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Obtaining and characterization of some peach (*Persica vulgaris*) carotenoidic extracts

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Abstract

The aim of this work is to obtain some carotenoidic extracts from peach (*Persica vulgaris*) pulp and pericarp, Red haven variety, and to determine by reverse phase- high pression liquid chromatography (HPLC) the content of β-carotene and total carotenoids. Also, were determined by atomic absorption spectrometry, 12 mineral elements (K, Na, Ca, Mg, Fe, Mn, Cu, Zn, Pb, Co, Cr, Ni) of raw materials and carotenoidic extracts obtained. The results showed that the peach pericarp are richer in β-carotene and total carotenoids than peach pulp. All macroelements from carotenoidic extracts are found in much lower concentrations than in raw materials. Concentrations of Na, Ca, Mg, Zn and Pb in the peach pulp and in the peach pulp extract are lower than the concentrations of these elements in the peach pericarp, respectively in the peach pericarp carotenoidic extract. Fe, Cu, Cr and Ni, however, are better represented in the peach pulp, respectively in the carotenoidic extract from it. Mn and Co are not present either in raw materials and in carotenoidic extracts.

Keywords: carotenoids, extracts, mineral elements, RP-HPLC, atomic absorption spectrometry, peach

1. Introduction

Peach (*Persica vulgaris*), *Rosaceae* familly, is a fruit tree, being the third fruit-growing species, after apple and plum, as economic importance. The peach was brought to India and Western Asia in ancient times. Alexander the Great introduced the fruit into Europe after he conquered the Persians. Then it was brought to the Americas by Spanish explorers in the 16th century [1].

Fresh fruits contain: water (85-89%), proteins(0,7%), sugars (5,0-12,9%), organic acids(0,3-1,4%), vitamins: A, B1, B2, B3, B5, B6, B9, C and E., carotenoids, minerals (calcium, chlorine, copper, iron, phosphorus, iodine, magnesium, manganese, sodium, potassium, sulfur, zinc), essential oil containing: linalool, esters, acetic acid, caprylic, aldehydes [1].

Carotenoids are responsible for the pleasing yellow, orange or red color of many foods. Whereas the carotenoids are ubiquitous in nature, they are an interdisciplinary research topic in chemistry, biochemistry, biology, medicine, physics and other branches of science [2,3]. The role of some of these compounds as provitamine A precursors has been known for years. Other beneficial effects to human health have been more recently atributed to carotenoids, such as enhancement of the immune response and reduction of the risk of degenerative diseases such as cancer, cardiovascular diseases, cataract and macular degeneration, these actions not

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being restricted to the provitamins A. Thus, the consumption of carotenoid-rich foods is widely recommended [2,4-7].

The principal carotenoids of foods are β-carotene, β-cryptoxanthin, lycopene, lutein and violaxanthin. Except for violaxanthin, these are also the principal carotenoids found in the human plasma, and together with zeaxanthin, are the carotenoids most studied in terms of human health [2]. In food industry the carotenoidic extracts are used some food products additivation, having role of antioxidant substances and natural colorants [8,9]. Also, carotenoids extracts are routinely used in the pharmaceutical industry (as an ingredient of various chemotherapeutic products) and cosmetics [10,11].

As showed above, in the purified carotenoidic extracts are keep a part of the mineral elements contained in the raw material, this being captured, some to a greater extent, to a lesser extent other, by the carotenoids macromolecules [12], this being important for the various possible uses of these extracts.

The purpose of this paper is to get some carotenoidic extracts of peach pulp and pericarp and to characterize them in terms of β-carotene and total carotenoids content (using RP-HPLC method), as well as concentrations of various mineral elements (K, Na, Ca, Mg, Fe, Mn, Cu, Zn, Pb, Co, Cr, Ni).

2. Materials and methods

2.1. Carotenoids extraction. As raw material was used peach pericarp and pulp (*Red Haven* variety), purchased from local market. The fruits of this variety are of medium size, with red pericarp and yellow, firmly pulp.

