The Annals of the University Dunarea de Jos of Galati
Fascicle VI – Food Technology (2017), 41(1), 41-49

ORIGINAL RESEARCH PAPER

IMPROVED CAROTENOID EXTRACTION FROM BULGARIAN TOMATO PEELS USING ULTRASONICATION

TSVETKO PROKOPOV*, MILENA NIKOLOVA, DONKA TANEVA

University of Food Technologies, 26 Maritsa Blvd., 4000 Plovdiv, Bulgaria
*Corresponding author: *tsvetko_prokopov@abv.bg*

Received on 30th September 2016
Revised on 15th December 2016

Ultrasound-assisted extraction (UAE) of carotenoids from tomato peels of two of the most widely used Bulgarian industrial tomato cultivars was investigated in this study. The carotenoid content in raw tomato peels was established by HPLC analysis. The application of UAE was compared to conventional organic solvent extraction, where the carotenoid content of the samples was spectrophotometrically determined. The effects of the extraction time and temperature on the carotenoid content of the extract were studied. It was found that the application of UAE led to 1.5 to 3.0-fold shortening of the extraction time and increase in the carotenoid content compared to the conventional extraction using acetone. The total carotenoid, lycopene and β-carotene contents increased by 27.2±1.1%, 11.9±0.7% and 28.2±0.1% respectively for tomato peels of the Stella cultivar, and by 23.9±2.4%, 15.3±0.8% and 26.5±1.0% respectively for the Karobeta cultivar. The increase in the UAE temperature from 20°C to 40°C resulted in extraction time reduction of up to 5 min and 22.1±0.6 and 24.4±1.2% increase in the lycopene content of the extract from Stella and Karobeta cultivars, respectively. The results of this study clearly demonstrate the advantages of UAE compared to the conventional solvent extraction of tomato carotenoids.

Keywords: tomato, ultrasound, extraction, carotenoid, yield

Introduction

Food processing wastes and by-products represent a major disposal problem for the industry. Nowadays, food wastes are regarded as a source of valuable nutraceuticals (Galanakis, 2012). The commercial processing of tomatoes produces a large amount of waste at various stages. The tomato peeling operation applied in the processing industry generates tomato skin and outer pericarp tissue, thus creating significant environmental problems. Industrial tomato wastes aroused the great interest of researchers and manufacturers concerning carotenoid extraction from this low cost material (Strati and Oreopoulou, 2011).

The carotenoid amount in industrial tomato cultivars depends on different factors such as genotype, agricultural practices, soil, climatic factors, harvesting date, degree

of maturity and post-handling. The reported lycopene amount in tomato peels ranges from 5 to 100 mg/100g (Lenucci *et al*., 2010; Hdider *et al*., 2013; Ilahy *et al*., 2016). It is well known that tomato carotenoids are present in the chromoplast. Lycopene crystals are enclosed into newly synthesized membranes originating by introflections of the inner membrane plastid envelope (Simkin *et al*., 2007). The primary cell wall of tomato is composed mainly of cellulose, hemicelluloses and a large amount of pectic polysaccharides, so the use of cell wall degrading techniques to disrupt this polysaccharide network will facilitate the release of the intracellular contents.

The conventional extraction of tomato carotenoids includes the use of common organic solvents and solvent mixtures (Strati and Oreopoulou, 2014). Improved methods such as enzyme-assisted, microwave-assisted and supercritical CO_2 extractions have been applied recently for decreasing the solvent consumption, shortening the extraction time and increasing the carotenoid extraction yield (Lianfu and Zelong, 2008; Zuorro *et al*., 2011; Lenucci *et al*., 2015). Fewer studies on carotenoids are being conducted via ultrasound-assisted extraction (UAE).

The traditional techniques used for the solvent extraction of natural products are associated with poor extraction efficiency. Ultrasound can be effectively used to improve the extraction rate by increasing the mass transfer rates and the possible cell wall rupture due to the formation of microcavities leading to higher product yields at reduced processing time and solvent consumption. The controlling mechanism of ultrasound-assisted extraction is generally attributed to mechanical, cavitation and thermal effects, which can result in cell wall disruption, particle size reduction and enhanced mass transfer across cell membranes (Vilkhu *et al*., 2008; Shirsah *et al*., 2012). Recently, UAE has been applied to carotenoid extraction from different tomato wastes (Eh and Siang, 2012; Konwarth *et al*., 2012; Kumcuoglu *et al*., 2014; Luengo *et al*., 2014).

