EFFECT OF SUBSTRATE PRETREATMENT ON THE SUPERCRITICAL CO_2 EXTRACTION OF TOMATO

Edgar L. Uquiche[a], Gonzalo A. Núñez[b], José M. del Valle[b]* & Jaime Ortíz[c]

[a] Department of Chemical Engineering
Universidad de la Frontera, P.O. Box 54-D, Temuco, Chile

[b] Department of Chemical and Bioprocess Engineering
Pontificia Universidad Católica de Chile, P.O. Box 306, Santiago, Chile

[c] Department of Food Science and Chemical Technology
Universidad de Chile, P.O. Box 233, Santiago, Chile

Email: delvalle@ing.puc.cl

Abstract. Carotenoids are an important group of natural pigments with biological activity. Tomato is an important source of carotenoid pigments, especially lycopene, of particular interest because of its potent antioxidant activity. Supercritical CO_2 ($scCO_2$) extraction was explored in this work for the recovery of tomato carotenoids, with an emphasis on the selection of an appropriate pretreatment (optimal pretreatments tend to be substrate-specific). Because densification by extrusion or pelletization can destroy cell walls and other microstructural barriers to mass transfer within the substrate, on one hand, and increase bulk density of the other, and these two factors may contribute to improved process economy, the objective of this work was to evaluate the effect of conditioning of substrate (tomato flakes) by extrusion or pelletization on $scCO_2$ extraction rate and yield of tomato oleoresins and carotenoids. Milled tomato flakes were used as a control. True and bulk densities were measured to differentiate treated materials. Six-hour extractions were carried out at 60 ºC and 50 MPa using a superficial $scCO_2$ velocity of 0.8 mm/s for each substrate. Total carotenoid pigment content of oleoresin samples was quantified at 470 nm by spectrophotometry. Because pretreatments affected significantly extraction rate and yield, this work provides new information about application of densification as pretreatment to obtain substrate specially adapted to supercritical extraction process.

Keywords: microstructure; oleoresin; pretreatment; supercritical CO_2 extraction; tomato.

1. Introduction

Currently, consumers are demanding healthier foods for avoiding diseases associated with poor nutrition. Due to this, the food industry is continuously looking for new ingredients for functional and value-added foods having antioxidant, antimicrobial, and anti-cancer activities, among others. One of those ingredients are carotenoids. Carotenoids are natural pigments that give yellow, orange, or red colour to fruits, vegetables, and plants [1]. Moreover, the antioxidant activity of carotenoids helps preventing cardiovascular diseases and cancer [2]. Among carotenoids, lycopene is of special interest because of its particular functional properties [3].

Lycopene is a 40-carbon, lineal, highly unsaturated, hydrophobic molecule that is soluble in organic solvents [4]. The main natural source of lycopene is tomato, which contains 0.088 to 0.42 mg per kg of fresh product [4]. Lycopene content is five times higher in tomato skin than tomato pulp [5] and varies depending on genetic, environmental, and culturing factors [6]. Lycopene is sensitive to light, heat, and oxygen, which cause isomerizations (lycopene is normally in a trans-configuration in biological products) and oxidations with the end result of losses of bioactivity, decolouration, and off-odours in foods [7].

The recovery of bioactive compounds in foods requires appropriate extraction technologies, being supercritical CO_2 ($scCO_2$) extraction an excellent alternative. Conventional extraction of tomato by organic solvent is disadvantageous because the process (heat, oxygen) degrades carotenoids and leaves behind traces

of toxic solvent. Supercritical CO_2 ($scCO_2$) extraction is an attractive alternative of conventional extraction, because CO_2 has a relatively low critical temperature and pressure, and it is non-toxic, non-flammable, non-corrosive, and inexpensive. There are several reports in literature on the extraction of tomato using $scCO_2$ as solvent that were reviewed by Zuknik *et al.* [8]. Table 1 summarizes results of these laboratory-scale studies [3,9-16] (there have been no studies at pilot-plant or larger scale). Most studies use tomato processing by-products as substrate, and grinding and drying as pre-treatments. With a few exceptions [9-11] extraction pressures were limited to≤40 MPa, despite clear indications of a positive effect of pressures on extraction [3,11-14]. The effect of temperature on extraction was evaluated in all cases but two [12,15]. Extractions generally improve with temperature [3,9-11,13]; the single exception being a study in which an intermediate temperature (60 ºC) was the best [14]. One study explored two combinations of extraction temperature and pressure, because it aimed at clarifying the effect of co-solvents (ethanol and canola oil) on extraction [15]. Densification by extrusion or pelletization can destroy cell walls and other microstructural barriers to mass transfer within the substrate, on one hand, and increase bulk density of the other, and these two factors may contribute to improved process economy. None of these pretreatments have been studied for tomato. As an aside, it is relevant mentioning that claims of optimal conditions in Table 1 are questionable in cases where the so-called optimum is in the border of the experimental region tested, as is the case in most studies [3,11-13].

