THE EFECT OF OLEIC ACID DURING THE EXTRACTION OF CAROTENOIDS FROM TOMATO PULP WITH LIQUID CARBON DIOXIDE

Dafina Karaj[1], Altin Mele[2], Elmira Mehmeti[3], Erinda Prifti, Vilma Gurazi[4]
[1]*Department of Chemistry, Faculty of Mathematical and Physical Engineering, University of Polytechnic of Tirana. Tirana, Albania*
[2]*Department of Chemistry, Faculty of Natural Science, University of Tirana, Tirana, Albania*
[3]*Department of Toxicology and Quality, Food Safety and Veterinary Institute, Tirana, Albania*
[4]*Department of Industrial Chemistry, Faculty of Natural Science, University of Tirana, Tirana, Albania*
Email: dfn_karaj@yahoo.com

Abstract

In this study, the effect of oleic acid during the extraction of lycopene and β-carotene from tomato pulp was investigated. Also, the kinetics of the extraction of carotenoids from tomato pulp, using as solvent the liquid carbon dioxide under its liquid-vapor equilibrium conditions was study. The experiments were carried out in a Jennings-type autoclave after the Soxhlet principle with and without modifier at 299 K and 64 bar. The extraction yields and the lycopene and β-carotene content of the liquid CO_2 extracts were determined after 0.5, 1, 3, every three hours, up to 39 hours of extraction in the presence of oleic acid as modifier. HPLC-DAD was used for the quantification of the lycopene and β-carotene in the extracts. The highest amount of trans-lycopene is extracted after 12 hours of extraction (0.26 μg/g sample) and β-carotene (0.33 μg/g sample). Only after 12 hours of extraction the amount of lycopene and β-carotene extracted from tomato pulp in presence of oleic acid, becomes higher that when no modifier is used. The presence of modifier increased the total amount of lycopene extracted but decreased the total amount of β–carotene.
Keywords: tomato, lycopene, β-carotene, near critical liquid CO_2, oleic acid.

Introduction

Carotenoids are an important group of lipid-soluble pigments widely distributed in nature, responsible for the yellow, orange and red colors of flowers, fruits, vegetables and plants (Petito et al., 2016). Tomato contains carotenoids in high amount (around 5.1–6.3 mg/100 g fresh weight), the main constituent is lycopene (70–80%) which provides the intensive red colour in tomato fruit. Numerous carotenes can be found above lycopene, like: phytoene (5.3%), phytofluene (2.8%), β-carotene (3.7%), lutein (2.0%), etc (Vagi et al., 2006). Lycopene and other carotenoids are found mostly in the outer pericarp (McCollum et al., 1955) with tomato skin containing 12 mg lycopene/100 g skin (wet basis) (Al-Wandawi et al., 1985).

Epidemiologic studies suggest that consumption of tomato and tomato-based products reduces the risk of chronic diseases such as cardiovascular disease and cancer, due to its high antioxidant activity of lycopene and provitamin A of β-carotene (Stajčić et al., 2015).Besides their biological properties, carotenoids are utilized as natural antioxidants for the formulation of functional foods or as additives in food systems to elongate their shelf-life (Nobre et al., 2009).

Extraction of carotenoids from vegetal sources is usually carried out using organic solvents, e.g., n-hexane, acetone, chloroform, ethanol, etc. These hazardous solvents possess many disadvantages, such as toxicity, disposal, difficult separation from final product (presence of solvent traces) and the need to work at high temperatures. Supercritical fluid extraction using CO_2 is a suitable alternative to the conventional extraction techniques of biological products, since this solvent allows working at moderate temperatures, is non-toxic

and easily separated from the extract (Nobre et al., 2009). The removal of lycopene and β-carotene from different tomato matrixes by several solvents are reported in the literature and presented in a review (Saldana, M. D.A et al., 2012). The effect of additional co-solvents to the SC-CO_2 like chloroform, ethanol, water, n-hexane, olive oil, hazelnut oil etc, is studied and also presented in the review of Temelli.

The use of near critical liquid CO_2 under liquid-vapor equilibrium condition as solvent for plant extraction has been reported in the literature and is shown that the extraction by liquid CO_2 can be more selective then the extraction by supercritical CO_2 (Mele et al., 2013, Naik et al 1989).

The addition of olive oil as modifier increases the extractability of lycopene and decreases the extractability of β-carotene from tomato (skin and pulp) when is using as solvent the liquid CO_2 under its liquid-vapor equilibrium conditions Olive oil, when used as a modifier improves lycopene extraction by swelling the matrix and increasing the mass transfer rate (Karaj et al., 2013).

