SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF LYCOPENE FROM INDUSTRIAL TOMATO WASTE

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Abstract: Cultivation of tomatos is an important agro-economic activity in the European Union (EU). Tomato processing industry entails the generation of large volumes of organic waste in the form of tomato seeds and skin. The tomato waste obtained from the manufacture of tomato juice was collected from "Orhei-Vit" JSC, Orhei, Republic of Moldova. Initially, tomato waste was dried from an initial humidity of 80.0% to a final moisture content of 6.5%. In order to increase the contact surface with carbon dioxide, the tomato waste was milled. Using the full factorial orthogonal experimental design method, was created the planning matrix in real variables, obtaining 15 extraction regimes by varying the parameters: temperature (36–73 °C), pressure (18–42 MPa) and time (24–96 min). Under laboratory conditions, samples of CO_2 extracts from tomato waste were obtained at different extraction parameters. The regression equation allowed the optimization of the response using the gradient ascension method, thus establishing the optimal extraction parameters of the bioactive compound – lycopene.
Key words: Tomato waste, supercritical CO2 extraction, lycopene

Introduction

With the increase in world trade of tomato products, the tomato processing industry is also expanding. As a consequence (result), the amount of by-products: tomato seeds and skin grows. At present, only a small amount of tomato by-products are sold at low prices and used as feed or fertilizer, but the remaining seeds are thrown away, thus wasting resources and polluting the environment.

Tomato seeds are an excellent source of macronutrients: 28% fat and 29% protein, and micronutrients: linoleic acid and other unsaturated fatty acids, high levels of essential amino acids such as lysine; without toxic ingredients or nutritional inhibitors. Therefore, the way of extracting nutrients from tomato by-products in order to obtain new products and greater economic value is a challenge [9].

Tomatoes and tomato waste, respectively, are highlighted by an increased content of lycopene, a powerful antioxidant. The lycopene molecule is the longest of all carotenoids, which like the β-carotene, the human body does not synthesize. Although it has a structure similar to that of the well-known antioxidant β-carotene, its antioxidant activity is at least 5 times higher. Lycopene protects cells against DNA damage and lipid peroxidation, and intervenes in reducing the risk of certain cancers (prostate, digestive, bladder, lung, skin) [10]. The antioxidant activity of lycopene is due to the capture of singlet oxygen from biological systems.

Lycopene is widely used in the alimentary industry as an antioxidant and natural colorant. As antioxidant it is active in non-polar environments, in oils, fats, foods with a lipid content. It has the ability to protect lipids from oxidative degradation by inactivating the reactive forms of oxygen. Namely, the double bonds of the molecules ensure the addition of reactive oxygen [14].

According to the European Food Safety Authority (EFSA Journal 2011; 9 (4): 2031 Scientific opinion on the substantiation of health claims related to lycopene according to Article 13, paragraph 1 of Regulation (EC) No 1924/2006) the Dietary Reference Intake (DRI) of lycopene to have antioxidant effect or a normal cardiac function (in case of cardiovascular disease) is 5-15 mg / day. According to the recommendations of the Guidebook MP 2.3.1. 19150-04 of 2004, (Recommended levels of biologically active substances), the DRI of lycopene is 5-10 mg (the maximum daily dose is 15 mg) [8].

This paper presents the content of lycopene in CO_2 extracts from tomato waste, obtained at different extraction regimes. Based on the obtained data, it is determined the influence of the extraction parameters: temperature, pressure and time on lycopene concentration in the fat-soluble CO_2 extracts from tomato waste.

Materials and methods

Materials

Tomato waste was collected from the industrial scale production of tomato juice at "Orhei-Vit" JSC, Orhei, Republic of Moldova. With the purpose of being used as raw material, tomato waste with an initial moisture content of 80.09 % was dried by the conductive method in Biosec Domus B5 dryer to a final moisture content of 6.50 %. One of the basic criteria for carrying out the supercritical CO_2 extraction is that the raw material subjected to the extraction of the lipid fraction has a humidity of maximum 10…12% [4]. In order to increase the contact area with the carbon dioxide, to achieve a more efficient extraction, both quantitatively and qualitatively, the tomato waste was milled. The lipid content in dried tomato waste is 10.5% [4]. Carbon dioxide is intended for use in the food industry. The reagents used in analyzes: hexane, ethanol and acetone, meet the quality requirements.

Methods

The supercritical extraction with carbon dioxide from tomato waste was carried out under laboratory conditions at the HA 120-50-01C pilot plant within the Practical Scientific Institute of Horticulture and Food Technology. The technical parameters of the installation are: Pmax=50 MPa (500 atm), Tmax = 75°C, volume of the extractor vessel – 1.0 l and maximum extract volume – 0,6l [4].

