JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Environmentally Friendly Lycopene Purification from Tomato Peel Waste: Enzymatic Assisted Aqueous Extraction

Stefano Cuccolini,[†] Antonio Aldini,[†] Livia Visai,[§,#] Maria Daglia,[⊥] and Davide Ferrari*[,#,⊗]

[†]John Bean Technologies SpA, Via Mantova 63/A, 43100 Parma, Italy

[§]Department of Molecular Medicine and Center for Tissue Engineering (CIT), University of Pavia, Pavia, Italy

[#]Nanotechnology Laboratory, Salvatore Maugeri Foundation IRCCS, via Salvatore Maugeri 4, Pavia, Italy

[⊥]Department of Drug Sciences, Pavia University, Via Taramelli 12, 27100 Pavia, Italy

[⊗]Department of Biochemistry and Molecular Biology, University of Parma, Parco Area delle Scienze 23/A, 43124 Parma, Italy

ABSTRACT: The antioxidant and anticancer properties of lycopene make it an ideal component for daily food supplements. For this reason this study investigated the possibility of extracting lycopene from tomato waste peels using a green chemistry protocol devoid of organic solvent. Cells are lysed thanks to a combination of pH changes and hydrolytic enzyme treatments. The lycopene-containing chromoplasts are then precipitated by lowering the pH and isolated through a centrifugation step. At this stage the lycopene content of the isolated chromoplasts shows a 10-fold increase (3−5% w/w, dry basis) with respect to untreated tomato peels. A further improvement in lycopene concentration is obtained by a second enzymatic treatment using a protease cocktail. This catalytic step eliminates unwanted proteins, bound to the chromoplasts, but not essential for their stability. The final product shows a lycopene content around 8−10% (w/w, dry basis), which represents a 30-fold increase with respect to the lycopene concentration of the untreated peels.

KEYWORDS: *lycopene, green chemistry, carotenoids, enzyme, tomato peel waste*

INTRODUCTION

Several million tons of tomatoes are processed every year to produce a variety of tomato products including canned tomatoes, juices, sauces, purees, and pastes. After the United States and China, Italy is the third largest world manufacturer, with approximately 4.6 million tons of tomatoes processed per year. [1] Tomato processing results in large amounts (up to 3% by weight of the fresh tomatoes) of industrial waste (generally called tomato pomace) composed mainly of tomato peels, pulp, and seeds in a proportion depending on the product being produced. Tomato pomace has no commercial value and is currently disposed of or used as animal feed. However, it contains several bioactive compounds. In particular, the abundance of lycopene in the peel suggests the possibility of utilizing it as a cheap source of this interesting molecule, which, in contrast, is commercially very expensive.

Lycopene, which is responsible for the deep red color of ripe tomatoes,[2] is a tetraterpenic hydrocarbon with 13 carbon−carbon double bonds, 11 of which are conjugated. The high degree of conjugation makes it one of the most potent natural antioxidants.[3] Free radicals can cause damage to both the structure and function of cell membranes, DNA, and proteins causing many degenerative diseases such as cancer, atherosclerosis, cataracts, and age-related macular degeneration, as well as premature aging.

In recent years, dietary carotenoids such as lycopene have attracted considerable attention as potentially beneficial phytochemicals in the light of epidemiological studies linking the consumption of foods rich in lycopene with a reduction in the risk of developing certain types of cancer.[4−8] In particular, the amount of lycopene in the blood has been shown to be inversely proportional to the incidence of prostate tumors.[9] For this reason lycopene is in high demand by the pharmaceuticals industry as well as by the food and cosmetics industries.[10] Moreover, in recent years, environmental concerns and sustainability issues have gained much attention. For this reason the need to devise new strategies for the efficient management of agro-industrial wastes has become a priority. [11] An interesting opportunity is to use tomato pomace for the extraction of value-added products such as lycopene in a similar manner as for the already marketed products proanthocyanidins obtained from grape seeds and pectin present in citrus peels or apple pomace.[12]

Commercial lycopene is available as standardized tomato extracts or from chemical synthesis. Natural lycopene is produced mainly by extraction and concentration from whole tomato fruits, which are grown specifically for this purpose. Although lycopene is very expensive, market trends indicate a growing demand for natural lycopene, 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.[13] A projection of future demand of lycopene highlights that the current production from whole tomato fruits is small and needs to be increased.

Thanks to its lycopene concentration of up to 5 times higher than in the pulp,[14] tomato peels and tomato pomace represent

Special Issue: IX Italian Congress of Food Chemistry

Received: June 28, 2012
Revised: September 14, 2012
Accepted: September 24, 2012
Published: September 24, 2012

a very promising alternative source of lycopene. However, the available extraction technologies do not seem to allow its rapid and efficient recovery from the tomato peel tissue. For example, using supercritical CO_2 at 60 °C and 30 MPa, the total lycopene extracted was only about 50% of that present in the processed waste.[15] Similarly, extraction by conventional food-grade organic solvents, such as hexane, ethanol, and ethyl acetate, is low under conditions preserving the activity of the carotenoid in vivo. In addition, lycopene extracted with the latter technique contains residual organic solvents which, even though they are minimal and within the concentration range allowed by the corresponding Pharmacopoeia, might still affect human health, especially if present in daily food supplement.

