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Enhancement of lycopene extraction from tomato peels by enzymatic treatment

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Four food-grade enzyme preparations (Citrozym CEO and Ultra L, Peclyve EP and LI) with pectinolytic, cellulolytic and hemicellulolytic activities were investigated to assess their suitability for improving lycopene recovery from tomato peels. After a preliminary screening, the influence of solvent type and enzyme incubation time on the extraction efficiency was studied. Under the best conditions (1-h enzyme incubation followed by a 3-h solvent extraction at 40 °C) up to 440 mg of lycopene per 100 g of dry tomato peels were obtained. Experiments on the peel fraction of tomato processing waste confirmed the significant increase in yields resulting from the use of enzymes. Overall, lycopene recovery from the enzymatically treated material was between 70 and 98%, while yields from the untreated peels were in the range of 3–40%.

1. Introduction

In recent years lycopene, the major carotenoid pigment found in ripe tomato fruits and responsible for their characteristic red colour, has been the focus of considerable attention for its potential health benefits (Shi et al. 2002; Rao and Rao, 2004). Results from epidemiological and experimental studies support the view that lycopene may provide protection against cardiovascular disease and certain types of cancer (Giovannucci, 2005; Omoni and Aluko, 2005). Beneficial effects are believed to arise from its antioxidant properties which, in turn, are related to the extensive conjugation of double bonds in the molecule (Figure 1).

Because of the growing demand for natural lycopene, considerable interest has been directed to the possibility of obtaining lycopene from tomato processing waste. Tomato skins can, in fact, contain up to 5 times more lycopene than the pulp (Sharma and Le Maguer, 1996). However, the available solvent extraction technologies do not seem to allow a fast and economic recovery of the carotenoid. For example, only about 50% of total lycopene was extracted from tomato processing waste using supercritical CO_2 at 60 °C and 30 MPa (Sabio et al., 2003). Similar results were obtained by Rozzi et al. (2002) with supercritical CO_2 at 86 °C and 34.5 MPa. Low extraction efficiencies can be ascribed to the difficulty for the solvent to penetrate the compact tomato peel tissue and solubilize the pigment, which is deeply embedded within the chromoplast membrane structures (Harris and Spurr, 1969).

To overcome the above limitations, we have explored the possibility of using cell-wall degrading enzymes as a means for enhancing lycopene recovery from tomato peel tissue. With a view to industrial exploitation, commercial enzyme preparations and

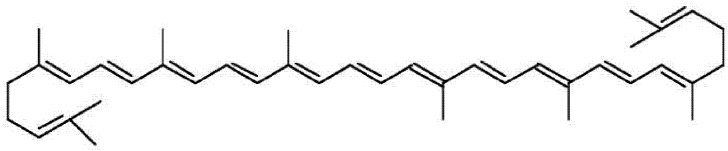

Figure 1 – Molecular structure of lycopene.

organic solvents approved for food applications were utilized. The results obtained indicate that a mild enzymatic treatment can lead to the almost complete recovery of lycopene from tomato peels or from the peel fraction of tomato processing waste.

2. Experimental

2.1 Materials

Fresh ripe tomatoes were purchased from a local market and stored at 4 °C for a maximum of 2 days before use. Tomato processing wastes were supplied by DESCO SpA (Terracina, Italy) and Az. DE LUCA (Anzio, Italy). As soon as obtained they were divided into small lots and frozen at −30 °C.

Citrozym CEO and Citrozym Ultra L were from NOVOZYMES (Denmark) and had a declared activities of 9,500 PGU/mL and 4,500 PECTU/mL, respectively. Peclyve EP and Peclyve LI were from LYVEN (France). All preparations were produced from *Aspergillus* strains and their main activities were pectinolytic.

Acetone, ethanol, ethyl acetate and hexane were obtained from CARLO ERBA (Italy). Their purities were greater than 99.7%, 99.5%, 99% and 95%, respectively.

2.2 Methods

Sample preparation and characterization

Ripe tomato fruits were immersed in boiling water for 1–2 min and, after being rapidly cooled, were hand-peeled.. The peels were partially dried in air for a few hours and stored at 4 °C. Just before use an appropriate amount of frozen tomato waste was thawed. The skins were hand-separated from the seeds and other impurities.

Each peel sample was characterized for moisture and total lycopene content. Moisture was determined by oven drying at 105 °C to constant weight. Total lycopene content was evaluated according to the procedure of Fish et al. (2002).

Lycopene assay

Lycopene concentration in the extracting solvents was determined by spectrophotometric measurement at room temperature in the wavelength range 350–600. A double-beam UV–VIS spectrophotometer (Perkin–Elmer Lambda 25) and quartz cells of 1-cm path length were used. The absorption spectra of the extract, independently of the solvent used and/or application of enzymes, displayed the three characteristic peaks of lycopene at around 445, 472 and 503 nm (Figure 2). To minimise interference from other carotenoids measurements were made at 503 nm, using a molar extinction coefficient of $1.585\ 10^5\ M^{-1}cm^{-1}$ (Merck et al., 1989).

