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EFFECT OF TREATMENT PARAMETERS ON THE CAROTENOID EXTRACTION FROM TOMATO PEELS OF BULGARIAN INDUSTRIAL VARIETIES

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Abstract: *Tomato by-products are an attractive source of natural carotenoids. This study was carried out to investigate the extractability of carotenoids from tomato peels of two Bulgarian industrial varieties, named "Stela" and "Karobeta", and to assess the effects of the extraction parameters (time, extraction steps, temperature, solid/liquid ratio and solvent type) on the yield of carotenoids. Individual carotenoid identification in dried tomato peels was carried out by using a HPLC system. The content of total carotenoids, lycopene and β-carotene in the obtained extracts was measured spectrophotometrically. Carotenoid recovery was significantly ($p < 0.05$) affected by the investigated extraction parameters. The maximum amounts of total carotenoids (111.58±2.11 and 76.59±2.13 mg/100g), β-carotene (54.69±2.15 and 64.70±1.42 mg/100g) and lycopene (49.07±0.19 and 6.51±0.57 mg/100g) extracted from dried tomato peels of "Stela" and "Karobeta" varieties, respectively, were obtained by using acetone as solvent, for three successive extraction steps of 30 min, at 40°C and solid/liquid ratio of 1:30. It was experimentally established that tomato peels of "Stela" variety are suitable for lycopene and β-carotene recovery, while those of "Karobeta"variety for the recovery of β-carotene only.*

Keywords: *vegetable, processing, waste, by-products, lycopene, β-carotene, extractability*

1. Introduction

The food processing industry produced large quantity of waste by-products. One-tird of the used product in fruit and vegetable processing was reported that being discarded which creates a significant environmental problem [1].
Today, food wastes including fruit and vegetable by-products are considered as a cheap source of valuable components since the existent technologies allow the recovery of target compounds and their recycling inside food chain as functional additives in different products [2].
For example, tomato is a food product containing large amounts of high added-value compounds such as carotenoids, mainly in the form of lycopene and β-carotene. At present, large quantities of tomato skin and outer pericarp tissue are discarded as a waste product from the peeling operation. About 10-40 % of the total tomato processed for tomato products are as skins and seeds. The quantity of wastes generated during tomato production and processing, combined with

the beneficial characteristics of components of these wastes justifies the great interest of researchers and manufacturers in extracting carotenoids from tomato wastes [3].

By-products of tomato processing are attractive source of valuable bioactive components and colour pigments. These products can be used as functional foods, dietary supplements and can also be applied to cosmetic and pharmaceutical products. Extraction with organic solvents is a well-established method in the food industry. As the main tomato carotenoids are lipid-soluble, common organic solvents such as acetone, hexane, ethanol, ethyl acetate, methanol, petroleum ether and solvent mixtures in different ratios (e.g. 50:50 hexane-ethyl acetate, hexane-acetone, 50:25:25 hexane-acetone-ethanol) have been tested for carotenoids extraction. Although carotenoids extraction yields obtained from tomato by-products and comparison of efficiency among different solvents are presented in the literature [3-8] there are limited data about the effect of treatment parameters on the carotenoids extraction from tomato by-products including the influence of tomato varieties. There are needs for further research concerning optimization of tomato carotenoids extraction and factors affecting carotenoids recovery from tomato by-products which could be applied on a commercial scale.

This study was carried out to investigate the extractability of carotenoids from peels of two Bulgarian industrial tomato varieties and to assess the effects of extraction parameters, such as time, successive extraction steps, temperature, solid/liquid ratio and type of organic solvent on the yield of total carotenoids, lycopene and β-carotene.

2. Materials and methods

2.1. Raw materials

Two Bulgarian industrial tomato varieties named “Stela” and “Karobeta” were used in present study. Raw tomatoes were grown under field conditions within the Maritsa Vegetable Crops Research Institute, Plovdiv, Bulgaria. Tomatoes were harvested from the selected plants in technological maturity for each variety and were transported to the laboratory within 24 h.

