Eur Food Res Technol (2008) 228:153–158
DOI 10.1007/s00217-008-0897-8

SHORT COMMUNICATION

Improved lycopene extraction from tomato peels using cell-wall degrading enzymes

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Received: 3 January 2008 / Revised: 12 May 2008 / Accepted: 17 May 2008 / Published online: 10 June 2008

Abstract Four commercial enzyme preparations with pectinolytic, cellulolytic and hemicellulolytic activities were tested for their ability to enhance lycopene extraction from tomato peels. Screening experiments were performed at 25 °C by subjecting the peels to a 4-h enzyme incubation followed by 1-h hexane extraction. Peclyve EP and LI were the most efficient, with an almost 20-fold increase in extraction yield. Peclyve LI was used to evaluate the influence of solvent type and enzyme incubation time on lycopene recovery. Hexane, ethyl acetate and the mixture hexane/acetone/ethanol 50:25:25 (v/v) were used as solvents. Under the best extraction 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. The percentage recoveries were in the range of 3–30%, for the untreated peels, and 77–98% for the enzymatically treated material.

Keywords Enzymes · Pectinase · Lycopene · Tomato peels · Tomato waste · Solvent extraction

Introduction

Lycopene is a lipophilic carotenoid pigment found in tomatoes and other red fruits such as watermelon, guava and pink grapefruit [1]. The high degree of conjugation of double bonds in the molecule makes lycopene one of the most potent antioxidants, with a singlet-oxygen-quenching ability twice as high as that of β-carotene and 10 times higher than that of α-tocopherol [2]. Although more in-vitro and in-vivo studies are needed to assess the real potential of lycopene and its effectiveness relative to other lipophilic antioxidants, increasing evidence suggests that it might have a role in the prevention of a variety of chronic diseases associated with oxidative stress [3, 4].

Commercial lycopene is available as standardized tomato extract or from chemical synthesis. Market trends indicate a growing demand for the former product, because of its natural origin and the presence of other phytochemicals, such as β-carotene, phytoene and phytofluene, which are believed to act synergistically with lycopene [5].

Large amounts of solid residues consisting primarily of ripe tomato peels and seeds are generated annually by the tomato processing industry. This material is currently disposed of as a solid waste or used as animal feed, but the abundance of lycopene in the peel fraction of the waste suggests the possibility of utilizing it as a cheap source of lycopene. As highlighted in the very recent review on vegetable waste treatment by Arvanitoyannis and Varzakas [6], the use of fruit or vegetable residues as a source of valuable phytochemicals offers great promise in the management of these wastes.

Tomato skins can contain up to five times more lycopene than the pulp [7]. However, despite such a high content, the available extraction technologies do not seem to allow an efficient 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 [8]. Yields increased to 73% when the temperature was raised to 80 °C [8]. Similar results were obtained by Rozzi et al. [9], who used supercritical CO_2 at 86 °C and 34.5 MPa to recover 61% of the lycopene contained in tomato peels.

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Low extraction efficiencies can be ascribed to the difficulty for the solvent molecules to penetrate the compact tomato peel tissue and solubilize the pigment, which is deeply embedded within the chromoplast membrane structures [10]. In theory, the extraction efficiency could be improved by using more severe extraction conditions, but the risk for lycopene to undergo oxidative degradation would proportionally increase [11].

Cell-wall degrading enzymes have been successfully used to favor the release of a variety of components, including vegetable oil [12], non-volatile grape aroma precursors [13] and carotenoids [14, 15], from vegetable tissues. Recently Choudhari and Ananthanarayan [16] have investigated their use for improving the extraction of lycopene from tomatoes. These authors used two enzymes, cellulase and pectinase, and found that both of them allowed a significant increase in lycopene recovery from various tomato materials. However, while pectinase was more effective than cellulase for whole tomatoes, tomato peels and fruit pulper waste, the opposite was true for industrial waste.

Since tomato peel is a highly structured plant material containing many different polysaccharide components, such as cellulose, hemicelluloses and pectins [17], we have explored the possibility of using mixed enzyme preparations with pectinolytic, cellulolytic and hemicellulolytic activities to enhance lycopene extraction. With a view to industrial exploitation, we used commercial enzyme preparations and organic solvents such as ethyl acetate and hexane that are approved for food applications in most countries. The results obtained indicate that a mild enzymatic treatment by these preparations can lead to a rapid and almost complete recovery of lycopene from tomato peels, even at temperatures close to ambient.

