

ENRICHMENT OF NATURAL PRODUCTS USING AN INTEGRATED SOLVENT-FREE PROCESS: MOLECULAR DISTILLATION

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Molecular distillation is a potential process for separation, purification and/or concentration of natural products, usually constituted by complex and thermally sensitive molecules, such as vitamins. Furthermore, this process has advantages over other techniques that use solvents as the separating agent, avoiding problems of toxicity. This process is characterized by short exposure of the distilled liquid to high temperatures, by high vacuum (around 10^{-4} mmHg) in the distillation space and by a small distance between the evaporator and the condenser (around 2 cm). The productivity of the process is high, considering the concentration of high added value compounds. In this work, the molecular distillation process was applied to enrich borage oil in gamma-linolenic acid (GLA) and also to recover tocopherols from deodorizer distillate of soybean oil (DDSO), since these are components of interest for food and pharmaceutical industries.

KEYWORDS: gamma-linolenic acid, vitamin E, molecular distillation, deodorizer distillate of soybean oil, borrage oil

INTRODUCTION

Molecular distillation shows potential in the separation, purification and/or concentration of natural products, usually constituted by complex and thermally sensitive molecules, such as vitamins and polyunsaturated fatty acids, because it can minimize losses by thermal decomposition. Furthermore, this process has advantages over other techniques that use solvents as the separating agent, avoiding problems with toxicity. In this process, the distilled liquid continuously passes downward over the heated evaporating cylinder, evaporates partially and the vapors then condense on the cooled condenser placed close to the evaporating cylinder. A sufficiently low pressure, around 10^{-4} mmHg, is reached in the evaporator. Therefore, evaporated molecules can pass through the distillation gap to the condenser freely. The distillation of thermally unstable mixtures of liquids with low vapor pressure occurs without thermal decomposition at a reduced distillation temperature (lowered by 200–250°C compared to that at normal pressure), with short residence times of the distilled liquid on the thermally exposed surface (about 10^{-1} to 10^{1} s), and at a small evaporating cylinder–condenser distance (20–50 mm). The evaporation of the liquid on the evaporating cylinder is a key step in the molecular distillation. The distilled liquid flows over the cylinder as a thin wiped-film with a thickness of

0.05–2 mm (Lutisan et al., 2002). Under typical conditions of this process, e.g., short residence time and low temperature, distillation of heat-sensitive materials is accompanied by only negligible thermal decomposition (Cvengros et al., 2000).

In this work, the molecular distillation process was applied to enrich borage oil in gamma-linolenic acid (GLA) and also to recover tocopherols from deodorizer distillate of soybean oil (DDSO), since these are components of interest for food and pharmaceutical industries. In higher animals, the ingestion of particular unsaturated fatty acids, such as GLA, is necessary for a great variety of their physiological and cellular functions, since they are unable to synthesize all the fatty acids they need. Inadequate intakes lead to various dysfunctions due to deficiencies of these fatty acids in particular cellular locations. Tocopherols are important for their Vitamin E activity and antioxidant property (Winters, 1990).

ENRICHMENT OF GAMMA-LINOLENIC ACID

LIPASE-CATALIZED HYDROLYSIS

Borrage oil was used as raw material for the GLA enrichment. Firstly, selective hydrolysis reactions were carried out using the commercial lipase Lipolase® 100 L, Type EX (Novozymes, Araucaria, PR, Brazil), which is a food-grade liquid preparation of a microbial lipase from *Thermomyces lanuginosus* produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism. The standard conditions used in the reactions were: 20 g of borrage oil from SP Farma, SP, Brazil (22.1% of GLA), 20 g of water, 40 KLU of enzyme/g of oil, stirring the reaction media at 500 rpm. One KLU (Kilo Lipase Unit) is the amount of enzyme which liberates 1 mmol per minute of titratable butyric acid from tributyrin under a set of standard conditions (Novozymes, 2005). The reaction temperature was fixed at 55°C. The most of the saturated and monounsaturated fatty acids was converted into free fatty acids (FFA), which was separated by Molecular Distillation. Thus, the GLA was concentrated into a mixture of residual monoacylglycerols (MG), diacylglycerols (DG) and triacylglycerols (TG). The composition of lipid classes was determined by high-performance size exclusion chromatography, according to Fregolente et al. (2005). Fatty acid composition was determined by gas-liquid chromatography, according to the AOCS official method Ce 1e-91.

