BIOACTIVE COMPOUNDS OF PUMPKIN JUICE ASSORTMENTS

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Abstract
The purpose of this study was to obtain and characterize of some fresh pumpkin juices with addition of water, walnuts, honey, cinnamon and fresh peppermint leaves. Total soluble content, vitamin C by titration with a 2,6-dichlorophenolindophenol sodium, antioxidant capacity by FRAP assay and total phenolic content by Folin-Ciocalteu method were analyzed. Vitamin C content ranged from 0.09 to 0.15 mg/mL, with 0.13 mg/mL in individual pumpkin juice; the juice assortment without walnuts has the lower vitamin C content. Among the investigated juices, the pumpkin juice assortment with all ingredients have the higher amount total polyphenol content (4.19 mM GAE/mL) and the total antioxidant capacity (2.53 mM Fe^{2+}/mL). We observed that the fresh pumpkin juice assortments are a real source of bioactive compounds.

Introduction
Pumpkin (*Cucurbita moschata*) is a species originating in either Central America or northern South America [17]. Pumpkin is highly nutritious and antioxidant-rich vegetable widely grown all over the world. The autumn star, pumpkin is a vegetable that grows for seeds, fruits and flowers. It has a rich content of phenolic compounds and vitamin A and a very low calorie level [9]. It is a rich source of vitamin C that is associated with the prevention of various degenerative, cardiovascular and neurological diseases. It has been used as a medicine in many countries in Central and North America [15]. Pumpkin is poor in taste and carbohydrates but high in antioxidants and it is mixed with fruit or vegetable to improve its nutritional and sensory properties [1]. Pumpkin is a fruit which is healthy and functional, being rich in phenolic compounds, flavonoids and vitamins, and has a low energy [5] showed that a pumpkin-rich diet could reduce blood glucose levels. The World Health Organisation (WHO) estimates that worldwide consumption of fruits and vegetables is only 20–50% of the recommended daily minimum of 400 g per person [16].

Experimental
Juice preparation
The pumpkin fruits and other ingredients were obtained from Timisoara, local markets. Was used linden honey and fresh peppermint leaves. Pumpkin was cut into pieces which were handily peeled and the seeds were discarded. The peeled pumpkin was squeeze immediately using a fruit and vegetable home scale juicer Myria MY4002, then analyzed immediately.
So, were prepared four juice assortments: PJ1 (control sample) - juice prepared of 100 g pumpkin with 100 mL water; PJ2 - juice prepared of 100 g pumpkin with 100 mL water, 10 g walnuts and 1 g honey; PJ3 - juice prepared of 100 g pumpkin with 100 mL water, 1 g honey and three peppermint leaves; PJ4 - juice prepared of 100 g pumpkin with 100 mL water, 10 g walnuts, 1 g honey, 0.5 g cinnamon and three peppermint leaves. Peppermint has a strong sweetish and fresh cooling taste and improves the pumpkin juices taste.

Chemical analysis

The fresh juices were analyzed in terms of total soluble substances (TSS), vitamin C, total antioxidant capacity (TAC) and total phenolic content (TP).

Total soluble substances (TSS) were determined with DR201-95 KRUSS digital handheld refractometer by Carl Zeiss Jena and the results were reported as ^{0}Brix at 20^{0}C [2].

The **Vitamin C** evaluation in pumpkin juice has been carried out following 2,6-dichlorophenolindophenol sodium assay [10] and has been measured in pumpkin juices (mg/mL).

For determination the total antioxidant capacity and total phenolic compounds the samples were subjected to alcoholic extraction. So, 1 mL of each juice sample was added to 9 mL ethanol 45% (v/v) and after 1 h the solution was filtered.

The **total antioxidant capacity** (TAC) has been measured by FRAP (ferric reducing antioxidant power) assay (Benzie & Strain, 1996) [3]. 0.5 mL of hydroalcoholic extract diluted in the ratio 1:10 (v/v) in distilled water has been added to 2.5 mL FRAP reagent: 10 mM TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) solution - diluted in HCl 40 mM, 20 mM $FeCl_3 \cdot 6H_2O$ solution and 300 mM sodium acetate buffer at pH 3.6 in the ratio of 1:1:10. FRAP reagent was prepared freshly. Absorbance was read after 30 minutes at the wavelength of 593 nm using as standard $FeSO_4$ aqueous solution. Correlation coefficient (r^2) for calibration curve was 0.9890. Total antioxidant capacity was expressed as mM Fe^{2+}/mL.

Total phenolic content (TP) of juice samples were determined by the Folin–Ciocalteau method [14] using gallic acid as a standard for the calibration curve ($r^2 = 0.9980$). Briefly, 0.5 mL of hydroalcoholic extract samples diluted in the ratio 1:10 (v/v) in distilled water was mixed to 2.5 mL of Folin-Ciocalteu reagent diluted 1:10 (v/v) in distilled water. Then, was added 2.0 mL of 7.5% sodium carbonate solution. The results were read at 750 nm after 2 hours with the UV-VIS Spectrophotometer SPECORD 205 by Analytik Jena. The results were calculated and expressed in mM gallic acid equivalent (GAE)/mL.

All determinations were performed in triplicate.

Statistical analysis

Pearson correlation was conducted using Microsoft Office Excel 2010 for Windows.

Results and discussions

The values of some chemical characteristics of pumpkin juice assortments are presented in Table 1.

