Use of cell-wall degrading enzymes to improve lycopene extraction from tomato processing waste

Roberto Lavecchia and Antonio Zuorro
Department of Chemical Engineering, "Sapienza" University
Via Eudossiana, 18 – 00184 Roma (Italy)

Pectinase and a food-grade enzyme preparation with pectinolytic, cellulolytic and hemicellulolytic activities were used to improve lycopene extraction from tomato peels. Experiments were carried out at 25°C for a total duration of around 24 h. The plant material was first contacted with the enzyme solution, at a concentration of 1 to 2.5% (v/v), and then extracted with hexane.
Recovery yields as high as 300 mg of lycopene per 100 g of dry material were obtained. Yields from untreated peels were of the order of 25 mg/100 g (dry basis). A spectrophotometric characterization of the hexane extracts showed that the enzymatic treatment has no apparent detrimental effect on the lycopene extracted from the peels.

1. Introduction

Lycopene, the carotenoid which gives tomatoes and other red fruits their characteristic color, is one of the most powerful antioxidants and singlet oxygen-quenching agents. A large body of evidence from clinical and experimental studies supports a role for lycopene in reducing the risk of cardiovascular disease and some forms of cancer (Rao and Rao, 2004; Giovannucci, 2005).
The lycopene market is experiencing a rapid growth, both in value and volume, with the highest demand in the area of nutraceuticals and functional foods. As a result, the search for new sources of the pigment and new extraction technologies is becoming an urgent need. In Italy more than 100,000 tons of tomato processing waste are produced yearly. The waste material consists primarily of tomato seeds and peels, with the latter representing one of the richest source of lycopene. According to Sharma and Le Maguer (1996), tomato skin can contain up to 5 times more lycopene than tomato pulp. Problems, however, arise when attempting to recover lycopene from such material. They are mainly due to the peculiar localization of the pigment in the peel, where it is present in the form of clusters of elongated crystals incorporated into thylakoid membranes (Shi et al., 2002). Such an environment makes the pigment highly protected against degradation but hardly accessible to solvent molecules. So, very low recovery yields are obtained when conventional solvent extraction procedures are used.
To overcome the above limitations, we investigated the possibility of enhancing the efficiency of extraction by enzymes, such as pectinase, cellulase and hemicellulase, capable of degrading the cell structures. These enzymes have been successfully used in a variety of applications, including oil recovery from raw materials (Dominguez et al., 1994) and extraction of natural compounds from plant tissues (Barzana et al., 2002).

In this work we report on the use of a food-grade enzyme preparation derived from *Aspergillus niger* as a means for improving lycopene recovery from tomato peels. The main objective of the study was to provide preliminary assessment of the potential of an enzyme-based technology for turning tomato waste into a valuable feedstock for lycopene production.

2. Experimental

2.1 Materials

Fresh ripe tomatoes of the commercial variety *Roma* were purchased from a local market and stored at 4°C for a maximum of 2 days before use.

Citrozym Ultra L was obtained from Novozymes (Bagsvaerd, Denmark). It is a food grade enzyme preparation derived from a selected strain of *Aspergillus niger* and containing pectinolytic, cellulolytic and hemicellulolytic activities. The claimed activity was 3500 FDU 55°C/ml.

Pectinase (EC 3.2.1.15) was purchased from Sigma-Aldrich (St. Louis, MO), as an aqueous solution in 40% glycerol. The claimed activity was 5 U per mg of protein, where 1 U represents the quantity of enzyme that liberates 1 μmol of galacturonic acid from polygalacturonic acid per min at 25°C and pH 4.

Just before use the enzymes were dissolved in distilled water by stirring at room temperature for a few minutes.

Acetone, ethanol and n-hexane were from Carlo Erba (Milano, Italy) with purities greater than 99.7%, 99.5% and 95%, respectively.