Materials and methods

Materials

Four commercial liquid enzyme preparations were used: Citrozym CEO and Citrozym Ultra L, from NOVOZYMES (Denmark), with declared activities of 9,500 PGU/mL and 4,500 PECTU/mL, respectively; Peclyve LI and Peclyve EP, from LYVEN (France). They were all produced from *Aspergillus* strains and the main activities of all were pectinolytic.

Acetone, ethanol, ethyl acetate and hexane were purchased from CARLO ERBA (Italy). Their purities were greater than 99.7, 99.5, 99 and 95%, respectively. Butylated hydroxytoluene (BHT) with a purity of >99% was from Sigma–Aldrich Chemie GmbH (Germany).

Sample preparation and characterization

Fresh ripe tomatoes were purchased from a local market and stored at 4 °C for a maximum of 2 days before use. 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 on tissue paper at room temperature (20–22 °C) in the dark for 4–5 h. Then they were wrapped in aluminum foil and stored at 4 °C for not more than 24 h before use.

The moisture content of the peels was determined by oven drying at 105 °C to constant weight. The total lycopene content was determined by a slight modification of the procedure described by Fish et al. [18]. In particular, instead of the single-stage extraction used by the authors, three consecutive extractions were performed on each tomato peel sample in order to achieve complete removal of lycopene from the plant material. Briefly, 1 g of fresh and finely ground tomato peels were placed into amber flasks, to which 25 mL of 0.05% (w/v) BHT in acetone, 25 mL of ethanol and 50 mL of hexane were rapidly added. The flasks were magnetically stirred for 15 min, after which time 15 mL of deionized water were poured into the flask to allow phase separation to occur. Stirring was continued for 5 min and then the system was left at room temperature for further 5 min. Finally, a sample of the upper hexane layer was taken and analyzed for lycopene content. The process was repeated three times and the total lycopene content was calculated as the sum of the values obtained in each extraction stage.

Lycopene assay

Lycopene concentration in the extracting solvents was determined spectrophotometrically by a double-beam UV-VIS spectrophotometer (Perkin-Elmer Lambda 25). The absorption spectra of lycopene in hexane and ethyl acetate were nearly identical and independent of application of enzymes, with the three characteristic peaks of lycopene at around 445, 472 and 503 nm (Fig. 1). Prior to analyze the lycopene content in the ternary mixture hexane–acetone–ethanol 50:25:25 (v/v), excess water (about 15/100 mL of the mixture) was added so as to obtain two clear and well separated phases: an upper organic layer, containing lycopene and consisting almost entirely of hexane, and an aqueous layer consisting of acetone, ethanol and water. Samples were then taken from the upper hexane layer.

Spectrophotometric measurements were made at 503 nm and the pigment was quantified using a molar extinction coefficient of $1.585\ 10^5\ M^{-1}\ cm^{-1}$ [19].

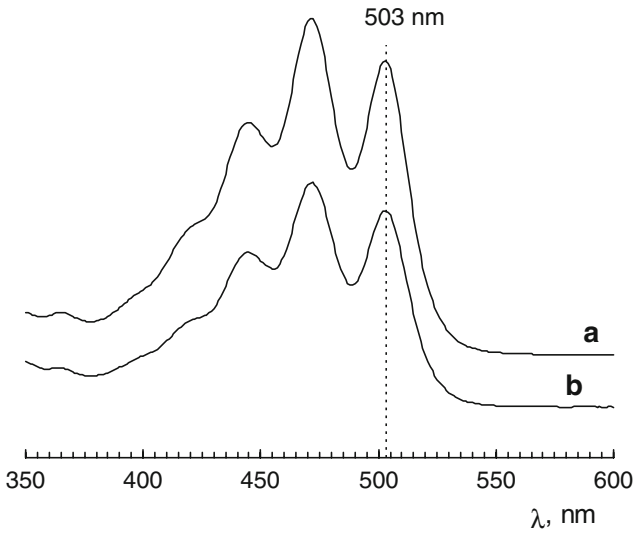

Fig. 1 VIS absorption spectra of hexane extracts from (**a**) enzyme-treated and (**b**) untreated tomato peels

Screening of enzyme preparations

The four enzyme preparations were screened for their ability to enhance lycopene extraction from the peels.

Partially dehydrated tomato peels, obtained as described in the sample preparation section, were broken by hand into small pieces (5–8 mm). 0.2 g of this material and 3.5 mL of the aqueous 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 screw-top 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 analyzed for lycopene content. Throughout all operations the flasks were kept in the dark and exposure to air during handling was minimized.