Low extraction yield might be explained by the natural lycopene location. Lycopene is predominantly found in the tomato peel chromoplasts, deeply embedded within the membrane structures, where its biosynthesis increases sharply during the ripening process, as the chloroplast undergoes transformation to chromoplast.[16] Low extraction efficiencies can be attributed to the difficulty of solvent molecules to penetrate the compact tomato peel tissue, which is a highly structured material containing many different polysaccharide components, such as cellulose, hemicelluloses, and pectins.[17] An improved extraction efficiency cannot be achieved by using more severe extraction conditions because of the consequent oxidative degradation of the pigment.[18]

To degrade the plant cell-wall rich in cellulose and pectins, enzymatic pretreatment of agro-materials with cellulases and pectinases is an already established approach with many applications for recovering valuable biological compounds. For example, grape aroma precursors[19] and carotenoids, including lycopene, have been extracted from their compact highly structured plant tissue from a variety of plant materials.[20,21] However, enzymatic assisted lycopene extraction techniques currently proposed still include the use of organic solvents, implicating the risk of organic solvent impurities when such lycopene is used in daily supplement pills.[21,22]

In the light of the above considerations, we have explored the possibility of devising a lycopene extraction method, for industrial applications, completely devoid of organic solvents. What we propose in this study is an environmentally friendly and, to our knowledge, innovative process to obtain a concentrated tomato product having a high amount of lycopene. The purpose of the method is to isolate the chromoplasts present in tomato peels, where lycopene is stable because it is still incorporated into its natural medium (lipoproteins), by exploiting hydrolytic enzymes in combination with aqueous solutions at different pH values, obtained with food grade approved acids and bases. Our method leads to the accumulation of the chromoplasts by discarding most of the other cell components. The lycopene present in the final product is still incorporated in the chromoplast and therefore protected against oxidation.

The whole process can be divided into two macrophases called 1 and 2. Macrophase 1 aims at the efficient opening of the tomato peel cell wall and at the isolation of the chromoplasts. In this phase we show that a pretreatment of the tomato peels with a NaOH solution increases the accessibility of the cell wall, where the substrate for the hydrolytic enzymes are located. Macrophase 2 aims at "carving" the isolated chromoplasts by using a protease cocktail such as pancreatin, which greatly increases the concentration of lycopene in the final product by eliminating unwanted soluble molecules such as proteins, peptides, and lipids not necessary for the stability of the chromoplasts containing lycopene.

MATERIALS AND METHODS

Reagents. Tomato peels were the discard material of an industrial steam peeling machine from ARP Soc. Agr. Coop. (Podenzano, PC, Italy). Collected peels were stored in plastic bags and frozen at −20 °C.

Cellulyve 50LC (lot 4yy1082; cellulose activity = 240 CMC/g, 32 CCU/g), Peclyve LI (lot 4yu1090; pectinase activity = 1300 PG/g, 400 PE/g) from *Aspergillus*, and Prolyve 1000 (lot 4yy2062; protease activity = 3000 PAL/g) from *Bacillus licheniformis* were purchased from Lyven (Colombelles, France; http://www.lyven.com/). A few experiments were performed with different lots of enzyme, and no differences were observed, proving the lot-to-lot consistency of the industrial enzymes. All enzyme preparations were in liquid form and stored at 4 °C as suggested by the manufacturing company. Optimal working conditions and the main enzymatic activities of the above-mentioned enzymes are listed in Table 1.

Table 1. Main Activities and Optimal Working Conditions of the Enzyme Preparations Used in This Study

preparation	main activity[a]	opt temp (°C)	opt pH
Cellulyve 50LC	C1, C2, C3	45	4.0−5.0
Peclyve LI	PG, PM, C1	45	4.0−5.0
Prolyve 1000	P	60	9.0−10.5

[a]C1, cellulase; C2, β-glucosidase; C3, cellulase 1,4-β-cellobiosidase; PG, polygalacturonase; PM, pectin methylesterase; P, alkaline protease.

Sodium hydroxyde (NaOH), hydrochloric acid (HCl) 37%, tetrahydrofuran (THF), methanol, petroleum ether (40−60 °C), acetone, butylated hydroxytoluene (BHT), magnesium carbonate, sodium phosphate anhydrous, and sodium chloride were from Carlo Erba Reagenti (Italy).

