CHEMICAL ENGINEERING TRANSACTIONS
VOL. 38, 2014
A publication of AIDIC
The Italian Association of Chemical Engineering
www.aidic.it/cet
Guest Editors: Enrico Bardone, Marco Bravi, Taj Keshavarz

ISBN 978-88-95608-29-7; **ISSN** 2283-9216
DOI: 10.3303/CET1438060

Use of Cell Wall Degrading Enzymes for the Production of High-Quality Functional Products from Tomato Processing Waste

Antonio Zuorro*, Roberto Lavecchia, Franco Medici, Luigi Piga

Dipartimento di Ingegneria Chimica, Materiali e Ambiente, Sapienza University, Via Eudossiana 18, 00184 Roma, Italy
antonio.zuorro@uniroma1.it

The feasibility of using tomato pomace, the solid waste resulting from the industrial processing of tomatoes, to produce a tomato oleoresin and a lycopene-enriched seed oil was investigated. The oil was obtained by cold-pressing the seeds, while the oleoresin was produced by pretreating the peel fraction of the waste with cell wall degrading enzymes. The latter consisted of polygalacturonase, pectin methylesterase, cellulase and hemicellulase. The enzymatic treatment followed by hexane extraction and solvent evaporation allowed the production of an oleoresin with a lycopene content of about 7% by weight. The oleoresin was incorporated in different proportions into tomato seed oil so as to obtain a functional oil with a lycopene content ranging from 30 to 600 ppm. The lycopene-enriched oil was characterized by official analytical methods and its potential use in the nutraceutical and cosmetic sectors was discussed.

1. Introduction

According to statistics from the World Processing Tomato Council, over 30 million tons of tomatoes are processed annually worldwide to produce tomato juice, ketchup, canned tomatoes and many other products (WPTC, 2013). During tomato processing, a waste known as tomato pomace is generated. This material represents about 5% by weight of the processed tomatoes and consists mainly of tomato peels, pulp residues and seeds. Tomato pomace has no commercial value and is currently disposed of as a solid waste or used, to a limited extent, for animal feeding.

However, a careful examination of the characteristics of tomato pomace reveals that it is a rich source of nutrients and valuable phytochemicals. In particular, important phenolics and carotenoids are present in the peel fraction of the waste. In addition, tomato seeds contain about 30% oil of high nutritional quality (Eller et al., 2010). Among tomato components, lycopene has attracted the greatest attention in recent years for its potential health benefits (Kong et al., 2010).

Lycopene (ψ,ψ-carotene) is an acyclic tetraterpenic hydrocarbon with 13 carbon–carbon double bonds, 11 of which are conjugated (Figure 1). The high degree of conjugation makes lycopene one of the most potent natural antioxidants, with a singlet-oxygen quenching ability twice as high as that of β-carotene and 10 times higher than that of α-tocopherol (Di Mascio et al., 1989). Evidence also indicates that other tomato phytochemicals such as β-carotene, phytoene and phytofluene are capable of synergistically enhancing its antioxidant activity (Shixian et al., 2005). During fruit ripening, lycopene accumulates in the outer skin layer, where carotenoid levels above five times higher than in the pulp are generally found. However, attempts to recover it by either conventional (Strati and Oreopoulou, 2011) or supercritical (Sabio et al., 2003) extraction have met only limited success. One reason is to be found in the compactness of the tomato peel tissue, which hinders solvent penetration and transport to the lycopene-containing chromoplasts. Furthermore, lycopene is tightly bound to membranous structures inside the organelle. As a result, harsh and potentially damaging extraction conditions are required to achieve a satisfactory recovery.

Please cite this article as: Zuorro A., Lavecchia R., Medici F., Piga L., 2014, Use of cell wall degrading enzymes for the production of high-quality functional products from tomato processing waste, Chemical Engineering Transactions, 38, 355-360
DOI: 10.3303/CET1438060

In past years we found that the efficiency of lycopene recovery from tomato peels can be significantly increased by pretreating it with cell wall degrading enzymes (Lavecchia and Zuorro, 2008). Similar results were obtained using tomato paste as the lycopene source (Zuorro and Lavecchia, 2010).
In this study we have investigated the feasibility of using a commercially available enzyme preparation with cellulolytic, hemicellulolytic and pectinolytic activities to produce a tomato oleoresin and a lycopene-enriched seed oil from tomato pomace. The oil was extracted from the seed fraction of the waste and was subsequently enriched in lycopene by incorporation of a tomato oleoresin obtained from the enzymatically treated peels.