Table 1. The chemical characteristics of pumpkin juice assortments

Characteristics	Juice			
	PJ1	PJ2	PJ3	PJ4
TSS [°Bx]	11.42	12.54	11.86	14.22
Vitamin C [mg/mL]	0.13	0.15	0.09	0.14
TAC [mM Fe^{2+}/mL]	0.67	2.10	0.42	2.53
TP [mM GAE/mL]	2.57	3.51	2.42	4.19

As shown in Table 1, the highest content of TSS was found in PJ4. It can be seen that the level of TSS content varies directly proportional to the amount of ingredients added to the pumpkin juice, as follows: PJ4 > PJ2 > PJ3 > PJ1. According to Romanian standard [12] the TSS values of juices varies from 5.20 to 11°Bx which is slightly larger than the results obtained in our study. After international standard [6] TSS value range between 10-20°Bx. Vitamin C and beta-carotene are present in abundance in pumpkin and impart high

antioxidant potential to it [9]. Vitamin C is highly sensitive to oxidation and leaching into water-soluble media during vegetables processing [4, 11]. The retention of ascorbic acid is often used as an estimate for the overall nutrient retention of food products [8, 11]. The vitamin C in the juice PJ2 (0.15 mg/mL) and PJ4 (0.14 mg/mL) has values close to those of natural pumpkin juice (0.13 mg/mL) whereas PJ3 show lower values (0.09 mg/mL) than PJ1. This is the only juice assortment that does not contain walnuts; the walnuts (*Juglans regia*) contain very large amounts of vitamin C – 1.3 to 1.7 mg/100g [13].
The phenolic composition of vegetables is dependent on commodity, cultivar, maturity stage and postharvest conditions. Since phenolic compounds are antioxidants, they are subject to oxidation during storage and processing of foods [11] Phenolic constituents is very important in the plant because of their scavenging ability due to their hydroxyl groups. Phenolic compounds are powerful chain breaking antioxidants and has been reported are associated with antioxidant activity [15]. The content of polyphenols is influenced by the content of ingredients added to the pumpkin juice. The data indicated that the TP of fresh pumpkin juice PJ1 (2.57 mM GAE/mL) decreased to 2.42 mM GAE/mL in PJ3 and increased to 3.51 mM GAE/mL in PJ2 and to 4.19 mM GAE/mL in PJ4.
A similar trend was seen in TAC. FRAP value of PJ1 (0.67 mM Fe^{2+}/mL) dropped to 0.42 mM Fe^{2+}/mL in PJ3 and increased to 2.10 mM Fe^{2+}/mL in PJ2 and to 2.53 mM Fe^{2+}/mL in PJ4, as reported in Table 1.
Difference in TAC and TP values is attributed to ingredients mixed in each juice assortments, including walnut. As is known, walnut contains two times more antioxidants than other hard shell nuts [13].

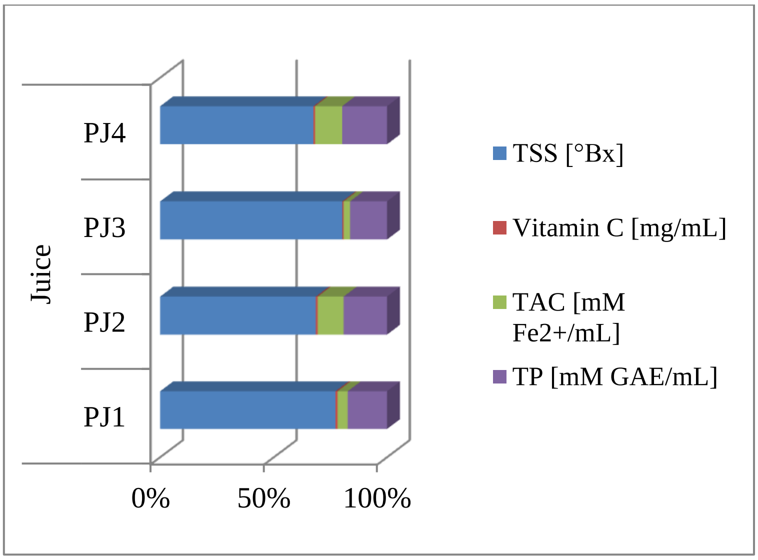

Figure 1. The percentage of chemical characteristics of pumpkin juice assortments

In the graphical representation of Figure 1, the TSS and vitamin C is a major percentage of the chemical characteristics of pumpkin juice assortments.
Full results of the statistical analysis methods used are reported in Table 2. The correlation of all chemical characteristics was positively [7]. Vitamin C are strong correlation with TAC (r = 0.788) and TP (r = 0.716). TAC has high correlation with TP (r = 0.985); it confirms, again, the strongly correlated between this two antioxidant compounds.

Table 2. The Pearson correlation among chemical characteristics of pumpkin juice assortments

	TSS [°Bx]	Vitamin C [mg/mL]	TAC [mM Fe^{2+}/mL]	TP [mM GAE/mL]
TSS [°Bx]	1			
Vitamin C [mg/mL]	0.450	1		
TAC [mM Fe^{2+}/mL]	0.881	0.788	1	
TP [mM GAE/mL]	0.943	0.716	0.985	1

Antioxidant activity has been attributed to total phenolic content which have been found to be strongly correlated [11].

Conclusion

The mixed vegetable juice prepared from pumpkin, water, walnuts, honey, cinnamon and peppermint leaves showed a high amount of antioxidant compounds.

I noticed that there is a close correlation between total antioxidant capacity and total phenolic content.

In conclusion, the fresh pumpkin juices are a real source of bioactive compounds.

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