2.2 Methods

Sample preparation

After removal of damaged parts and washing, whole tomato fruits were immersed in boiling water for 1-2 min. Then they were cooled under tap water and hand peeled. The peels were dried in air for a few hours and then stored at 4°C. The moisture content of the peels was determined by oven drying to constant weight at 105°C.

Lycopene content of tomato peels

The amount of lycopene contained in tomato peels was determined by a slight modification of the procedure of Sadler et al. (1990). Experiments were made using a hexane/ethanol/acetone solution (50:25:25 v/v) as the extracting medium. Total lycopene content of the peels was evaluated from the amounts obtained in three consecutive extractions and was expressed as mg of lycopene per 100 g of dry matter. Each assay was performed at least in duplicate.

Lycopene assay

Lycopene concentration in the organic solvent was determined spectrophotometrically. Absorption spectra were recorded at room temperature in the wavelength range 350-600 nm, using a double-beam UV-VIS spectrophotometer (Perkin-Elmer Lambda 25) and quartz cells of 1-cm path length.

VIS spectra showed the three characteristic peaks of lycopene at around 445, 472 and 503 nm (Figure 1). To minimise interference from other carotenoids measurements were made at 503 nm (Fish et al., 2002).

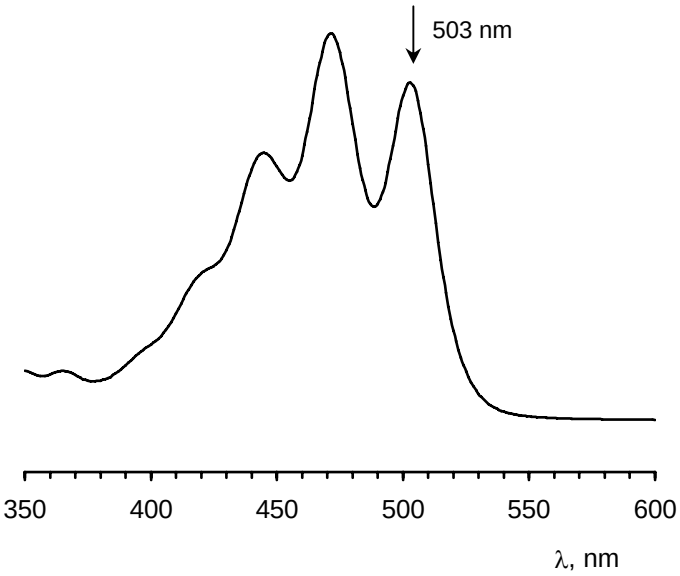

Figure 1 – Absorption spectrum of lycopene in hexane extracts.

Enzyme-mediated extraction

1 to 2 g of partially dehydrated peels, obtained as described in the sample preparation section, were initially charged into 50-mL screw-top conical flasks. The flasks were placed in a water bath at 25°C and magnetically stirred. 10 mL of an aqueous enzyme solution were then added and incubated for about 20 h. After this time, 30 mL of hexane were poured into the flasks and kept under stirring, at the same temperature, to allow for lycopene solubilization. Enzyme concentrations of the pre-treating solution were 1% (v/v) Pectinase and 1% or 2.5% (v/v) Citrozym. Two extraction times were considered: 3.5 and 7 h. When the extraction was completed, the agitation was stopped and two liquid layers (the aqueous and the organic phases) formed. A sample of the hexane supernatant was taken and analysed for lycopene content.

The following control experiments were made: *(i)* hexane extraction of peels pretreated with bidistilled water, in the same amount as the enzyme solution; and *(ii)* hexane extraction of peels not subjected to either water or enzymatic pretreatment.

All runs were performed at least in duplicate and the results were averaged.

Characterization of lycopene extracts

To highlight possible detrimental effects of the enzymatic treatment on lycopene, the peel extracts were characterized spectrophotometrically. After the extraction was completed, aliquots of the organic phase were taken and diluted in hexane. The absorption spectrum was recorded between 350 and 600 nm, using hexane as the blank. From each spectrum the following indices, representing the ratios between molar extinction coefficients at the peak maxima, were determined: $\varepsilon_{445}/\varepsilon_{472}$ and $\varepsilon_{503}/\varepsilon_{472}$.