Homogenization of Tomato Peel. Frozen tomato peels were thawed just before use, resuspended in water, and triturated at 12000 rpm with an OV5homogenizer from VELP Scientific (Usmate (MB), Italy). Triturated peels were centrifuged at 2400*g* for 15 min, and the supernatant was discarded.

Humidity Content. The humidity content of the samples was determined using an infrared thermobalance (ORMA Thermored model) at a constant temperature of 105 °C until no changes in weight were observed.

Determination of Lycopene Content. The amount of lycopene in different samples was determined spectrophotmetrically using a Cary 50 UV−visible spectrophotometer (Agilent Technologies). To minimize the absorbances from other carotenoids, the lycopene concentration was calculated on the basis of the absorbance at 505 nm using an extinction coefficient of 150 mM^{-1} cm^{-1}.[23]

The lycopene to be quantified was extracted using the method of Hart and Scott with some modification.[23] A 0.5 g aliquot of wet homogenized sample was added to 0.1 g of magnesium carbonate and stirred in a 200 mL beaker after the addition of a 1:1 methanol /THF solution (200 mL). After 45 min, the solution was filtered on a glass microfiber (1.6 μm Büchner filter) under vacuum. The solution was then poured in a separating funnel with the addition of 50 mL of a 10% sodium chloride solution and 50 mL of petroleum ether containing 0.1% BHT. After vigorous mixing, the upper organic layer is poured in an Erlenmeyer flask in the dark, whereas the lower layer is used for a second extraction by adding 50 mL of petroleum ether containing 0.1% BHT. The same procedure is repeated until the upper layer appear colorless. The organic phase is then saturated with anhydrous sodium sulfate to eliminate residual water and then dried using a rotary evaporator. The dried material is dissolved in acetone, filtered using hydrophobic PTFE filters (0.5 μm), and diluted in an amount suitable for an efficient spectrophotometric measurement.

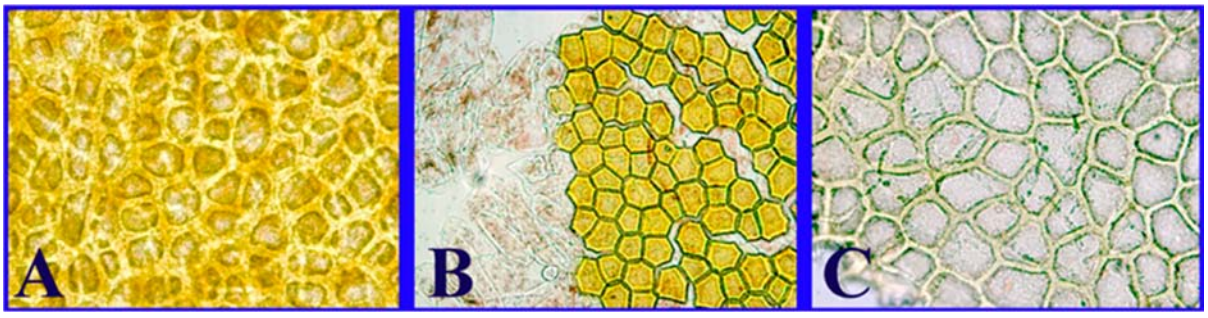

Figure 1. (A) Optical microscope view of tomato peels before any treatments; (B) after treatment with 4% NaOH; (C) after both NaOH (4%) and enzymatic treatments (3%).

NaOH Treatment. A 300 g aliquot of homogenized peel waste was resuspended in a beaker with 450 mL of aqueous solution containing NaOH at different concentrations. The temperature was set at 70 °C, and the solution was magnetically stirred for 2 h. At the end of the 2 h reaction, the pH was lowered to 2.2 by adding concentrated HCl. The mixture was then centrifuged at 2400*g* for 15 min using an IEC CL10 centrifuge from Thermo Scientific, and the supernatant was discarded.

Cell Lysis by Enzymatic Treatment. Either homogenized tomato peels or the pellet collected after the NaOH treatment was resuspended in distilled water and brought to the optimal pH and temperature for the enzymatic treatment as indicated by the manufacturing company (see Table 1). The concentrations of the enzymes (Cellulyve 50 CL and Peclyve LI) were expressed as the percent in weight of the wet pellet (for instance, 3% is an amount of added enzyme equal to the 3% in weight of the wet material to be hydrolyzed, before the resuspension in water). The temperature was set to 45 °C, and the solution was magnetically stirred in the dark for 4 h. The mixture was then sieved on a 100 μm sieve. After the pH had been lowered to 2.2 with 3% HCl, the solution was centrifuged at 2400*g* for 40 min using an IEC CL10 centrifuge from Thermo Scientific. The supernatant was discarded.

Filtration. After cell lysis, the solution was filtered with a Certified Test Sieve Giuliani, series ASTM, 106 μm.

