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# Biochemical Composition of Some Local Pumpkin Population

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**Abstract**

This study aims at investigating the nutritional value of the pumpkin fruits from different local populations of the *Cucurbita pepo* L., *C. maxima* Duch. and *C. moschata* Duch. ex Poir species. The chosen cultivars were: P1, P2, P3, P4, P5, P6 of local populations from Oltenia, Romania namely from Dolj and Olt counties. The pumpkin culture was established in the teaching field of the Faculty of Horticulture in Craiova. The chemical analysis of the pumpkin fruits was carried out using standard methods. There were determined: the dry matter content, the soluble substance content, the total carotenoid content, the antioxidant activity through the DPPH and Trolox method. There have also been studied the correlation coefficients between the quality parameters using the multiple correlation coefficients. The results show differences in the studied quality parameters. There was observed that the "Local population 6" has the highest total carotenoid content of 6.587 mg/100 f.m. and the local population 1 has the highest antioxidant activity of 0.284 mmol/l Trolox.



*Keywords*: *Cucurbita maxima* Duch; *Cucurbita moschata* Duch. ex Poir.; carotenes; antioxidant activity â

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## 1. Introduction

The pumpkin (*Cucurbita moschata* Duch.) is included into the category of functional vegetables because it is rich in phenolics, flavonoids, vitamins (including β-carotene, vitamin A α-tocopherol, and vitamin C), amino acids and carbohydrates (Zhang et al., 2000, 2002; Wang et al., 2002). This species has multiple uses as fruits, vegetables and also therapeutic and medicinal properties (Malik et al., 2010; Adams et al., 2011) but also in the traditional Chinese medicine (Zhang and Sheng, 2003). According to Padulosi (1998) it is very important and necessary to know and use less known species in order to meet the needs of food for the future generations. This species is considered to be the one that meets all the conditions for a healthy diet (Gajewski et al., 2008).

The genus Cucurbita includes five domesticated varieties from which three: *Cucurbita pepo* L., *Cucurbita maxima* D. and *Cucurbita moschata* Duch. ex Poir. are very important globally both economically and for human consumption (Whitaker and Davis, 1962; Robinson and Decker-Walters, 1997). Because the pumpkin cultivars differ in terms of nutritional value, the breeders and scientists seek the most valuable and adequate for nutrition genotypes.

The experimental studies suggest that a diet with a higher content of carotenoids protects against the development of certain types of cancer (e.g. lung, skin, uterine, cervical, and gastrointestinal cancer), macular degeneration, cataracts, and other illnesses related to oxidation and radical free.

There is a growing trend among consumers to eat food with high nutritional value, even if it is not very popular. The pumpkin is included in this category having a very high ornamental and nutrition value so that the consumers began to appreciate the species as a healthy and valuable component for various dishes. The pumpkins are considered valuable vegetables primarily due to the higher carotenoid content and low energy value. Therefore, it has become highly appreciated in the losing weight diets. The biochemical composition of pumpkin regulates the metabolism and has a detoxifying and gently dehydrating effect.

This variety has many healthy properties that make the pumpkin to overcome the most vegetables. The pumpkin itself is a vegetable with a high yield, easy to grow and therefore cheap.

However, few studies refer to the promotion of total carotenoids, polyphenols or of the antioxidant activity of the pumpkin. Thus, the objective of this study was to determine the nutritional value of some pumpkin cultivars from Oltenia and to present the properties of this species for the human consumption.

## 2. Materials and methods

The study was conducted using 6 local pumpkin populations: two varieties of *Cucurbita moschata* Duch. ex Poir (1 and 6), two of *Cucurbita maxima* Duch (2 and 4) and one of *Cucurbita pepo* L. cultivar 5. These were grown in the teaching field of Horticulture Faculty of Craiova located in the south- western part of Romania (44°19'N and 23°48' E, a very favourable area for growing this species).

### *2.1. Samples preparation*

A quantity of 100 g of each sample was broken up in blenduire to mix. 5 grams of homogenized sample (pulp) was mixed with 10 ml 70% methanol, intensely agitated for 10 minutes using a vortex. The extraction of phenolic compounds was carried out by keeping the mixture in an ultrasonic bath for 70 minutes. The mixture was filtered, and to the residue solid was added 5 ml of 70% methanol and the extraction procedure repeated.

Obtained the two extracts were combined and analyzed using protocols specific work to determine the total polyphenol, PT and antioxidant capacity, CA.

Total dry substance was determined in an oven at 105 $^{0}$C to constant weight (ISO 751: 2000). Soluble dry substance was determined by refractometer and expressed in $^{0}$Brix.

