ARTICLE
FOOD BIOTECHNOLOGY
DOI: 10.2478/V10133-010-0028-0 FB

MILD ENZYMATIC METHOD FOR THE EXTRACTION OF LYCOPENE FROM TOMATO PASTE

A. Zuorro, R. Lavecchia
University of Rome "La Sapienza", Department of Chemical Engineering, Materials & Environment, Roma, Italy
Correspondence to: Roberto Lavecchia
E-mail: roberto.lavecchia@uniroma1.it

ABSTRACT

Four commercial enzyme preparations containing cell-wall degrading enzyme activities were tested for their ability to facilitate lycopene extraction from tomato paste. At 25°C, up to 75.6% of the lycopene present in the tomato product was extracted by a two-stage procedure that consisted of a preliminary 5 hour enzyme incubation followed by a 3 hour solvent (hexane or ethyl acetate) extraction. Increasing the duration of the enzymatic treatment to 12-18 h allowed the recovery of 85-90% of total lycopene. The highest extraction yields were achieved by using enzyme preparations with polygalacturonase and pectin methylesterase in addition to pectin lyase or cellulase activities.

Biotechnol. & Biotechnol. Eq. 2010, **24**(2), 1854-1857

Keywords: lycopene, enzymes, tomato paste, cell-wall polysaccharides, bioprocessing

Introduction

A growing body of evidence suggests that lycopene, the major carotenoid pigment found in ripe tomato fruits and one of the most powerful natural antioxidants, can provide protection against cardiovascular disease and some epithelial cancers (12). This has lead to an increased interest for the development of nutritional supplements and functional food products containing this carotenoid (17). Lycopene-enriched vegetable oils (3) and quail eggs (14) are just two examples of the products under investigation. Natural lycopene, however, is very expensive and current production from whole tomato fruits is small relative to the expectations of future demand. As a result, alternative raw materials for the obtainment of lycopene are actively being investigated.

Tomato processing waste and tomato paste are among the most interesting and rich sources of lycopene. The peel fraction of tomato waste contains large amounts of lycopene, up to five times more than the pulp (16), but its high moisture levels and susceptibility to microbial spoilage make storage and processing of this material quite problematic. On the other hand, tomato paste is much more resistant to spoilage and easy to store and process, but lycopene extractability by conventional food-grade organic solvents is extremely low, at least under the conditions that normally preserve the activity of the carotenoid *in vivo*. One main reason is to be found in the cellular localization of lycopene, which is deeply embedded within the chromoplast membrane structures (6). In addition, the progressive removal of water during the manufacturing process leads to a partial collapse of the network of polysaccharide material, hindering solvent penetration and lycopene extraction. Although these limitations could be partly overcome by using more severe process conditions, the risk for lycopene to undergo degradation would proportionally increase (17, 18).

In light of the above considerations, we have explored the possibility of using cell-wall degrading enzymes, i.e., enzymes that are capable of hydrolyzing the main polysaccharide components of the plant structures where the pigment accumulates, as a mild and efficient means to facilitate the recovery of lycopene from tomato paste. These enzymes have successfully been used to facilitate the release of phenolic compounds (7), non-volatile grape aroma precursors (2), capsaicinoids (15) and some types of carotenoids (1, 4) from a variety of plant materials, but no attempt has so far been made to assess their suitability in the case of tomato paste. In this paper, with a view to industrial applications, we have focused our attention on a method utilizing commercial enzyme preparations and organic solvents, such as hexane and ethyl acetate that are approved for food applications in most countries.

Materials and Methods

Simple, double and triple concentrated tomato pastes were obtained from an Italian producer (Mutti SpA, Italy). The following enzyme preparations, all from fungal sources and in liquid form, were used: Citrozym CEO and Ultra L, from Novozymes (Denmark), Peclyve EP and LI, from Lyven (France). Their main activities and optimal temperature and pH, as indicated by the manufacturers, are reported in **Table 1**. Acetone, ethanol, ethyl acetate and n-hexane were purchased from Carlo Erba (Italy), with purities greater than 99.7%, 99.5%, 99% and 95%, respectively. Butylated hydroxytoluene (BHT) with purity greater than 99% was from Sigma-Aldrich Chemie GmbH (Germany).

Prior to extraction, tomato pastes were characterized for moisture content and total lycopene. Moisture content was determined by oven drying at 105°C, while the amount of total lycopene was evaluated according to procedure of Fish

et al., based on the use of the mixture hexane-acetone-ethanol 50:25:25 (v/v) as extracting solvent and BHT (0.05% w/v in acetone) as antioxidant (5). The developed enzymatic method consists in: **(a)** contacting tomato paste with an aqueous enzyme solution for a time sufficient to degrade the tomato plant tissue; **(b)** adding a suitable lycopene solvent to the so obtained suspension; and **(c)** recovering lycopene from the solvent by simple solvent evaporation, so as to obtain a lycopene-rich tomato oleoresin, the standard commercial product on the market.