Lycopene Concentration by Enzymatic Treatment. The isolated chromoplasts obtained after the enzymatic cell lysis were resuspended in 6 volumes of distilled water. The enzyme Prolyve 1000 was added to a concentration of 12% of the weight of the wet pellet. The mixture was brought to the optimal pH and temperature, as indicated by the manufacturing company (Table 1), magnetically stirred, and kept in the dark for 3 h. To collect the hydrolyzed chromoplasts, 3% HCl was added to the mixture to a final pH of 2.2, and the solution was centrifuged at 2400*g* and room temperature for 60 min using an IEC CL10 centrifuge from Thermo Scientific. The supernatant was discarded.

HPLC Analysis. The analysis was performed using a Waters model 712 Wisp autosampler, a Waters model 510 solvent delivery pump (Millipore), and a Waters model 490E programmable multiwavelength detector (Millipore) connected to two modular interfaces of a Waters System Interface Module, which transferred a signal to an HPLC management program Maxima 820, Chromatography Workstation, Dynamic Solution version 3.3. The column system comprised a 10 mm, 5 μm, metal-free guard ODS-2 prefilter (Alltech) linked to a 100 × 4.6 mm Partisil 5 ODS column (Whatman), connected to a 250 × 4.6 mm, 5 μm, Vydac 20 L TP54 standard-bore C18 analytical column (The Separation Group, Inc., Hesperia, CA, USA) modified by the replacement of metal frits with "bio-compatible" frits.

RESULTS AND DISCUSSION

The first step in devising an environmentally friendly method for the extraction of lycopene from tomato peel waste was the opening of the tomato peel cells, which, being the border between the internal fruit and the external world, is extremely resistant. By using an optical microscope we were able to monitor the extent of cell opening and therefore to follow the fate of their lycopene rich content. We noted that the untreated tomato peel cells were extremely compacted (Figure 1A) and likely to be not easily accessible by the hydrolytic enzymes. Therefore, we decided to test different conditions to loosen the highly structured composition of the peels. We noted that among the tested conditions (data not shown) only treatment with NaOH at a concentration of at least 1 N and a temperature of 70 °C for 2 h was able to dissolve the "waxy" layer responsible for cementing the tomato peel cells. However, such treatment does not open the cell wall, leaving the cells intact and full of their red content (Figure 1B). This was confirmed by the spectrophotometric analysis of the NaOH supernatant solution obtained after centrifugation of the treated tomato peels. As shown in Figure 2 the spectrum has no

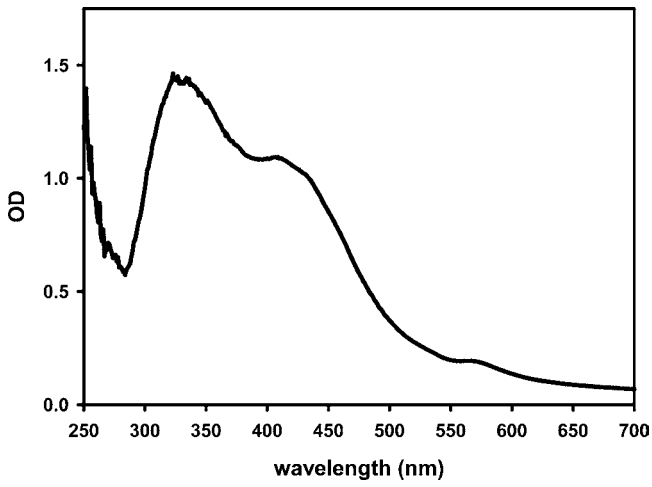

Figure 2. UV−vis spectra of the supernatant solution obtained after centrifugation of tomato peels previously treated with 4% NaOH at 70 °C for 2 h.

evident peaks at 260 and 280 nm, meaning that no proteins or DNA have "escaped" from the cell. On the other hand, the clear band around 340 nm and its shoulder between 400 and 500 nm clearly show the presence of polyphenolic compounds (but not lycopene) in the solution, as confirmed by its yellow color.

Macrophase 1. We first tested the ability of the two hydrolytic enzymes (cellulase and pectinase) to open the tomato peel cells without pretreating them with NaOH. Cellulase hydrolyzes the 1,4-β-D-glycosidic linkages in cellulose and is mainly responsible for cell opening, whereas pectinase breaks down pectin, a polysaccharide found in the cell walls. Following the manufacturer's instructions we resuspended the

dx.doi.org/10.1021/jf3027815 | J. Agric. Food Chem. 2013, 61, 1646−1651

homogenized peels in a solution at pH 4.5 and 50 °C with 3% of cellulase. After a 4 h treatment, the solution was sieved to eliminate large cell debris and unbroken cells. The sieved solution was then amended with HCl to a pH of 2.2. At such pH the chromoplasts are insoluble and can be isolated with a centrifugation step. The concentration of lycopene in the collected chromoplasts was measured as described under Material and Methods. The analysis showed a lycopene concentration around 10 times higher than that found in the starting material and a yield around 60% of the total lycopene present in the starting material (data not shown). Increasing the concentration of cellulase to 6% and/or the time to 6 h did not improve either the lycopene concentration or the yield of extraction. To test the effect of pectinase, the same procedure was performed with the addition of 3% of the second hydrolytic enzyme. The addition of pectinase shows a positive effect on the final lycopene yield, which increases from 60 to 80% (Figure 3), whereas the lycopene concentration is unaffected. This is consistent with the fact that pectinase improves cell lysis, whereas it has no effect on the composition of the collected chromoplasts.