2.2. Preparation of tomato peels

Raw tomatoes were blanched at 95^{o}C for 2 min, cooled on tap water and hand peeled. The obtained tomato peels were subsequently air dried at 25±1^{o}C, ground in a laboratory mill (Bosh MKM 6003, Germany) and sieved through a 1.00 mm sieve. Moisture content of ground dry tomato peels was determined by gravimetric method at 105^{o}C and was 4.61±0.21 % and 5.30±0.32 % for “Stela” and “Karobeta” variety, respectively. The obtained dry ground material was kept in glass jars closed with aluminium caps and wrapped with aluminium foil at -20^{o}C until conduction of experiments.

2.3. Chemicals

Acetone, n-hexane, ethanol, methanol, tetraclormethane, acetonitrile and methyl tert-butyl ether (MTBE) in analytical grade were purchased from Sigma (Germany). Used standards of lutein, lycopene and β-carotene were purchased from Extrasynthese (France).

2.4. Carotenoids extraction

The extraction of carotenoids was performed into 250 mL conical glass flask wrapped with aluminium foil. The flask was plased in a temperature-controlled (±1^{o}C) water bath and continuously agitated with magnetic stirrer (VELP

Scientifica Aluminium Hot Plate Stirrer-ARE, Italy) at 400 rpm. Ground dry tomato peels (1.00 g) were plased in the extraction flask and stirred with extraction solvent at different extraction conditions, such as time (5, 10, 15, 30 and 40 min), temperature (20, 30, 40 and 50^{o}C) and tomato peels/solvent ratio (1:5, 1:10, 1:20, 1:30 and 1:50). The obtained extract was vacuum filtered through filter paper MN640de and analysed for carotenoids content.

The effect of extraction steps was examined as follows: Successive extractions with acetone were conducted under the same conditions as above for 30 min each at 20^{o}C and peels/solvent ratio 1:10. At the end of the first extraction step, the mixture was vacuum filtered, the residue was collected, put again in the flask and re-extracted with another quantity of acetone. The whole procedure was repeated four times to complete the successive extraction steps. The carotenoids content of the extract in each successive extraction were determined.

When the effect of type of organic solvent was studied the obtained extracts were vacuum filtered, the liquid fraction was vacuume dried (t < 40^{o}C), redisolved in acetone and analysed for carotenoids content.

2.5. Determination of carotenoids content

Total carotenoids, lycopene and β-carotene contents in the extracts were measured spectrophotometrically (UV-VIS Helios Omega Spectrophotometer, Thermo Fisher Scientific, Madison, WI, USA) at λ_{max} 448 and 472 nm against acetone as blank, according to Manuelyan [9]. Carotenoids content was expressed as mg/100g dry weight.

2.6. Carotenoids analysis

For the identification of individual carotenoids dryed tomato peels were analysed by HPLC system (Waters, Milford, USA) composed of a UV-VIS deterctor (Waters 2487 Dual λ), a Waters 1525 binary pump and thermostat (LCO 102), according to the method proposed by [10]. The HPLC system was equipped with a (Supelco Discovery HS) C_{18} column (25 cm x 4.6 mm, 5 μm particle). A 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^{o}C and detection was carried out at 270 nm and 290 nm. The analysis of the chromatographic data was carried out on a Breeze 3.30 (Waters, Milford, USA) software.

The identification of major carotenoids in tomato dry peels was carried out by comparing the retention times and absorption spectra with reference standards as described by [10]. The calibration curves were linear from 5 to 50 μg/mL ($r^2 > 0.99$).

The percentage of HPLC identified carotenoids in dried tomato peels was calculated as the ratio of the concentration of each carotenoid (based on the respective standard curve) to the sum of all identified carotenoids in the chromatogram, multiplied by 100 [4].

2.7. Statistical analysis

All experiments were run in triplicate. The data were analysed and presented as mean values with standard deviation. Statistical analysis was conducted by using of Statgraphics Centurion XVI Version 16.2.04 software (Statpoint Technologies Inc., USA). The analysis of variance technique, incl. Lavene's test, ANOVA, and Duncan's Multiple Range Test were

used to determine significant differences at 95 % confidence ($p < 0.05$) level.