2. Experimental

2.1 Chemical and enzymes

Acetone, ethanol, hexane and butylated hydroxytoluene (BHT) were purchased from Carlo Erba (Milano, Italy). Natural lycopene standard (10% by weight, from tomatoes) was obtained from LycoRed Natural Products Industries Ltd. (Beer-Sheva, Israel). Peclyve LI, an enzyme preparation with polygalacturonase (EC 3.2.1.15) and pectin methylesterase (EC 3.1.1.11) main activities and minor cellulase and hemicellulase activities, was from Lyven S.A. (Colombelles, France). The product was in liquid form and was kept in the dark at 4 °C until use.

2.2 Tomato processing waste

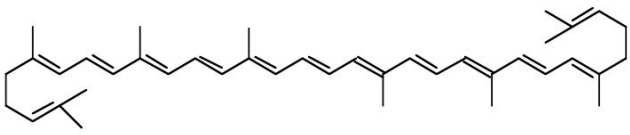
Tomato pomace was obtained from a tomato processing company in southern Lazio (Latina, Italy). The seeds were separated from the peels and the pulp residues by sedimentation in water. After recovery, the two main fractions (peels and seeds) were rinsed thoroughly in water and left to dry in air for a few hours. Tomato seeds were further dried at 50 °C for 12–15 h and stored in the dark at room temperature.

2.3 Seed oil extraction

Seed oil extraction was carried out using a continuous laboratory press extractor (Komet CA59G, IBG Monforts, Germany) with a nominal capacity of 3–5 kg seeds per hour and an installed power of 1.1 kW. Typically, 5 to 6 kg of dried tomato seeds were processed and the collected oil was allowed to decant and filtered on paper prior to storage.

2.4 Production of tomato oleoresin and lycopene-enriched oil

A Pyrex glass device with a working volume of about 1 L was used to produce the tomato oleoresin (Figure 2). The extractor was provided with a thermostated water jacket and a mechanical stirrer (two-blade impeller of diameter 60 mm). The stirring speed was 300 rpm and the other conditions were set to values close to those found as optimal in a previous study (Zuorro et al., 2013). More specifically, 50 g of partially dehydrated tomato peels and 250 mL of the enzyme solution at 0.05 g mL^{-1} were first loaded into the extractor. The suspension was stirred for 3 h at 25 °C. Then, 400 mL of hexane were added and the system was kept under stirring for further 3 h. After this time, the solvent was recovered and evaporated in a rotary evaporator (Rotavapor R-215, BÜCHI, Switzerland). The resulting residue, that is, the tomato oleoresin, was weighed and analysed for lycopene content as reported in a previous paper (Zuorro et al., 2011). The lycopene-enriched oil was prepared by dissolving, under gentle stirring, appropriate amounts of the oleoresin into the seed oil.
Lycopene concentration in the enriched oil samples was determined by a double-beam UV-VIS spectrophotometer (Lambda 25, Perkin Elmer, USA). The absorption spectrum of lycopene exhibits three characteristic peaks at around 456, 483 and 518 nm. To minimize interference from other carotenoids, measurements were made at 518 nm using a molar extinction coefficient of 1.294×10^5 $M^{-1}cm^{-1}$. This value was determined from a calibration curve obtained by dissolving the lycopene standard in the oil.



