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Enhanced Lycopene Recovery from Tomato Processing Waste by Enzymatic Degradation of Plant Tissue Components

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Abstract – *Eight commercial enzyme preparations with pectinolytic, cellulolytic, hemicellulolytic and proteolytic activities were tested for their ability to enhance lycopene extraction from tomato peels. Screening experiments were performed at 40 °C by subjecting the peels to a 1-h enzyme incubation followed by 1-h hexane extraction. The resulting yields were between 51 and 195.9 mg of lycopene per 100 g of dry tomato peels, while the value obtained for the untreated peels was 23.7 mg /100 g. Synergistic and antagonist effects were observed when different enzyme products were used in 50:50 (v/v) combination. The preparations richest in cellulase and pectinase were the most efficient, with an up to 10-fold increase in extraction yield. A statistical analysis of factors affecting lycopene extraction revealed that enzyme dosage was the most influential, followed by temperature and enzyme incubation time. A strong interaction was also found between the latter two variables.*

Keywords: *Bioprocessing, Cell Wall Polysaccharides, Enzymes, Lycopene, Plant Tissue, Tomato Processing Waste*

I. Introduction

In recent years, environmental concerns and sustainability issues have prompted efforts to devise new strategies for the efficient management of agro-industrial wastes [1]. An interesting opportunity is offered by their use as raw materials for the extraction of value-added products such as dietary fibers, antioxidants or other substances with positive health effects [2], [3]. Proanthocyanidins from grape seeds and pectin from citrus peels or apple pomace are examples of products that can be obtained and are already marketed [4].

More than 30 million tons of tomatoes are transformed annually into a variety of tomato products ranging from canned tomatoes to tomato paste and ketchup. After the USA, Italy is the second largest world manufacturer, with 4.6 million tons of tomatoes processed in 2007 [5]. Tomato processing produces huge amounts (up to 3% by weight of the fresh tomatoes) of a solid waste generally known as pomace. It consists primarily of tomato peels and seeds, in a proportion depending on the product being produced. Tomato pomace has no commercial value and is currently disposed of as a solid waste or used as animal feed. However, it contains several bioactive compounds such as dietary fiber, vitamins and a number of phytochemicals that might be of interest for the pharmaceutical, cosmetic and food industries. One of such compounds is lycopene, the pigment that gives tomatoes their characteristic red color [6]. Chemically, lycopene (ψ,ψ–carotene) is a tetraterpenic hydrocarbon with 13 carbon–carbon double bonds, 11 of which are conjugated (Fig. 1).

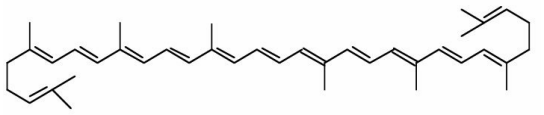

Fig. 1. Chemical structure of lycopene

The high degree of conjugation gives the molecule the ability to inactivate free radicals and other deleterious oxygen species, making it one of the most potent natural antioxidants. Its efficiency of singlet oxygen quenching has been shown to be 10 times higher than that of α-tocopherol and twice as high as that of β-carotene [7]. These properties have been invoked to explain the observed association between the consumption of lycopene-rich foods and the reduced risk of cardiovascular disease and some cancers [8], [9].

Natural lycopene is very expensive and is currently produced by extraction and concentration from whole tomato fruits that are specifically grown for this purpose. Although tomato peels are particularly rich in lycopene, with levels that are up to five times higher than in the pulp [10], the available extraction technologies do not seem to provide a rapid and efficient recovery of the pigment from the tomato peel tissue. For example, only about 50% of total lycopene was extracted from tomato processing waste using supercritical CO_2 at 60°C and 30 MPa [11]. To increase the yield to 73%, a temperature of 80 °C was necessary [11]. Similar results were obtained

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by Rozzi et al. [12], who used supercritical CO_2 at 86 °C and 34.5 MPa to recover 61% of the lycopene contained in the peels.

