Phenolic compounds and carotenoids in pumpkin fruit and related traditional products

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Abstract

Pumpkin fruit is used in a diet since ancient times especially in rural communities. The major contributory factors of nutritional and medicinal value of pumpkins are carotenoids, polysaccharides, vitamins, minerals, and phenolic compounds. Due to a very large fruit that it is not easy to consume a whole as well as short shelf-life of fresh-cut pumpkin, different ways of conserving and processing are performed. In our study, total carotenoids, total phenolics and individual phenolics in fresh pumpkin and pumpkin traditional products such as sweet in wine, jam and juice, which are typical for northern parts of Serbia, were studied. Total carotenoids ranged from 27.6 µg/g of pumpkin sweet in wine to86.3 µg/g of fresh fruit, while the amount of total phenolics varied between 93.0 µg GAE/g of pumpkin juice and 905.9 µg GAE/g of fresh fruit. Eight phenolic compounds were identified in the investigated samples and among them phenolic acids dominated. Among flavonoids, flavanon glycoside hesperidin was detected.

Keywords: *Cucurbita maxima*, jam, juice, sweet, phenols, carotenoids, LC/MS.

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Pumpkin (*Cucurbita pepo*, *C. moschata*, *C. maxima* and *C. mixta*) is one of the vegetables used in healthy diets as well as in traditional medicine in many countries. Since ancient times, it has been essential in the diet of rural communities. Nowadays, it is cultivated both, for fruit and seeds that are used in a variety of ways. Fruit is regarded by consumers due to its sweet and mild taste as well as high nutritive value [1]. Also, pumpkin flesh is widely used as a component in a variety of products for children and adults [2]. Pumpkin seeds have also high nutritional and medicinal value. Cold pressed pumpkin seed oils are rich source of phytosterols, tocopherols and squalene [3].

The major contributory factors of nutritional and medicinal value of pumpkin fruit are high total content of carotenoids with >80% of β-carotene [4,5] as well as presence of pectin and non-pectin polysaccharides, minerals (potassium, phosphorus, magnesium, iron, and selenium), vitamins (C, E, K, thiamine (B1) and riboflavin (B2), piridoxine (B6)), dietary fiber, phenolic compounds (flavonoids, phenolic acids) and other substances beneficial to human health [1,6–8].

Due to such diverse chemical composition, a lot of biological activities are attributed to pumpkin. Jin *et al.* [9] showed that a pumpkin-rich diet could reduce blood glucose. Similar result was obtained by Zhao *et al*. [10] who showed increased levels of serum insulin, reduced blood glucose levels and improvement of glucose tolerance in mice by pumpkin polysaccharides. Also, hypocholesterolemic, antibacterial, antiinflammatory and antitumor activities were reported [11].

One of the problems with consuming pumpkin is that the fruits are big and it is not easy to consume a whole pumpkin in a day even by a whole family. On the other hand, shelf-life of fresh-cut pumpkin is very short, and it tends to deteriorate during storage. So, different ways of conserving and processing of pumpkin are reported among them processing into jams, puree, juice pickles and dried products and it is also used as a base for soups and desserts [12–14]. There are a few works about engineering of the pumpkin processing [15,16], but the results on the effects of processing on pumpkin phenolic compounds are limited [17].

The aim of this study was the analysis of total carotenoids, total phenolics and individual phenolic compounds in traditional products of pumpkin such as sweet in wine, jam and juice, which are typical for northern parts of Serbia, together with analyses in fresh pumpkin fruit. LC/MS analysis was used for qualitative analysis of individual phenolic compounds.

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EXPERIMENTAL

Material

Traditional pumpkin products (sweet in wine, jam and juice) were obtained from household, family Stojanovic, Vojvodina, Serbia. These products are unique in wider area and they are prepared according to the old, traditional recipes and technology that has been handed down in the family for generations. Local variety of pumpkin (*Cucurbita maxima* L., Cucurbitaceae), which is grown in the household was used for products preparation. The pumpkins were washed with potable water, peeled and cut into small pieces. Prior further processing, average sample of fresh pumpkin fruit was used for chemical analysis. After processing, products were stored in cold, dry and dark place. Five samples of each type of traditional pumpkin products were randomly chosen, mixed and average sample was used further for analysis.

Total phenolics

Samples of fresh pumpkin fruit and sweet in wine were grained prior extraction and homogenized. A mass of 10 g of each sample was extracted with 20 mL of solvent mixture H_2O/MeOH (1:1) for 30 min using the ultrasonic bath. From those extracts 3 dilutions were made (depends of the sample, experimentally determined to be in a linear range $A = f(c)$) and used for obtaining total phenolics). Total phenolic content was determined using the method of Folin–Ciocalteu reagent (FCR) with slight modifications [18]. An aliquot (200 µL) of each extract was mixed with 1000 µL of 1:10 dilution of Folin-Ciocalteu reagent. After 6 min 800 µL of sodium carbonate (75 g/L) was added. After 2 h of incubation in the dark at room temperature, absorbance was measured at 740 nm. Gallic acid (0–100 mg/L) was used for the preparation of the calibration curve. Results are expressed as µg of gallic acid equivalents (GAE) per g of fresh fruit or product, or per mL of juice. Analyses were performed in triplicates and the results are expressed as mean ± standard deviation.