3. Results and discussion

3.1. Carotenoids identification

HPLC analysis of dried tomato peels of the two investigated varieties indicated that the main carotenoid was β-carotene, while lycopene and lutein are also presented (Table 1). This is contrary to the results obtained of [3], who reported lycopene as the main carotenoid of processing tomato waste composed of skins and seeds. Tomato peels of "Stela" variety were riched in β-carotene (61.8 %) and lycopene (35.3 %), while these of "Karobeta" variety were riched in β-carotene (86.7 %).

Table 1

Carotenoids concentration (%) in dried tomato peels

Tomato varieties	Carotenoids concentration (%)		
	Lycopene	β-carotene	Lutein
Stela	35.34	61.78	2.86
Karobeta	8.09	86.72	5.17

3.2. Effect of extraction time

The effect of time on carotenoids extraction from dried tomato peels was investigated by conducting experiments during the extraction with acetone in solid:liquid ratio 1:10 at 20°C.

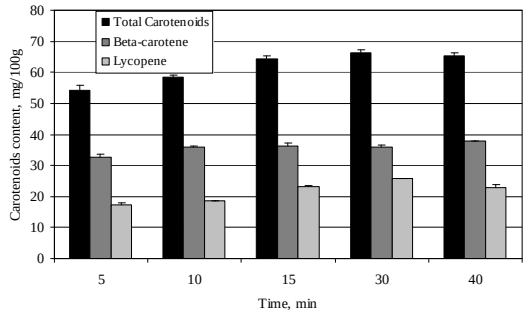

Fig.1. Effect of time on carotenoids extraction (peels/acetone 1:10, t = 20°C) from dried tomato peels of "Stela" variety

The obtained results are shown in Figure 1 and Figure 2 for "Stela" and "Karobeta" variety, respectively.

As it was observed, carotenoids content of the extracts depended on extraction time, showing a high intial rate of extraction that decreased with time until an almost equilibrium was riched. The experimental results indicated the suitable extraction time of 30 min for total carotenoids, lycopene and β-carotene extraction from peels of the two tomato varieties. This is in accordance with the results obtained by another research [3].

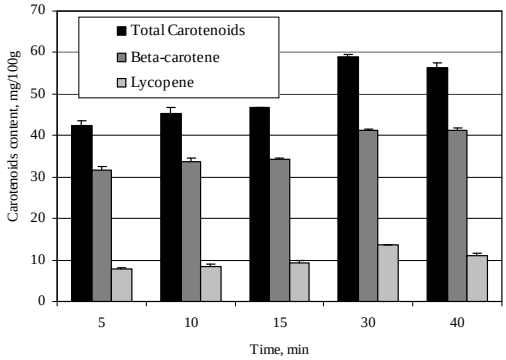

Fig.2. Effect of time on carotenoids extraction (peels/acetone 1:10, t = 20°C) from dried tomato peels of "Karobeta" variety

3.3. Effect of extraction steps

The effect of successive extraction steps on carotenoid extraction is illustrated in Table 2. It is clearly indicated that the total carotenoids, lycopene and β-carotene contents in the obtained extracts were significantly ($p < 0.05$) affected by the number of extractions. As it was observed, no significant differences ($p < 0.05$) or in some cases decreasing was obtained for the carotenoids contents between third and fourth extraction steps. Triple extraction was found as adequate for all experiments.

3.4. Effect of temperature

The carotenoids content obtained by three successive extraction of 30 min each with acetone in solid/liquid ratio 1:10 at

temperatures ranging from 20 to 50°C is presented in Table 3. Limiting factor for the choise of extraction temperature was the boiling point of acetone (56°C) and the need to avoid isomerisation and/or oxidation of carotenoids. The increase in extraction temperature generally increased the extractability of carotenoids. The contents of total carotenoids, lycopene and β-carotene in the obtained extracts were significantly ($p < 0.05$) higher at 40°C than at 20°C. However, when the extraction temperature rose to 50°C, an important extractability of carotenoids was not noticed. Finally, at 50°C decreasing of the carotenoids content was oserved, excluding the content of β-carotene in the extract from tomato peels of "Karobeta" variety. Following the obtained results, a suitable extraction temperature of 40°C was chosen for adequate extraction of carotenoids.