First- and second-order derivative spectra were obtained from absorption spectra by UV WinLab™ software (Perkin-Elmer).

3. Results and Discussion

The initial lycopene content of tomato peels was between 385 and 415 mg/100 g (dry basis), while the moisture content ranged from 81.6 to 84.3%, depending on the sample considered.

The amount of lycopene recovered from the peels was calculated from the absorbance of the hexane extracts at 503 nm and the volume of the organic phase. Extraction yields were expressed as mg of lycopene per 100 g of dry plant material.

Preliminary examination of the data showed that extraction times of 3.5 or 7 h did not result in significant differences in lycopene recovery, suggesting that, under the experimental conditions used, 3-4 h are sufficient to solubilize lycopene from the enzymatically treated peels.

For samples not subjected to enzymatic treatment, lycopene recovery was of the order of 25 mg/100 g (dry basis). This result was independent of whether or not the plant material was contacted with water prior to addition of the organic solvent. Conversely, very high extraction yields were observed when the peels were enzymatically treated (Figure 2). On the average, lycopene recovery was 280 ± 20 mg/100 g, which corresponds to a more than 10-fold increase with respect to control values. A closer inspection of the results reveals that increasing the Citrozym Ultra concentration from 1 to 2.5% (v/v) had only a limited effect on extraction, with yields going from 272 to 302 mg/100 g. Moreover, treatment by the two enzymes gave nearly identical results (272 mg/100 g for Citrozym Ultra and 264 mg/100 g for Pectinase).

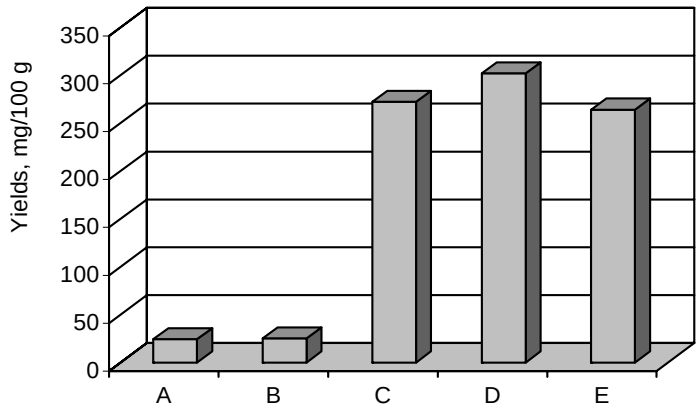

Figure 2 – Recovery yields of lycopene from untreated and enzymatically treated tomato peels (A: no pretreatment; B: water pretreatment; C: 1% v/v Citrozym pretreatment; D: 2.5% v/v Citrozym pretratment; E: 1% v/v Pectinase pretreatment).

Table 1 – Spectral indices of lycopene absorption spectra in extracts obtained from untreated and enzymatically treated tomato peels.

Peel Treatment	$\varepsilon_{445}/\varepsilon_{472}$	$\varepsilon_{503}/\varepsilon_{472}$
Pure water	0.688	0.873
Citrozym Ultra 1% (v/v)	0.693	0.880
Pectinase 1% (v/v)	0.693	0.875

Considering that Citrozym Ultra is an enzymatic preparation with pectinolytic, cellulolytic and hemicellulolytic activities, while Pectinase has mainly a pectinolytic activity, this result would seem to indicate that the pectin network within the tomato skin tissue is the main responsible for the low yields of extraction from untreated peels. When pectic substances are enzymatically degraded, disintegration of the plant tissue occurs, with lycopene becoming easily accessible to solvent molecules.