Effect of extraction conditions on yields

These experiments were made using Peclyve LI, the preparation yielding the greatest improvement in lycopene extraction. The effects of solvent type and enzyme incubation time were investigated. The solvents included hexane, ethyl acetate and the ternary mixture hexane/acetone/ethanol (50:25:25 v/v). This latter was chosen because of its proven efficacy for the extraction of carotenoids from plant material [20]. The enzyme incubation time was varied between 1 and 15 h.

Extraction runs were carried out according to the procedure described in the previous section. The experimental conditions were the same, except for the temperature, which was 40 °C, and the extraction time, which was set at 3 h. These values were modified with respect to the previous ones (25 °C and 1 h) in order to increase the efficiency of extraction, minimizing at the same time the loss of lycopene due to oxidation. Throughout all operations the flasks were kept in the dark and exposure to air during handling was minimized.

Results and discussion

Extraction yields were expressed as mg of lycopene per 100 g of dry plant material. Values reported in the following are means obtained from two to three independent experiments. For each set of extraction conditions, control runs were also performed using water instead of the enzyme solution.

The moisture content of the peels was between 80 and 85 wt%, and the total lycopene content was 450 ± 21 mg/100 g of dry material. Since the latter was obtained from measurements on different tomato peel samples, the observed variation in values can be attributed to differences in fruit ripeness and/or to the intrinsic heterogeneity of the plant material used.

Screening of enzyme preparations

Results shown in Fig. 2 indicate the remarkable extent to which lycopene recovery was increased by the enzymatic treatment. While untreated controls gave yields as low as 18 mg of lycopene per 100 g of dry tomato peels, extraction from enzyme-treated samples yielded from 95 to 356 mg/100 g. Of the four preparations examined, Peclyve EP and LI were the most efficient, with recoveries of 318 and 356 mg/100 g, respectively. On average, these values correspond to an almost 20-fold increase with respect to the untreated peels.

The observed enhancement of extraction efficiency can be explained by considering that pectin, cellulose and hemicellulose are the major polysaccharide components of tomato peel tissue [17] and that the enzyme preparations used contained, in addition to pectinolytic activity, cellulolytic and hemicellulolytic activities. Accordingly, when the peels are enzymatically treated, improved solvent penetration and lycopene dissolution are expected to occur as a result of cell separation and degradation of cell-wall components. 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 peel tissue is dependent on fruit variety and ripening stage [21], a preliminary

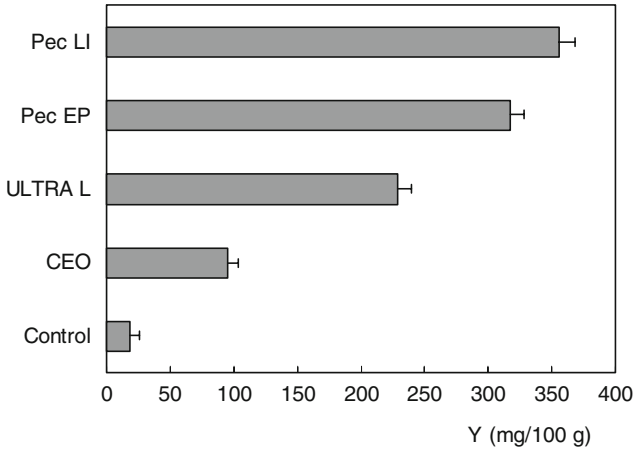

Fig. 2 Extraction yields (Y) of lycopene from untreated and enzyme-treated tomato peels. 'Pec EP' and 'Pec LI' denote the enzyme preparations Peclyve EP and Peclyve LI ($T = 25$ C; incubation time, 4 h; solvent, hexane; extraction time, 1 h)

screening of enzyme preparations should always be performed on the specific material used.

Effect of extraction conditions on yields

Table 1 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, 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 that were 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 [10]. Although the underlying mechanisms are still under investigation, 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 [22]. The reduction in extraction yields observed at prolonged incubation times could therefore be a reflection of the progressive lycopene loss due to oxidation.

Assuming a total lycopene content of 450 mg/100 g of dry tomato peels (the average of the values determined for the material used), percentage recoveries at 1 h incubation time can be easily calculated. As can be seen from Fig. 3, lycopene recovery from enzymatically treated peels was 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.