3.5. Effect of solid/liquid ratio

Solid/liquid ratio is another factor which affects the extraction of carotenoids. An equilibrium between the use of high and low solid/liquid ratios, involving a balance between high costs and solvent wastes, on the one side, and avoidance of insufficient mixing and saturation effects, on the other side, has to be found to optimized value [5].

Table 2

Carotenoids content (mg/100g) in extracts from dried tomato peels (peels/acetone 1:10, 30 min extraction, t = 20°C) depending of successive extraction steps

Parameter	Tomato variety	Extraction steps			
		1	2	3	4
Total carotenoids, mg/100g	Stela	31.84±0.09^{a}	44.45±1.05^{b}	54.17±1.00^{c}	54.58±0.82^{c}
	Karobeta	12.05± 0.07^{a}	14.78±0.43^{b}	23.92±1.78^{c}	20.38±1.26^{d}
Lycopene, mg/100g	Stela	6.03±0.34^{a}	10.59±0.30^{b}	12.62±0.44^{c}	12.71±0.35^{c}
	Karobeta	0.61±0.06^{a}	0.66±0.03^{a}	1.62±0.07^{b}	1.44±0.21^{b}
β-carotene, mg/100g	Stela	24.07±0.81^{a}	34.16±1.02^{b}	41.44±0.57^{c}	42.11±1.52^{c}
	Karobeta	10.80±0.23^{a}	13.96±0.27^{b}	22.27±1.71^{c}	18.94±1.05^{d}

The values are mean of three replicates ± SD. Values bearing different lowercase letters (a, b, c, d) in the same row differ significantly ($p < 0.05$).

Table 3

Carotenoids content (mg/100g) in extracts from dried tomato peels (peels/acetone 1:10, triple extraction of 30 min) depending of temperature

Parameter	Tomato variety	Temperature of extraction, °C			
		20	30	40	50
Total carotenoids, mg/100g	Stela	10.17±1.38^{a}	23.62±1.65^{b}	31.16±1.21^{c}	26.92±1.10^{d}
	Karobeta	19.28±0.28^{a}	21.45±0.25^{b}	23.35±0.55^{c}	23.59±0.32^{c}
Lycopene, mg/100g	Stela	1.36±0.31^{a}	3.54±0.37 ^{b}	5.29±1.08^{c}	3.36±0.28^{b}
	Karobeta	1.59±0.07^{a}	2.79±0.36^{b}	3.61±0.03^{c}	1.33±0.03^{a}
β-carotene, mg/100g	Stela	8.08±0.97^{a}	18.41±1.17^{b}	27.4±1.10^{c}	21.66±0.75^{b}
	Karobeta	16.34±0.19^{a}	17.15±0.12^{b}	18.21±0.43^{c}	20.39±0.53^{d}

The values are mean of three replicates ± SD. Values bearing different lowercase letters (a, b, c, d) in the same row differ significantly ($p < 0.05$).

Table 4 presents the effect of solid/liquid ratio on carotenoids extractability. The increased organic solvent volume increased the carotenoids extractability up to solid/liquid ratio of 1:30, than at ratio 1:50 it decreased. The solid/liquid ratio of 1:30 produced significantly ($p < 0.05$) higher contents of total carotenoids, β-carotene and lycopene in all samples in comparison with the other ratios.

Table 4

Carotenoids content (mg/100g) in extracts from dried tomato peels (triple extraction of 30 min with acetone, t = 20°C) depending of solid/liquid ratio