Although the process is not yet optimized, we note that even under the very mild conditions used (ambient temperature and extraction times of a few hours), unexpectedly high lycopene yields were obtained. Assuming an initial lycopene content of 400 mg/100 g, percentage yields result ranging from 66% to 75.5%. In comparison, yields from untreated peels were of only 6%.

Turning to the effects of treatment on the spectral features of lycopene, we report in Table 1 the ratios $\varepsilon_{445}/\varepsilon_{472}$ and $\varepsilon_{503}/\varepsilon_{472}$ calculated from the VIS absorption spectra of hexane extracts. As can be seen, the values of both indices are almost identical for the three samples, with variations of less than 0.8%. Figures 3 and 4 show first- and second-order derivative spectra (i.e., $dA/d\lambda$ and $d^2A/d\lambda^2$ against λ) for the same samples.

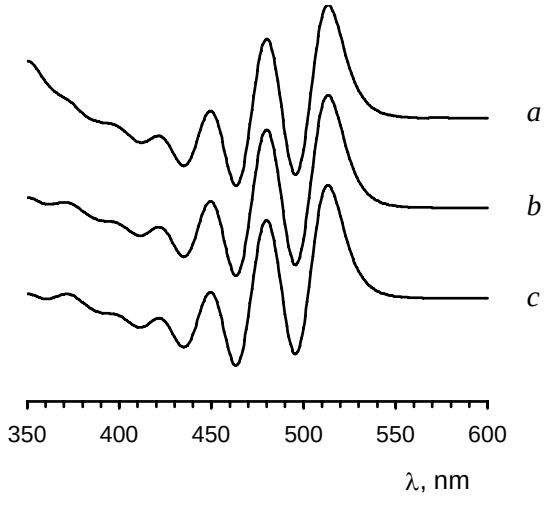

Figure 3 – First-order derivative spectra of lycopene in extracts obtained from: (a) untreated, (b) 1% v/v Citrozym-treated and (c) 1% v/v Pectinase-treated peels.

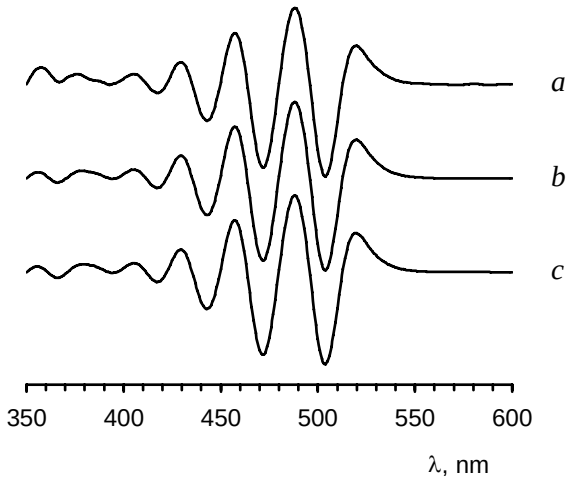

Figure 4 – Second-order derivative spectra of lycopene in extracts obtained from: (a) untreated, (b) 1% v/v Citrozym-treated and (c) 1% v/v Pectinase-treated peels.

As is known, the higher resolution of these spectra allows an easier detection of molecular rearrangements or other structural modifications of the absorbing compound. A comparison of the spectra of lycopene from untreated or enzymatically-treated peels does not evidence any significant change in the region 350-600 nm. We can then infer that the enzymes used and/or the cell-wall degradation products resulting from their activity do not have prominent effects on the molecular structure of lycopene.
From the above results the following conclusions can be drawn: (1) Recovery yields of lycopene from tomato peels can be greatly increased by the use of cell-wall degrading enzymes, even at low temperature and short extraction times. (2) Commercial enzyme preparations with pectinolytic, cellulolytic and hemicellulolytic activities can be advantageously used for this purpose. (3) The use of these enzymes does not seem to have detrimental effects on the lycopene extracted from the peels.

4. References

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