Figure 1 shows the conversion of TG into DG, MG and FFA. One can note that, in the first 60 min, the TG concentration decreases drastically, owing to their conversion into DG, MG and AGL. In this time interval, the DG concentration reaches a maximal value of 28% and then starts decreasing up to approximately 20%, after 300 min of reaction. This maximal DG concentration occurs owing to the conversion of DG into MG and FFA. The MG concentration also seems to present a maximal value within the first 15 min, due to the conversion of MG into FFA and glycerol (GL). The FFA concentration increases up to approximately 38% after 300 min.

Table 1 shows the GLA enrichment during the reaction course. It can be seen that, after 180 min, the GLA concentration was 1.5 times higher than the GLA concentration of the raw material. It means that the lipase acted selectively, enriching the remaining

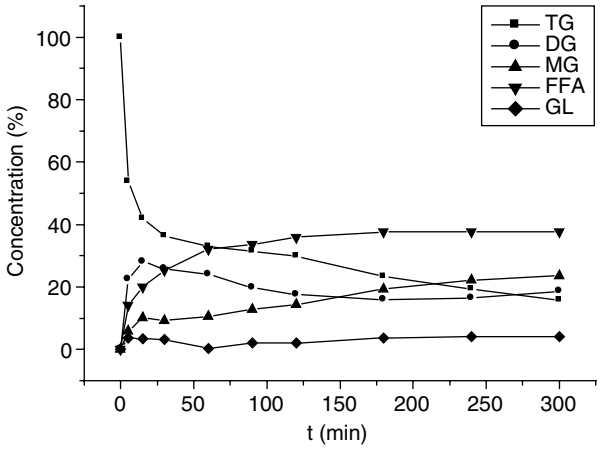

Figure 1. Lipid class composition during the reaction course

acylglycerols in GLA. One may conclude the GLA concentration in the acylglycerols is significantly higher than the GLA concentration in the FFA fraction. Although, this tendency of enrichment seems to stop at 300 min of reaction, since at 300 min, the GLA concentration is lower than the GLA concentration at 180 min, evidencing that, at the experimental condition studied, the maximal GLA concentration is around 33.6% (1.5 higher than the initial concentration).

Table 1. Enrichment of GLA

		Fatty acid composition[a]						
t (min)	Fraction (%)	16:0	18:0	18:1	18:2	18:3	20:0–24:1	Enrichment of GLA
0	Acilglycerols ≈100	10.6	3.2	17.6	36.9	22.1	9.2	
60	Acilglycerols = 67.7	8.3	2.7	16.5	34.8	29.5	7.5	1.3
60	FFA = 32.0	13.1	4.1	17.9	38.6	14.5	10.5	
120	Acilglycerols = 61.9	9.3	3.0	15.9	34.7	28.6	7.5	1.3
120	FFA = 35.9	11.6	4.5	17.7	38.7	16.8	9.8	
180	Acilglycerols = 58.6	7.9	2.4	15.9	32.1	33.6	6.9	1.5
180	FFA = 37.7	11.7	4.7	18.4	38.0	16.9	5.1	
300	Acilglycerols = 58.0	9.5	3.5	15.8	35.0	27.7	7.3	1.3
300	FFA = 37.7	11.2	4,0	15.8	37.4	19.9	9.1	

[a]16:0 Palmitic acid; 18:0 Stearic acid; 18:1 Oleic acid; 18:2 Linoleic acid; 18:3 γ-Linolenic acid.

Table 2. Composition of the starting material used in the distillation step

		Fatty acid composition[a]					
t (min)	Fraction (%)	16:0	18:0	18:1	18:2n-6	18:3	20:0–24:1
60	Acilgliceróis = 77.5	8.5	2.7	16.0	35.3	28.7	7.7
60	AGL = 22.5	13.1	5.0	17.7	38.6	14.3	10.4

[a]For abbreviations see Table 1.