From the storage tank, the carbon dioxide is pumped through the heat exchanger into the extractor vessel with raw material – tomato waste. Using the pressure and temperature control system the required extraction pressure and temperature are created in the extractor vessel. Once the supercritical CO_2 and the feed reach equilibrium in the extraction vessel, through the manipulation of pressure and temperature to achieve the operating conditions, the extraction process proceeds. The mobile phase, consisting of the superctitical CO_2 fluid and the solubilized components, is transferred to the separators I and II where the fluid is reduced by decreasing the pressure of the system. The extract precipitates in the I or II separator while thesuperctitical CO_2 fluid is either released to the atmosphere or recycled back to the extractor [1, 4].

Determination of Lycopene content

The lycopene from tomato by-products is extracted using hexane: ethanol: acetone mixture (2:1:1) (v/v) following the Sharma and Le Maquer method, exposed by Alda [5]. One gram of the homogenized samples, and 25 ml of hexane: ethanol: acetone mixture, which was placed into the rotatory mixer for 30 min, adding 10 ml of distilled water and the stirring was continued for another 2 minutes. The solution was then left to separate into two distinct layers, polar and non-polar. The absorbance was measured at 502 nm, using hexane as a reference sample. The lycopene concentration was calculated using its specific extinction coefficient (E 1%, 1 cm) of 3150 in hexane at λ=502 nm. The concentration of lycopene is expressed in mg/100 g product.

$$C = \frac{E}{3.15} \cdot \frac{20}{m} \tag{1}$$

C – lycopene content, mg/100g
m – mass of product sample, g
E – extinction coefficient.

Statistical analysis

Variance analysis of the results was carried out by least square method with application of Microsoft Office Excel program. Differences were considered statistically significant if probability was greater than 95% ($q < 5\%$). All assays were performed at room temperature, $20 \pm 1\ ^{0}C$. Experimental results are represented according to standard rules.

Results and discussion

In order to determine practical values of the process parameters, it is necessary to establish interdependencies capable of describing both the nature and the extent of the influences of the input factors, so it is foreseen to determine a mathematical model. For *the planned experiments*, to the influence factors were assigned two levels of variation: *a upper level* x_{sup} and *a lower level* x_{inf}. These two levels are chosen at equal distance from the *central level* x_0 of the influence factor, also called *base level* or *zero level*.

The zero level indicates the value of the influence factors around which experimental modeling was desired. The interval limited by the lower and upper values of the influence factors defines *the experimental field*. All influence factors can take values within this range of variation [3, 7].

During the supercritical-CO_2 extraction from tomato waste was examined the oscillation of three process parameters, namely temperature, pressure and time. Respectively, it was obtained the matrix in which the variable parameters of the process were encoded by X_1, X_2 and X_3 and were noted the minimum, center and maximum values that will be used in supercritical carbon dioxide extraction of liposoluble substances, including lycopene.

Table 1. *The classical matrix of assigning the values of influence factors*

Factor	Coding	Min. (-)	Center	Max. (+)
Temperature, *°C*	X_1	40	55	70
Pressure, *MPa*	X_2	20	30	40
Time, *min.*	X_3	30	60	90

Each input factor was assigned a coded variable. The variation range, from minimum (-) to maximum (+), was chosen in accordance with the characteristics of the CO_2-extraction plant, so that all experiments were achievable.

In order to have relevant results on the CO_2 extraction process of lycopene from tomato waste, two parallel experiments of the 15 regimes of extractions were performed, at temperature, pressure and time parameters to maximum, minimum and center values and combinations thereof. When selecting the extraction parameters, were taken into account the characteristics of the pilot plant type HA 120-50-01C, (P_{max} = 50 MPa, T_{max} = 75 ° C, V_{cel} = 1,0 l) [12], the parameters required to ensure the supercritical state of carbon dioxide (P_{cr} = 7.377 MPa, T_{cr} = 30.978 ° C, ρ_{cr} = 467.6 kg / m^3) [10, 13], but also that these parameters do not affect the quality of the extraction products. The lycopene concentration in the CO_2 extract of tomato waste, obtained with supercritical carbon dioxide, was taken as the output variable.

Knowing the factors that influence the CO_2 extraction process of lycopene from tomato waste, it can be chosen an optimum.