Total carotenoids

Samples of fresh pumpkin fruit and sweet in wine were grained prior extraction and homogenized. For all samples 1 g was extracted with 50 mL of methanol which was the solvent that gave the best results for the extraction of carotenoids [19]. Total carotenoids were determined using earlier developed method and were calculated and expressed in µg/g of the sample. Analyses were performed in triplicates and the results are expressed as mean ± standard deviation.

LC/MS analysis

Sample preparation for analysis: Each sample (100 g) was homogenized, diluted with water (100 mL), and then this solution was extracted three times with ethyl acetate (100 mL). The organic phases were combined and evaporated to dryness in a rotary vacuum evaporator. Obtained ethyl acetate extracts (10 mg) were dissolved in 1 mL of methanol, filtered (0.45 µm filter) and analyzed by LC/MS techniques. Apparatus: liquid chromatograph Agilent 1200 series, Agilent Technologies, with a degasser, a binary pump, an autosampler, a thermostated column compartment and a DAD detector, coupled with a 6210 time-of-flight LC/MS system (Agilent Technologies); column: Zorbax Eclipse Plus RR C-18, 150 mm×4.6 mm id., (1.8 µm); mobile phase: A – 0.5% formic acid in water and B – acetonitrile; combination of gradient and isocratic modes of elution: 0–20 min, 5–16% B, 20–28 min, 16–40% B, 28–40 min, 40–90% B, 40–45 min, 90% B, 45–46 min, 90–5% B, 46–51 min, 5% B; flow rate: 0.95 mL/min. The injection volume was 5 µL, and the column temperature 40 °C. DAD-MSD conditions: spectral data for all the signals were accumulated in the wavelength range of 190–450 nm, and chromatograms were recorded at wavelengths of 230, 260, 280 and 340 nm. The total ion chromatograms were recorded using the following parameters: ion source (ESI) polarity, negative; capillary voltage, 4000 V; gas temperature, 350 °C; drying gas, 12 L/min; nebulizer pressure, 45 psig (310.26 Pa); fragmentor voltage, 140 V, mass range 100–1500 m/z. A personal computer system running Mass Hunter Workstation software was used for data acquisition and processing. Identification: compounds were identified by comparing retention times (t_R), UV and MS spectra with those of the reference standards (protocatechuic acid, *p*-hydroxybenzoic acid, vanillin, *p*-coumaric acid and hesperidin), or on the basis of the exact mass measurement and literature data (salicylic acid, eriodictyol 7-neohesperidoside, and abscisic acid).

RESULTS AND DISCUSSION

Total carotenoids

Cucurbita species are known as a good source of carotenoids [4]. Total carotenoid content was analyzed in traditional products typically produced in north part of Serbia (Table 1). Fresh pumpkin fruit contained the highest amount of total carotenoids (86.3 µg/g FW), thus presenting a valuable source of this bioactive compounds. Murkovic *et al.* [20] investigated a carotenoid content in different varieties of three *Cucurbita* species grown in Austria (*C. pepo*, *C. maxima* and *C. moschata*)

Table 1. Total carotenoids and total phenolics in fresh pumpkin fruit and pumpkin traditional products; results are expressed on fresh mass basis as mean ± standard deviation of triplicate analysis

Sample	Total carotenoids µg/g	Total phenolics µg GAE/g
Pumpkin fruit	86.3±1.9	905.9±13.9
Pumpkin sweet in wine	27.6±0.8	227.2±8.0
Pumpkin jam	63.9±1.6	769.1±14.1
Pumpkin juice	28.6±1.1	93.0±6.0

and it was similar as in our sample. On the other hand, Provesi *et al*. [21] reported lower amounts of carotenoids in *C. moschata* and *C. maxima* from Brasil (*cca*. 40 and 30 μg/g, respectively). Total carotenoids content in pumpkin is known to be strongly influenced by the cultivar type as well as by the conditions of cultivation [22].

Processing affected the amount of total carotenoids, but the smallest decrease was noticed in jam in which about 70% of carotenoids content was preserved. Also, Provesi *et al*. [21] showed that decrease of carotenoids in pumpkin puree (similar product to jam) was less than 50%. On the other hand, decrease of total carotenoids in pumpkin sweet in wine as well as in pumpkin juice was more pronounced (for almost 70%). Total carotenoids content in pumpkin products are strongly influenced by the technological processes applied, specifically thermal processes [11,14]. The highest retention of carotenoids was obtained when vegetables were cooked almost without water and the lowest retention of carotenoids was associated with the use of a large amount of water during cooking [23]. Moreover, Provesi and Amante [14] stated that processing of pumpkin may cause oxidation and/or isomerization of carotenoids, which affect biological activity and colour. The most important factors that lead to this loss are temperature and contact with oxygen and light.