Lycopene (ψ,ψ-carotene)

CAS number	502-65-8
Molecular formula	$C_{40}H_{56}$
Molecular weight	536.87
Density	0.889 g mL^{-1}
Melting point	173 °C

Figure 1: Chemical structure and properties of lycopene

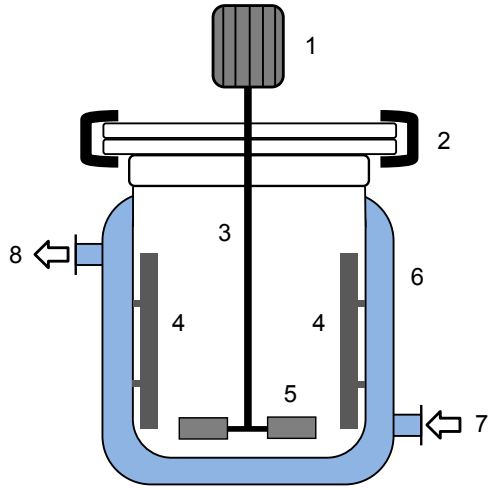


Figure 2: Schematic diagram of extraction vessel and components for the production of tomato oleoresin

3. Results and discussion

3.1 Characterization of tomato processing waste

The moisture content of tomato pomace was 84.2 ± 1.9 wt%. It consisted of about 78% peels and pulp residues, while the remaining 22% were seeds. The total lycopene content of tomato peels was 325 ± 13 mg/100 g dw.

3.2 Seed oil extraction

The oil content of tomato seeds, as determined by the NGD method B4–1976, was 29.3% (on a dry weight basis). Cold pressing of the seeds resulted in about 58% oil recovery, which corresponds to a production of approximately 170 kg of oil per ton of dry seeds. The physicochemical properties of the oil and its fatty acid composition are reported in Tables 1 and 2.

Table 1: Physicochemical properties, tocopherol content and total sterols of tomato seed oil

Property	Units	Value
Free acidity (as oleic acid)	wt%	1.45
Peroxide value	mEq/kg	2.6
Refractive index	–	1.4733
Total tocopherols	mg/kg	1204.8
α-tocopherol	mg/kg	30.8
γ-tocopherol	mg/kg	1168.3
δ-tocopherol	mg/kg	5.7
Total sterols	mg/kg	4732.0

Table 2: Fatty acid composition of tomato seed oil

Fatty acid	Chemical structure	Composition (wt%)
Myristic (C14:0)	$CH_3(CH_2)_{12}COOH$	0.12
Palmitic (C16:0)	$CH_3(CH_2)_{14}COOH$	14.12
Palmitoleic (*cis*-9 C16:1)	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$	0.34
Stearic (C18:0)	$CH_3(CH_2)_{16}COOH$	5.93
Oleic (*cis*-9 C18:1)	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	22.36
Linoleic (*cis,cis*-9,12 C18:2)	$CH_3(CH_2)_3(CH_2CH{=}CH)_2(CH_2)_7COOH$	54.52
Linolenic (*cis,cis,cis*-9,12,15 C18:3)	$CH_3(CH_2CH{=}CH)_3(CH_2)_7COOH$	2.06
Arachidic (C20:0)	$CH_3(CH_2)_{18}COOH$	0.44
Eicosenoic (*cis*-10 C20:1)	$CH_3(CH_2)_8CH{=}CH(CH_2)_8COOH$	0.11

Free acidity (FA) and peroxide value (PV) are important quality parameters for seed and fruit oils. FA is a measure of the extent to which hydrolysis liberates fatty acids from their parent triglyceride molecules, while PV is an indicator of oxidative stability, as it is related to the amount of peroxides present in the oil. As is known, peroxides are unstable compounds that tend to degrade into secondary oxidation products such as aldehydes, ketones and conjugated dienes. Oxidation can cause the development of off-flavors and negatively affect the nutritional properties of the oil. Accordingly, the lower the PV, the better the oil quality and the higher its shelf life. In most cases, oils become rancid when their PV reaches a value between 20 and 40 mEq of active oxygen per kg of oil. For virgin or cold-pressed oils, guidelines from the Codex Alimentarius Commission recommend a maximum level of 15 mEq O_2/kg (CAC, 2005). From Table 1 it can be seen that tomato seed oil had low free acidity (FA = 1.45 wt%) and peroxide value (PV = 2.6 mEq/kg). The level of residual hexane in the oil samples, as determined by GC analysis, was <1 ppm.

