

ULTRASOUND-ASSISTED EXTRACTION OF LYCOPENE AND β -CAROTENE FROM TOMATO-PROCESSING WASTES

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ABSTRACT

Ultrasound-assisted extraction (UAE) of lycopene and β -carotene from tomato-processing wastes were investigated. Hexane:acetone:ethanol (2:1:1 v/v/v) including 0.05% (w/v) butylated hydroxyl toluene (BHT) was used as a solvent, with 1:35 w/v solid liquid ratio at $15\pm 5^\circ\text{C}$. Ultrasonic power (50, 65, 90W) was applied in UAE for 1-30 min. Conventional organic solvent extraction (COSE) was applied under the same solvent and temperature conditions for 10-40 min.

UAE was more effective and required a shorter time than COSE. Maximum lycopene and β -carotene yields were obtained using 90W ultrasonic power for 30 and 15 min, respectively.

Keywords: lycopene, β -carotene, ultrasound assisted extraction, tomato processing wastes

1. INTRODUCTION

The tomato is popular because of its healthy composition and consumption both in raw and processed forms all over the world. The essential role of carotenoids, being a major dietary source of vitamin A, is having remarkable effects on the immune response and intercellular communication (TAYLOR *et al.*, 2000; SU *et al.*, 2002; SUN *et al.*, 2010). Studies show that antioxidant-rich diet reduces or prevents risks of epithelial and prostate cancers, cardiovascular diseases and cataracts (TONUCCI *et al.*, 1995; ARAB and STECK, 2000; STAHL and SIES, 2005; CAPANOGLU *et al.*, 2008; LIANFU and ZELONG, 2008).

Lycopene and β -carotene are used in industry for their wide color range between yellow and red (SABIO *et al.*, 2003; ALDA *et al.*, 2009). However, for their great solubility in oil and fat, both of them are used as a natural colorant in the food, cosmetics, and pharmaceutical industries. β -carotene is used as pro-vitamin A, animal feed, an additive in cosmetics, multivitamin constituent and anti-oxidant (BEN-AMOTZ and FISHLER, 1998). The basic carotenoid in tomato is lycopene, and it is known for its high anti-oxidant capacity. Depending on protective properties of lycopene against cancer and oxidants, lycopene is one of the important components used in pharmaceuticals and cosmetic formulations (KUMCUOGLU *et al.*, 2014; DOLATABADI *et al.*, 2016).

Various amounts and type of wastes at various stages appear during tomato processing. Dry pomace consists of 44% seed and 56% pulp and skin (KAUR *et al.*, 2008; LAVELLI and TORRESANI, 2011; DOLATABADI *et al.*, 2016). A major part of waste is composed of seed and skin, which contain five times more lycopene than tomato pulp (SHI *et al.*, 1999; TAYLOR *et al.*, 2000). Since the skin is the major source of lycopene, it must be separated from other parts for better extraction (GEORGE *et al.*, 2004; KAUR *et al.*, 2008). Total lycopene content in tomato pulp and tomato skin varies between 90 to 190 mg/kg and about 120 mg/kg in fresh weight, respectively, while β -carotene is found to be about 3 mg/kg (BAYSAL *et al.*, 2000; ALDA *et al.*, 2009; POOJARY and PASSAMONTI, 2015; KUMCUOGLU *et al.*, 2014). Besides having high antioxidant activity, lycopene degrades and isomerizes under light irradiation and high temperature treatments (CHEN *et al.*, 2009). To isolate all-*trans*-lycopene and avoid isomerization, suitable and fairly controlled conditions should be satisfied. Extraction of lycopene is applied by classic solvent extraction (COSE) method traditionally (LIANFU and ZELONG, 2008). But in traditional extraction methods, long process time and high amounts of solvent are needed. Decreasing the solvent consumption, shortening the extraction time, increasing the extraction yield, and enhancing the quality of extracts can be reached by new methods such as ultrasound-assisted (UAE), microwave-assisted, or supercritical extraction (WANG and WELLER, 2006; FANTIN *et al.*, 2007; WANG *et al.*, 2008).

