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Kinetics of lycopene and β-carotene extraction from tomato skin in presence of oleic acid by near critical liquid CO_2

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***Abstract**: In this study we investigated the extraction of lycopene and β-carotene from tomato skin using near critical liquid carbon dioxide in the presence of oleic acid as modifier.*
The experiments were carried out in a Jennings-type autoclave after the Soxhlet principle with and without modifier at 299 K, using as solvent liquid carbon dioxide under its vapour pressure of 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 24 hours of extraction. HPLC-DAD was used for the quantification of the lycopene and β-carotene in the extracts. The presence of the modifier improves the extractability of lycopene (1.16-1.45 μg/g sample) but strains that of β-carotene (1.6-0.96 μg/g sample). Using the oleic acid as co-solvent has a beneficial role in the stability of cis isomer of lycopene (0.19-0.067 μg/g sample).

***Keywords**: Lycopene, β-carotene, oleic acid, extraction, near critical liquid CO_2.*

I. INTRODUCTION

Of all the tomato carotenoids, β-carotene has the highest provitamin A activity, while lycopene has potent antioxidant activity [1] [2]. These carotenoids have been the subject of numerous studies to demonstrate their health benefits, in terms of cancer, cardiovascular disease, reduces of prostate cancer, etc. [2][3][4]. Lycopene in tomato represent more than 85% of the total carotenoids. The concentration of lycopene in tomato varies from 30 to 200 mg/kg in the fresh fruit and from 430 to 2950 ppm on a dry basis [3].
Extraction of lycopene and β-carotene from tomato using several liquid solvents as hexane, dichloromethane, water, acetone and their mixtures is widely reported in the literature [5][6]. Supercritical CO_2 extraction of lycopene and β-carotene from tomato with and without co-solvents (ethanol, methanol, and acetone) and modifiers (olive oil, hazelnut oil) is extensively studied as well [3][2][7].
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 [8][9].
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 [10].
The main objective of this study was to investigate the kinetics of the extraction of lycopene and β-carotene from tomato skin using as solvent near critical liquid CO_2 with and without addition of oleic acid as co-solvent and its influence on the lycopene / β-carotene ratio.

II. EXPERIMENTAL

2.1 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.

2.2 Liquid CO_2 extraction

Two different procedures were tested: the extraction of tomato skin by a Soxhlet-type process via periodic solvent recycling with and without co-solvent (oleic acid). In both case the extraction with liquid CO_2 is done in a Jennings-type autoclave, shown schematically in Fig. 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 [9]. 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 parts of this autoclave are shown as in figure 1 and explained by numbers and letters.

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 64 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.

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 to 24 and to 30 hours in presence of co-solvent. 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^oC.

3.3 Solubility of fatty acid in liquid CO_2.

Knowledge of the solubility of pure fatty acids of olive oil is very essential for understanding the effect of the olive oil in the extraction process. The solubility of pure oleic (C18:1), stearic (C18:0) and palmitic (C16:0) acids in near critical liquid CO_2 was measured in a Jennings-type autoclave, described below, at temperatures 300 K and pressures 6.3 MPa and with the same amount of CO_2 as during the tomato extractions.

The amount of fatty acids dissolved in liquid phase was determined gravimetrically, as the difference of the mass of acid before and after the dissolution.

3.4 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 [3]. 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 [4].

In all cases β-apo-8'-carotenal (5.7 min) was used as the internal standart and the chromatograms were monitored at 450 and 475 nm, shown in Fig.2. The content of lycopene and β-carotene in the extract were estimated by comparing the peaks areas with their respective standards.

III. RESULTS AND DISCUSSION

The solubility for oleic, stearic and palmitic acid in liquid carbone dioxide at T = 300 K at pressure 63 MPa are shown in Table 1.

The highest obtained solubility was found for oleic acid compared to the two others acids, for that reason was selected to be used in the CO_2 extraction, because it means that higher amounts of oleic acid would be present in the liquid CO_2 extraction process of tomato to change the solubility of lycopene and β-karotene.

Extractions have been carried out at the same temperature and pressure conditions without modifier and with oleic acid (3.3%) in order to verify its role in the process.

It is proven that the addition of 2 % olive oil at 299 K in extraction of tomato skin with liquid carbon dioxide increased slightly the extracted amounts of lycopene but decreased drastically the extracted β-carotene [10]. The same thing happens when oleic acid was we used oleic acid as co-solvent, we see that the amount of β-carotene is decreased.

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 20 hours of extraction, as shown in Fig. 3 and Fig. 4.

That is, the presence of the modifier probably promotes a better transport and a better solubility of the pigment from solid phase into the liquid phase [3].