The objective of this study was to investigate the effect of oleic acid as modifier during the extraction of lycopene and β-carotene from tomato pulp using as solvent near critical liquid CO_2.

Materials and methods

Samples and chemicals

The tomato skin was provided by local tomato sauce producer in Ballsh, Albania and was sun dried. It was ground using a laboratory mill, which contains 34% of particle size greater than 1 mm and 66% of particle size smaller than 1 mm. Its remaining moisture content of 16 % was determined by a Sartorius Moisture Analyzer, Model MA 45. Food grade carbon dioxide 99.5 % pure was purchased from Messer-Griesheim. The reagent grade methanol and tetrahydrofuran were purchased from Fluka, while oleic acid from Merck. The lycopene, β-carotene and apo-8'-carotenal reference standards were purchased from Sigma-Aldrich.

Liquid CO_2 extraction

Two different procedures were tested: the extraction of tomato pulp by a Soxhlet-type process via periodic solvent recycling with and without modifier (oleic acid). In both case the extraction with liquid CO_2 is done in a Jennings-type autoclave, shown schematically in Figure 1. Some modifications in the construction of the autoclave used here, done by Lentz, consist in enabling the visual control of Soxhlet apparatus inside it, through a small glass window on its upper cover (Naik et al., 1989). The autoclave is made out of stainless steel, especially resistant towards chemically aggressive substances. It has an outer diameter of 80 mm, a wall thickness of 17mm and a height of 300mm. In the upper screw able cover, a capillary with a high pressure valve for loading and discharging the solvent, a capillary with a pressure gauge, a window and a cooling finger are welded. Through the window one can observe the liquid CO_2 solvent condensing on the cooling finger, and the siphon of the glass apparatus inside the autoclave when it loads and empties. The window is made of synthetic sapphire (thickness 10mm, diameter 12 mm), which are sealed by an O-ring (28mm×2 mm). The 25mm thick upper and lower covers of the autoclave are sealed with O-rings (63mm×2 mm).

The solvent inside the autoclave can be recycled periodically according to the Soxhlet principle autoclave and the apparatus must be operated under the conditions of liquid–vapor equilibrium, below the critical temperature and critical pressure of CO_2. Soxhlet apparatus inside the autoclave is made out of two glass parts: the upper one is used to hold the tomato sample and is equipped with a siphon to remove periodically the liquid CO_2 from the sample and the second is a collection beaker used as a reservoir for the extract collection and the evaporation of CO_2.

The extraction of tomato sample with liquid CO_2 was performed under its liquid-vapor equilibrium conditions at temperature of 299 K which corresponds to equilibrium pressures of 63 bar. The CO_2 is near its critical state ($T_{critical}$ = 31.1^oC and $P_{critical}$= 73.8 bar) but still in the liquid–vapor equilibrium conditions and therefore an extraction after the Soxhlet principle is possible.

In order to create a temperature gradient inside, the bottom of the autoclave was immersed in a heating water bath at and the cooling finger was connected to a cooling bath of ethylene glycol. Consequently liquid CO_2 evaporates from the bottom of the autoclave and condenses at the cooling finger, dropping afterwards into the tomato material. When the upper glass is completely filled, it empties through the siphon and liquid CO_2 flows into the lower glass. This procedure is repeated periodically, in the same way as in the classical Soxhlet-type apparatus.

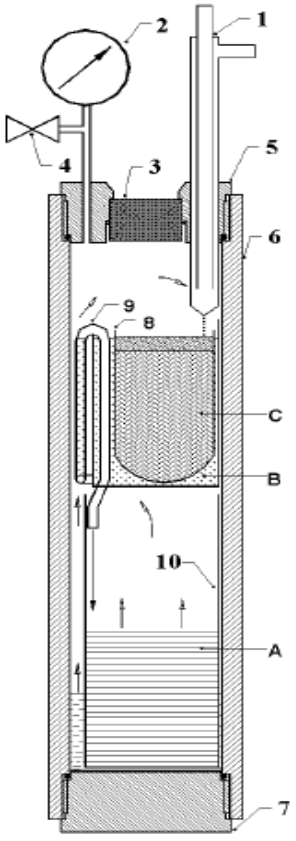

Figure 1: High pressure apparatus for Soxhlet extraction by liquid CO_2: 1. Cooling finger, 2. Pressure gauge, 3. Window, 4. Valve, 5. Upper cover, 6. Steel cylinder, 7. Lower cover, 8. Extraction thimble, 9. Siphon, 10. Collection beaker, A. Product, B. Extract solution, C. Extraction material