### *2.2. The determination of total carotenoids*

The weighed samples, having been put separately in 95% in acetone (50 ml for each gram), were homogenized with Braun MR 404 Plus for one minute. The homogenate was filtered and was centrifuged using the Hettich Universal 320/320R centrifuge at 2500 rpm for ten minutes. The supernatant was separated and the absorbance were read at 400-700nm on Cary 50 spectrophotometer. It was recorded that Chlorophyll a showed the maximum absorbance at 662 nm, chlorophyll b at 646 nm and total caroten at 470 nm. The value of these pigments was calculated using the method described by Dinu et al., (2013).

*2.3. Determination of antioxidant activity* in equivalent Trolox and ascorbic acid equivalent.

In order to determine the antioxidant activity of the extract of pumpkin pulp was used the method described Hatano et. al (1988) with some modifications (Cosmulescu and Trandafir, 2012). Reagents used: Methanol (Merck), AcidAscorbic; Trolox (Merck), DPPH (Sigma-Aldrich). To establish the calibration curve for Trolox were prepared seven Trolox solutions with content: 0.0; 0.5; 1.0; 1.5; 2.0; 2.5; 3.5 mm. To establish the calibration curve for ascorbic acid standards were prepared with ascorbic acid content of between 25-200 ppm.

Way of working:

The working protocol includes the following steps: mix 50 ml and 3 ml sample DPPH methanol solution (0.004%). Samples obtained were mixed vigorously and left in the dark for 30 min. After incubation the absorbance was measured at 517 nm was determined that by using the two calibration curves. For determinations was used a spectrophotometer Evolution 600 UV-Vis Spectrophotometer.

The antioxidant capacity was measured in the extracts diluted in methanol (1:4) using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay as described by Hatano et al. (1988), wisome modifications (Cosmulescu and Trandafir, 2012). Briefly, each methanol diluted extract (50 μL) was mixed with 3 ml of a 0.004% (v/v) DPPH methanolic solution. The mixture was incubated for 30 min at room temperature in the dark and the absorbance was measured at 517 nm on Varian Cary 50 UV–Vis spectrophotometer (Varian Co., USA). The inhibition of the DPPH radical by the samples was calculated according to the following formula: DPPH scavenging activity (%) = $[1 - \text{Abs.sample}/\text{Abs.blank}] \times 100$. The DPPH scavenging activity was subsequently calculated with respect to the 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), which was used as a standard reference. Six calibration solutions of Trolox (0.5, 1, 1.5, 2, 2.5 and 3.5 mM) were also tested to establish a standard curve. A blank control of methanol/water mixture was run in each assay. All assays were conducted in triplicate. Results were expressed as milimoles Trolox equivalents per liter.

*2.4.Total phenolic content*

The amount of total phenolic compounds in the pumpkin fruits extract was determined colorimetrically with Folin-Ciocalteu reagent by using the method described by Singleton and Rossi (1965) with some modifications. To 1 ml extracts (diluted 1:10 with ultrapure water), 1 ml bidistilled water (blank), 1 ml of each standard solution were introduced in laboratory flasks of 25 ml and added every 5 ml reactive Folin-Ciocalteu (diluted 1:10 with ultrapure water). After 2 min, 4 ml of sodium carbonate solution 7.5% was added and they were kept in the incubator during 2 h at the room's temperature.

The absorbance was measured at 765 nm by using a model evolution 600, double beam scanning UV-visible spectro-photometer, PC control with VISION pro software. A standard curve was prepared by using 50, 100, 150, 200 and 250 mg/L solutions of gallic acid in methanol and water (60:40, v/v). Gallic acid was used as the reference standard and the results (total phenolic content) were expressed as gallic acid equivalents (GAE) in milligrams per liter.

*2.5. Statistically analyses*

The experimental results were interpreted statistically by ANOVA (Stat graphics Plus software) using analysis of variance single factor. Tukey LSD test was used to show that values differ significantly at $P = 0.05$. The correlation coefficients between caught quality parameters were performed by multiple correlation coefficients.

## 3. Results and discussions

As stated in the literature, the most important biochemical components of the pumpkin fruits are: total dry matter, soluble solid content and content of carotenoids (Danilchenko et al., 2002). Thus, for the six samples of pumpkin there were determined the following: total dry matter content, soluble solid content, refractive index, total carotene content, the total polyphenols and antioxidant activity in the equivalent of Trolox and ascorbic acid.

The high content of **soluble dry substance** corresponds to an accumulation of sugars which it is very important in terms of the sensorial aspect (Gajc-Wolska et al., 2005).