Sound waves produced by an ultrasonic probe at frequencies greater than human hearing cause a mechanical impact, allowing greater penetration of solvent into the plant body, known as the "sponge effect." Another effect of ultrasonic power is producing high-energy cavitation bubbles containing solvent vapor. These bubbles implode near cell walls causing very high local temperatures, pressure increase and cell wall destruction, which eases mass transfer from cell to solvent and enhances micro-streaming. The combination of these effects intensifies solvent penetration and satisfies sufficient mixing for extracting high amounts of active components. Besides easing extraction, ultrasound may also produce free radicals within the cavitation phenomenon such as highly reactive hydroxyl radicals in water-contained solutions (TOMA *et al.*, 2001; VINATORU, 2001; MASON and LORIMER, 2002; WANG *et al.*, 2008; JERMAN *et al.*, 2010; SUTKAR *et al.*, 2010).

The aim of this study was to evaluate the effects of time and ultrasonic intensity in UAE of lycopene and β -carotene from tomato-processing wastes and to compare the efficacy between UAE and COSE.

2. MATERIALS AND METHODS

2.1. Material

Tomato wastes used in this study were supplied from a tomato-paste-manufacturing pilot plant (Ege University, Agricultural Faculty Izmir, Turkey, 2012). The raw material was dried, from 75% to 4.90 ± 2.50 % moisture content, in a vacuum drier at 40°C for 24 h. Before extraction process, dried raw material containing 48.80 ± 4.70 % skins and % 51.20 ± 3.10 seeds was grounded in a laboratory scale hammer mill (Armfield Hammer Mill, England) and then subjected to sieving. The average particle size of the powder was determined by screen analysis. Samples with 286 ± 24 μm average size were used in the extraction process. Then, the samples were packed under vacuum and stored at 40°C until the extraction process.

2.2. Methods

2.2.1 Conventional Organic Solvent Extraction (COSE)

Extraction of lycopene and β -carotene was carried out according to the method of Sadler *et al.* (1990) and modified as described by PERKINS-VEAZIE *et al.* (2001). The mixture of solvents hexane: methanol: acetone (2:1:1 v/v), containing 0.05% (w/v) BHT (butylated hydroxyl toluene), were used to extract carotenoids from the sample. A 1.14 g dried sample was placed into a 50 mL flask, and a 40 mL solvent mixture was added to satisfy 1:35 (w/v) ratio. Then, these flasks were agitated continuously in a shaking water bath. Extractions were applied at 15 ± 5 °C temperature for 10, 20, 30 and 40 minutes. After the extraction process 15 ml of cold distilled water was added after the extraction process and then suspension was centrifuged by 1000 rpm at 5°C. The solution was then allowed to stand for 5 min for separation of polar and non-polar layers. Non-polar phase was used for lycopene and β -carotene determination. Filtered non-polar phases were first dried under nitrogen flow and then kept at -40°C in amber bottles as described in previous study (KUMCUOGLU *et al.*, 2014) for 6 months before subjected to HPLC analyses.

2.2.2 Ultrasound Assisted Extraction (UAE) Method

A high-intensity ultrasonic probe with maximal input power of 400 W and operating frequency of 24 kHz (Model UP400S, Dr. Hielscher, Germany) equipped with a H14 sonotrode (Dr. Hielscher, Germany) was used for the extraction experiments. Solvent composition and liquid-solid ratio were the same as applied in COSE. Ultrasonic probe was immersed 7 cm into the solution from the top of the 150 ml flask. Ultrasonic treatments were performed for 1, 2, 5, 10, 15, 20 and 30 min. In this study, ultrasonic power levels and corresponding ultrasonic intensities were 50, 65, 90 W and 32.50, 42.25, 52 W/cm² respectively. During UAE experiments, the temperature of samples was kept at 15 ± 5 °C by using indirect cold water circulation system. For lycopene and β -carotene determination, the same procedure was applied as the one implemented in COSE.