The most widely used Bulgarian tomato cultivars in the canning industry are Stella and Karobeta. Tomato peels of the first cultivar are rich in lycopene and β-carotene, whereas those of the second cultivar are rich in β-carotene. The optimal conditions for organic solvent extraction of carotenoids from the peels of these two tomato cultivars were established in our previous study (Nikolova *et al*., 2014).

On the basis of the above considerations, we explored the feasibility of using ultrasound irradiation as a means of improving carotenoid extraction from tomato peels. This study was designed to investigate the ultrasound treatment effects on the carotenoid extraction from peels of the two Bulgarian industrial tomatoes cultivars.

Materials and methods

Raw materials and chemicals

The Bulgarian tomato cultivars Stella and Karobeta were grown under open-field conditions at the Maritsa Vegetable Crop Research Institute, Plovdiv district, Bulgaria. Fresh red-ripe tomatoes were blanched at 95°C for 2 min, cooled in tap water and hand peeled. The tomato peels obtained were subsequently air-dried at 25±1°C, ground in a laboratory mill (Bosh MKM 6003, Germany) and sieved

through a 1.0 mm sieve. The moisture content of the dry ground tomato peels was determined gravimetrically at 105°C and was found to be 4.61±0.21%. The resultant material was kept in glass jars closed with aluminium foil at -20°C in dark condition until the start of the experiments. HPLC grade acetone and methanol, tetrachloromethane (THM), acetonitrile and methyl tert-butyl ether (MTBE) of analytical grade were purchased from Sigma, Germany. Lycopene and β-carotene standards were supplied by Extrasynthese, France.

Conventional organic solvent extraction of carotenoids

The carotenoid extraction was performed in a 250 ml conical glass flask wrapped with aliminium foil. The flask was placed in a temperature-controlled water bath and continuously agitated with a magnetic stirrer (VELP Scientifica, Italy) at 400 rpm. A 1.00 g sample was weighed, placed in the extraction flask and stirred with acetone at a solid/liquid ratio of 1:30 for 5, 10, 15 and 20 min at 20±1°C. The extracts obtained were vacuum filtered through MN640de filter paper and analyzed for carotenoids content determination.

Ultrasound-assisted extraction of carotenoids

The UAE of carotenoids was performed in a 100 ml conical flask wrapped with aluminium foil. A 1.00 g sample of dried tomato peels was weighed and placed in the extraction flask. The extraction was carried out with 30 ml acetone in an ultrasonic bath VWR USC 100TH (45 kHz) for 5, 10, 15 and 20 min at 20 and 40±2°C. The resultant extracts were filtered through MN640de filter paper and analyzed for carotenoids content determination.

Determination of carotenoids content

The total carotenoid, lycopene and β-carotene contents of the extracts were determined spectrophotometrically (UV-Helios Omega, USA) and expressed as mg of extracted carotenoid per 100 g of dry material weight, according to the procedure of Manuelyan (1991).

HPLC carotenoid analysis

Individual carotenoid identification and quantification were carried out according to the procedure described by Nikolova *et al.*, (2014). Briefly, a sample of 0.1 g of dried tomato peels was put in a vessel, protected from light, and mixed with 5 ml of THM/Methanol (3:1 v/v) containing 0.5% BHT as antioxidant. The extraction procedure was performed in an ultrasound bath (VWR, USC 100TH, 45 kHz) for 15 min at 20±2°C. After extraction, 1 ml of 10% sodium chloride solution was added to the sample and mixed by careful shaking. The extract was centrifuged at 5000 U/min (Janetzki Model T23, Poland) for 10 min and the THM phase was separated, passed through a column packed with anhydrous sodium sulphate and collected in a volumetric flask of 5 ml. The 20 µl of the solution was injected for the HPLC analysis. The HPLC system (Waters, Milford, USA) composed of a UV-VIS detector (Waters 2487 Dual), a Waters 1525 binary pump, thermostat (LCO 102) and Suspelco Discovery HS C_{18} column (5 µm, 25 cm x 4.6 mm) was used. Mobile phases of methanol:acetonitrile in ratio 8:2 (A) and MTBE (B) with following gradient elution were used: 95 % (A) and 5 % (B) initially, 95 % (A) and 5 % (B) in