Reagents used for carotenoids extraction were: ethanol (extraction solvent, p.a., 96%, Chimopar, Bucharest), petroleum ether (extraction solvent, p.a., 40-67 ˚C, Chimopar, Bucharest), acetone (extraction solvent, p.a., Chimopar, Bucharest), BHT (t-butylated hydroxytoluene to prevent oxidative degradation of carotenoids, p.a., Merck), potassium hydroxide (for extracts saponification, p.a., Merck), sodium chloride (p.a., Chimopar, Bucharest), anhydrous sodium sulphate (to remove traces of water from extracts, p.a., Chimopar, Bucharest). Final purified extracts were taken and dissolved in petroleum ether from Merck company**.**

Washed peach fruit pericarp was removed with a knife and was weighing a certain amount of pericarp and pulp respectively (table 1.) which was then triturated with a little quartz sand and acetone in a porcelain mortar. Well triturated plant material was introduced into a 1000 ml flask with glass stopper and flat bottom, further subjecting to the extraction with a mixture petroleum ether: acetone: ethanol 96% (6:2:2) in wich was added BHT (0,1% from raw material) to prevent carotenoids oxidation [13]. Extraction was repeated several times with a new solvent mixture until it remained colorless. After each addition of the mixed solvent, the flask was vigorously shaken about. 5 minutes. The extracts were then subjected to combined carotenoid concentration under vacuum at 35 ˚C in a rotary evaporator (model RV-05, basic 1-B, Shimadzu Japan), to a lower volume (15-20 ml).

►*Sterols removal from the extract.*Primary extract obtained was treated with 40 ml petroleum ether and left overnight (16 hours) at a temperature of -10 ˚C [2].Precipitated sterols were removed by centrifugation for 10 minutes at 2000 rpm (centrifuge model Universal 32 R, Hettich, Germany). The supernatant was then concentrated under vacuum rotary evaporator at 35 ˚ C to a lower volume (10-15 ml).

►*Extract saponification.*After removal of sterols, the extract was saponified to remove lipids and esters, by treatment with 40 ml of 20% potassium hydroxide alcoholic solution and leaving overnight (16 hours) at room temperature under nitrogen and in the dark [2,3]. Carotenoids were then reextrase with petroleum ether in a 500 ml separating funnel, washed several times with a saturated solution of sodium chloride and then with distilled water until complete removal of soap and alkali. Combined ether extracts were passed over anhydrous sodium sulphate to remove traces of water and then concentrated under vacuum at 35 ˚ C in rotary evaporator until complete removal of solvent. Carotenoids obtained were redissolved in a volume of petroleum ether and kept in brown bottles at -20 ˚ C, under nitrogen, to be then subjected to analysis. *Note*: to prevent photochemical degradation of carotenoids, all operations were conducted in rooms with low light at room temperature.

Were carried out 3 parallel tests from the same quantity of raw material and working under the same conditions.

Table 1. shows the quantities of raw materials and purified carotenoidic extracts from peach peel and pulp.

Table 1.The quantities of raw materials and carotenoid extracts from peach peel and pulp (*Persica vulgaris*) Red Haven variety

Nr. crt.	Sample	$m_{raw\ material}$ (g)	$m_{pure\ extract}$ (g) (average)
1.	Peach pulp	300,00	0,2001
2.	Peach pericarp	100,00	0,1852

*2.2. RP-HPLC analysis of the extracts.*In order to determine the concentration of β-carotene and total carotenoids in the samples, all carotenoid extracts were analyzed by reversed phase- high performance liquid chromatography (RP-HPLC). For this was used an Agilent 1100 HPLC chromatograph (Agilent, USA) with a Zorbax SB-C18 column with dimensions: 250 x 4.6 mm, 5µm diameter particles. As eluent we used a mixture of acetonitrile: methanol (20:80), both reagents from company Merck&Co., Inc, New Jersey. We worked at an eluent flow of 1 ml / min, column temperature 30 ° C and wavelength of 450 nm. Samples were injected and 20 µl and for determination of β-carotene concentration in the samples we used a calibration curve (figure 1.) obtained with standard β-carotene of >97% puriry, from Sigma Chemical Company.