Through the sapphire window we could observe that the time for one Soxhlet cycle is

10 min (one filling, and one drainage via siphon). Extraction was interrupted after 0.5, 1, 3, 6, every three hours, up 39 hours. After each extraction the CO_2 was released from autoclave and the bottom glass with the solvent free extract was weighted. By weight difference of the bottom glass before and after extraction, the mass of extracted was determined and the yield of the extraction was calculated as the ratio in percent of the mass of the extract against the mass of plant material.

The extraction was carried out also in the presence of oleic acid as modifier. Approximately of 1g of acid was added directly to the sample, before each extraction with liquid CO_2. By interrupting the extraction after several time periods and weighing the lower glass, the mass of the extract and the yield of extraction were determined.

Each time, 30 g of tomato sample were placed in the extractor and were extracted with liquid CO_2. In order to minimize the decomposition and oxidation of the extracted compounds, all samples were dissolved in THF, collected in 10 ml brown sample vials to prevent UV-activated degradation, and stored at -20^{o}C

HPLC analysis

The identification and quantification of β-carotene and lycopene were analyzed by high performance-liquid chromatography (HPLC) equipped with Eclipse C18 column (3.5µm, 3x150 mm) connected with Diode Array Detector as reported by Vasopollo with some modification (Vasapollo et al., 2004). A mixture of methanol, THF and water (84:10:6) was used as a mobile phase to a flow rate of 1.0 ml/min for the first 5 minutes and after this the ratio of mixture is (67:27:6) with a flow rate of 1.5 ml/min (20 µl injection volume). The peaks of trans-lycopene and β-carotene were identified by comparing the retention times (10.7 and 12.6 min, respectively) with those of their standard compounds. As cis-lycopene was identified the peak coming immediately after the trans-lycopene based on the results reported from Topal where a similar HPLC method is used for the carotenoids separation (Topal et al., 2006).

In all cases β-apo-8'-carotenal (5.7 min) was used as the internal standart and the chromatograms were monitored at 475 nm, shown in Figure 2. The content of lycopene and β-carotene in the extract were estimated by comparing the peaks areas with their respective standards.

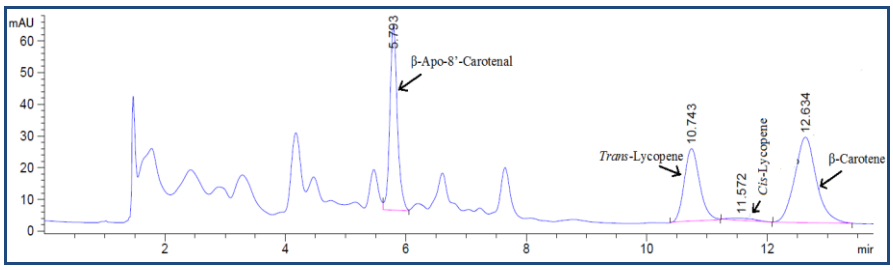

Figure 2. HPLC analyses of the extracts of tomato pulp after 15 hours of extraction without modifier.

Results

Extractions of lycopene and β-carotene from tomato pulp have been carried out at the same temperature and pressure conditions, in absence and present of oleic acid (3.3%) in order to verify its role in the process.

Using vegetable oils as modifier, the lycopene is solubilised in the vegetable oil, which is extracted as well (Strati et al., 2014). It is proven that the addition of 2 % olive oil at 299 K in extraction of tomato pulp with liquid carbon dioxide increased slightly the extracted amounts of lycopene but decreased drastically the extracted β-carotene (Karaj et al., 2013).

The same thing happens when oleic acid was used as modifier, it seems that the amount of β-carotene is decreased and the amount of lycopene is increased. The selection of oleic acid as modifier was done after determination of solubility of three fatty acid of olive oil constituted. Oleic acid has the higher solubility in liquid carbon dioxide compare with palmitic and stearic acid (Karaj et al., 2015).