3 min, 80 % (A) and 20 % (B) in 4.5 min, 65 % (A) and 35 % (B) in 10 min, 95 % (A) and 5 % (B) in 15 min. The flow rate was maintained at 1 ml.min^{-1}, the column temperature at 30°C and detection was carried out at 270 nm and 290 nm. The analysis of the chromatographic data was conducted on a Breeze 3.30 (Waters, Milford, USA) software. The determination of major carotenoids in tomato peels was carried out by comparing the retention times and absorption spectra with reference standards. The calibration curves were linear from 5 to 50 μg/ml (r^2 > 0.99). The results were expressed as mg of extracted carotenoid per 100 g of dry material weight.

Statistical analysis

All experiments were run in triplicate. The data were analyzed and presented as mean values with standard deviation. The statistical analysis was conducted using SigmaPlot 11.0 software. Statistical techniques, incl. Lavene's test, ANOVA and Duncan's Multiple Range Test, were applied to determine the significant differences at 95% confidence ($P < 0.05$) level.

Results and discussion

The carotenoid content of tomatoes is influenced by agricultural practices, soil, climatic factors, fruit growth, harvesting date, degree of maturity and post-harvest handling (Lenucci *et al.*, 2010; Hdider *et al.*, 2013). Our results indicated that the main carotenoids contained in tomato peels from the Bulgarian cultivar Stella were β-carotene (293.40±0.42 mg/100g), lycopene (167.90±0.55 mg/100g), and lutein (13.60±0.22 mg/100g), whereas those from the Karobeta tomato cultivar were β-carotene (155.50±0.33 mg/100g), lycopene (14.50±1.26 mg/100g), and lutein (9.30±0.16 mg/100g).

The results of the conventional organic solvent extraction of tomato carotenoids are presented in Table 1.

Table 1. Conventional organic solvent extraction of tomato carotenoids (solid/liquid ratio 1:30, 20°C)

Tomato cultivar	Extraction time, min			
	5	10	15	20
	Lycopene, mg/100g			
Stella	9.06±0.16^{a}	11.68±0.08^{b}	13.59±0.45^{c}	11.94±0.40^{b}
Karobeta	9.36±0.43^{a}	8.85±0.64^{b}	9.82±0.30^{c}	10.70±0.25^{c}
	β-carotene, mg/100g			
Stella	31.07±0.54^{a}	35.55±0.27^{b}	38.94±1.17^{c}	39.70±1.33^{c}
Karobeta	28.48±1.32^{a}	28.53±0.33^{b}	31.73±1.70^{c}	32.58±0.80^{c}
	Total carotenoid, mg/100g			
Stella	43.42±0.37^{a}	50.78±0.37^{b}	56.84±0.21^{c}	55.53±1.86^{c}
Karobeta	40.69±1.88^{a}	40.19±0.33^{a}	44.68±1.50^{b}	46.54±1.13^{b}

The data are means ± standard deviation of three independent replicates.

$^{a-c}$The values with different letters indicate significant differences (P<0.05).

The difference between the content of total carotenoid and the sum of lycopene and β-carotene contents can be explained by the presence of other carotenoids into the extracts in addition to lycopene and β-carotene. The obtained data showed that carotenoids extraction was in increasing trend up to 15 minutes and after that lycopene, β-carotene and total carotenoid contents did not vary significantly. This can be explained by osmotic balance. The osmotic pressure between the inside and the outside of the cell reached equilibrium easily. Carotenoid extraction decreased at this equilibrium point due to a decreased driving force (Kumcuoglu *et al*., 2014). Strati and Oreopoulou (2011) found that the carotenoid extraction was controlled by diffusion phenomena and the extraction rate decreased with the extraction time to reach equilibrium.