The total tocopherol content was about 1200 mg/kg, a value that is among the highest for seed oils (CAC, 2005). We note that γ-tocopherol was the most abundant homologue, accounting for about 97% of total tocopherols, followed by α- and δ-isoforms (2.57 and 0.47%, respectively). The importance of tocopherols is primarily related to their antioxidant activity, as these components are capable of interrupting the free-radical chain reactions responsible for oxidation by donating hydrogen from their phenolic group (Choe and Min, 2009). In addition, degradation of γ-tocopherol leads to the formation of two compounds, γ-tocopherol biphenyl-dimer and γ-tocopherol ether-dimer, which are still effective as antioxidants, while α-tocopherol degrades into products exhibiting low or no antioxidant activity (Kochhar, 2000).

The total sterol content was over 4700 mg/kg. We also found (data not reported) that β-sitosterol and stigmasterol were the major sterols in tomato seed oil. Phytosterols are widely recognized as important components of healthy diets. Among them, β-sitosterol has been shown to protect against oxidative stress via modulation of antioxidant enzymes and to possess cholesterol-lowering properties (Loizou et al., 2010).

Examination of the fatty acid composition of the oil indicates that about 80% of total fatty acids were unsaturated (Table 2). Linoleic (C18:2) and oleic (C18:1) acids were the major unsaturated components, followed by linolenic (C18:3) and palmitoleic (C16:1) acids. The predominant saturated fatty acids were palmitic (16:0) and stearic (C18:0) acids. Therefore, tomato oil falls in the linoleic-oleic acid oils category and can be considered similar, in terms of fatty acid composition, to sunflower and soybean oils (Ryan et al., 2008).

The proximate analysis of the seed cake showed that it was rich in protein (35.5 wt%) and crude fiber (13.2 wt%). The oil content was about 15 wt% and the ash content 3 wt%. The major amino acids determined after protein hydrolysis were Glu, Arg and Asp. Furthermore, all essential amino acids except Trp were present, which supports the use of this byproduct for food applications such as the enrichment of cereal products or low-lysine foods.

3.3 Production of tomato oleoresin and lycopene-enriched oil

Tomato oleoresin was produced in the batch extractor by submitting the peel fraction of the waste to the enzymatic treatment and subsequent solvent extraction. The conditions for the two steps are listed in Table 3. On completion of extraction, the solvent was recovered, vacuum evaporated and the solid residue analysed for lycopene content. The production rate of oleoresin was 27 ± 1.8 mg per g of dry peels and its lycopene content was 7.2 ± 0.2 wt%. Finally, the oleoresin was incorporated into the oil in amounts appropriate to achieve the desired lycopene enrichment levels. In Figure 3 pictures are shown of the oleoresin and some oil samples of different enrichment degrees.

Table 3: Experimental conditions for the production of oleoresin from the peel fraction of tomato waste

Treatment step	Parameter	Value
Enzyme incubation	Temperature	25 °C
	Fresh peel weight	50 g
	Volume of enzyme solution	250 mL
	Enzyme dosage	0.25 g g^{-1}
	Incubation time	3 h
Solvent extraction	Temperature	25 °C
	Solvent	Hexane
	Solvent volume	400 mL
	Extraction time	3 h

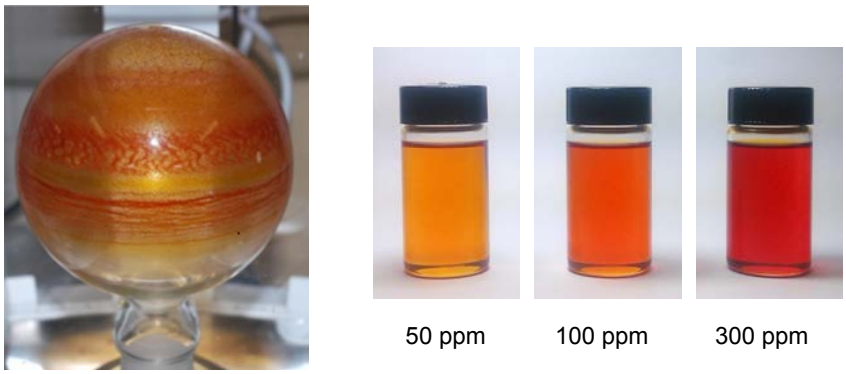


Figure 3: Tomato oleoresin deposited on the inner wall of the evaporation flask (left) and seed oil samples with increasing degrees of lycopene enrichment (50,100 and 300 ppm)