TABLE 1

Main activities and optimal temperature (T_{OPT}) and pH (pH_{OPT}) for the enzyme preparations used

Preparation	Main activities	T_{OPT} (°C)	pH_{OPT}
Citrozym CEO	Polygalacturonase	40	5.5
Citrozym Ultra L	Polygalacturonase	50	4.5
Peclyve EP	Polygalacturonase Pectin methylesterase Pectin lyase	50	4.5
Peclyve LI	Polygalacturonase Pectin methylesterase Cellulase	45	4.0-5.0

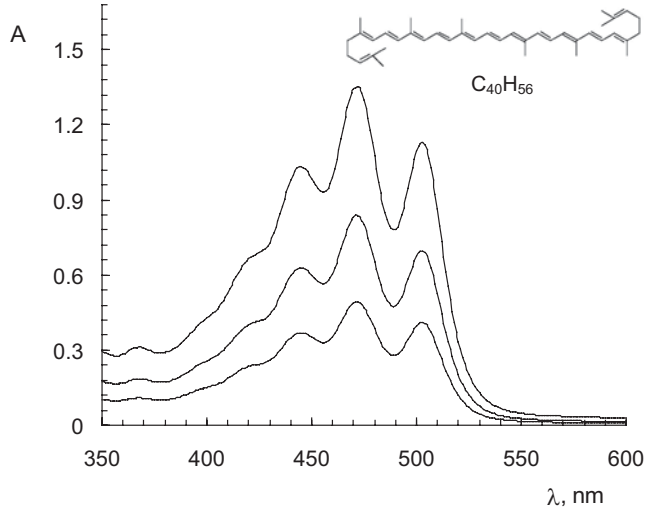

Fig. 1. Visible absorption spectra of lycopene in hexane extracts at different pigment concentrations

In a typical experiment, about 4 g of tomato paste were contacted with 5 mL of the enzyme solution in thermostated (25 or 40°C) and magnetically stirred flasks. The reaction mixture was prepared by dissolving 0.2 mL of the liquid enzyme preparation in 4.8 mL of bidistilled water. The incubation time was in the range of 3-12 h, depending on the temperature and the duration of the successive solvent extraction step. At the end of pretreatment, 30 mL of hexane or ethyl acetate were poured into the flasks and the system was kept under stirring for further 3 h. After this time, a sample of the liquid was withdrawn, filtered and analysed spectrophotometrically for lycopene content. An example of the resulting absorption spectra in hexane is displayed in **Fig. 1**, which shows the three characteristic peaks of lycopene at around 445, 472 and 503 nm. To minimize interference from other carotenoids, lycopene concentration was determined at 503 nm, using a molar extinction coefficient of 1.585 10^5 $M^{-1}cm^{-1}$ (10).

Results and Discussion

Characterization of the three tomato pastes gave results, which are summarized in **Table 2**. The moisture content was between 67.5 and 80.7%, while the average lycopene content was around 48 mg/100 g, a value that largely supports the possibility of using this product as a source of lycopene.

TABLE 2

Moisture and total lycopene content for the three used tomato pastes

Tomato paste	Moisture (wt %)	Lycopene content (mg/100g)
Simple concentrated	80.7±0.5	43.11±1.88
Double concentrated	74.0±0.4	48.80±1.97
Triple concentrated	67.5±0.4	52.60±0.96

The extraction yield was calculated as:

$$Y = \frac{cV_L}{m_S} \quad (1)$$

where c is the concentration of lycopene in the solvent, V_L is the volume of the liquid and m_S is the amount of solid. Y was expressed as mg of lycopene per 100 g of tomato paste.

Lycopene extraction from the three tomato pastes provided very similar results, in terms of the influence of process conditions on yields, suggesting that the basic structure of these materials and the properties of the membrane-associated carotenoid were essentially the same. Two types of control experiments were carried out: **(a)** direct solvent extraction from the tomato paste materials, and **(b)** solvent extraction after pretreatment of the tomato paste with pure water. These latter were aimed at highlighting possible beneficial effects arising from the contact of tomato paste with water. The results of control runs were compared with those for the enzymatically treated samples. No appreciable difference was observed in the overall results by replacing hexane with ethyl acetate as extraction solvent. A representative example of the obtained results using the simple concentrated product is shown in **Fig. 2**. Under the considered conditions here (T=25°C; incubation time: 5 h; extraction time: 3 h) control experiments gave yields of 1.7±0.8 mg/100 g, for the unpretreated samples, and 10.9±1.1 mg/100 g for the water-pretreated samples. Pretreatment by the enzyme preparations resulted in significantly higher yields. In particular, with respect to the unpretreated samples, lycopene recovery increased by a factor ranging from about 10 (Citrozym Ultra L) to 20 (Peclyve LI and EP). Peclyve LI and EP were the most efficient preparations yielding, respectively, 32.6±1.7 and 31.7±1.4 mg of lycopene per 100 g of tomato paste. If we consider a total lycopene content of 43 mg/100 g for this material, percent extraction yields of, respectively,

75.6% and 73.5% are calculated. For comparison, yields from the unpretreated samples were slightly less than 4%.