The results of tomato carotenoid UAE are presented in Table 2. The data analysis showed that after 5 minutes of extraction using UAE, the carotenoid content increased exponentially. Similarly to conventional solvent extraction, the carotenoid content increased gradually until reaching a maximum at 10 – 15 minutes and finally remained constant or decreased at 20 minutes. The initial sharp increase of the extraction rate in both UAE and conventional solvent extraction was due to the large carotenoid concentration gradient between the solvent and the plant cells at the beginning of the extraction. This gradient decreased with the increase in the extraction time due to the increased mass transfer caused by ultrasonic treatment. Consequently, the extraction of carotenoids from the inside of the cell gradually became more difficult. Furthermore, the solvent saturation was a limiting extraction factor. All of these led to a delay in the extraction rate, which resulted in carotenoid content reduction. A similar trend was observed by other researchers for carotenoid extraction from *Spirulina plantaris* and tomato wastes (Deyb and Radhod, 2013; Luengo *et al*., 2014; Kumcuoglu *et al*., 2014).

Table 2. Ultrasound-assisted extraction of tomato carotenoids (45 kHz, solid/liquid ratio 1:30, 20°C)

Tomato cultivar	Extraction time, min			
	5	10	15	20
	Lycopene, mg/100g			
Stella	11.54±0.44^{a}	13.07±0.01^{b}	13.61±0.48^{b}	12.20±0.70^{b}
Karobeta	10.07±0.34^{a}	10.20±0.45^{b}	11.32±0.58^{c}	10.89±0.37^{c}
	β-carotene, mg/100g			
Stella	39.84±0.44^{a}	37.26±0.68^{b}	36.54±0.59^{b}	34.56±0.44^{c}
Karobeta	34.81±0.70^{a}	36.09±0.68^{b}	34.29±0.19^{a}	34.12±0.15^{a}
	Total carotenoid, mg/100g			
Stella	55.23±0.04^{a}	54.11±0.66^{b}	53.93±0.66^{b}	50.26±0.26^{c}
Karobeta	47.81±0.05^{a}	49.80±1.13^{b}	49.03±0.39^{b}	48.40±0.55^{b}

The data are means ± standard deviation of three independent replicates.

$^{a-c}$The values with different letters indicate significant differences ($P<0.05$).

The data on UAE showed that the maximum lycopene content for the Stella and Karobeta cultivars was reached after 10 and 15 minutes of sonication, whereas the

highest total carotenoid content for the Stella and Karobeta cultivars was achieved after 5 and 10 minutes of sonication. The maximum β-carotene content was obtained after 5 minutes of sonication for the tomato peels of the Stella cultivar and 10 minutes of sonication for those of Karobeta cultivar. The statistical processing of the results showed that apart from the ultrasonic treatment time, the tomato cultivar type also affected the carotenoid extraction. Our results demonstrated that ultrasonication led to shortening of the extraction time by 1.5 to 3.0 times for the achievement of the maximum carotenoid extraction compared to the conventional solvent extraction. After ultrasound treatment for 5 minutes in total carotenoids and β-carotene and for 10 minutes in lycopene extraction from tomato peels of the Stella cultivar, carotenoid contents increased by 27.2±1.1%, 28.2±0.1% and 11.9±0.7% respectively compared to conventional extraction for the same extraction time. The carotenoid content from the peels of the Karobeta cultivar increased by 23.9±2.4% (10 min extraction of total carotenoids), 26.5±1.0% (10 min extraction of β-carotene) and 15.3±0.8% (15 min extraction of lycopene) respectively. The results in Tables 1 and 2 clearly demonstrate the advantage of UAE compared to the conventional solvent extraction of tomato carotenoids.

Another important factor that affected UAE was temperature. The influence of the extraction temperature on the lycopene content during UAE of tomato peels from the Stella and Karobeta cultivars is presented in Figures 1 and 2, respectively. The results showed that at 40°C, the increase in the processing time led to a reduced lycopene content, whereas at 20°C, up to 15 minutes, the opposite effect was observed. The temperature increase from 20°C to 40°C reduced the extraction time from 15 to 5 minutes, when the maximum lycopene content was reached. The lycopene contents increased by 24.4±1.2% and 22.1±0.6% for the Stella and Karobeta tomato cultivars respectively after an increase in the temperature from 20°C to 40°C and ultrasound treatment for 5 minutes.