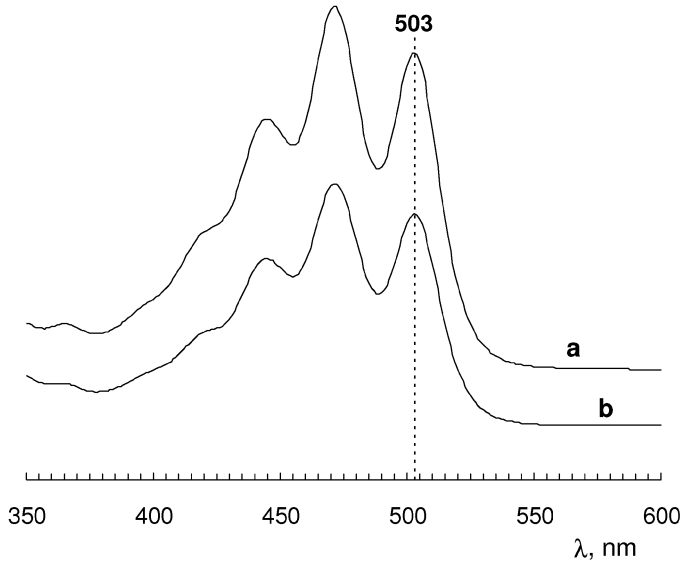

Figure 2 – VIS absorption spectra of hexane extracts from: (a) enzyme-treated and (b) untreated tomato peels.

Screening of enzyme preparations

The four enzyme preparations were screened for their ability to enhance lycopene extraction from tomato peel tissue using ripe tomato peels. 0.2 g of peels and 3.5 mL of an enzyme solution prepared by dissolving 0.1 mL of the commercial enzyme product in 3.4 mL of distilled water were initially charged into 50-mL conical flasks. The flasks were magnetically stirred and incubated at 25 °C for 4 h. 30 mL of hexane were then poured into the flasks and the system was kept under agitation, at the same temperature, for further 1 h. After this time, stirring was stopped and the aqueous and organic phases allowed to separate. A 2-mL sample of the hexane layer was taken and analysed for lycopene content.

Study of the influence of extraction conditions on yields

Peclyve LI, the preparation yielding the greatest improvement in lycopene extraction, was used to investigate the effects of solvent type and enzyme incubation time on yields. Hexane, ethyl acetate or the ternary mixture hexane/acetone/ethanol (50:25:25 v/v) were used as solvent. The latter was chosen because of its proven efficacy for the extraction of carotenoids from plant material (Olives Barba et al., 2006). The enzyme incubation time was varied between 1 and 15 h.

Lycopene extraction was carried out according to the procedure described in the previous section. The temperature and the extraction time were set at 40 °C and 3 h, respectively. These values were found to be a good compromise between the efficiency of extraction and the loss of lycopene due to oxidation.

3. Results and Discussion

Extraction yields were expressed as mg of lycopene per 100 g of dry plant material. Depending on the material considered, the moisture content was between 85 and 95 wt%, while the total lycopene content was between 280 and 540 mg/100 g dw (Table 1). Differences in the lycopene content for the two tomato processing wastes may be due to the different ripeness of the tomatoes used and/or the storage conditions of the material.

Screening of enzyme preparations

Lycopene extraction by the enzyme preparations tested gave the results shown in Table 2. As is evident, all four preparations increased the extraction yields. Peclyve EP and LI were the most efficient, with recoveries of 317.6 and 356 mg/100 g dw, respectively. These values correspond to an almost 20-fold increase with respect to the untreated peels.

The observed increase in yields can be explained by considering that pectin, cellulose and hemicellulose are the major polysaccharide components of tomato peel tissue (Gross, 1984). Accordingly, a more or less considerable fraction of these components can be expected to be degraded by the enzymes used, thus favouring solvent penetration and lycopene dissolution. The higher efficiency of Peclyve LI suggests that this preparation has the best activity profile, *i.e.*, the best combination of type and concentration of hydrolyzing enzymes for the tomato peels used. However, since the polysaccharide composition of tomato peels is dependent on fruit variety and ripening stage (Wakabayashi, 2000), a preliminary screening of enzyme preparations should always be performed on the specific material to be processed.

Table 1 – Characterization of tomato peels and tomato processing wastes.

Material source	Moisture (wt %)	Lycopene content (mg/100 g dw)
Ripe tomatoes	85.0	450 ± 21
DESCO SpA	95.0	280 ± 16
Az. DE LUCA	90.5	540 ± 30

Table 2 – Screening of enzyme preparations. Y represents lycopene recovery and Y_0 refers to control.