By keeping an eye on the industrial application of the method and therefore to the economical aspect of it, we lowered the concentration of the two enzymes (which represent expensive reagents of the process) in a series of experiments. Keeping their ratio at 1, we wanted to verify whether we could reach the same results using a smaller amount of biocatalyst. As shown in Figure 3, lowering the amount of cellulase and pectinase results in a lower yield of lycopene in the isolated chromoplast but also in a lower lycopene concentration. If the lower yield can be explained by a smaller fraction of disrupted cells, the same is not true for the lowered concentration. A possible explanation is the fact that a low enzyme concentration is able to open mainly the cells of the pulp, still attached to the peel (which have a lower lycopene content), leaving most of the peel intact. This would explain the lower lycopene concentration of the collected chromoplasts.

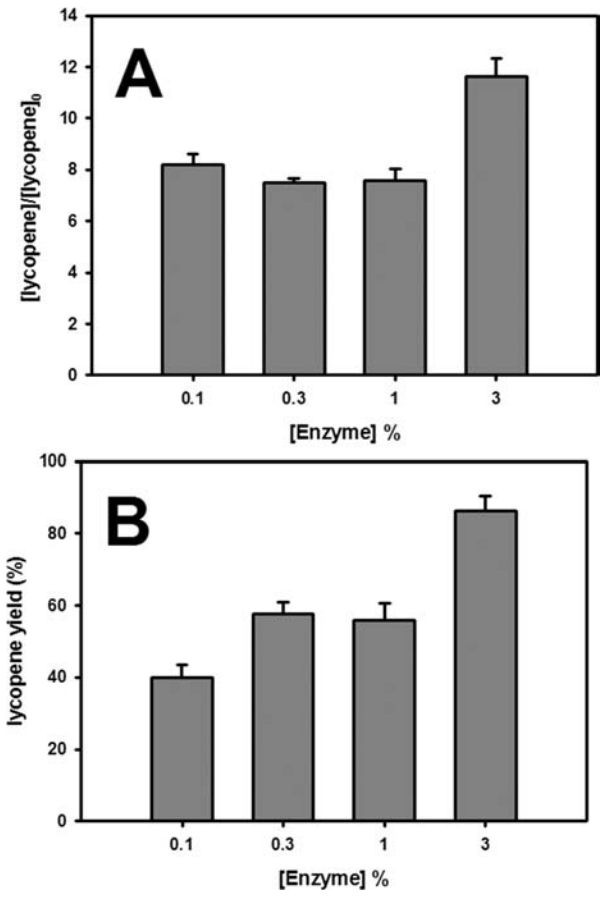

Figure 3. Enzymatic treatment without NaOH pretreatment: (A) fold increase in lycopene concentration expressed as the ratio [lycopene]/[lycopene]$_0$, where [lycopene]$_0$ is the initial lycopene concentration in the homogenized peel and [lycopene] is the concentration of lycopene in the final product obtained after enzymatic treatment; (B) effect of different concentrations of hydrolytic enzymes on the yield of lycopene. 100% is the total amount of lycopene present in the initial homogenized peels.

The effect of the NaOH pretreatment mentioned above was tested by resuspending the homogenized tomato peels in a solution at different concentrations of NaOH at 70 °C and measuring the concentration and yield of lycopene of the collected chromoplasts after the enzymatic treatment using the optimal enzyme concentration (3%). As shown in Figure 4 a NaOH concentration ranging between 0.4 and 4% increases the yield to 100% compared to 80% of the control (in the absence of NaOH). In contrast, an 8% concentration of NaOH results in an dramatic decrease in lycopene yield, which can be due to an aggressive effect of the NaOH on the stability of lycopene. With regard to the lycopene concentration, Figure 4 clearly shows that 4% of NaOH gives the highest value. Further increase to 8% results in a lower lycopene concentration, probably for the reasons discussed above.