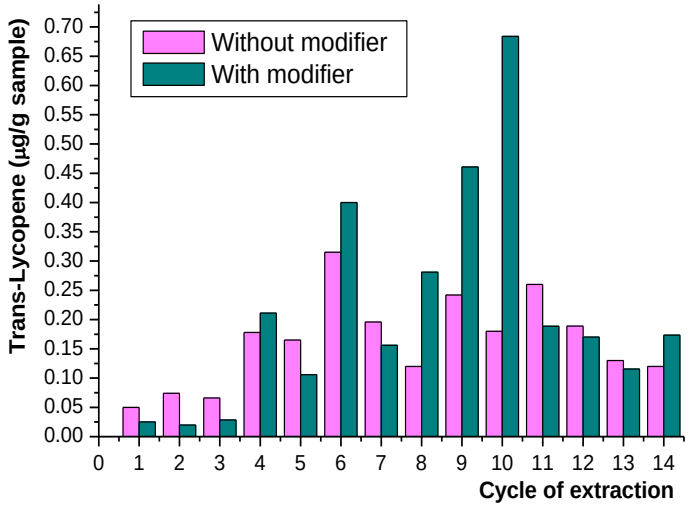

Figure 3. Change in the content of lycopene in liquid CO_2 extracts with the cycle of extraction, after Soxhlet extractions carried out under liquid-vapor equilibrium conditions at 299 K and 6.3 MPa with and without oleic acid as modifier.

The amount of lycopene extraction from tomato by liquid CO_2 in presence of oleic acid, becomes higher that when no modifier is used, only after 24 hours of extraction, as shown in Figure 3. That is, the presence of the modifier probably promotes a better transport and a better solubility of lycopene from solid phase into the liquid phase (Vasopollo et al., 2004). Lycopene in the absence of oleic acid was extracted during the first hours of extraction. The opposite occurs when it was extracted in presence of oleic acid.

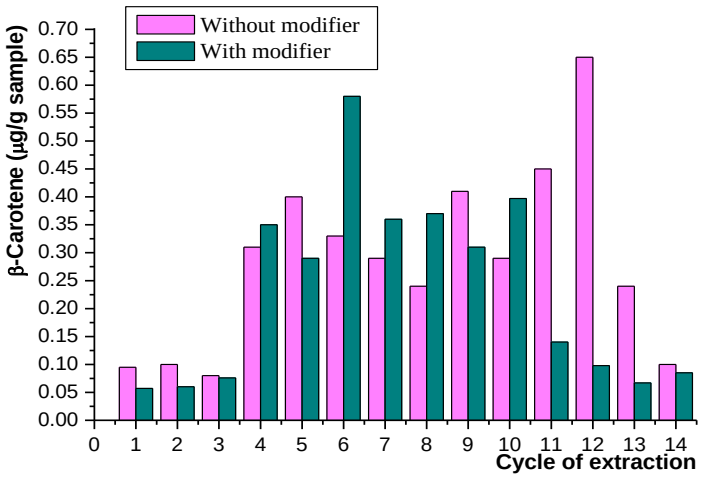

Figure 4. Change in content of β-carotene in liquid CO_2 extracts with cycle of extraction,

after Soxhlet extractions carried out under liquid-vapor equilibrium conditions at 299 K and 6.3 MPa with and without oleic acid as modifier.

During the first hours of extraction, the amount of lycopene extracted is very low but after 18 hours it's increased and reached the highest value. Whereas, in the absence of oleic acid, the higher amount of lycopene extracted was reached after 12 hours of extraction.

The addition of olive oil surprisingly decreases the extracted amount of β-carotene, like as the addition of oleic acid. Almost the entire amount of β-carotene extracted was reached after 24 hours of extraction, while in the presence of modifier it extend throughout the time of extraction, as shown in Figure 4. The amount of β-carotene extracted is same during the first hours of extraction, using or not the oleic acid as modifier. The last hours of the extraction make the difference in the amount of β-carotene extracted.

Besides the advantages of having an improvement in the yields of the lycopene extraction, the content of cis-lycopene decreases drastically in liquid CO_2 extracts when oleic acid is added, as shown in Figure 5.