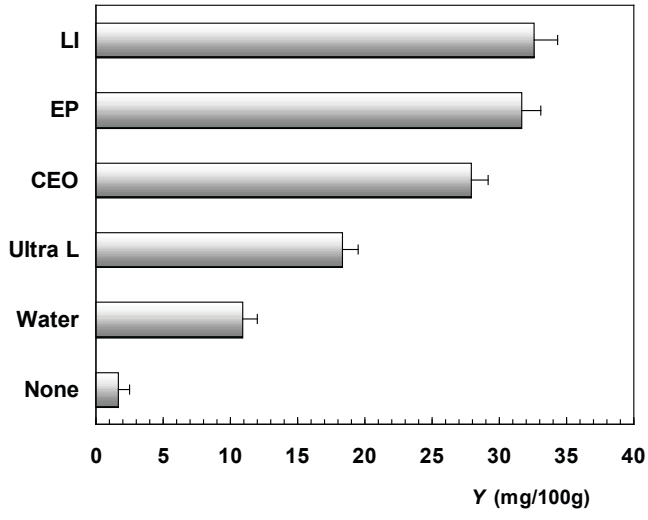

Fig. 2. Lycopene extraction yields from untreated (None), water-treated (Water) and enzyme-treated (LI, EP, CEO, Ultra L) samples of simple concentrated tomato paste. Experimental conditions: T=25°C; incubation time: 5 h; extraction time: 3 h; solvent: n-hexane

Regarding the results from control experiments, it is worth noting that the pretreatment of tomato paste with pure water provided some beneficial effect on yields, which were increased by about 6-fold as compared to the untreated ones (see **Fig. 2**). This promoting effect could be attributed to the swelling of plant tissue by water molecules. Water, in fact, is an effective swelling-inducing agent, due to its small molar volume and high hydrogen bonding capability (11). Swelling makes the tomato paste matrix less compact, favouring the diffusion of the extracting solvent.

Lycopene extraction yields were further increased by varying the incubation time and/or the temperature. At 25°C, an increase in the duration of enzymatic treatment of up to 12-18 h allowed the extraction of about 85-90% of total lycopene. At 40°C, 4-6 h were sufficient to achieve the same yields. This effect is probably a result of the temperature-induced increase in enzyme activity, being the optimal temperatures for the enzyme preparations used between 40 and 50°C (see **Table 1**). Also supporting such a view is the observation that no apparent improvement in yield was seen for samples that did not undergo enzymatic treatment.

The observed enzyme-induced enhancement in extractability can be explained by the fact that tomato tissue is rich in cellulose, hemicellulose and pectin, and that the used preparations have cellulolytic, hemicellulolytic and pectinolytic activities. According to current views of plant cell-wall architecture, cellulose, a linear polymer of β-1,4-linked glucose, and hemicelluloses (xyloglucans and xylans) form a fairly rigid network that interacts with a gel-like matrix of hydrated pectic substances (13). Since lycopene is segregated within these structures, the degradation of polysaccharide components improves solvent penetration into the product, increasing the amount of recovered lycopene.

Comparative screening of the enzyme preparations indicated that Peclyve LI and EP were the most efficient among those tested. Examination of **Table 1** reveals that Peclyve preparations contain, in addition to polygalacturonase which is common to all of them, pectin methylesterase and pectin lyase (or cellulase) activities. It can therefore be argued that the association polygalacturonase-pectin methylesterase provides the best exploitation of enzyme activities, in terms of degradation of the structural components of tomato paste. However, since the polysaccharide composition of tomato products or tomato fruit parts may depend on the type of product, the fruit variety and the ripening stage, the optimal enzyme formulation should be assessed case by case (8, 9).

Conclusions

The results from this study clearly demonstrate that the recovery of lycopene from tomato paste can be greatly enhanced, even at ambient temperature, by the use of cell-wall degrading enzymes. Of course, the cost of enzymes should be carefully considered when assessing the economic feasibility of the process on a larger scale. However, it should be emphasized that the enzyme preparations used in this work are not expensive, being industrially produced for large-scale food applications, and that the dosage recommended by the manufacturers for similar uses is fairly low. These considerations, and the fact that tomato paste is an abundant and rich source of lycopene, lend strong support to the use of enzymes as a convenient means for the obtainment of functionally active natural lycopene from this tomato product.

Acknowledgements

We thank MUTTI SpA (Parma, Italy) for their kind gift of tomato paste products.

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