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 Fig. 5

IV. FIGURES AND TABLES

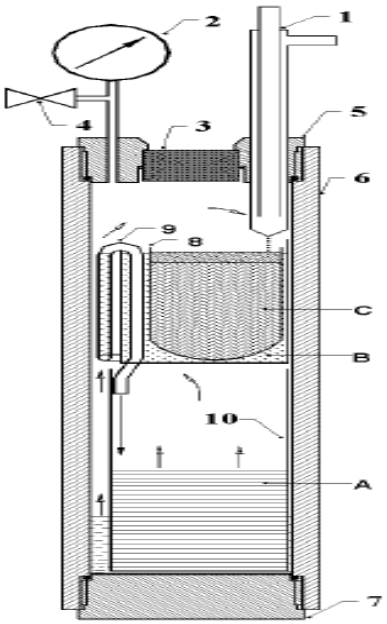

Figure 1: High pressure apparatus for Soxhlet extraction by liquid CO_2

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

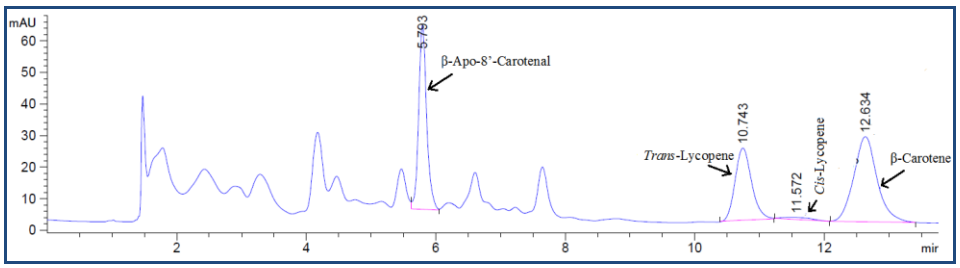

Figure 2. HPLC analyses of the extracts of tomato skin after 15 hours of extraction without co-solvent

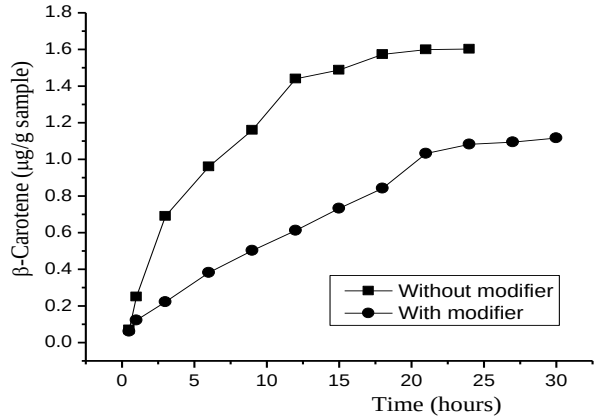

Figure 3. Change in content of β-carotene in liquid CO_2 extracts with time, after Soxhlet extractions carried out under liquid-vapor equilibrium conditions at 300 K and 6.3 MPa with and without oleic acid as modifier.

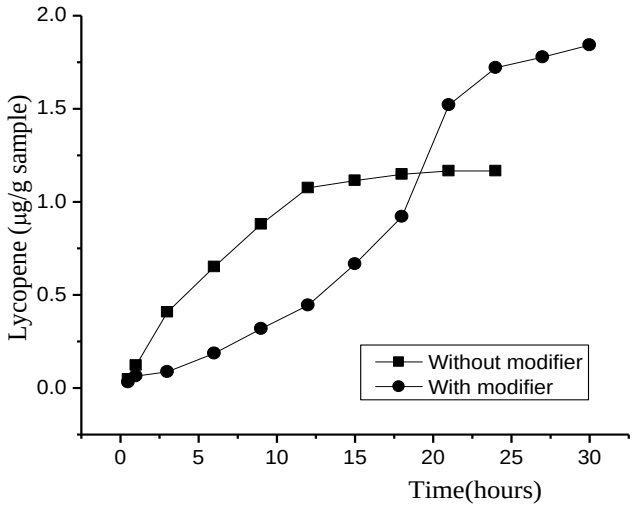

Figure 4. Change in the content of lycopene in liquid CO_2 extracts with the time, after Soxhlet extractions carried out under liquid-vapor equilibrium conditions at 300 K and 6.3 MPa with and without oleic acid as modifier.

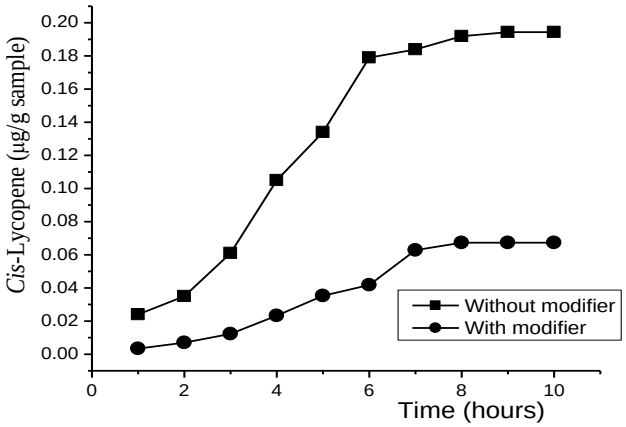

Figure 5. Change in the content of cis-lycopene in liquid CO_2 extracts with the time, after Soxhlet extractions carried out under liquid-vapor equilibrium conditions at 300 K and 6.3 MPa with and without oleic acid as modifier.

Table.1 Solubility of Fatty Acid in liquid CO_2

		Solubility (mg/g CO_2)
Oleic Acid	**(C18:1)**	1.35
Stearic Acid	**(C18:0)**	0.25
Palmitic Acid	**(C16:0)**	0.11

V. CONCLUSIONS

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

The presence of modifier increased the amount of lycopene extracted but decreased the amount of β –carotene.

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.

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