In the light of these results we designed the final protocol for macrophase 1, which is composed of a 2 h pretreatment of the homogenized tomato peels at 70 °C with 4% NaOH, followed by a pH lowering by the addition of HCl and a centrifugation step to recover the precipitated chromoplasts.[24] The pellet is resuspended in a buffer at pH 4.5 containing 3% of both cellulose and pectinase. The reaction proceeds for 4 h at 50 °C, and the solution is then filtered to eliminate insoluble fiber or cell debris. After the filtration step, the pH is lowered again to pH 2.2, by the addition of HCl, and a subsequent centrifugation step will collect the precipitated chromoplasts. At the end of macrophase 1 we obtain a pellet with a lycopene content of 3−5% (approximately 15-fold more concentrated than the initial product) and a yield near 100%. Visual inspection of the tomato peel cells after macrophase 1 (Figure 1C) clearly shows how the cells are devoid of their red content.

Statistical analysis using the ANOVA test shows that the improvement in lycopene concentration is significant ($p < 0.05$) when using a NaOH concentration of 4% and an enzyme concentration of 3%.

Macrophase 2. The product obtained after macrophase 1 is a collection of chromoplasts in which lycopene and other carotenoids are embedded into lipoproteins. To increase the lycopene concentration of the extract, we decided to exploit a cocktail of unspecific hydrolytic enzymes, which, according to the U.S. patent from Koch et al.,[24] will cleave proteins unnecessary for the stability of the chromoplasts. Such reaction will produce small and therefore soluble peptides and/or amino acids, which can be removed thanks to a centrifugation step. The pellet from macrophase 1 was resuspended in 6 volumes of distilled water and, following the manufacturer's instructions, brought to pH 9−10 using NaOH and a temperature of 60 °C, before the addition of the enzyme cocktail Prolyve 1000. The amount of enzyme added was 12% of the wet weight of the

dx.doi.org/10.1021/jf3027815 | J. Agric. Food Chem. 2013, 61, 1646−1651

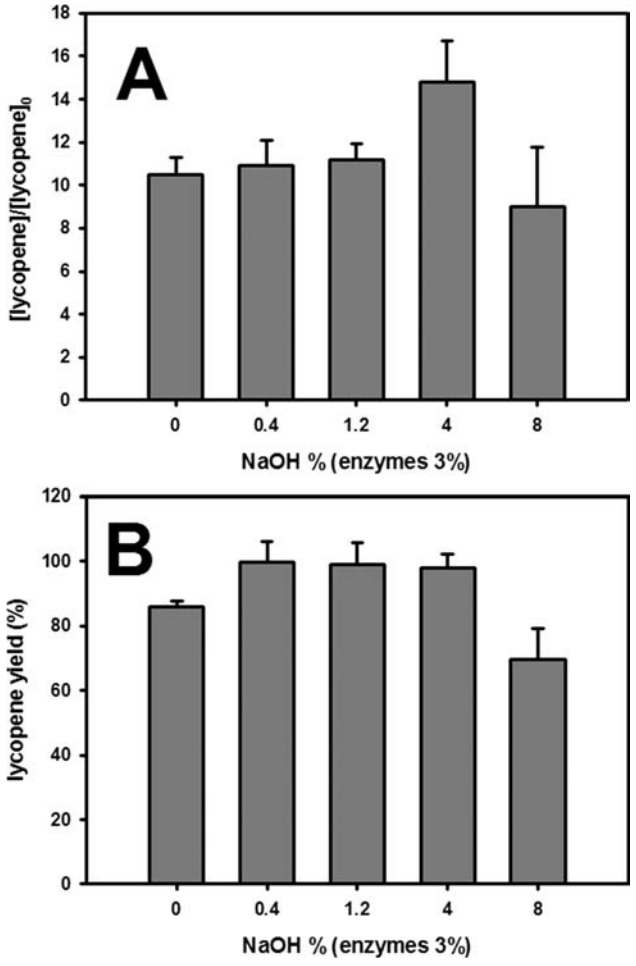

Figure 4. Enzymatic treatment with NaOH pretreatment: (A) fold increase in lycopene concentration expressed as the ratio [lycopene]/[lycopene]$_0$, where [lycopene]$_0$ is the initial lycopene concentration in the homogenized peel and [lycopene] is the concentration of lycopene in the final product obtained after NaOH pretreatment followed by enzymatic treatment using a concentration of enzymes equal to 3%; (B) effect of different concentrations of NaOH on the yield of lycopene. Peels are pretreated with different concentrations of NaOH followed by enzymatic treatment using a concentration of enzymes equal to 3%. 100% is the total amount of lycopene present in the initial homogenized peels. NaOH concentration (w/v) is expressed as the grams of NaOH per 100 mL of final solution.