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 [13].

Extraction yields could theoretically be enhanced by using more severe process conditions, but the risk for lycopene to undergo oxidative degradation would proportionally increase [14].

In this study we have explored the possibility of using enzymes to improve the recovery of lycopene from the peel fraction of tomato processing waste. In particular, we focused our attention on cell-wall degrading enzymes, i.e., enzymes that are capable of hydrolysing the major polysaccharide components of plant cell walls. These enzymes have been successfully employed to facilitate the release of vegetable oils [15], [16], non-volatile grape aroma precursors [17] and carotenoids [18]-[20] from plant materials.

The main aim of our investigation was to determine whether the available commercial enzyme preparations could be used for effectively degrading the tomato peel tissue and, hence, for increasing the extractability of lycopene from tomato processing waste. For this purpose we screened some food-grade enzyme preparations, either singly or in combination, and analysed the statistical influence of the main process variables to evaluate their contributions to the overall extraction efficiency.

II. Experimental

II.1. Materials

Deep-red tomatoes were purchased from a local market and stored at 4 °C for a maximum of 2 days before use. Samples of tomato processing wastes were obtained from ASSO.PRO. (Guglionesi, CB, Italy), Azienda DE LUCA (Anzio, RM, Italy) and DESCO SpA (Terracina, LT, Italy). They were put in plastic bags as soon as received and stored at –20 °C.

Eight enzyme preparations from fungal sources were used: Peclyve EP, LI and LVG; Cellulyve 50L and 50LC; Prolyve PAC 30L, from Lyven (France), Citrozym CEO and Ultra L, from Novozymes (Denmark).

The main activities and the optimal temperature and pH, as indicated by the manufacturers, are reported in Table I.

All preparations were in liquid form and were diluted with distilled water prior to use.

Acetone, ethanol and hexane were purchased from Carlo Erba (Italy), with purities greater than 99.7%, 99.5% and 99%, respectively. Butylated hydroxytoluene (BHT) with purity greater than 99% was from Sigma-Aldrich Chemie GmbH (Germany).

TABLE I
MAIN ACTIVITIES AND OPTIMAL TEMPERATURE AND PH OF THE ENZYME PREPARATIONS USED

Preparation	Main Activities	T_{OPT} / °C	pH_{OPT}
Peclyve EP	PG, PM, PL	50	4.5
Peclyve LI	PG, PM, C1	45	4.0–5.0
Peclyve LVG	PG, PM, PL	45–50	4.5
Cellulyve 50L	C1, C2, C3	55	4.0–4.5
Cellulyve 50LC	C1, C2, C3	55	4.0–4.5
Prolyve PAC 30L	AP	55	2.5–3.0
Citrozym CEO	PG	40	5.5
Citrozym Ultra L	PG	50	4.5

PG, Polygalacturonase; PM, Pectin Methylesterase; PL, Pectin Lyase; C1, Cellulase; C2, β-glucosidase; C3, Cellulose 1,4-β-cellobiosidase; AP, Acid Protease

II.2. Lycopene Assay

Lycopene concentration in the extracting solvent was determined spectrophotometrically at room temperature using 1-cm path length quartz cuvettes and a double-beam UV-VIS spectrophotometer (Perkin-Elmer Lambda 25).

Absorption spectra of hexane extracts displayed the three characteristic peaks of lycopene at around 445, 472 and 503 nm (Fig. 2). To minimise interference from other carotenoids measurements were made at 503 nm, using a molar extinction coefficient of $1.585\ 10^5\ M^{-1}cm^{-1}$ [21].

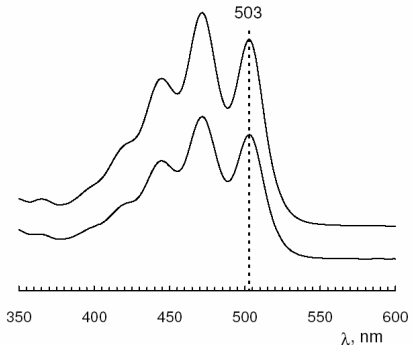

Fig. 2. Visible absorption spectra of lycopene in hexane extracts

II.3. Sample Preparation and Characterization

Appropriate amounts of frozen tomato waste were thawed just before use. The skins were separated by hand from the seeds and other impurities. Fresh tomato fruits were hand peeled.