2.2.3 Lycopene and β -carotene Determination

Solvent-free extracts were dissolved in HPLC-grade hexane and then analyzed by high-performance liquid chromatography (HPLC 1200 Agilent Technologies/USA) using a

diode array detector (DAD) at 475 nm by applying BARBA *et al.* (2006) procedure. All extracts were filtered through a 0.22 μm filter membrane before injection to the HPLC column. Extracts were diluted with HPLC grade hexane to fit the concentrations to the calibration curves. The calibration curves were determined by injecting 20 μl samples of 2.5, 5.0, 7.5, 10 and 20 mg/kg of pure β -carotene (95% synthetic, HPLC grade-Sigma Chemical Co.) and samples of 20, 30, 50, 80 and 100 mg/kg of pure lycopene (90-95% from tomato, Sigma Chemical Co.). Correlation coefficients of calibration curves were 0.9975 and 0.9979, respectively.

C_{18} column (10 μm , 3x300 cm Waters/USA) was used for the separation. The column temperature was 30°C. Methanol: acetonitrile (90:10 w/w) was used as a mobile phase at a flow rate of 0.9 ml/min, while 20 μl hexane phase was injected for each sample. At the beginning of analyses of each extract, the column was washed with mobile phase in order to remove pigments other than lycopene and β -carotene, which are not soluble in this polar solvent. Identification of carotenoids in the extracts was done by comparing their retention time with the retention time of their standards. Results were calculated as mg/kg of dry weight.

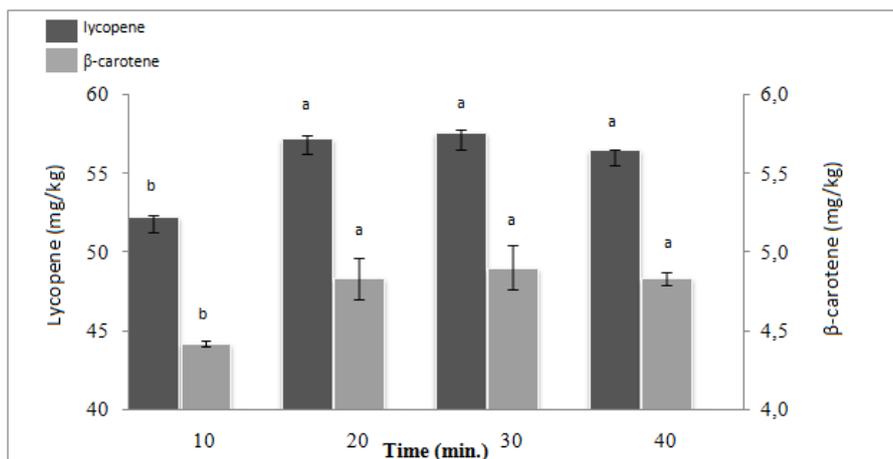
2.2.4 Statistical analysis

All measurements were carried out in triplicate. The results were expressed as the mean value \pm the standard deviation. Data were subjected to statistical analysis using SPSS 16.0 (SPSS Inc. Chicago, IL, USA), and analysis of variance (ANOVA) was applied using generalized linear model to determine the single and multiple effects of the parameters on the conditions by comparing the mean values. All significant differences were reported at $p < 0.05$. Duncan's multiple range test (MRT) was used in post hoc analysis for multiple comparisons.

3. RESULTS AND DISCUSSIONS

3.1 Conventional Organic Solvent Extraction (COSE)

Effect of extraction time for lycopene and β -carotene in COSE is given in Fig 1. It has been observed that the amount of extracted lycopene and β -carotene increased with time at the beginning of the extraction period. Statistical evaluation of the results showed that time was an important factor in extraction of lycopene and β -carotene ($p < 0.05$). There is an increase in yield for 10 min and 20 min extraction periods ($p < 0.05$) while longer extraction periods such as 30 min and 40 min yields were similar ($p > 0.05$) for both lycopene and β -carotene yields. This can be explained by driving force decrease in osmotic balance; as the diffusion of carotenoids from material to the solution in COSE takes place slowly so that the osmotic pressure between the inside and the outside of the cell easily reached equilibrium (SUN *et al.*, 2011; KUMCUOGLU *et al.*, 2014).