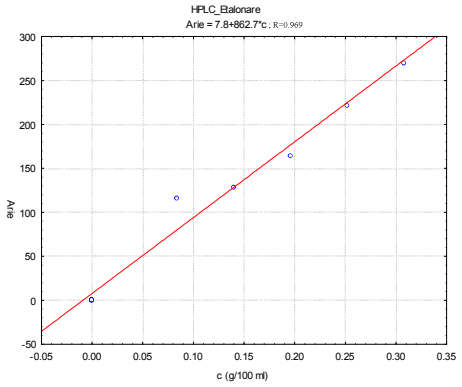

Figure 1. Calibration curve for standard β-carotene

2.3. Analysis of the mineral elemants content of raw materials and carotenoidic extracts

The method is based on measuring, by atomic absorption spectrometry, of the mineral elements concentration in the acid extract obtained from ash sample of plant product. The analytical process includes two steps: dry mineralization and determination, by atomic absorption spectrometry, respectively by flame emission spectrometry (in the case of Na and K elements) [14,15].

Organic substance from plant material or extract is oxidized by oxygen in the air in a furnace heated progressively to 500 ^{0}C temperature, where is maintained for 6-8 hours. The method used was that described in STAS 5954/1-86, adapted to the specific plant products analysis.

In porcelain capsules, previously well cleaned and brought to constant mass by drying at 105 ° C, were weighed approximately 1,000 g of raw material, respectively 0.200 g carotenoid extract. Sample capsules are placed in an oven at 50-60 ° C where they remain about 8 hours. It then raises the temperature at 105 °C and held for 5-6 hours. After this period of time capsules are removed from the oven and place samples in a furnace termoreglabil, at cold. Raise the temperature gradually to 200-250 °C and held until complete carbonization. It then raises the oven temperature to 500 °C and ash for 6-8 hours until a white ash. The ash samples which showed black dots, representing traces of organic matter, were treated with 1 ml of concentrated nitric acid, dried in a sand bath and then were calcined at 500 °C still 2 hours. After cooling, the ash is treated with 0.5 ml double distilled water and 1 ml 6N hydrochloric acid and evaporate to dryness on a sand bath, the operation is repeated twice. Thereafter, the residue is dissolved in 5 ml portions of 0.5 N HCl, passing quantitatively into a 50 ml flask, which is brought to volume with 0.5 N HCl. Finally, the content is filtered in a perfectly dry flask through a dry filter paper and free of heavy metals. In parallel, prepare a blank sample for check.

Mineral elements are dosed from the hydrochloride solution by spraying in the air-acetylene flame and measuring the absorbance, emission respectively, at a wavelength characteristic of each analyzed element. For calibration device, prepare sets of standards, of different concentration, in HCl 0,5 N solution. It was used an atomic absorption spectrometer monofascicul Varian Spectra AA 110 type (Mulgrave, Australia), PC controlled.

For each sample were determined following mineral elements: Na, K, Ca, Mg, Fe, Mn, Cu, Zn, Pb, Co, Cr, Ni.

The final content of mineral elements are calculated using the relationship:

C (mg/kg or ppm) = a x f /m

where:
f – dilution factor,
a – machine readable element content in mg/l,
m – sample weight.

Preparation of calibration solutions. Before each series of determinations- were made, from concentrated standard solution of the determined element, five sets of calibration solutions, to covering the range of concentration which follow to be determined. Calibration solutions were prepared simultaneously with spectrophotometric determinations.

Measurements. Except for samples extraction in flame phase, done manually, the remaining operations are controlled by PC.

3. Results and discussions

3.1. β-Carotene and total carotenoids content of samples. The results obtained on the carotenoid content of the extracts are presented in tables 2 and 3 and RP-HPLC chromatograms of each carotenoidic extract are presented in figures 2 and 3.