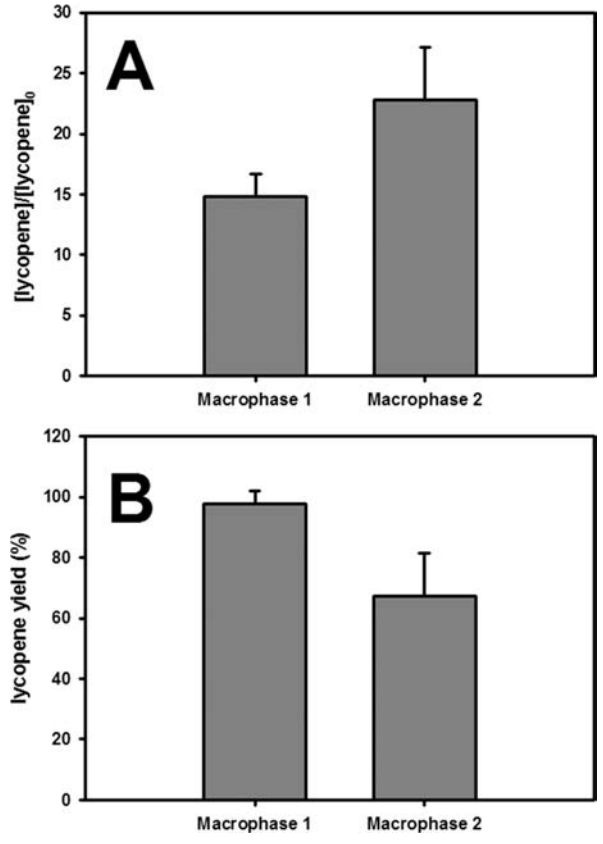

Figure 5. Comparison between macrophases 1 and 2; (A) fold increase in lycopene concentration expressed as the ratio [lycopene]/[lycopene]$_0$, where [lycopene]$_0$ is the initial lycopene concentration in the homogenized peel and [lycopene] is the concentration of lycopene in the final product obtained after either macrophase 1 or 2; (B) effect on the yield of lycopene. 100% is the total amount of lycopene present in the initial homogenized peels.

pellet from macrophase 1. The mixture was magnetically stirred for 3 h in the dark. At the end of the proteolytic reaction, the chromoplasts were collected by lowering the pH to 2.2, with HCl, followed by a centrifugation step.

As shown in Figure 5, the pellet recovered after macrophase 2 has a lycopene concentration around 25-fold that of the tomato peels. As expected, such a value is much higher than that obtained after macrophase 1, which is around 15 (Figure 5A). This result can be explained by the fact that the proteolytic treatment has hydrolyzed several proteins in the chromoplasts, generating small soluble peptides, which have been eliminated through the centrifugation step, leaving the lycopene unaffected. In contrast, the yield decreases from a complete recovery after macrophase 1 to a 60−80% recovery after macrophase 2 (Figure 5B). This result can be explained by the fact that the proteolytic treatment may, in some cases, affect chromoplast stability by cleaving structurally critical proteins. In this case some lycopene might be released in solution as a lycopene/protein soluble complex, which will be then eliminated in the centrifugation step. As shown in Figure 5A, the final product has a lycopene content about 25-fold more concentrated than the raw initial material. Because tomato peel has a lycopene content around 3000−5000 ppm, the final product will have a lycopene concentration around 120 000 ppm (12%) on dry basis. The macrophase 2 process increases the lycopene concentration about 2-fold with respect to the product obtained after macrophase 1. Such an increase was consistently repeated in 10 different experiments with a Student's t test showing a significant difference with a residual error of <5%. An HPLC analysis performed on the product obtained after macrophase 2 (chromatogram not shown) shows that the final product contains also a significant amount (10000 ppm each, on dry basis) of tocopherols, phytoene, β-carotene and phytofluene, whereas sugars (glucose, fructose, and sucrose) are basically absent.

In conclusion, we have developed a "green chemistry" protocol, devoid of organic solvent, allowing the concentration of lycopene extracted from tomato peels. Our methodology exploits hydrolytic enzymes and pH changes to obtain a final product having a 20−30-fold increase in lycopene content with respect to the initial raw material. We believe that such a methodology represents an economic alternative to the CO_2

dx.doi.org/10.1021/jf3027815 | J. Agric. Food Chem. 2013, 61, 1646−1651

extraction of lycopene to be used especially for daily food supplements.

AUTHOR INFORMATION

Corresponding Author

*Phone: + 39 0521 905137. Fax: + 39 0521 905151. E-mail: davide.ferrari@unipr.it.

Funding

This work was supported by a grant from John Bean Technologies SpA, Parma, Italy.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are very grateful to Prof. Anna Torelli and Dr. Corrado Zanni for their help with the optical microscope.

REFERENCES

(1) WPTC. *World production estimate as of 1 June 2008*, release 38; http://www.wptc.to/releases/releases38.pdf (accessed April 2012).

(2) Schieber, A.; Stintzing, F. C.; Carle, A. By-products of plant food processing as a source of functional compounds − recent developments. *Trends Food Sci. Technol.* **2001**, *12*, 401−413.