Different letters on bars indicate significant differences ($p < 0.05$)

Figure 1: Effect of Extraction Time for Lycopene and β -carotene in COSE

In this study, the amount of extracted lycopene and β -carotene in dried samples were between 52.21- 57.19 mg/kg dry weight and between 4.42-4.90 mg/kg, respectively. In previous studies, similar results were found; extracted lycopene values of fresh tomatoes were reported between 8.5 - 136 mg/kg (LUGASI *et al.*, 2003; KARAKAYA, 2007; KUMCUOGLU *et al.*, 2014). The lycopene values in tomato skin were determined between 25.5 - 141 mg/kg, and extracted β -carotene values in fresh tomatoes varied between 0.5-9.5 mg/kg, depending on genetic, agronomic, climatic factors and processing conditions (RAO *et al.*, 1998; RAO and AGARWAL, 1999; BAYSAL *et al.*, 2000; TOOR and SAVAGE, 2005; BRAVO *et al.*, 2012).

3.2 Ultrasound Assisted Extraction (UAE)

The results of the general linear model indicates that extraction time, ultrasonic power and their composite effects are significant for both lycopene and β -carotene yields as given in Table 1, and the applied model seems significant to describe extraction for each carotenoid ($p < 0.05$).

Table 1: ANOVA of response for ultrasound-assisted extraction experiments for lycopene and β -carotene

Lycopene				β -carotene			
Source	SS	DF	P	Source	SS	DF	P
Corrected Model	14296.275 ^a	20	<0.0001	Corrected Model	49.510 ^b	20	<0.0001
Intercept	179.031.254	1	<0.0001	Intercept	1447.072	1	<0.0001
power	6.725.223	2	<0.0001	power	12.436	2	<0.0001
time	6.828.752	6	<0.0001	time	32.242	6	<0.0001
power * time	742.299	12	<0.0001	power * time	4.832	12	<0.0001
Error	282.484	42		Error	1.359	42	
Total	193610.013	63		Total	1497.941	63	
Corrected Total	14578.759	62		Corrected Total	50.869	62	
a. R Squared = .981 (Adjusted R Squared = .971)				b. R Squared = .973 (Adjusted R Squared = .961)			

SS, sum of squares; DF, degrees of freedom, significant effects, $\alpha=0.05$.

Table 2: Identification and Chromatographic Data for Lycopene and β -carotene after UAE.

Component	Power (W)	Extraction time (min)						
		1	2	5	10	15	20	30
Lycopene	50	28.44 ^{cE} ±1.68	31.72 ^{cD} ±0.69	37.39 ^{cC} ±3.48	43.00 ^{cB} ±2.20	44.20 ^{cAB} ±0.03	47.03 ^{cA} ±0.28	48.05 ^{cA} ±0.65
	65	35.79 ^{bE} ±1.43	39.23 ^{bD} ±1.37	57.49 ^{bC} ±5.75	60.02 ^{bB} ±3.73	65.34 ^{bAB} ±0.08	62.93 ^{bA} ±0.24	63.00 ^{bA} ±0.56
	90	35.89 ^{aE} ±2.52	56.34 ^{aD} ±0.42	67.34 ^{aC} ±3.38	70.07 ^{aB} ±2.20	73.73 ^{aAB} ±0.04	75.90 ^{aA} ±0.07	76.87 ^{aA} ±0.33
carotene	50	3.46 ^{cE} ±0.16	3.56 ^{cD} ±0.10	4.05 ^{cC} ±0.41	4.15 ^{cB} ±0.13	4.72 ^{cA} ±0.05	5.00 ^{cA} ±0.02	5.24 ^{cA} ±0.07
	65	3.07 ^{bE} ±0.10	3.57 ^{bD} ±0.06	4.71 ^{bC} ±0.26	4.99 ^{bB} ±0.08	5.28 ^{bA} ±0.03	5.52 ^{bA} ±0.01	5.66 ^{bA} ±0.06
	90	3.92 ^{aE} ±0.08	5.00 ^{aD} ±0.05	5.30 ^{aC} ±0.29	6.01 ^{aB} ±0.20	6.12 ^{aA} ±0.07	5.92 ^{aA} ±0.01	5.41 ^{aA} ±0.03

Data are means \pm standard deviation. For every compound, different apices (capital letters) in a row indicate significant difference with respect to extraction time; different apices in a column (small letters) indicate significant differences with respect to ultrasonic power. $p<0.05$, Duncan's multiple range test.