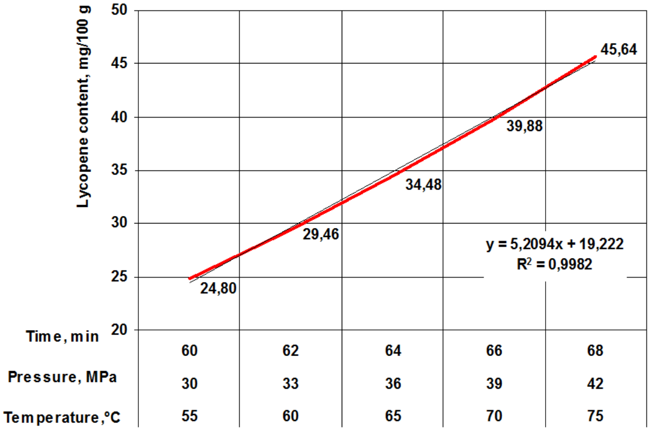

Fig. 1. *The calculated lycopene content according to the obtained equation at envisaged parameters with the recalculated step*

Analyzing the data from Figure 2, it is noted that increasing the temperature by 5.0°C, the pressure by 3.0 MPa and the extraction time by 2.0 minutes, the lycopene content increases with 4.66 to 5.76 mg/100 g of extract.

In order to obtain the graphs of response surfaces in the final form of regression equation: $\widehat{Y} = 24,80 + 7,48X_1 + 6,29X_2 + 1,37X_3 + 1,66X_1^2$ the parameters X_1, X_2, X_3 are replaced with the expressions (2):

$$X_1=(T-T_0)/\Delta T; \text{ or : } X_1=(T-55)/15$$
$$X_2=(P-P_0)/\Delta P; \text{ or : } X_2=(P-30)/10 \quad (2)$$
$$X_3=(t-t_0)/\Delta t; \text{ or : } X_3=(t-60)/30$$

Therefore, the final form of the second degree equation (3) describing the lycopene CO_2 extraction from tomato waste is:

$$\widehat{Y} = 24,80 + 7,48(T-55)/15 + 6,29(P-30)/10 + 1,37(t-60)/30 + 1,66((T-55)/15)^2 \quad (3)$$

The plot of response surface of lycopene concentration in the CO_2 extracts from tomato waste, at 1 constant input factor (for minimum and maximum values) and 2 variable inputs were modeled.

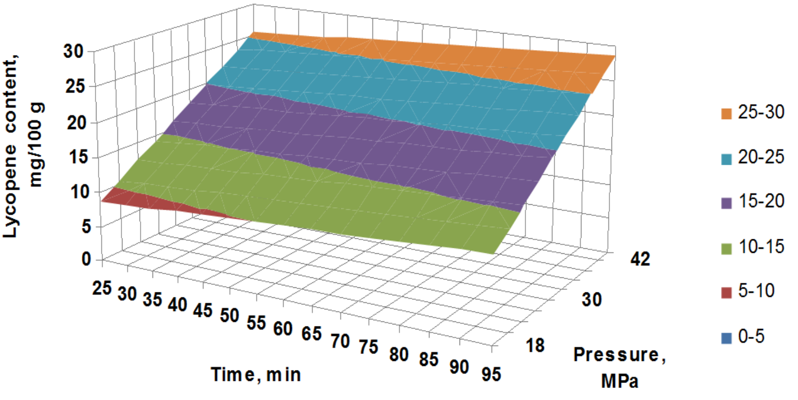

Fig. 2. *The response surface plot of tomato waste CO_2 extraction: lycopene content vs. extraction pressure and time at constant temperature 35 °C*

At constant temperature T = 35 °C (Fig. 2), the minimum and maximum lycopene content calculated by the obtained formula (4) is:

$$\widehat{Y}_{min} = 8.63 \text{ mg/100 g, at P=18 MPa, t=25 min.}$$
$$\widehat{Y}_{max} = 28.81 \text{ mg/100 g, at P=45 MPa, t=95 min.} \quad (4)$$

According to the mathematical model, at 75 °C a maximum lycopene content of 48.76 mg/100 g is obtained at P = 45 MPa and t = 95 min, and the minimum lycopene content of 28.58 mg/100 g at P = 18 MPa, t = 25 min. If the pressure is constant, namely for P_{min} = 15 MPa (Fig. 4), the minimum lycopene concentration is equal to 6.74 mg/100 g (35 °C and 25 min) and the maximum concentration is up to 29.89 mg/100 g (75 °C and 95 min).

