E.A., and Adams, D.O. 2003. Measurement of Polymeric Pigments in Grape Berry Extracts and Wines Using a Protein Precipitation Assay Combined with Bisulfite Bleaching. Am. J. Enol. Vitic. 54:301-306.

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# Does extra-cellular DNA play a role in biofilm formation of *Listeria monocytogenes* EGDe?

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## 1. Summary

The foodborne pathogen, *Listeria monocytogenes*, is known to persist in food processing plants, and it has been reported that some strains are capable of forming biofilms. The mechanisms have not been elucidated but seem to depend on factors such as temperatures and inducing compounds. The occurrence of extra-cellular DNA (eDNA) in the matrix of biofilms has been reported to influence the initial attachment and/or the biofilm structure of *Pseudomonas* and *Streptococcus* species. This encouraged the investigation of a possible role for eDNA during biofilm formation. The results showed that eDNA affects both cellular attachment and biofilm development of *Listeria monocytogenes* EGDe. These data are further supported by DNaseI treatment of two other known biofilm forming *L. monocytogenes* strains (LO28 and 412). Confocal laser scanning microscopy images of flow-cell biofilms and macroscopic images show that the biofilm can be removed effectively by addition of DNaseI. This general biofilm inhibitory effect of DNaseI has not been described previously for other species. These results suggest that extra-cellular DNA plays an essential role in attachment and maintenance of a *L. monocytogenes* biofilm at essentially all stages of biofilm development. This conclusion is relevant in connection with sanitation processes in food production plants.

Keywords: *Listeria monocytogenes*, biofilm, eDNA, attachment, sanitation

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# Enzymatic synthesis of lipophilic flavonoid derivatives in organic solvents containing ionic liquids

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## 1. Summary

Ionic liquids have no volatile organic compounds/ solvents discharged into the atmosphere and adjustable solubility properties. Some ILs could be the suitable media for flavonoids ester synthesis. Flavonoids like esculin have received keen attention due to their antioxidant, antimicrobial and anticarcinogenic properties. Flavonoids have some limitations in practical use by their low solubility and unavailability in hydrophobic media where lipid oxidation happens. The solubility of flavonoids can be increased by using ILs as media in the enzymatic synthesis reaction of flavonoids and fatty acids. The reaction rate and the reaction equilibrium may be affected during the flavonoids ester synthesis. This project used the concept of conventional solvent engineering to study the mixing system of IL and organic solvent system.

The aim of this study was to search for suitable organic solvents to mix with polar ionic liquids. Theses polar ionic liquids didn't have any enzyme activity in the lipophilic flavonoid reaction. The enzymatic model system consists of palmitic acid and esculin as substrates and uses commercial immobilized lipase (Novozym 435) from Candida antarctia. HPLC is used for the analysis.

Keywords: Esculin, Palmitic acid, ILs / organic solvent system, enzymatic synthesis

## 2. Extended Abstract

Compared with t-pentanol and t-butanol, acetone had the highest bioconversion 92% (72 h, 40°C) and initial enzyme activity (0, 046 mmol esculin /g enzyme /h). Seven different polar ionic liquids were used in acetone containing either1%, 5% or 10% ILs for the enzymatic synthesis. The experiments showed that the acetone mixed with 1% Methyltrioctylammonium trifluoroacetate ([MTOA].TAF), 1%1-Ethyl-3-methylimidazolium n-octysulfate ([EMIM].OctSO4) and 1%1-Ethyl-3-methylimidazolium 2(2-methoxyethoxy) ethylsulfate ([EMIM].MDEG.SO4) had lower initial enzyme activity than pure acetone media. Acetone with 1% [MTOA].TAF had the highest conversion (59%) in contrast to the other mixing systems with either 1%[EMIM].OctSO4 (24%) or 1%[EMIM].MDEG.SO4 (39%). The other 4 ILs mixed with acetone didn't have any enzyme activity. These results are being reported for the first time and are very useful to categorize ILs and to

understand the influences of ILs' nature and structure on the enzyme activity. Among the 7 ILs we tested, we observed their influence on the enzyme activity: [MTOA].TAF> [EMIM].MDEG.SO4> [EMIM].OctSO4> [DMIM].DMP = [EMIM].ES = [EMIM].OTos = [HMIM].CL.

## References

Katsoura, M.H., Polydera, A.C., Tsironis, L., Tselepis, A.D. and Stamatis, H. (2006) *Journal of Biotechnology,* 491-503.

Ardhaoui, M., Falcimaigne, A., Engasser, J.M., Moussou, P., Pauly, G. and Ghoul, M. (2004) *Journal of Molecular Catalysis B: Enzymatic,* 24, 63-67.

# Author Index

## References


Gani, R., Jiménez-González, C. and Constable, D. J. C., (2005) *Computers and Chemical Engineering*, 29,1661-1676.

Reichardt, C., *Solvents and Solvent Effects in Organic Chemistr*y, VCM Publishers, UK (1988).