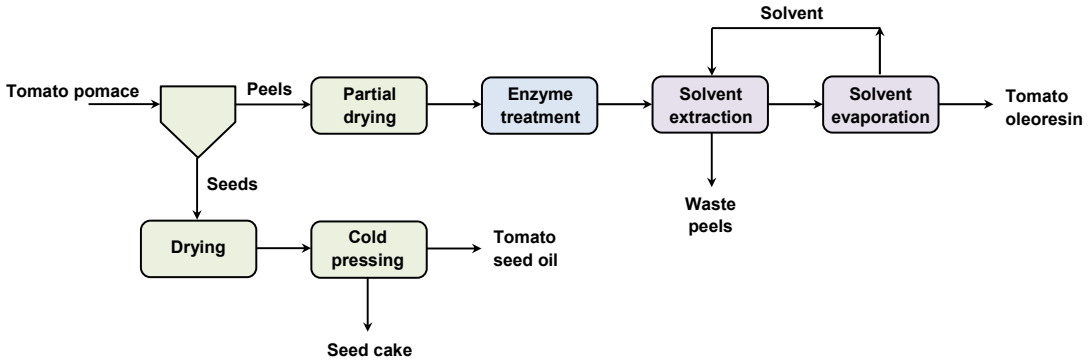


Figure 4: Layout of the process for the production of oleoresin and seed oil from tomato pomace

Oil samples with lycopene levels ranging from 30 to 600 ppm were prepared by adding from 0.42 to 8.4 g of oleoresin per kg of oil. This range of values was chosen in view of possible applications of these products in the nutraceutical and cosmetic fields.

A daily intake of 5–7 mg lycopene is considered sufficient to counteract the effects of oxidative stress and prevent some chronic diseases (Rao and Shen, 2002). It is also known that dissolving lycopene in fats allows a significant increase in bioavailability and hence in efficacy (Fielding et al., 2005). If the lycopene-enriched tomato seed oil were to be used as a source of lycopene, 8 to 12 g of the product with a lycopene content of 600 ppm would be sufficient to provide the recommended daily intake. This amount could, of course, be reduced in the presence of additional dietary sources of lycopene.

In the cosmetic field, the lycopene-enriched oil could be used, alone or in combination with sunscreen products, for skin photoprotection (Anunciato and da Rocha Filho, 2012). In fact, since excessive exposure to UV radiation results in increased ROS production, topical application of antioxidants could be an effective means to reduce skin damage.

From the above, it seems reasonable to say that the lycopene-enriched oil and the tomato oleoresin could find application in the functional-food and cosmetic sectors.

3.4 Industrial implementation of the technology

A simplified layout of the proposed technology is shown in Figure 4. Tomato pomace is first separated into the peel and seed fractions, which are dried to the appropriate moisture content. The seeds are pressed to yield the vegetable oil and the cake, while the peels are subjected to the enzymatic treatment. Then, the treated material is contacted with the solvent to recover lycopene and the other lipophilic components. From this operation, waste peels are produced together with the solvent phase. The solvent is vacuum-evaporated and recycled. Evaporation leads to the formation of tomato oleoresin, which can be used as produced or incorporated into the seed oil to obtain a lycopene-enriched product.

Based on the experimental results of this study, we performed some simulations to estimate the amount of enriched oil obtainable by the above-described technology. In these calculations, an amount of waste ranging from 1,000 to 10,000 tons per year and a seed-to-peel weight ratio from 10% to 50% were

assumed. The production volume estimates for oleoresin and seed oil ranged from 1.5 to 30 t/y and 4 to 200 t/y, respectively, depending on the characteristics of the starting waste. It was also found that only small amounts of oleoresin (from approximately 0.2 to 12%) were required to produce functional oils with a high lycopene level (100 to 600 ppm). Considering the high selling price of tomato oleoresin, which is currently obtained from whole tomatoes, it can therefore be expected that its production from tomato pomace in combination with the enriched oil can further increase the economic value of the initiative.