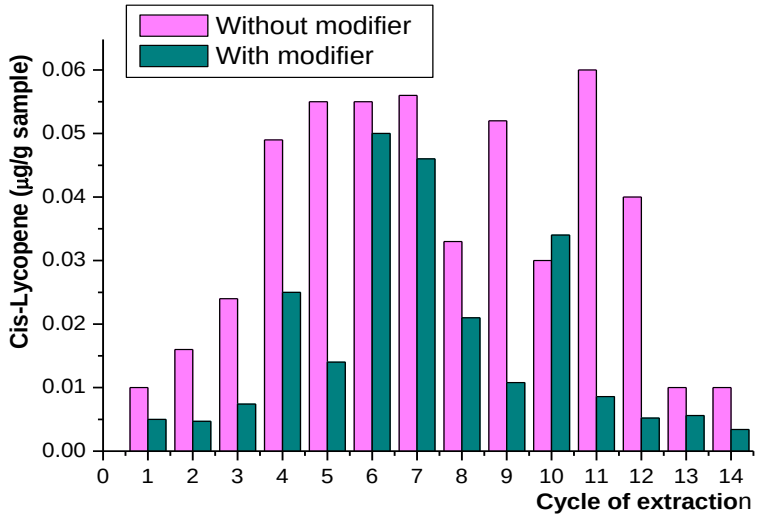

Figure 5. Change in the content of cis-lycopene in liquid CO_2 extracts with cycle of extraction, after Soxhlet extractions carried out under liquid-vapor equilibrium conditions at 299 K and 6.3 MPa with and without oleic acid as modifier.

Conclusions

The near critical liquid-CO_2 extraction of lycopene and β-carotene from the dried tomato pulp with and without modifier was experimented.

Oleic acid as one of the ingredients of olive oil increased the amount of lycopene extracted but decreased the amount of β –carotene.

The stability of cis-lycopene is increased with the presence of acid oleic during the extraction.

References

Petito N., Dias D., Costa V., Falcão D., Araujo K., (2016). Increasing solubility of red bell pepper carotenoids by complexation with 2-hydroxypropyl-b-cyclodextrin. Food Chemistry, 208, 124–131

Vagi, E., Simandi, B., Vasarhelyine, K. P., Daood, H., Kery, A., Doleschall, F., et al. (2007).

Supercritical carbon dioxide extraction of carotenoids, tocopherols and sitosterols from industrial tomato byproducts. Journal of Supercritical Fluids, 40, 218–226.
McCollum, J.P., (1955). Distribution of carotenoids in the tomato. Food Res. 20, 55–59.
Al-Wandawi, H., Rahman, M.A., Shaikhly, K.A., (1985). Tomato processing wastes as essential raw materials source. Journal Agriculture. Food Chemistry. 33, 804–807.
Stajčić et al., C´ etkovic´ G, C˘ anadanovic´-Brunet J, Djilas S, Mandic´ A, C˘ etojevic´-Simin D., (2015). Tomato waste: Carotenoids content, antioxidant and cell growth activities. Food Chemistr, 172, 225–232
Nobre, B. P.; Palavra, A. F.; Mendes, R. L., 2004. Supercritical Fluid Extraction of Carotenoids from Tomato Industrial Wastes. 7th Italian Conference on Supercritical Fluids and Their Applications,Trieste, Italy; N 22.
Saldana, M. D.A., Temelli. F., S. Guigard, E., Tomberli, B., Gray, C.G. (2012). 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, 1-8.
Mele A., Lentz H., Mele A., Feizimayr E., Abazi S., Bauer R.(2013): Extraction of rotundifuranandcasticin from chaste tree fruid by near critical liquid carbon dioxide.The journal of supercritical fluids. Vol. 79, Fq. 123-126.
Naik S.N, Lentz H., Maheshvari R.C. (1989): Extraction of perfumes and flavours from plant materials with liquid carbon dioxide under liquid–vapor equilibrium conditions. Fluid Phase Equilibria 49, 115–126.
Karaj D., Mele A., Abazi S., Mehmeti E., (2013). The Liquid CO_2 extraction of Lycopene and β-Carotene from tomato. 6-th Internacional Symposium on the High Pressure on Technology, 247-251
Vasapollo, G., Longo, L., Rescio, L., Ciurlia, L. (2004): Innovative supercritical CO_2 extraction of lycopene from tomato in the presence of vegetable oil as co-solvent.The Journal of Supercritical Fluids. Nr. 29, fq. 87-96
Topal, U., Sasaki, M., Goto, M., Hayakawa, K., 2006. Extraction of lycopene from tomato skin with supercritical carbon dioxide: Effect of operating conditions and solubility analysis. J. Agric. Food Chem. 54, 5604–5610.
Strati, I. F., & Oreopoulou, V. (2014). Recovery of carotenoids from tomato processing by-products-a reviw.Food Research International, 65, 311-321.
Karaj, Mele A. (2015). Kinetics of lycopene and β-carotene extraction from tomato skin in presence of oleic acid by near critica.l liquid CO2. International Journal of Engineering Inventions, Volume 4, Issue 7, 1-5