The content of the pumpkin fruits varied widely (Table 1). Regarding the content of the pumpkin fruits at the studied genotypes, it ranged from 12.6 $^0$Brix at cultivar 1 to 5.51$^0$Brix at cultivar 6. According to Murkovic et al. (2002), Gajc-Wolska et al., (2005); Gajewski et al., (2008) and Zinash et al., (2013), the genotypes influence significantly the content of the mentioned elements and the total carotenoids. On the carrot, Hoza Gh (2008) - through analysis of biochemical composition of several cultivars of carrot - obtained values of 7.8-11.4%.

Table1. Quality parameters of pumpkin cultivars

| Genotypes | T.D.M (%) | S.S.M $^0$Bx | Humidity (%) | Refractive index |
|---|---|---|---|---|
| Population 1 | 11.08ab | 12.6a | 89.0ab | 1352ns |
| Population 2 | 8.40bc | 11.4ab | 88.9ab | 1355ns |
| Population 3 | 8.46bc | 9.4abc | 91.6a | 1348ns |
| Population 4 | 7.53c | 8.2bc | 92.5a | 1337ns |
| Population 5 | 8.80bc | 7.6bc | 91.2ab | 1344a ns |
| Population 6 | 12.42a | 5.51c | 87.6b | 1341a ns |
| LSD (0,05) | 3.43 | 4.01 | 3.76 | 18.42 |

TDS – total dry substance; SDS- soluble dry substance
ns- not significant

Regarding the **total dry substance** content, the obtained results have indicated significant differences between the samples. They ranged between 7.53% at cultivar 4 and 12.42% at cultivar 6. The highest values were recorded by the cultivars 1 and 6 while the other 4 cultivars had similar values. Agnieszka Nawirska-Olszańska (2011) studied various combinations of pumpkin puree enriched with Japanese Guti, raspberries, cherries, apples, and the content of TDM. At the pumpkin confirmed our findings. Zinash, et al., (2013) study conducted on 20 pumpkin fruits recorded total dry matter values ranging between 6.0 and 11.0% which confirmed the values obtained in the present study. Hoza Gh. (2008) measured the total dry substance in 6 cultivars of carrot, species with high content in nutrients and similar influence - those of pumpkin and the obtained values were 9.8 to 10.3% - on first harvest and 12.8-13.3% - from the last harvest.

**The humidity** ranged between 87.6 and 92.5% with differences between the studied samples (Table 1). These values have been confirmed by other authors too (Fedha, et al., 2010). According to a study conducted by Lee and Lim (2011) on some pumpkin cultivars in Spain, the humidity of fruits ranged between 85 and 97%. Determining this parameter is very important because the flour can be obtained from the pulp of the pumpkin and in this process, the humidity is very eloquent for the processors.

The antioxidant activity of vegetable species is a very important quality parameter in terms of nutrition.

The **total carotene** content recorded values between 4.200 and 6.587 mg/100 g f.m. with the highest values at the cultivar 6 and the lowest values at the cultivar 4 (Table 2). These values are confirmed by other researchers too who carried out observations at different varieties of pumpkin grown with nitrogen-based fertilizer or with organic fertilization (Anita Biesiada et al., 2009; Agnieszka Nawirska-Olszańska et al., 2011) or who were interested in vegetable species with high carotene content such as pumpkin (Danilchenko, 2002; Murkovic et al., 2002; Gajewski et al., 2008). According to Paulauskiene et al., (2006) who conducted a study on 11 varieties of pumpkin of Lithuanian origin, they have found significant differences between varieties with values that ranged between 0.90 and 11.25 mg 100 $g^{-1}$. *Cucurbita moschata* which includes cultivars 1 and 6 had the highest values, values confirmed by other authors too (Azevedo-Meleiro and Rodriguez-Amaya, 2007; Kandlakunta et al., 2008; Kurz et al., 2008).

In another study previously carried out, twenty-two varieties of *C. moschata* have been evaluated and the reported results are equivalent to those in the present study (Azizah et al., 2009; Lucia Maria Jaeger de Carvalho et al., 2014).

**The antioxidant activity**, determined through the Trolox method, expressed in mmol/L, ranged from 0.108 mmol/L for cultivar 5 to 0.284 mmol/L for cultivar 1 (Table 2). Also regarding the antioxidant activity determined by the ascorbic acid content expressed in mg/L, the previously results are confirmed. These values are confirmed by other authors too (Paulauskiene et al., 2006; Gajewski et al., 2008).

The antioxidant activity expressed in content of ascorbic acid had the highest content at cultivar 1 with a value of 79.539 mg/L and the lowest values at cultivars 5 and 4 with values of 44.261 and 44.767 mg/L. Among the other 3 cultivars there were no significant variations.