MOLECULAR DISTILLATION

After the lipase-catalized step, the reaction products were submitted to molecular distillations in order to separate FFA from the acylglycerols. The molecular distillator used was a centrifugal distillator from Myers Vacuum Inc. (Kittanning, PA), with an evaporation surface area of 0.0046 m^2. The feed and condenser temperatures were maintained at 40°C and 30°C respectively. The typical pressure of the system was 0.12 mmHg and the evaporator rotation velocity was 1350 rpm. The composition of the starting material used to study the distillation step is shown in Table 2.

Figure 2 shows the effect of the evaporator temperature on the GLA concentration. It is clear that the GLA concentration in the distillate stream is close to the GLA concentration in the FFA fraction of the starting material, because this stream is rich in FFA (above 60% of FFA in all the experiments). In analyzing the results obtained for the residue stream, it can be seen that the GLA concentration of this stream is similar to

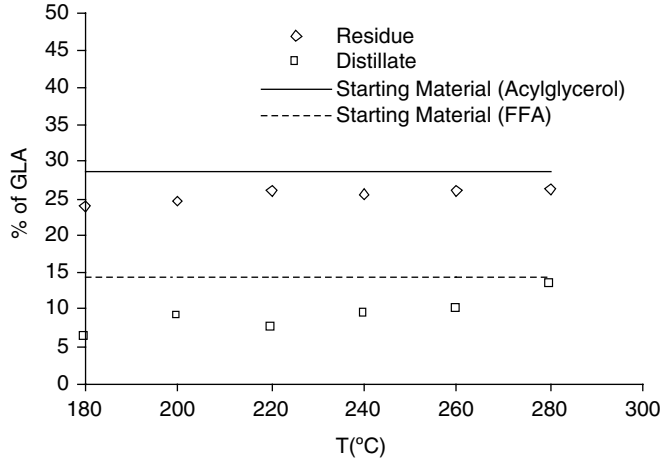

Figure 2. Enrichment of GLA obtained in the molecular distillation

the GLA concentration in the acylglycerol fraction of the starting material. This happens owing to the low concentration of FFA in this fraction (below 16% in all the experiments).

Therefore, it can be concluded that the molecular distillation process is appropriate to separate FFA from products of lipase-catalyzed reactions. Furthermore, it can be seen that the GLA enrichment level obtained in the integrated process (lipase-catalyzed hydrolysis and molecular distillation) depends strongly on the enrichment of GLA in the acylglycerol fraction of the reaction.

RECOVERY OF TOCOPHEROLS

METHODOLOGY: SIMULATION *VERSUS* EXPERIMENTAL

Simulation

DDSO is a complex mixture due to its large number of components. It includes thermal sensitive molecules such as tocopherols. Some physical properties can not be experimentally determined without the decomposition of these sensitive molecules. Consequently, it is very hard to find their physical properties in a database and they must be estimated and/or predicted before the simulation. Firstly, it was necessary to create hypothetical components using the UNIFAC group contribution (a tool of the Commercial Simulator HYSYS^{TM}), to estimate some physical properties, e.g., critical pressure, critical temperature, critical volume and acentric factor. These properties will be necessary to calculate other properties, e.g., mean free path, enthalpy of vaporization, mass diffusivity, vapour pressure, liquid density, heat capacity, thermal conductivity, viscosity of the system to be studied (DDSO) to insert in DISMOL simulator, which allows to simulate the molecular distillation process. All explanations on the equations used, on the solution methods and on the routine of solution are described in Batistella and Maciel (1996). The DISMOL simulator requires, besides the components and mixture properties, equipment, process and systems characteristics that are simulation inputs. Evaporation rate, temperature and concentration profiles, residence time, stream compositions and flow rates are the output from the simulation. All calculations of these properties are described in Moraes et al. (2004). There is no discussion about equilibrium, because the molecular distillation is a non-equilibrium process.