(3) Di Mascio, P.; Kaiser, S.; Sies, H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch. Biochem. Biophys.* **1989**, *274*, 532−538.

(4) Block, G.; Patterson, B.; Subar, A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutr. Cancer* **1992**, *18*, 1−29.

(5) Miller P. E.; Snyder, D. C. Phytochemicals and cancer risk: a review of the epidemiological evidence. *Nutr. Clin. Pract.* **2012**, Aug 9 [Epub ahead of print].

(6) Steinmetz, K. A.; Potter, J. D. Vegetables, fruit, and cancer prevention: a review. *J. Am. Diet Assoc.* **1996**, *10*, 1027−1039.

(7) Giovannucci, E. L. Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. *J. Natl. Cancer Inst.* **1999**, *91*, 317−329.

(8) Omoni, A. O.; Aluko, R. E. The anti-carcinogenic and anti-atherogenic effects of lycopene: a review. *Trends Food Sci. Technol.* **2005**, *16*, 344−350.

(9) Teodoro, A. J.; Oliveira, F. L.; Martins, N. B.; Maia, G. A.; Martucci, R. B.; Borojevic, R. Effect of lycopene on cell viability and cell cycle progression in human cancer cell lines. *Cancer Cell Int.* **2012**, *12* (1, Aug 6), 36 [Epub ahead of print].

(10) Borguini, R. G.; Torres, E. A. F. D. Tomatoes and tomato products as dietary sources of antioxidants. *Food Rev. Int.* **2009**, *25*, 313−325.

(11) Arvanitoyannis, I. S.; Varzakas, T. H. Vegetable waste treatment: comparison and critical presentation of methodologies. *Crit. Rev. Food Sci. Nutr.* **2008**, *48*, 205−247.

(12) Laufenberg, G.; Kunz, B.; Nystroem, M. Transformation of vegetable waste into value added products: (A) the upgrading concept; (B) practical implementations. *Bioresour. Technol.* **2003**, *87*, 167−198.

(13) Shixian, Q.; Dai, Y.; Kakuda, Y.; Shi, J.; Mittal, G.; Yeung, D.; Jiang, Y. Synergistic anti-oxidative effects of lycopene with other bioactive compounds. *Food Rev. Int.* **2005**, *21*, 295−311.

(14) Sharma, S. K.; Le Maguer, M. Lycopene in tomatoes and tomato pulp fractions. *Ital. J. Food Sci.* **1966**, *8*, 107−113.

(15) Sabio, E.; Lozano, M.; Montero de Espinosa, V.; Mendes, R. L.; Pereira, A. P.; Palavra, A. F.; Coelho, J. A. Lycopene and β-carotene extraction from tomato processing waste using supercritical CO_2. *Ind. Eng. Chem. Res.* **2003**, *42*, 6641−6646.

(16) Harris, W. M.; Spurr, A. R. Chromoplasts of tomato fruits. II. The red tomato. *Am. J. Bot.* **1969**, *56*, 380−389.

(17) Gross, K. C. Fractionation and partial characterization of cell walls from normal and non-ripening mutant tomato fruit. *Physiol. Plant* **1984**, *62*, 25−32.

(18) Xianquan, S.; Shi, J.; Kakuda, Y.; Yueming, J. Stability of lycopene during food processing and storage. *J. Med. Food* **2005**, *8*, 413−422.

(19) Bautista-Ortín, A. B.; Martínez-Cutillas, A.; Ros-Garcia, J. M.; López-Roca, J. M.; Gómez-Plaza, E. Improving color extraction and stability in red wines: the use of maceration enzymes and enological tannins. *Int. J. Food Sci. Technol.* **2005**, *40*, 867−878.

(20) Barzana, E.; Rubio, D.; Santamaria, R. I.; Garcia-Correa, O.; Garcia, F.; Ridaura Sanz, V. E.; López-Munguía, A. Enzyme-mediated solvent extraction of carotenoids from marigold flower (*Tagetes erecta*). *J. Agric. Food Chem.* **2002**, *50*, 4491−4496.

(21) Zuorro, A.; Lavecchia, R. Mild enzymatic method for the extraction of lycopene from tomato paste. *Biotechnol. Biotechnol. Equipment* **2010**, *24*, 1854−1857.

(22) Papaioannou, E. H.; Karabelas, A. J. Lycopene recovery from tomato peel under mild conditions assisted by enzymatic pretreatment and non-ionic surfactants. *Acta Biochim. Pol.* **2012**, Mar 17 [Epub ahead of print].

(23) Hart, D. J.; Scott, J. Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of vegetables and fruits commonly consumed in the UK. *Food Chem.* **1995**, *54*, 101−111.

(24) Koch, L.; Sandor, M.; Kalman, T.; Attila, P.; Victorovich, B. S. Natural carotenoid concentrates from plant material and a process for preparing the same. U.S. Patent 5,962,756, Oct 1999.