Table 2. β-Carotene and total carotenoids content in peach pulp, Red Haven variety

Peak No.	Retention time (min)	Area (mAU)	A%	Compounds	Concentration (µg/g)
3	3,852	131,00	5,50	β-Caroten	11,40
Total carotenoids					207,38

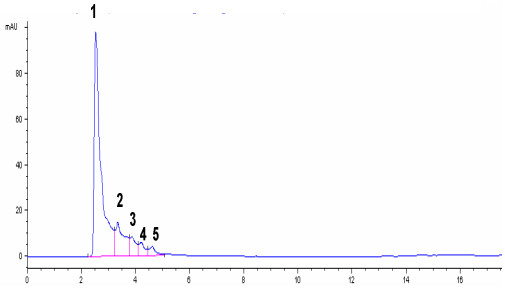

Figure 2. RP_HPLC chromatogram for peach (*Persica vulgaris*) pulp carotenoidic extract

It could be observed that peach pulp, *Red Haven* variety, presents an high content of total carotenoids (207,38 µg/g), in which β-carotene is in proportion of 5,5% (11,40 µg/g). The obtained values are higher than that of literature data relating to other varieties of peaches [2,16].

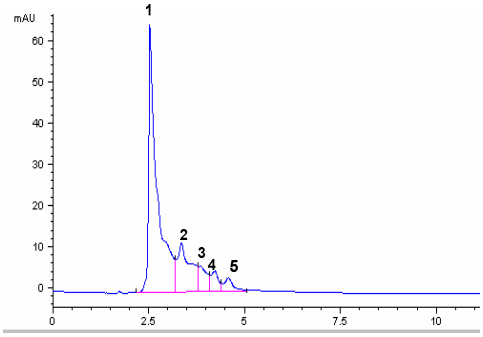

Figure 3. RP_HPLC chromatogram for peach (*Persica vulgaris*) pericarp carotenoidic extract

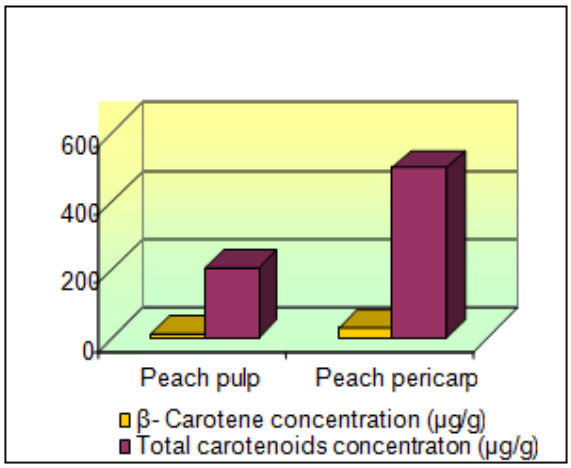

Figure 4. The content of β-carotene and total carotenoids from peach pulp and pericarp, Red Haven variety

3.2. Mineral elements content of samples. The results obtained on the mineral elements content of raw materials and carotenoidic extracts, are presented in table 4, figures 5 and 6.

Table 4. Mineral elements content in the raw materials and in carotenoidic extracts

Sample	Peach pericarp	Peach pericarp extract	Peach pulp	Peach pulp extract
Code	PP	ExPP	MP	ExMP
K	3472,8	1465,2	3490	1423
Na	472	45,8	343,9	31,2
Ca	1033,8	125,4	586,5	10,2
Mg	886,75	132,3	354,79	12,23
Fe	71,5	35,4	28,33	81
Mn	0	0	0	0
Cu	0,096	0,028	0,178	0,103
Zn	2,26	0,984	1,49	0,82
Pb	0,29	0,248	0,27	0,23
Co	0	0	0	0
Cr	0,067	0,008	0,261	0,013
Ni	0,167	0,098	0,184	0,1

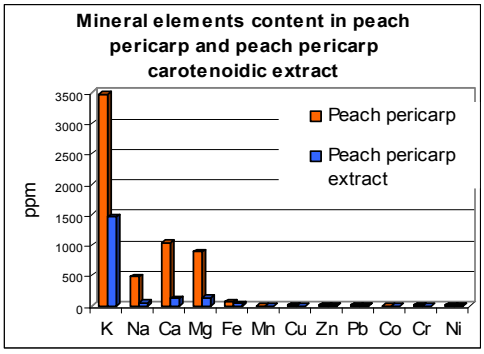

Figure 5. Graphical representation of mineral elements content (ppm) from peach pericarp and peach pericarp carotenoidic extract

Although the same carotenoids were identified in the pulp and in the Red Haven peach pericarp, the pericarp has a much higher content of total carotenoids (500.05 µg / g). β-Carotene is in proportion of 6,14 % (30,70 µg/g)- figure 4.