Enzyme preparation	Y (mg/100 g dw)	Y/Y_0 –
Citrozym CEO	95.3	5.3
Citrozym Ultra L	228.5	12.7
Peclyve EP	317.6	17.6
Peclyve LI	356.0	19.8

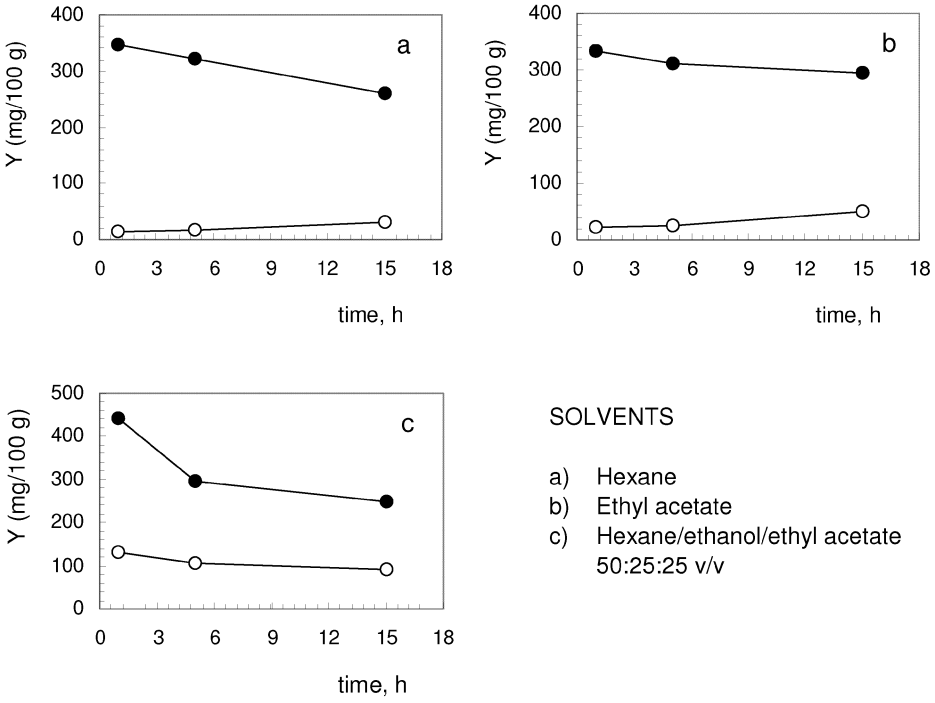

Figure 3 – Influence of enzyme incubation time on lycopene recovery (Y, mg/100 g dw) from untreated (open circles) and enzyme-treated (solid circles) tomato peels using different extraction solvents (T = 40 °C; enzyme preparation: Peclyve LI; extraction time: 3 h).

Study of the influence of extraction conditions on yields

Figure 3 shows the influence of extraction conditions on the recovery of lycopene from peels pretreated by Peclyve LI, the enzyme preparation with the best performance. As can be seen, and in line with what was found in screening tests, the enzymatic treatment increased significantly the extractability of lycopene. Overall, the extraction yields were 3- to 25-fold higher than those from the untreated material.

For all solvents, the highest recovery was achieved with an enzyme incubation time of 1 h. Under these conditions, lycopene extraction yields in hexane, ethyl acetate and the mixture hexane/acetone/ethanol 50:25:25 were 346.4, 334.3 and 440.2 mg/100 g dw, respectively. Increasing the incubation time resulted in a progressive reduction in yields. This suggests that the enzymatic degradation of cell-wall components is very fast and occurs within the first hour of incubation. Therefore, the vast majority of lycopene molecules contained in the plant tissue is likely to be rapidly released from the protective chromoplast structures and exposed to the conditions of the external environment. Because of their high reactivity, the released lycopene molecules can