Table 2. Compounds identified in pumpkin fruit and pumpkin traditional products by LC/MS

t_R in LC/ DAD, min	t_R in ESI ToF, min	Molecular formula	Exact mass	Compound
6.20	6.31	$C_7H_6O_4$	154.0266	Protocatechuic acid
6.42	6.58	$C_{16}H_{18}O_9$	354.0951	Chlorogenic acid
8.79	8.92	$C_7H_6O_3$	138.0317	Salicylic acid
9.21	9.36	$C_7H_6O_3$	138.0317	*p*-Hydroxibenzoic acid
16.58	16.75	$C_8H_8O_3$	152.0473	Vanillin[a]
17.21	17.46	$C_9H_8O_3$	164.0473	*p*-Coumaric acid
22.19	22.47	$C_{27}H_{32}O_{15}$	596.1741	Eriodictyol-7-neohesperidoside
25.34	25.64	$C_{28}H_{34}O_{15}$	610.1898	Hesperidin

[a]Detected only in pumpkin jam

Total phenolics

Also, total phenolic content was determined in fresh pumpkin fruit as well as in pumpkin products. As it was previously shown for total carotenoids, pumpkin fruit was the most abundant in phenolics (Table 1). Obtained result was in accordance with literature data as Azizah *et al*. [24] detected similar amounts of total phenolics (90 mg GAE/100 g). However, Nawirska-Olsza *et al*. [25] detected lower amounts of total phenolics in pumpkin (24 mg/100 g FW). As it could be expected, in pumpkin products lower but still significant amounts of phenolics were determined and the registered reductions were probably due to the thermal processing. Pumpkin jam was the richest product in total phenols, pointed that this type of processing provides a product of high value. The lowest amount of total phenolics was detected in juice and it was almost 2.5 times lower than in jam. Content of total phenols is important parameter because it is usually correlated with antioxidant activity.

LC/MS analysis

Eight compounds were identified, and among them phenolic acids dominated (Table 2). Protocatechuic and chlorogenic acid are polyphenols which are widely distributed in variety of plant species known as the sources of potent antioxidants such as green tea. Also, Dragovic-Uzelac *et al.* [26] reported the presence of chlorogenic acid in *C. pepo*, *C. maxima* and *C. moschata*. Among flavonoids, hesperidin, glycoside of hesperetin, characteristic for citrus fruits was detected. Hesperidin shows different biological activities such as antioxidant, anti-inflammatory and anticancer activities [27,28]. Eriodictyol-7-neohesperidoside is also found in citrus fruits. It is strong antioxidant since it has catechol moiety in B ring and 5-OH group in A ring (capable for chelating metal cations with carbonyl group in position 3). Vanillin was noticed only in the pumpkin jam probably due to its addition according to the recipe.

CONCLUSION

Consumption of fruits and vegetables has been increased rapidly due to awareness regarding their health benefits for humans. Carotenoids and phenolics are among the phytochemicals, believed to reduce the risk of developing degenerative and chronic diseases. The results of our study indicated that tested pumpkin fruit as well as products made from pumpkin fruit, *i.e.*, jam, sweets in wine, and juice, are a valuable source of carotenoids and phenolics. Although total carotenoids and total phenolic content in pumpkin products are strongly influenced by the applied technological processes, specifically thermal processes, about 70% of carotenoids and 85% of total phenolics were preserved in pumpkin jam compared to fresh pumpkin fruit. Thus, obtained results pointed out possibilities for better utilization of such a nutritionally rich underutilized pumpkin products especially in the periods with the lack of fresh fruits.

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ИЗВОД

ФЕНОЛНЕ КОМПОНЕНТЕ И КАРОТЕНОИДИ У ПЛОДУ ТИКВЕ И ТРАДИЦИОНАЛНИМ ПРОИЗВОДИМА ОД ТИКВЕ

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(Научни рад)

Тиква се користи у исхрани од давнина, посебно у руралним срединама. Главни фактори који доприносе нутритивним и здравственим вредностима тикве су каротеноиди, полисахариди, витамини, минерали, и фенолна једињења. Због веома великих плодова као и кратког рока трајања свеже сечених кришки тикве, користе се различити начини чувања и прераде. У нашим истраживањима испитивали смо садржај укупних каротеноида и укупних фенола и присуство појединачних фенолних једињења у свежем плоду тикве, као и у традиционалним производима од тикве који су типични за северне делове Србије, као што су џем, слатко у вину и сок. Садржај укупних каротеноида се кретао од 27,6 μg/g слатка од тикве у вину до 86,3 μg/g свежег плода тикве, док се садржај укупних фенола кретао између 93,0 μg GAE/g сока тикве до 905,9 μg GAE/g свежег плода тикве. Што се тиче појединачних фенолних једињења у испитиваним узорцима, коришћењем LC/MS технике, идентификовано је осам једињења, међу којима доминирају фенолне киселине. Од флавоноида, детектован је флаванонски гликозид хесперидин.

Кључне речи: *Cucurbita maxima* • Џем • Сок • Слатко • Феноли • Каротеноиди • LC/MS