The peels were partially dried in air for a few hours and stored at 4°C.

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. [22], which makes 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.

II.4. Screening of Enzyme Preparations

The eight enzyme preparations were screened, either singly or in combination, for their ability to enhance lycopene extraction from the peels.

Partially dehydrated peels, obtained from fresh tomatoes as described previously, were broken into small pieces (5–7 mm) and mixed by hand. 0.5 g of this material and 15 mL of a 2.5% by weight enzyme solution were initially charged into 50-mL screw-top conical flasks.

The flasks were magnetically stirred and incubated at 40 °C for 1 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.

Finally, a 2-mL sample of extracting solvent was taken and analysed for lycopene content.

At all stages of extraction the flasks were kept in the dark to minimize pigment degradation.

For the same reason exposure to air during handling was as short as possible.

II.5. Factor Influence Analysis

The enzyme preparation with the highest cell-wall degrading activity was used to evaluate the influence of the main process variables on lycopene recovery. Experiments were made on the peel fraction of an "average" reconstituted tomato processing waste obtained by mixing equal amounts of the three waste materials. In a previous study we found that temperature, incubation time and enzyme dosage were the most important factors affecting the extraction process on a qualitative level [23]. In order to quantitatively estimate their contributions to the overall extraction yield we used a three-factor two-level full factorial design. Four center-point replicates were also included, for a total of $2^3 + 4 = 12$ runs (Table II).

TABLE II
EXPERIMENTAL DESIGN LAYOUT

Run	Trial	X1	X2	X3
1	3	–1	–1	–1
2	2	+1	–1	–1
3	10	–1	+1	–1
4	7	+1	+1	–1
5	4	–1	–1	+1
6	1	+1	–1	+1
7	9	–1	+1	+1
8	6	+1	+1	+1
9	8	0	0	0
10	11	0	0	0
11	12	0	0	0
12	5	0	0	0

The "run" column indicates the formal order of runs in the experimental design, while the "trial" column shows the randomized order in which the experiments were carried out.

The factor levels were chosen so as to cover a range of values of practical interest (Table III).

Extraction runs were carried out as described in the previous section, using 0.5 g of peels, 10 mL of enzyme solution, 30 mL of hexane as solvent and an extraction time of 3 h.

Throughout all operations the flasks were kept in the dark and exposure to air was minimized.

TABLE III
FACTORS, CODES AND LEVELS FOR THE FACTORIAL DESIGN

Factor	Code	Levels		
		-1	0	1
Temperature (°C)	X1	25	37.5	50
Incubation time (h)	X2	1	2	3
Enzyme dosage (mg/g)	X3	50	150	250

III. Results and Discussion

Lycopene extraction yields were expressed as mg of pigment per 100 g of dry plant material.

Characterization of tomato peels gave the results summarized in Table IV.

Total lycopene content exhibited a quite large variability (between 227 and 546 mg/100 g), which could be due to differences in fruit maturity and/or to pigment loss during processing or storage.

TABLE IV
MOISTURE AND LYCOPENE CONTENT OF PEELS FROM FRESH TOMATOES AND TOMATO PROCESSING WASTES

Peel Source	Moisture (wt %)	Lycopene content (mg/100 g)
Fresh tomatoes	85.4	460 ± 15
Waste from ASSO.PRO.	86.7	539 ± 42
Waste from Azienda DE LUCA	90.5	546 ± 39
Waste from DESCO SpA	96.0	227 ± 34

III.1. Screening of Enzyme Preparations

Data in Table V show the remarkable extent to which the enzymatic treatment increased lycopene recovery. While untreated controls gave yields as low as 23.7 mg of lycopene per 100 g of dry tomato peels, extraction from enzyme-treated samples yielded from 51 to 195.9 mg/100 g.