undergo rapid oxidative degradation (Xianquan et al., 2005). Although the underlying mechanisms are only partially elucidated, it has been shown that lycopene oxidation leads to the formation of several cleavage products, including apo-lycopenals/ones and apo-carotendials, whose spectra are shifted to shorter wavelengths compared to that of lycopene (Caris-Veyrat et al., 2003). So, the reduction in extraction yields observed at prolonged incubation times could be a reflection of the progressive lycopene loss due to oxidation. The molecular structures of some oxidation products are depicted in Figure 4.
As regards the influence of solvent type, we note that using hexane or ethyl acetate did not produce significant differences in extraction efficiency. In contrast, the mixture hexane/acetone/ethanol 50:25:25 appeared to be much more effective, both in the presence and absence of enzymatic treatment. Since hexane is the only component of the mixture with a high affinity for lycopene, it follows that acetone and ethanol must play some auxiliary role in the overall extraction process. A possible explanation is that the two polar compounds, due to their small molar volume, high hydrogen bonding capability and large basicity, could cause the swelling of the plant tissue (Mantanis et al., 1995; Obataya and Gril, 2005), thus facilitating solvent penetration. In support of this hypothesis we note (see Figure 3) that beneficial effects associated with the ternary mixture are more evident when the structural integrity of the tomato peel tissue is preserved, *i.e.*, for untreated samples or, to a lesser extent, for short enzyme incubation times.
Considering that the tomato peels used had a lycopene content of 450 mg per 100 g dw, percentage recoveries at 1 h were of about 77%, 74% and 98% with hexane, ethyl acetate or the mixture hexane/acetone/ethanol 50:25:25 as the solvent. The corresponding values for untreated samples were 3%, 4.7% and 29%, respectively.

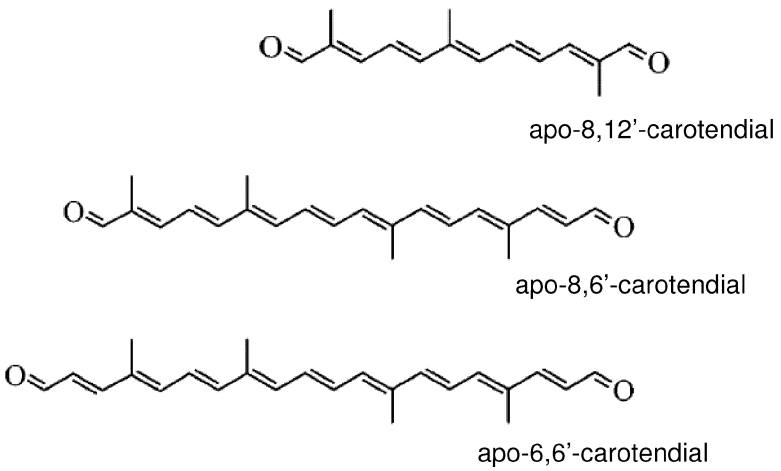

Figure 4 – Molecular structures of some apo-carotendials, cleavage products generated from lycopene oxidation (adapted from Caris-Veyrat et al., 2003).

Finally, Figure 5 shows lycopene extraction yields from the tomato processing waste supplied by Az. DE LUCA. Similar results were obtained with the waste from DESCO SpA. On the whole, recovery values were between 70 and 94% (and between 5 and 40% for the untreated material), which are very close to those for ripe tomato peels, indicating that the enzymatic procedure developed could also be applied to the peel fraction of tomato waste.

4. Conclusions

From the results of this study the following conclusions can be drawn: (1) Recovery of lycopene from tomato peels can be greatly enhanced, even at low temperatures and short incubation times, by the use of cell-wall degrading enzymes. (2) By proper selection of process conditions (*e.g.* 1 h incubation at 40 °C followed by a 3-h solvent extraction) lycopene can be almost completely extracted from the tomato peel tissue. (3) Conventional single or mixed organic solvents approved for food applications can be advantageously used.

Overall, the above points strongly support the possibility of using commercial enzyme preparations to obtain lycopene from the peel fraction of tomato processing waste. The utilization of this material as a source of lycopene could add significant value to the tomato processing chain, improving economic performance and decreasing disposal problems.

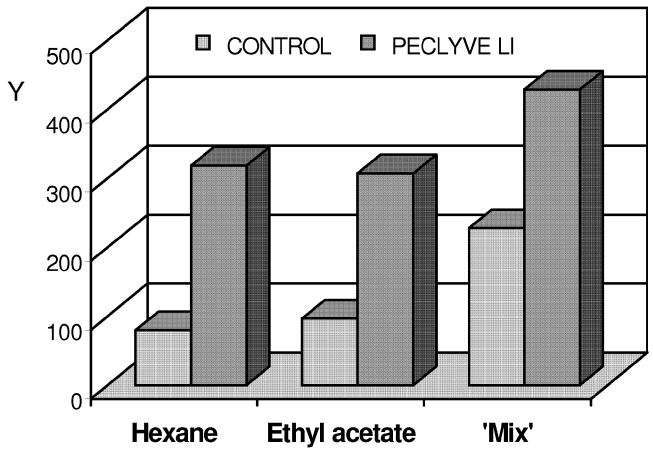

Figure 5 – Lycopene recovery (Y, mg/100 g dw) from untreated and enzyme-treated tomato processing waste after an incubation time of 1 h (T = 40 °C; enzyme preparation: Peclyve LI; extraction time: 3 h). 'Mix' denotes the mixture hexane/acetone/ethanol 50:25:25.

5. Acknowledgements

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