In relation to the equipment, it is necessary to know its dimension, the feed flow rate and its heating temperature. In this study, the equipment used was a falling film distillator and it was considered the DDSO composition described in Table 3a. As results, the concentrations and the exit rates of the distilled and of the concentrated streams, the evaporation rate and the time of distillation are obtained.

Experimental Procedure

The composition of the raw material used in the experiments is shown in Table 3b. The distillation was performed using a laboratory wiped film molecular distillator model KDL 5, GmbH UIC (Alzenau, Germany) which is a variation from falling film molecular distillation with agitation. The major part of the equipment was constructed with glass. The heating of the evaporator was provided by a jacket circulated with heated oil from

Table 3. DDSO Composition used in the simulation (**a**) and obtained experimentaly (**b**)

(**a**) Simulation		(**b**) Experimental[a]	
Components	% mass	Components	% mass
Palmitic acid	10.80	FFA as oleic acid	57.42
Stearic acid	2.40	Tocopherols	8.97
Linoleic acid	26.20	Phytosterols	7.69
Oleic acid	12.30	Triglyceride	8.00
Lauric acid	1.90	Diglyceride	4.30
Araquidic acid	4.20	Monoglyceride	13.62
Phytosterols	7.70		
Tocopherols	8.90		
Scalene	14.70		
Glyceride	10.90		
Total	**100.00**		**100.00**

[a]Martins, 2005.

an oil bath. The vacuum system included a diffusion and a mechanical pump. The surface area of the evaporator is 0.048 m^2 and the surface area of internal condenser was 0.065 m^2. The roller wiper speed inside the evaporator was fixed at 350 rpm.

The experiments were organized according to the following way: samples were melted to obtain a liquid and homogenous mixture necessary to feed it inside the equipment. The evaporator temperature selected in this work was 100°C. Firstly, the evaporator temperature was fixed and, then, the feed flow rate was varied from 0.1 to 0.7 kg/h. For each process condition, samples of both streams (distillate and residue) were collected and submitted to tocopherols analysis. The process pressure was maintained at 7.5×10^{-4} mmHg, the feed temperature at 50°C, the condenser temperature at 60°C, and the stirring at 350 rpm. The collected samples of the distillate and residue streams were kept in a freezer at −18°C, for further analysis.

FFA elimination from DDSO through molecular distillation is technologically viable due to the differences between molecular weights and vapor pressures of FFA and tocopherols (Table 4).

Due to the values of molecular weights and vapor pressures, it is expected that FFA be removed from DDSO in the distillate stream and tocopherols be concentrated in the residue stream at 120° and at 140°C, preferentially.

The composition of tocopherol was determined by normal phase high performance liquid chromatography (AOCS, 1990) using a modular equipment composed by Waters 515 HPLC pump (Mildford, MA), equipped with a fluorescence detector (Waters model 2475 multi fluorescence). The separation was conducted in a μporasil column 125 Å, with particle size of 10 μ and 3.9 × 300 mm of dimension (Waters, Ireland). The

Table 4. Molecular weights and vapor pressures of FFA and tocopherols[a]

Component	Molecular weight* (g/gmol)	Vapor pressure at 200°C* (mmHg)
FFA	180	4.00
Tocopherols	415	0.15

[a]Winters, 1986.

mobile phase used was hexane:isopropanol (99:01). The feed flow rate of mobile phase was set at 1.0 ml/min. The data processing was done by the Millenium software 2010 Chromatography Manager Software (Waters, Mildford, MA). The DDSO and samples of distillate and residue were dissolved in hexane (~1 mg/ml) and injected in the equipment. Each chromatografic run took about 10 min. This method determines α-, β-, γ- and δ-tocopherol individually. The tocopherols detected in the chromatograms of DDSO were identified comparing the retention time of these compounds with the retention time of standards tocopherols.

RESULTS AND DISCUSSION

The results of the preliminary analysis were presented in Figures 3 and 4.