Cellulyve 50L and 50LC, the preparations with a broad spectrum of cellulolytic activities, were the most efficient, with recoveries of 186.2 and 195.9 mg/100 g, respectively. Significant increases were also observed with Peclyve EP and LI, which provided about 140 mg/100 g. Values determined for the remaining preparations were between 51 and 88.3 mg/100 g.

TABLE V
LYCOPENE EXTRACTION YIELDS (Y) OBTAINED FROM THE SCREENING OF THE ENZYME PREPARATIONS. Y^* IS THE VALUE DETERMINED FOR THE UNTREATED MATERIAL

Enzyme Preparation	Y / mg/100 g	Y/Y^*
None	23.7 ± 2.5	1.0
Peclyve EP	139.8 ± 1.4	5.9
Peclyve LI	141.0 ± 5.7	5.95
Peclyve LVG	51.0 ± 5.1	2.15
Cellulyve 50L	186.2 ± 6.5	7.86
Cellulyve 50LC	195.9 ± 3.6	8.26
Prolyve PAC 30L	88.2 ± 4.9	3.72
Citrozym CEO	77.8 ± 5.0	3.28
Citrozym Ultra L	88.3 ± 2.4	3.72
A – Peclyve LI + Citrozym CEO	125.3 ± 4.6	5.29
B – Cellulyve 50LC + Peclyve LI	218.3 ± 5.1	9.21
C – Peclyve LI + Citrozym Ultra L	78 ± 4.8	3.29
D – Peclyve EP + Peclyve LI	130.6 ± 4.3	5.51

The above results clearly demonstrate the ability of all the products tested to improve the recovery of lycopene from tomato skins. The enhancement in extractability can be explained by the fact that the peel tissue is rich in cellulose, hemicellulose and pectin [24] and that the preparations used have cellulolytic, hemicellulolytic and pectinolytic activities. Increased solvent penetration and lycopene dissolution can therefore be expected to occur when cell-wall polysaccharides are enzymatically degraded.

According to current views of plant cell-wall architecture, cellulose, a linear polymer of β-1,4-linked glucose, and hemicelluloses, such as xyloglucans and xylans, form a fairly rigid network that is embedded in and interacts with a gel-like matrix of hydrated pectic substances [25], [26]. Bundles of cellulose molecules are aggregated together in the form of microfibrils composed of highly ordered crystalline domains and extended amorphous regions. Cell walls also contain small amounts of structural proteins involved in the reinforcement and assembly or restructuring of the wall [27].

The higher efficiency observed when using Cellulyve 50L and 50LC suggest that their activity profile allows the most effective degradation of the tomato peel tissue. Since these preparations are particularly rich in cellulase and hemicellulase, it can be speculated that the enzymatic disruption of the cellulose–hemicellulose network is mostly responsible for the observed increases in yield.

To explore possible interaction effects among the enzyme preparations, we performed additional experiments with four binary mixtures (A–D, in Table V) prepared by blending equal volumes of each product. The presence of such effects was assessed by comparison of the effective yields with those expected from a linear dependence of yield on mixture composition, i.e., in the absence of interactions. The results shown in Fig. 3 indicate synergistic effects for formulations A (Peclyve LI + Citrozym CEO) and B (Cellulyve 50LC + Peclyve LI), and antagonistic effects for C (Peclyve LI + Citrozym Ultra L) and D (Peclyve LI + Peclyve EP). In the first two cases the observed increases in yield were of about 15% and 30%, respectively. For the latter ones, decreases were of the order of 47% and 7.5%, respectively.