It may be interesting to compare our results with those of the only, to our knowledge, published study on the enzyme-assisted extraction of lycopene from tomato peels [16]. In this study it was found that, under optimized extraction conditions, cellulase and pectinase increased lycopene recovery by more than twofold and threefold, respectively. Since the enhancement that we observed was in the range of 3- to 25-fold, it can be inferred that mixed enzyme

Table 1 Influence of enzyme incubation time on the extraction yields of lycopene from enzyme-treated (Y) and untreated (Y_0) tomato peels using hexane, ethyl acetate and the mixture hexane/acetone/ethanol 50:25:25 as solvents ($T = 40$ °C; enzyme preparation: Peclyve LI; extraction time: 3 h)

Solvent	Time (h)	Y (mg/100 g)	Y_0 (mg/100 g)
Hexane	0.5	346.4 ± 12.2	13.6 ± 10.0
	5	322.8 ± 14.1	17.6 ± 12.6
	15	260.3 ± 15.0	30.8 ± 12.9
Ethyl acetate	0.5	334.3 ± 11.7	21.4 ± 10.1
	5	312.1 ± 12.6	25.4 ± 11.7
	15	295.1 ± 14.8	49.8 ± 12.0
Hexane/acetone/ ethanol 50:25:25	0.5	440.2 ± 13.9	130.7 ± 14.8
	5	297.0 ± 15.2	104.4 ± 13.6
	15	248.6 ± 16.4	89.5 ± 15.1

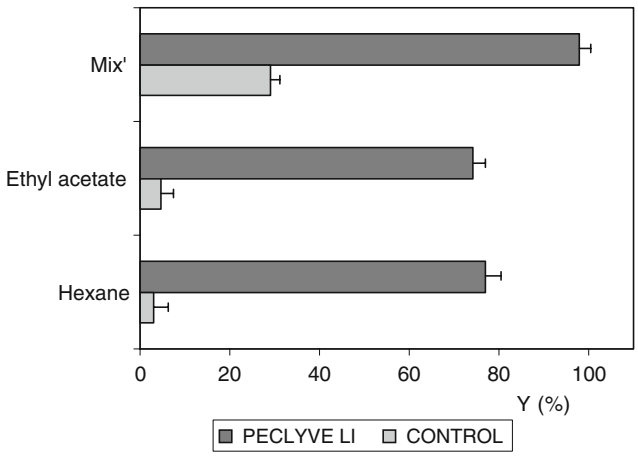

Fig. 3 Percentage yields ($Y\%$) of lycopene from untreated and enzyme-treated tomato peels after an incubation time of 1 h. 'Mix' denotes the mixture hexane/acetone/ethanol 50:25:25 v/v (T = 40 °C; Enzyme preparation: Peclyve LI; Extraction time: 3 h)

preparations may offer greater advantages over single classes of enzymes like cellulases or pectinases. The synergism resulting from the combined use of different cell-wall degrading enzymes has been evidenced for a number of plant materials, such as carrots, sweet potatoes and orange peels [23–25]. It seems therefore reasonable that the same should apply to tomato peels, which are made up of the same basic constituents [17, 26].

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 an 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, cause the swelling of the plant tissue [27, 28], thus facilitating solvent penetration. In support of this hypothesis we note (see Table 1) that the 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.

A last point to be made about the mixture hexane/acetone/ethanol 50:25:25 or other multi-component solvents is that, although their use within an analytical procedure is quite straightforward and easy to realize [20], some difficulties may be encountered when implementing the process on an industrial scale. The most apparent are the need to accurately control the mixture composition throughout the entire extraction process and the fact that, depending on the specific country legislation, one or more solvent components could not be permitted for food use.

Conclusions

From the results of this study two main 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 mixed enzyme preparations with pectinolytic, cellulolytic and hemicellulolytic activities. (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, with either single or mixed organic solvents.

From an industrial viewpoint, the use of enzymes may offer several advantages over conventional extractions. The most obvious are the use of smaller amounts of solvent and energy, for a specified degree of lycopene recovery, or the increase in yield, for given process conditions. Furthermore, compared with supercritical fluid extraction, lower capital costs and greater flexibility are expected, along with the possibility of using tomato waste material with any moisture content. Of course, the cost of enzymes is a key factor to consider when assessing the economic feasibility of the process on a large scale. In this connection, however, it should be considered that the enzyme preparations used in this study are not expensive, being commercially produced for industrial applications, and that the suggested dosage for vegetable or fruit liquefaction is of the order of a few hundreds of grams per ton of raw material.

Acknowledgments We thank NOVOZYMES A/S (Bagsvaerd, Denmark) and LYVEN S.A. (Colombelles, France) for their kind gift of enzyme preparations.

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