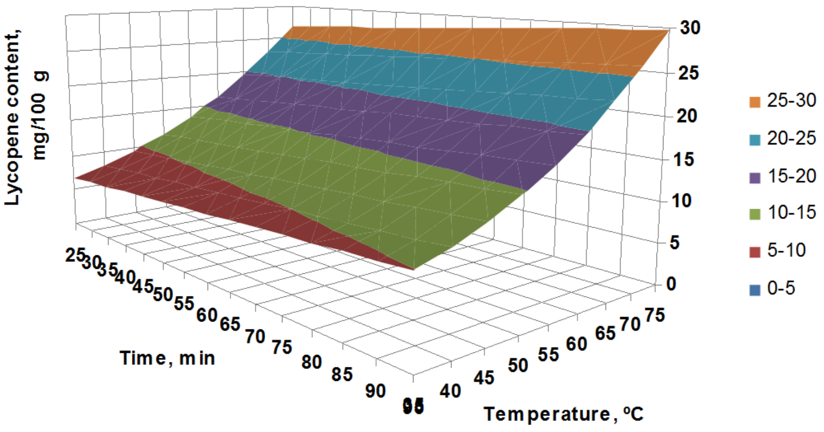

Fig. 3. *The response surface plot of tomato waste CO_2 extraction : lycopene content vs. extraction temperature and time at constant pressure 15 Mpa*

In the case when pressure is constant, for P_{max} = 45 MPa, the minimum lycopene concentration is 25.61 mg/100 g (35 °C and 25 min) and the maximum concentration reaches 48.76 mg/100 g (75 °C and 95 min). At a constant time of 25 minutes, the lycopene concentration would be at least 5.43 mg/100 g at 35 °C and 18 MPa, and at most 42.36 mg/100 g at 75 °C and 45 MPa (Fig. 4).

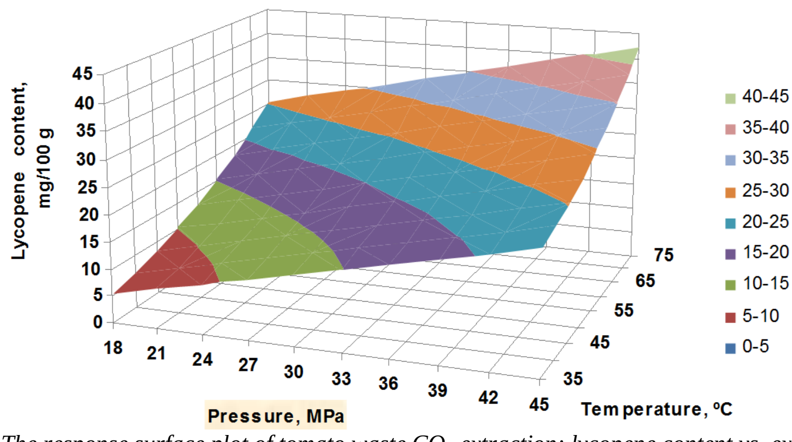

Fig. 4. *The response surface plot of tomato waste CO_2 extraction: lycopene content vs. extraction pressure and temperature at constant time 25 min*

For the duration of the 120 minute constant extraction, according to the response area obtained according to the final equation, the minimum concentration of lycopene is 18.45 mg/100 g at 35 °C and 18 MPa and the maximum concentration is 55.38 mg/100 g at 75 °C and 45 MPa.

The content of lycopene in the CO_2 extract from tomato waste varies between 10,80–47,12 mg/100 g, ie on average 28.96 mg/100 g of CO_2-extract, about 2–9 times higher than Recommended Daily Amount (RDA), 5–15 mg/day [8]. In order to provide the human body with 15% of the RDA [6] of lycopene, a portion of the consumed product must contain at least 0.75 mg of lycopene.

Conclusions

Tomato waste can be used as a secondary raw material for the extraction of lycopene in liposoluble CO_2 extract. For supercritical CO_2 extraction parameters: T=36–73 °C; P=18–42 MPa and t=24–96 min, the lycopene content in CO_2 fatty soluble extracts from tomato waste varies in the range from 10.80 to 47.12 mg/100 g. The greatest influence on the extracting process of lycopene in CO_2 extracts from tomato waste has the temperature, followed by pressure, and the duration of the process has the least influence. The final form of the second degree equation describing the CO_2 extraction of lycopene from tomato waste has been established. The optimal parameters of supercritical CO_2 extraction of lycopene from tomato waste are temperature 60–75 °C, pressure 33–42 MPa and time 62–68 min. The untapped potential for industrial tomato waste is in line with the current zero waste sustainability concept (Directive 2008/98/EC).

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