Table 1. Summary of SCFE of lycopene from tomato with pure CO_2 at laboratory scale in literature

Substrate	Pre-treatment	T (°C)	P (MPa)	Optimal condition	Ref.
Skin and seeds (by-product)	Grinding and drying	60 – 80	25 – 30	80 °C; 30 MPa	[3]
Dried skin	none	70 – 100	20 – 50	100 °C; 40 MPa	[9]
Skin and seeds (by-product)	none	32 – 86	13.8 – 48.3	86 °C; 34.5 MPa	[10]
Pulp, skin and seeds (by-product)	Grinding and drying	40 – 80	30 – 46	80 °C; 46 MPa	[11]
Pulp and skin	Grinding and drying	40	7.7 – 28.2	40 °C; 28.2 MPa	[12]
Dried skin and seeds	Grinding	40 – 100	20 – 40	100 °C; 40 MPa	[13]
Skin and seeds (by-product)	Grinding and drying	40 – 80	20 – 30	60 °C; 30 MPa	[14]
Pulp and skin	Freeze-drying	40; 70	40	70 °C; 40 MPa*	[15]

* With canola oil as co-solvent

The objective of this work was to study the effect the pretreatment of raw material (grinding, extrusion, and pelletizing) on the supercritical CO_2 extraction of tomato at 60 °C and 50 MPa.

2. Materials and methods

2.1. Substrate for extraction

Dehydrated tomato was acquired from Alto La Cruz Company (Santiago, Chile). This product was dried in a convection oven at 60 °C for 5 days down to around 9.7 g water per 100 g dry substrate, and coarsely milled for in a mortar and pestle to obtain particle between 1.00 and 1.41 mm (-12/+16 Mesh Tyler).

A fraction of milled substrate was treated in a laboratory single-screw extruder Haake PolyDrive 0-120 Nm (Thermo Electron, Karlsruhe GmbH, Germany), with 25:1 barrel length and diameter ratio, internal barrel diameter of 19 mm, 3:1 screw compression ratio and a die nozzle with 3 mm diameter. The extruder was fed mechanically, the screw was turned at 46 rpm, and the extrusion die was held at 80 °C. The barrel has three sections with electric heaters that were independently controlled. Compressed air was circulated around the barrel to maintain a precise control of the temperature of the barrel and die assembles.

Another fraction of the milled substrate was treated in a PP85 pelletizer (Pellet Pros Company, Dubuque, IA) which had a disc with 2.5 mm-holes, and a cutter that allowed adjustment of pellets length. In this device the material is sheared by two gear wheels that turn in its axis, crushed against the rotating disc, forced through the disc holes, and cut at required length at the exit. Finally, the pellets are placed on a tray to prevent warping as they cool.

Finally, extruded and pelletized substrates were milled to obtain a packed bed with particles of 1.00−1.41 mm, allowing a ratio between particle diameter and extraction vessel diameter ≤ 1/10 for all extractions.

2.2. Substrate characterization

The three substrates were dried in a convection oven set at 60 °C for 18 h and then placed in a desiccator with silica gel. All substrates had a moisture content (determined gravimetrically by drying in an oven for 15 h at 102 ºC) that ranged 5.5-5.7 g per 100 g dry substrate. The oleoresin content (determined gravimetrically by extraction with technical grade hexane in a Soxhlet apparatus for 10 h at 70 °C) was 2.1 g per 100 g dry substrate. The real density (ρ_r, kg of dry substrate per m^3 of dry substrate) of the three materials was measured by N_2-pycnometry using an Ultrapyc 1200e device (Quantachrome, Boynton Beach, FL). The bulk density (ρ_b, kg of dry substrate per m^3 of packed bed) of the pelletized substrate was estimated gravimetrically (weighing the material loaded in a graduated cylinder following a standardized tapping procedure) as the ratio between the weight of milled pretreated substrates and the volume of graduated cylinder. Total porosity (ε) of the pelletized substrate was determined using values of real density and bulk density ($\varepsilon_p = 1 - \rho_r/\rho_b$).