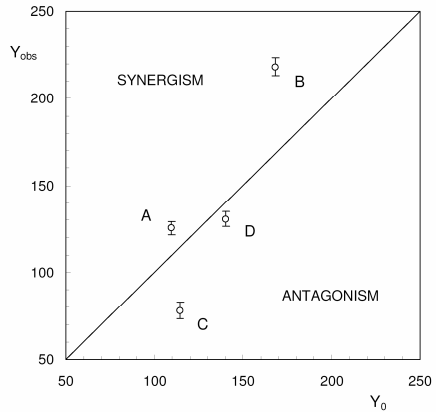

Fig. 3. Observed extraction yields (Y_{obs}) and values expected in the absence of interactions (Y_0) for the bynary mixtures A, B, C and D

The mixtures showing synergistic effects (A and B) contained cellulases and pectinases as main enzyme components, with a preponderance of cellulases in the more efficient of the two (B). Synergism resulting from the combined use of cellulases and pectinases has been evidenced in numerous studies, including enhancement of starch recovery from cassava waste [28], release of phenols into blackcurrant juice [29], improvement of phenolics content in olive oil [30] and extraction of carotenoids from orange peel, sweet potatoes and carrots [31]. Although the underlying mechanisms are not fully understood, synergistic effects are considered to be a reflection of the strong interaction of the cellulose–hemicellulose network with the surrounding pectic milieu [26], [32].

Negative enzyme interactions, such as those observed with mixtures C and D, are also reported in the literature [29], [33]. They are explained as the result of non-productive adsorption phenomena, i.e., the competitive adsorption of some enzyme molecules on substrate sites that they cannot attack. In fact, for the catalytic reaction to occur, enzymes need to adsorb on specific substrate regions but, if these sites are occupied by other enzyme molecules, their access can be sterically inhibited, causing the overall degradation rate to decrease.

III.2. Factor Influence Analysis

Table VI shows the influence of extraction conditions on the recovery of lycopene from the peel fraction of tomato processing wastes pretreated by Cellulyve 50LC + Peclyve LI 50:50 v/v - the enzyme preparation with the best performance. Percentage yields, calculated with respect to the total lycopene content, were between

51.9% and 82.3%, with an average value of 66.3%.

TABLE VI
PERCENTAGE YIELDS OF LYCOPENE EXTRACTION (Y%) EXPRESSED AGAINST TOTAL LYCOPENE CONTENT

Run	T / °C	τ / h	δ / mg/g	Y%
1	25.0	1.0	50	53.5
2	50.0	1.0	50	57.8
3	25.0	3.0	50	66.0
4	50.0	3.0	50	51.9
5	25.0	1.0	250	74.1
6	50.0	1.0	250	68.8
7	25.0	3.0	250	82.3
8	50.0	3.0	250	67.0
9	37.5	2.0	150	70.1
10	37.5	2.0	150	69.2
11	37.5	2.0	150	67.2
12	37.5	2.0	150	67.7

T, Temperature; τ, Incubation time; δ, Enzyme dosage

To evaluate the contributions of the three main factors (temperature, incubation time and enzyme dosage) and their interactions to the extraction yield we used the following equation:

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3 + a_{123}X_1X_2X_3 \quad (1)$$

where Y is the percentage yield of extraction and X_i are the factors under consideration (see Table III). a_1, a_2 and a_3 are the coefficients associated with the three main effects; a_{12}, a_{13} and a_{23} are those related to the binary interactions and a_{123} is the ternary interaction coefficient. Examination of Eq. (1) allows one to understand the meaning of these coefficients. So, if we consider the temperature, the first main factor, the value $a_1 \cdot 2$ represents its contribution to Y when it varies over a dimensionless range of 2 (in our case, from 25 to 50°C). Accordingly, the greater the numerical value of a coefficient the higher the influence of the associated factors. Positive (or negative) coefficients are indicative of a direct (or inverse) association between the factors and the dependent variable. Finally, the intercept a_0 is the predicted value of Y when all X_is equal zero, i.e., at the center of the experimental domain. The eight model coefficients were determined from the data of runs 1–8. Their values are presented in Table VII.

To assess the significance of the parameters we calculated the standard deviation of the experimental response from the four replicated center points, obtaining: $\sigma_y = 1.328$.