Analyzing the data obtained shows that the pericarp of the peach has a mineral elements content much higher than the carotenoid extract made from this, the macro-and microelements are largely away during the extraction process. Şi In this case, the best represented macroelement, both in raw material and the extract is potassium (3472.80 ppm - in peach pericarp, 1465.00 ppm respectively - in the peach pericarp extract). After potassium, the most abundant elements in the peach pericarp and in the carotenoid extract from it are calcium (in the pericarp 1033.80 ppm, 125.40 ppm respectively - in the extract) and magnesium (886.75 ppm - the pericarp, respectively 132,30 ppm - in the extract). Heavy metals are found in lower concentrations in the peach pericarp than the maximum limits prescribed by law [17], and the presence of manganese and cobalt has not been identified.

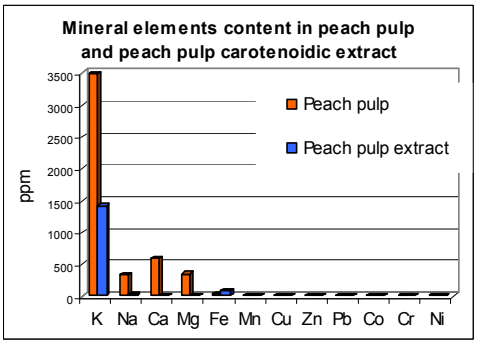

Figure 6. Graphical representation of mineral elements content (ppm) from peach pulp and peach pulp carotenoidic extract

The data for minerals in the peach pulp and peach pulp carotenoidic extract reveals that the extract is much poorer in all mineral elements, compared with the raw material, quantitatively predominant element, and in this case, both in pulp and in extract is also potassium. The quantities of potassium in peach pulp (3490.00 ppm) and carotenoid extract obtained from this (1423.00 ppm) are very close to concentration of this element in the pericarp, respectively pericarp crotenoidic extract. Concentrations of Na, Ca, Mg, Zn and Pb in the peach pulp and in the peach pulp extract are lower than the concentrations of these elements in the peach pericarp, respectively in the peach pericarp carotenoidic extract. Iron, copper, chromium and nickel, however, are better represented in the peach pulp, respectively in the carotenoidic extract from it, than in the peach pericarp and in the pericarp carotenoidic extract.

4. Conclusions

From analysis of this experimental researches results the following conclusions can be drawn:

- At the peach fruit (*Persica vulgaris*), *Red Haven* variety is noted that the pericarp is more rich in total carotenoids (500.05 mg/g fresh material) and β-carotene (30.70 mg /g fresh material) than pulp (207.00 mg / g fresh material, respectively 11.40 mg /g fresh material). The obtained values are higher than those from literature data, relating to other varieties of peaches.
- All macroelements from raw materials, are at levels that fall within the limits of literature. Macroelements from the carotenoidic extracts are at much lower concentrations than in raw materials, they largely expunged in time of the extraction process.
- Potassium is the best represented mineral elementin all samples, both in raw materials and in extracts.
- Heavy metals are found in lower concentrations in the peach pericarp and peach pulp than the maximum limits prescribed by law, and the presence of manganese and cobalt has not been identified.
- The Na, Ca, Mg, Zn and Pb content in the peach pulp and in the peach pulp extract are lower than in the peach pericarp, respectively in the peach pericarp carotenoidic extract.

Concentration of Fe, Cu, Cr and Ni, higher in the peach pulp, respectively in the carotenoidic extract from it, than in the peach pericarp and in the pericarp carotenoidic extract.

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