4. Conclusions

The results of this study demonstrate that tomato pomace can be effectively used for the production of a tomato oleoresin and a lycopene-enriched seed oil. We have also shown that the use of enzyme preparations with pectinolytic, cellulolytic and hemicellulolytic activities allows the recovery of lycopene and other bioactive components from the waste under extremely mild process conditions.
At present, considerable amounts of tomato processing waste are produced in the world and disposed of as solid waste or illegally discarded. As a result, its transformation into value-added products could not only be an economic opportunity for the agri-food sector but also contribute to environmental protection.

References

Anunciato T.P., da Rocha Filho P.A., 2012. Carotenoids and polyphenols in nutricosmetics, nutraceuticals and cosmeceuticals, J. Cosmet. Dermatol., 11, 51–54.

CAC (Codex Alimentarius Commission), 2005, CODEX Standard for named vegetable oils, CODEX STAN 210, Food and Agriculture Organisation of the United Nations, Rome, Italy.

Choe E., Min D.B., 2009, Mechanisms of antioxidants in the oxidation of foods, Compr. Rev. Food. Sci. Food Saf., 8, 345–358.

Di Mascio P., Kaiser S., Sies H., 1989, Lycopene as the most efficient biological carotenoid singlet oxygen quencher, Arch. Biochem. Biophys., 274, 532–538.

Eller F.J., Moser J.K., Kenar J.A., Taylor S.L., 2010, Extraction and analysis of tomato seed oil, J. Am. Oil Chem. Soc., 87, 755–762.

Fielding J.M., Rowley K.G., Cooper P., O'Dea K., 2005, Increases in plasma lycopene concentration after consumption of tomatoes cooked with olive oil, Asia Pac. J. Clin. Nutr., 14, 131–136.

Kochhar S.P., 2000, Stabilisation of frying oils with natural antioxidative components, Eur. J. Lipid Sci. Technol., 102, 552–559.

Kong K.W., Khoo H.E., Prasad K.N., Ismail A., Tan C.P., Rajab N.F., 2010, Revealing the power of the natural red pigment lycopene, Molecules, 15, 959–987.

Lavecchia R., Zuorro A., 2008, Enhancement of lycopene extraction from tomato peels by enzymatic treatment, Chemical Engineering Transactions, 14, 301–308.

Loizou S., Lekakis I., Chrousos G.P., Moutsatsou P., 2010, Beta-sitosterol exhibits anti-inflammatory activity in human aortic endothelial cells, Mol. Nutr. Food Res., 54, 551–558.

Rao A.V., Shen H., 2002, Effect of low dose lycopene intake on lycopene bioavailability and oxidative stress, Nutr. Res., 22, 1125–1131.

Ryan L., Mestrallet M.G., Nepote V., Conci S., Grosso N.R., 2008, Composition, stability and acceptability of different vegetable oils used for frying peanuts, Int. J. Food Sci. Technol., 43, 193-199.

Sabio E., Lozano M., Montero de Espinosa V., Mendes R.L., Pereira A.P., Palavra A.F., 2003, Lycopene and β-carotene extraction from tomato processing waste using supercritical CO2. Ind. Eng. Chem. Res., 42, 6641–6646.

Shixian Q., Dai Y., Kakuda Y., Shi J., Mittal G., Yeung D., 2005, Synergistic anti-oxidative effects of lycopene with other bioactive compounds, Food Rev. Int., 21, 295–311.

Strati I.F., Oreopoulou V., 2011, Effect of extraction parameters on the carotenoid recovery from tomato waste. Int. J. Food Sci. Technol., 46, 23–29.

WPTC (The World Processing Tomato Council), 2013, World production estimate as of 25 October 2013, <www.wptc.to/> accessed 13.12.2013.

Zuorro A., Fidaleo M., Lavecchia R., 2011, Enzyme-assisted extraction of lycopene from tomato processing waste, Enzyme Microb. Technol., 49, 567–573.

Zuorro A., Lavecchia R., 2010, Mild enzymatic method for the extraction of lycopene from tomato paste, Biotechnol. Biotechnol. Equip., 24, 1854–1857.

Zuorro A., Lavecchia R., Medici F., Piga L., 2013, Enzyme-assisted production of lycopene from tomato processing waste, Food Bioprocess Technol., 6, 3499–3509.