2.3. Supercritical Extraction

Supercritical CO_2 extractions were carried out in a Spe-ed SFE unit (Applied Separations, Allentown, PA). Depending on substrate type, between 25 and 30 g of substrate sample was loaded in the extractor (50–cm^3 vessel, 14–mm inner diameter) and extracted using 3.8 L NPT/min of 99.95%-pure CO_2 (Aga S.A., Santiago, Chile) (superficial CO_2 velocity = 0.8 mm/s). All experiments were carried out at 60 °C and 50 MPa. In all cases, a 20-min static extraction period was followed by a 6-h dynamic extraction when the expansion valve (kept at 130 ºC) was opened to adjust the flow rate of CO_2. The extract (oleoresin) was collected during extraction in pre-weighed glass vials (60–cm^3 capacity). Mass of oleoresin aliquots, was assessed gravimetrically by difference with cleaned and dried vials after removing co-extracted water in a desiccator with silica gel. A sample of oleoresin was dissolved in petroleum ether p.a. (Merck, Darmstadt, Germany) and total carotenoid content of extract (mg carotenoids/g extract) was quantified as lycopene at 470 nm by UV spectrophotometry in a SP-2000 UV-Vis spectrophotometer (Bausch-Lomb, USA) using a extinction coefficient ($E^{1\%}$) of 3450.

3. Results and Discussion

3.1. Substrate Characteristics

Table 2 summarizes the physical characteristics of the three substrates used in this study. There were not important differences in the real density of the substrates that ranged between 1457 and 1477 kg/m^3. There were small differences in bulk density in favor of the extruded and specially the pelletized product as compared to the milled product. This caused a limited decrease in total porosity as a result of extrusion and pelletization. We still need to differentiate the contributions of the interparticle and intraparticle space to the porosity that will require complimentary measurements of Hg penetrometry of the three samples, and that provides information about the intraparticle porosity. In any case, we believe that part of the advantages/disadvantages of extrusion and pelletization are partially overcome by the complimentary milling that reduced particle size to the level required to overcome differences in extraction brought about by undesirable axial dispersion phenomena when using large particles in small diameter laboratory extraction vessels. Because of that, we will carry out complimentary extraction experiments at the pilot plant level using particle diameter as an added variable.

Table 2. Physical characteristics of substrates

	Milled tomato	Extruded tomato	Pelletized tomato
Real density (ρ_r, kg/m^3)	1459.8	1476.9	1457.3
Bulk density (ρ_a, kg/m^3)	451.4	544.4	552.2
Total porosity (ε_t, -)	0.691	0.631	0.621

Authors believe that the applied pretreatments of extrusion and pelletization cause a destruction of the inner barriers to mass transfer in the original tissue (intact cell walls and membranes) and this will be evaluated by microscopy. The destruction of these barriers coupled with the maintenance of an intraparticle void space (characterized by Hg penetrometry) will facilitate diffusion and will positively impact $scCO_2$ extraction curves.

3.2. Supercritical Extraction

Figure 1 shows the cumulative extraction plot of oleoresins *versus* specific consumption of solvent, for each type of substrate. Up to using 2.4 kg CO_2/kg dry substrate, the curves overlap as expected for a solubility-controlled process. The apparent solubility estimated for this initial period is 4.21 g oleoresin/kg CO_2.