This value was then used to perform the Student's t test [34], which provided the following confidence interval at the probability level of 95% for the coefficients: [–1.494; +1.494].

Inspection of Table VII and the Pareto chart shown in Fig. 4 reveals that only four coefficients: a_1, a_2, a_3 and a_{12} can be considered significant.

TABLE VII
COEFFICIENT VALUES DERIVED FROM CORRELATION OF EXTRACTION YIELD DATA BY EQ. (1)

Coefficient	Effect	Value
a_0	–	65.184
a_1	Temperature	–3.804
a_2	Incubation time	1.639
a_3	Dosage	7.879
a_{12}	Temperature–Incubation time	–3.544
a_{13}	Temperature–Dosage	–1.339
a_{23}	Incubation time–Dosage	–0.001
a_{123}	Temperature–Incubation time–Dosage	1.036

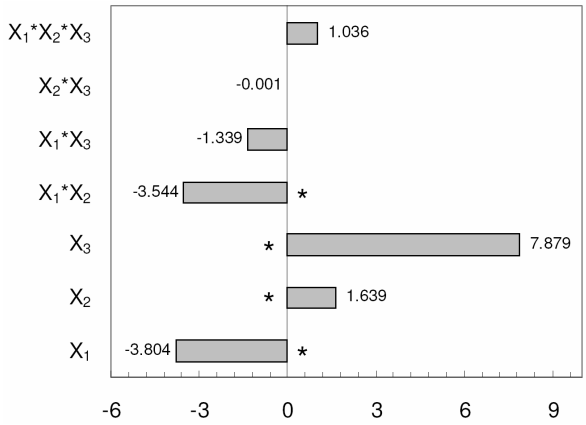

Fig. 4. Calculated coefficients for main and interaction effects. Significant effects are marked with an asterisk

Therefore, the only influential parameters for lycopene extraction are the three main factors: temperature (X_1), enzyme incubation time (X_2) and enzyme dosage (X_3), and the two-factor interaction temperature–incubation time (X_{12}).

The negative sign of a_1 indicates that the temperature has a negative effect on lycopene extraction. In particular, raising the temperature from 25 to 50°C causes a reduction of about 7.6 in the percentage yield. Since the enzyme preparations used in this study have an optimal temperature around 45–55°C, the observed decrease in yield is probably the result of an increased lycopene loss due to oxidation. It is, in fact, known that as long as lycopene remains within the tomato plant tissue its structural integrity is largely preserved [35]. By contrast, when lycopene is released from the protective chromoplast structures a rapid oxidative degradation occurs. In particular, lycopene degradation is greatly affected by temperature [14] and is accompanied by the formation of several cleavage products, including apo-lycopenals/ones and apo-carotendials [36].

As regards the other two main factors, enzyme incubation time and enzyme dosage, they both have a positive effect on extraction, with the latter showing a much stronger influence. Finally, there appears to be a significant negative interaction between temperature and enzyme incubation time, suggesting that an increase in temperature has a stronger influence on lycopene extraction at lower incubation times.

IV. Conclusion

From this study it can be concluded that the recovery of lycopene from the peel fraction of tomato processing waste can be greatly enhanced by the use of commercial enzyme preparations containing cellulase, pectinase and other minor activities. The results obtained indicate that an enzymatic treatment at temperatures close to ambient and low incubation times can increase the extraction yields by a factor from about 2 to 10.

The increased extractability of lycopene resulting from the enzymatic degradation of cell-wall polysaccharides may offer some important advantages over conventional non-enzymatic 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 are to be expected and much higher operation flexibility, for the possibility of using tomato waste material with any moisture content.

The results from the analysis of influential factors may provide some useful insights for future research studies and the successive cost-benefit evaluation. In regard to the latter point, and restricting consideration to the cost of enzymes, it should be emphasized that the enzyme preparations used in this work are not much expensive, being industrially produced for large-scale food applications.

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