With this preliminary analysis using the DISMOL simulator and experimental data, it was possible to shown that the simulated results agree with the experimental data. It is important to observe, although there are raw materials differences as showed in Tables 3a and 3b, it was reached a good agreement between simulated and experimental data (Figures 3a and 4a).

The main differences between simulated and experimental DDSO composition are related to FFA and glycerides concentration. Experimentally, FFA were measured

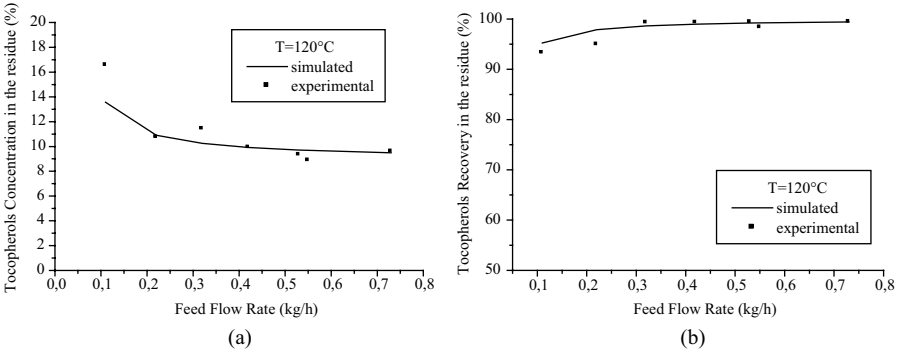

Figure 3. (a) Tocopherols concentration in the residue (%) versus feed flow rate (kg/h) and (b) Tocopherols recovery in the residue (%) versus feed flow rate (kg/h) at 120°C

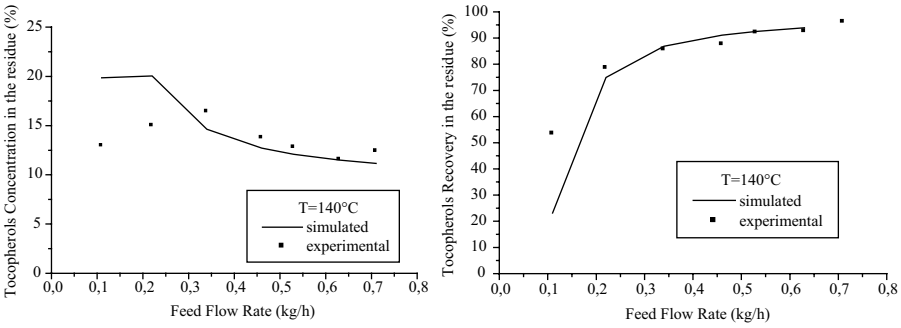

Figure 4. (a) Tocopherols concentration in the residue (%) versus feed flow rate (kg/h) and (b) Tocopherols recovery in the residue (%) versus feed flow rate (kg/h) at 140°C

by titration and calculated as if all FFA were composed just by oleic acid. The concentration of glycerides was estimated considering mono, di and triglycerides. The simulated raw material considered each FFA, e.g. palmitic, stearic, oleic, linoleic, lauric and araquidic acids, as a individual specie and glycerides as only one group of substances, including the mono, di and triglycerides.

These hypotheses can be responsible for possible deviations between simulated and experimental results, since the raw material composition influences the mixture properties used in the molecular distillation simulation. According to Figures 3b and 4b, it is possible to observe, that the tocopherols remain in the residue in these temperatures (120°C and 140°C). In Figures 1a and 2a, it was observed a deviation that could be explained by the irregular distribution of the film on the evaporator, due to the low feed flow rate.

CONCLUSIONS

In the case of the enrichment of gamma-linolenic acid, for the conditions studied, the maximal GLA concentration obtained in the lipase-catalyzed hydrolyses was around 33.6% (1.5 higher than the initial concentration), at 180 min of reaction. Molecular distillation was effective to separate FFA (poor in GLA) from the reaction products.

Also for recovering tocopherols and to eliminate FFA from crude DDSO, it was observed that the Molecular Distillation is a powerful process. Moreover, it is a clean technology and presents some advantages in relation to other conventional techniques, since it does not make use of solvents.

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