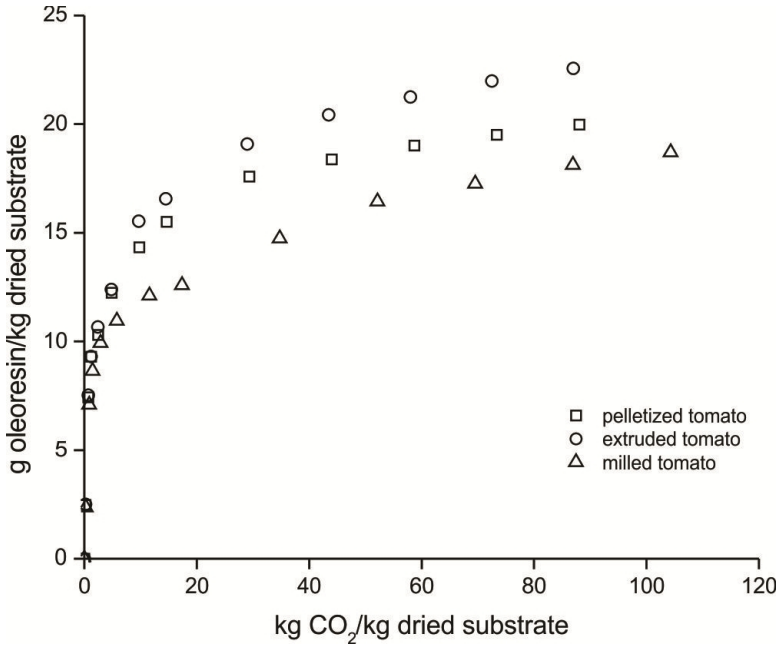

Figure 1. Cumulative curves for the $scCO_2$ extraction of tomato oleoresin at 60 °C and 50 MPa for three different pretreatments: pelletization, extrusion, and milling

Differences in extraction kinetics were observed after using >2.4 kg CO_2/kg dry substrate due mainly to the effect of the solid matrix on the solute diffusion to the interior of the particles [16]. Slow extraction is determined by slow diffusion from broken cells and very slow diffusion from intact cells. Extrusion and pelletization rupture tissues and deliver solutes from the inner of cells, on one hand, and restructure the substrate into a high-density matrix with interconnected pores, on the other. The destruction of inner mass transfer barriers and densification caused by these pre-treatments can maximize the volumetric yield extraction. Milling of the substrate does not ensure complete destruction of the cell walls and cell membranes, and in consequence the presence of intact cells in milled tomato would make that the mechanism of very slow diffusion prevail in the milled substrate. Therefore a smaller extraction rate is observed for milled tomato (Fig. 1).

The cumulative extraction yield of densified substrates was higher for extruded tomato (22.6 g/kg dry substrate) than pelletized tomato (20.0 g/kg dry substrate). Partial information collected up to now (Table 2) does not show differences that could explain the differences in extraction kinetics between these two substrates. We believe more information is required on the distribution and connectivity of pores of the two substrates, which can be backed up by microscopic observation.

For the extraction of total carotenoids (Fig. 2) there were not differences in the extraction kinetic for the different substrates until a CO_2 consumption of 10 kg CO_2/kg dry substrate. From the slope of this lineal extraction stage, it was estimated an apparent solubility of 5.8 mg carotenoids/kg CO_2. The maximum yields (mg carotenoids/kg dry substrate) reached in each extraction were 132.2 (milled tomato), 192.0 (extruded

tomato) and 89.7 (pelletized tomato). Extrusion of the substrate favored the supercritical extraction of carotenoids.

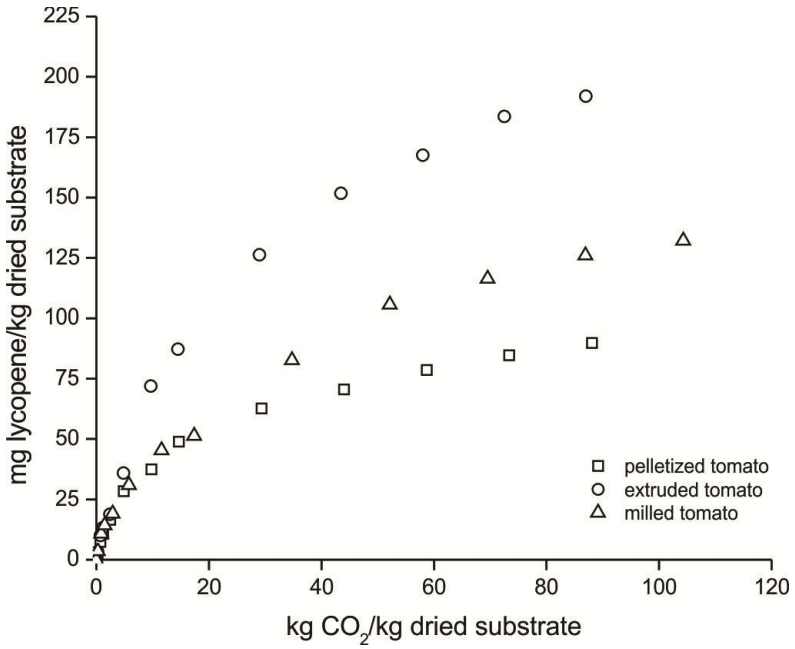

Figure 1. Cumulative curves for the $scCO_2$ extraction of lycopene from pretreated tomato at 60 °C and 50 MPa.