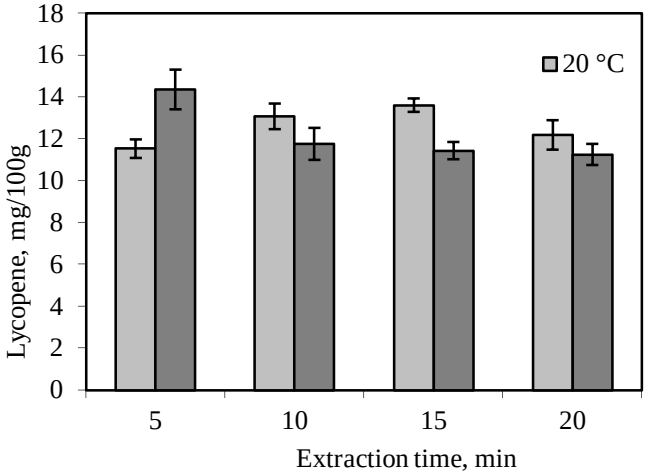

Figure1. Influence of temperature on UAE of lycopene from tomato peels of Stella cultivar (45 kHz, solid/liquid ratio 1:30)

These results are due to the fact that cavitation and thermal effects play an important role in UAE. The cavitation effect consists in the formation of bubbles and voids, whereas the thermal effect acts on the cellular structure and diffusion, thereby enhancing mass transfer from the inside of the cells to the solvent. At low temperatures, the thermal effect is negligible, therefore at the beginning the carotenoid extraction is low, then it gradually increases until it reaches equilibrium. With the increase in temperature, a combined thermal and cavitation effect occurs. On the other hand, the carotenoid solubility and diffusion increase at higher temperatures, which results in higher carotenoid extraction rate at the beginning of UAE compared to the extraction at lower temperatures (Shirsath *et al.*, 2012; Strati and Oreopoulou, 2014).

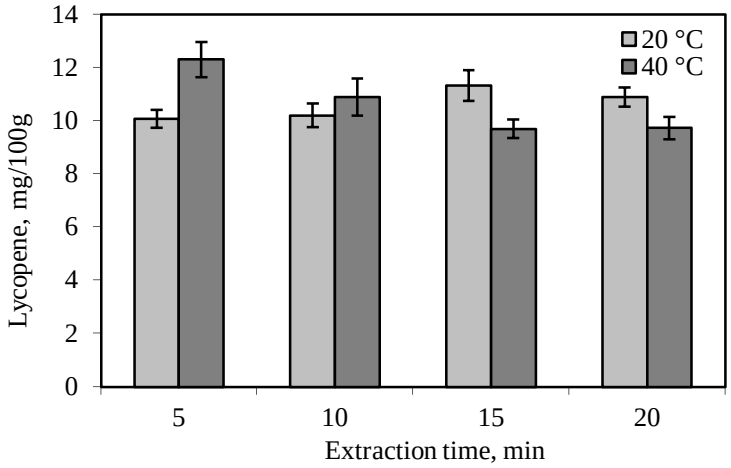

Figure 2. Influence of temperature on UAE of lycopene from tomato peels of Karobeta cultivar (45 kHz, solid/liquid ratio 1:30)

Conclusions

In this study the effects of the ultrasonication on the carotenoid extraction from tomato peels was investigated. The results indicated that UAE led to shorter extraction times and higher carotenoid content compared to the conventional organic solvent extraction. In conventional extraction, maximum carotenoid content was achieved at 15-minute extraction time, whereas in UAE the highest content was reached between 5 and 10 minutes of sonication.

An initial increase in the carotenoid extraction rate was observed in both conventional extraction and UAE. The carotenoid content did not vary significantly with time between 15 and 20 minutes for conventional extraction and 10 and 15 minutes for UAE.

The temperature increase from 20°C to 40°C applied during sonication reduced the extraction time of tomato carotenoid to 5 minutes and increased the lycopene content in the extract by 22.1±0.6% and 24.4±1.2% depending on tomato cultivar.

The results of this study clearly demonstrate that UAE is more effective than the conventional organic solvent extraction of carotenoids from tomato peels of two of the most widely used Bulgarian industrial tomato cultivars.

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