It was observed that the extraction yield increased exponentially in a few minutes (2 min), later increased gradually (10 min) and then became constant during extraction (Table 2). The initial sharp increase in the rate of extraction was due to the large β -carotene and lycopene concentration gradient between the extracting solvent and the cell. Later, the concentration gradient decreased as the extraction became difficult thanks to the interior location of cells. Similar results were observed for extraction of all-*trans*-lycopene from red grapefruit and lycopene from tomato-processing wastes (KUMCUOGLU *et al.*, 2014; XU and PAN, 2013). In previous studies, similar results were observed for ultrasonic extraction of all-*trans*-lycopene, and time was found to be the most important factor affecting extraction yield. It is reported that most of all-*trans*-lycopene could be extracted during the 1/3 of total extraction period (30 min), and then lycopene degradation and isomerization led to the reduction of lycopene amount due to the side effect of sonication called ultrasonic degradation (WANG and WELLER, 2006; JERMAN *et al.*, 2010; SUN *et al.*, 2010; XU and PAN, 2013; KUMCUOGLU *et al.*, 2014).

As the power increased from 50W to 65W, lycopene value increased significantly ($p < 0.05$), while the increase in β -carotene value between 50W and 65W was not significant ($p > 0.05$). But application of 90W had a significant effect on both lycopene and β -carotene contents ($p < 0.05$).

Cavitation and thermal effects play an important role in UAE. With an increase in power, more energy was getting transferred for cavitation, and this resulted in the increase in lycopene and β -carotene yield. At low ultrasonic intensities, thermal effect can be ignored because the heat produced by ultrasound may be completely diffused. As the ultrasonic intensity is further increased, the cavitation effect becomes less important compared to thermal effects during extraction of sensitive products such as carotenoids (SORIA and VILLAMIEL, 2010; SUN *et al.*, 2011; EH and TEOH, 2012). It was reported that during sonication the extreme physical conditions of temperature and pressure caused carotenoid isomerization (CHEN *et al.*, 2009). Besides enhancing extraction efficiency, high ultrasonic power could cause thermal degradation to thermally sensitive components such as β -carotene (LIANFU and ZELONG, 2008; ADEKUNTE *et al.*, 2010).

As a result, lycopene content increased with time from 15 min to 30 min at all ranges of ultrasonic power; β -carotene content started to decrease at 90W. This can be explained by sensitivity differences of lycopene and β -carotene in thermal effects. In previous studies, it was shown that lycopene was relatively resistant to thermal degradation compared to other carotenoids such as α -tocopherol and β -carotene (TAYLOR *et al.*, 2000).

4. CONCLUSIONS

In this study, various parameters affecting the COSE and UAE of lycopene and β -carotene were investigated. Maximum lycopene and β -carotene yields were obtained in UAE at 90W for 30 min and 90W for 15 min extraction time, respectively. UAE extraction yields were significantly higher ($p < 0.05$) than COSE for both lycopene and β -carotene yields except when ultrasonic power of 50W was applied. Extracted values of β -carotene obtained from 50W treatments at $15 \pm 5^\circ\text{C}$ after 5 min extraction were similar to the values from COSE at 20°C after 20 min extraction because the effect of heat was still a predominant factor for extraction efficiency of β -carotene at this ultrasonic intensity.

The results indicated that UAE was more effective and requires shorter time than COSE even at lower temperatures. The ultrasound was beneficial for extracting compounds from tomato waste while shortening extraction time and being able to extract heat-sensitive compounds by increasing mass transfer at a lower temperature.

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