Zinash et al., (2013) in a study on 20 pumpkin cultivars determined the ascorbic acid content which recorded values ranging between 4.81 and 9.10 mg/100 $g^{-1}$. Similar results were reported by Sudhakar et al., (2003), which showed that the ascorbic acid ranged from cultivar to cultivar. Many researchers have reported that the pumpkin is a valuable source of ascorbic acid, which plays a major role in nutrition, with vitamin C as antioxidants (Duke and Ayensu, 1985, Sudhakar et al., 2003) suggesting the potential of cultivars of the *Cucurbita* species.

The **total polyphenol** content expressed in mgGAE/L varied widely within the studied local populations. Thus the highest value was recorded at cultivar 1 with 159.696 mgGAE/L and the lowest value was recorded at cultivar 3 with 35.947 mgGAE/L. The cultivars 4 and 6 had similar values (Table 2).

The phenolic compounds are associated with the antioxidant activity and play an important role in the stabilization of the lipid peroxidation (Yen et al., 1995).

Table 2. Total carotene, phenolics and antioxidant activity of pumpkins cultivars

| Genotypes | Content of: | | | |
|---|---|---|---|---|
| | Total carotene (mg/100 g f.m.) | Total phenolis content* (mgGAE/L) | Antioxidant activity ** (mmol/L) | Antioxidant activity*** (mg/L) |
| Population 1 | 6.100$^{ab}$ | 159.696$^{a}$ | 0.284$^{a}$ | 79.539$^{a}$ |
| Population 2 | 5.900$^{ab}$ | 78.402$^{b}$ | 0.159$^{b}$ | 54.459$^{b}$ |
| Population 3 | 5.200$^{bc}$ | 35.947$^{f}$ | 0.156$^{b}$ | 54.355$^{b}$ |
| Population 4 | 4.200$^{c}$ | 41.367$^{e}$ | 0.127$^{d}$ | 44.767$^{d}$ |
| Population 5 | 4.620$^{c}$ | 56.246$^{c}$ | 0.108$^{e}$ | 44.261$^{d}$ |
| Population 6 | 6.587$^{a}$ | 46.607$^{d}$ | 0.135$^{c}$ | 50.651$^{c}$ |
| LSD 5% | **1.15** | **0.110** | **0.0046** | **0.619** |

*gallic acid equivalents; **Trolox equivalents ; *** ascorbic acid equivalents

The correlations between certain quality parameters of the pumpkin fruits are shown in Table 3.

The significantly positive correlations were found between the S.D.T. and total carotene. Other, distinct significantly positive correlations were found between the polyphenols content and the antioxidant activity determined by the Trolox method and between polyphenols and antioxidant activity equivalent to ascorbic acid. Also there were recorded positive correlations among the other chemical parameters, but insignificant ones. The literature confirms that when fruits and vegetables with a high content of carotene are consumed, a powerful antioxidant activity occurs in the body because between these two elements is a strong correlation.

Table 3.Correlations between chemical compounds analysed of pumpkin cultivars

| Specification | Total Caroten (mg/100 g f.m.) | Total polyphenols (mgGAE/L) | Antioxidant activity* (mMol/L) | Antioxidant activity** (mg/L) |
|---|---|---|---|---|
| S.D.T (%) | 0,882* | 0,378 | 0,398 | 0,450 |
| S.S.M ($^0$Bx) | 0,130 | 0,751 | 0,770 | 0,746 |
| Total carotene | - | 0,427 | 0,475 | 0,548 |
| Polyphenols | | - | 0,921$^{**}$ | 0,914$^{**}$ |
| Antioxidant activity* | | | - | - |

*r – P 5% = 0,81; **r – P 1% = 0,92; ***r – P 0,1% = 0,97

* Trolox equivalents ** ascorbic acid equivalents

There are opinions that the pumpkin fruits that have high sugar content and high total carotenoids are considered very good quality fruits (Corrigan et al., 2000) and they can be consumed raw.

## 4. Conclusions

The local pumpkin populations which were studied had a very diverse biochemical composition which demonstrates the different behaviour depending on the climate, species and genotype.

The highest S.S.M accumulation was recorded by the local populations 1, 2 and 3, the highest values of total carotenes were recorded by the local populations 1, 2 and 6, and the highest values of the antioxidant activity and total polyphenols were recorded by the population 1.

The pumpkin consumption in Romania could be promoted more aggressively in order to increase the content of vitamin A among the population, which has an antioxidant effect on free radicals in the body. More studies should be performed to identify the genotypes with a higher total carotene content, polyphenols content or high antioxidant activity.

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