Parameter	Tomato variety	Solid/liquid ratio				
		1:5	1:10	1:20	1:30	1:50
Total carotenoids, mg/100g	Stela	42.13±0.25^{a}	59.92±0.21^{b}	84.41±0.19^{c}	98.26±0.67^{d}	58.16±5.54^{b}
	Karobeta	26.40±0.46^{a}	27.02±0.18^{a}	29.26±0.30^{b}	64.08±0.25^{c}	27.17±0.32^{a}
Lycopene, mg/100g	Stela	6.92±0.18^{a}	10.75±0.55^{b}	13.65±0.64^{c}	16.94±1.01^{d}	12.50±1.19^{c}
	Karobeta	0.17±0.04^{a}	1.63±0.26^{b}	2.01±0.12^{c}	3.9±0.12^{d}	1.09±0.16^{e}
β-carotene, mg/100g	Stela	32.30±0.79^{a}	41.65±2.57^{b}	64.25±0.29^{c}	73.98±1.89^{d}	44.87±0.6^{b}
	Karobeta	24.81±0.18^{a}	23.63±0.34^{b}	25.21±0.22^{a}	56.06±1.40^{c}	23.88±0.98^{b}

The values are mean of three replicates ± SD. Values bearing different lowercase letters (a, b, c, d, e) in the same row differ significantly ($p < 0.05$).

3.6. Effect of extraction solvent

Solvent selection is usually considered as the most important factor [5]. The contents of total carotenoids, lycopene and β-carotene in the extracts obtained by three successive extractions of 30 min with two different solvents and one solvents mixture at 40°C and solid/liquid ratio of 1:30 are presented in Table 5. The extraction efficiency was affected by the solvent type and its polarity. As indicated in Table 5, acetone presented significant ($p < 0.05$) higher content of carotenoids extracted from tomato peels of "Karobeta" variety, compared to the hexane and hexane/acetone/ethanol mixture, possibly due to better penetration of the acetone to plant cells where carotenoids are enclosed. Concerning "Stela" variety, no significant differences ($p > 0.05$) was observed for carotenoids content extracted from tomato peels using acetone and hexane, respectively.

Table 5

Carotenoids content (mg/100g) in extracts from dried tomato peels (solid/liquid ratio 1:30, triple extraction of 30 min, t = 40°C) depending of used organic solvent

Parameter	Tomato variety	Type of solvent		
		Acetone	Hexane	Hexane/Acetone/ Ethanol 50/25/25
Total carotenoids, mg/100g	Stela	111.58±2.11^{a}	108.42±1.06^{a}	96.83±2.11^{b}
	Karobeta	76.59±2.13^{a}	69.14±1.07^{b}	65.95±0.01^{c}
Lycopene, mg/100g	Stela	49.07±0.19^{a}	49.05±0.21^{a}	41.18±2.35^{b}
	Karobeta	6.51±0.57^{a}	4.66±0.25^{b}	5.45±2.46$^{a\,b}$
β-carotene, mg/100g	Stela	54.69±2.15^{a}	51.78±0.77^{a}	48.86±0.39^{b}
	Karobeta	64.70±1.42^{a}	59.63±0.75^{b}	55.39±1.98^{c}

The values are mean of three replicates ± SD. Values bearing different lowercase letters (a, b, c) in the same row differ significantly ($p < 0.05$).

From the experimental results presented in Table 5 can be concluded that extraction of total carotenoids, lycopene and β-carotene from tomato peels of "Stela" and "Karobeta" varieties with acetone is adequate.

4. Conclusion

The extractability of carotenoids from dried peels of two Bulgarian industrial tomato varieties, named "Stela" and "Karobeta", was investigated. The effects of extraction time, extraction steps, temperature, solid/liquid ratio, two organic solvents and one solvents mixture on the contents of total carotenoids, lycopene and β-carotene in the obtained extracts were established. The maximum amounts of total carotenoids, lycopene and β-carotene extracted from tomato peels of the investigated tomato varieties were obtained in our experiments using acetone for three successive extractions of 30 min, at 40^{o}C and solid/liquid ratio of 1:30. Carotenoids contents in the extracts obtained at these optimal extraction conditions, for "Stela" and "Karobeta" varieties, respectively were as follow: total carotenoids of 111.58±2.11 and 76.59±2.13 mg/100g, β-carotene of 54.69±2.15 and 64.70±1.42 mg/100g and lycopene of 49.07±0.19 and 6.51±0.57 mg/100g. Experimentally was established that tomato peels of "Stela" variety are suitable for lycopene and β-carotene recovery, whiles these of "Karobeta"variety for the recovery only of β-carotene.

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