4. Conclusion

Densified (or compacted) tomato by extrusion or pelletization showed higher extraction yield of oleoresin from tomato. These pretreatments favor destruction on cellular barriers to mass transfer and facilitate intraparticle diffusion. However, it was not possible to explain these differences by means of the density measurements used in this work. Pelletization may have an unfavorable effect on the initial content of carotenoids in the substrate, contrary to extruded tomato and milled tomato, where higher extraction yields were reached.

Acknowledgements

The present work was funded by Chilean agencies Fondecyt (Project 111-1008).

References

[1] P. Astorg 1997, Food carotenoids and cancer prevention: an overview of current research, Trends Food Science Technology 8(1997) 406-413.
[2] C.O. Perera, Yen, G.M., Functional properties of carotenoids in human health, International Journal of Food Properties 10(2007) 201-230.
[3] E. Sabio, M. Lozano,V.M. de Espinosa, R.L. Mendes, A.P. Pereira, A.F. Palavra, J.A. Coelho, Lycopene and beta-carotene extraction from tomato processing waste using supercritical CO_2, Industrial & Engineering Chemistry Research 42(2003) 6641-6646.
[4] J.M. Roldán-Gutiérrez, M.D.L de Castro, Lycopene: The need for better methods for characterization and determination, Trac-Trends in Analytical Chemistry 26(2007) 163-170.
[5] S.K. Sharma, M. LeMaguer, Lycopene in tomatoes and tomato pulp fraction, Italian Journal of Food Science 8 (1996) 107-113.
[6] Y. Dumas, M. Dadomo, G. Di Lucca, P. Grolier, Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes, Journal of the Science of Food and Agriculture 83(2003) 369-382.
[7] L.K. Henry, G.L. Catignani, S.J. Schwartz, The influence of carotenoids and tocopherols on the stability of safflower seed oil during heat-catalyzed oxidation, Journal of the American Oil Chemists Society 75(1998) 1399-1402.

[8] M.H. Zuknik, N.A. NikNorulaini, A.K. Mohd Omar, Supercritical carbon dioxide extraction of lycopene: A review. Journal of Food Engineering, 112(2012) 253-262.
[9] U. Topal, M. Sasaki, M. Goto, K. Hayakawa, Extraction of lycopene from tomato skin with supercritical carbon dioxide: Effect of operating conditions and solubility analysis, Journal of Agricultural and Food Chemistry, 54(2006) 5604-5610.
[10] N.L. Rozzi, R.K. Singh, R.A. Vierling, B.A. Watkins, Supercritical fluid extraction of lycopene from tomato processing byproducts, Journal of Agricultural and Food Chemistry, 50(2002) 2638-2643.
[11] E. Vagi,B. Simandi, K.P. Vasarhelyine, H. Daood, A. Kery, F. Doleschall, B. Nagy, Supercritical carbon dioxide extraction of carotenoids, tocopherols and sitosterols from industrial tomato by-products, Journal of Supercritical Fluids, 40(2007) 218-226.
[12] M.S. Gómez-Prieto, M.M. Caja, M. Herraiz, G. Santa-María, Supercritical fluid extraction of all-trans-lycopene from tomato, Journal of Agricultural and Food Chemistry, 51(2003) 3-7.
[13] C. Yi, J. Shi, S.J. Xue, Y.M. Jiang, D. Li, Effects of supercritical fluid extraction parameters on lycopene yield and antioxidant activity, Food Chemistry, 113(2009) 1088-1094.
[14] B.P. Nobre, A.F. Palavra, F.L.P Pessoa, R.L. Mendes, Supercritical CO_2 extraction of trans-lycopene from Portuguese tomato industrial waste, Food Chemistry, 116(2009) 680-685.
[15] M.D.A. Saldaña, F. Temelli, S.E. Guigard, B. Tomberli, C.G. Gray, Apparent solubility of lycopene and β-carotene in supercritical CO_2, CO_2 + ethanol and CO_2 + canola oil using dynamic extraction of tomatoes, Journal of Food Engineering 99 (2010) 1-8.
[16] H. Sovová, Mathematical model for supercritical fluid extraction of natural products and extraction curve evaluation, Journal of Supercritical Fluids, 33(2005) 35-52.