



## Chemical composition of pumpkin (*Cucurbita maxima* D.) flesh flours used for food

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Received 28 June 2014, accepted 10 September 2014.

### Abstract

Pumpkin flours (*Cucurbita maxima* D.) are alternative horticultural products and functional properties of food components. The main aim of this study was to investigate the quality parameters of the pumpkin (*Cucurbita maxima* D.) fruit flesh flours of different cultivars: 'Justynka F<sub>1</sub>', 'Karowita' and 'Amazonka'. Standard methods were applied to determine dry matter, crude fibre, crude protein, crude fat, crude ash, the neutral dietary fiber (NDF), modified acid-detergent fibre (MADF), water-soluble carbohydrates (WSC) and carotenoids (β-carotene, lutein + zeaxanthin, lycopene). The pumpkin fruit flours of 'Justynka F<sub>1</sub>' accumulated significantly highest content of dry matter, crude ash, crude fiber, water-soluble carbohydrates (WSC). The highest amount of crude protein, crude fat and lutein + zeaxanthin were in the pumpkin fruit flours of 'Karowita'. The maximum of neutral detergent fiber (NDF) and modified acid-detergent fibre (MADF) was accumulated in 'Amazonka' pumpkin flour, respectively, 21.37, 20.13% DM, so that the flour of this pumpkin variety is most suitable to enrich food with dietary fiber.

**Key words:** Pumpkin, flesh, flours, quality, nutritional value.

### Introduction

Pumpkins can be processed into flour which has a longer shelf-life. This flour can be used for its flavour, sweetness, deep yellow-orange color and considerable amount of dietary fiber. It can be also used to supplement cereal flours in bakery products, soups, sauces, instant noodles and also as a natural coloring supplement for food<sup>28,29</sup>.

Currently Lithuanian consumers also buy more vegetables that are grown in small farms and have exclusive properties (organic products), vegetables of exceptional quality. In this way changes are inevitable in the cultivation of raw materials, their processing and marketing. Lithuanian climate is suitable for growing pumpkins as well. They grow well in the soil which is sheltered from the winds, in sandy loam or in clay, warming soil.

Pumpkins produce high yields in comparison with other vegetables and they are rated for the simple production technology<sup>14</sup>. The breeders have already created shrubby type of pumpkin plants. *Cucurbita maxima* is cultivated for flesh and seeds for human nutrition, either for direct consumption or for preparation of other foods such as syrups, jellies, jams, and purees. This vegetable can be processed in different ways. It can be baked, frozen, dried, crystallized, marinated or lyophilized<sup>10</sup>. The fruits of pumpkins have a lot of biologically active compounds : vitamin C, vitamin E, minerals, pectins and carotenoids. The beneficial influences of carotenoids on human health have proven by many researchers. In the human body carotenoids keep same chemical reactivity as in plants - catching free radicals and active atomic oxygen<sup>11</sup>. Carotenoids also potentially play an important role in human health by acting as biological antioxidants, protecting cells

and tissues from the damaging effects of free radicals and singlet oxygen. The protective role of xanthophyll pigments lutein and zeaxanthin have been recently added to the list of potentially beneficial nutrients for coronary heart diseases and stroke, cataract and macular degeneration (AMD)<sup>18</sup>. In China, Yugoslavia, Argentina, India, Mexico, Brazil, and America pumpkins are utilized in the pharmaceutical industry<sup>30</sup>.

Pumpkins are good sources of proteins and fibre. Proteins are irreplaceable, because other nutrients don't have nitrogen or amino acids. Many investigations have been reported which concerning the health benefits or the quantities of the fibre found in fruits and vegetables<sup>13, 25</sup>. Most of the research has concentrated on the physiological properties of fibres and how they influence the gastrointestinal tract. The fibre plays an important role in the prevention and cure of diabetes, obesity, atherosclerosis, heart diseases and colon cancer<sup>7,8</sup>. The structural polysaccharides are the major part of plant cell walls. The types of plant material that are included within the definitions of dietary fiber may be divided into two forms based on their water solubility: insoluble dietary fiber, which includes celluloses, some hemicelluloses and lignin and soluble dietary fiber which includes β-glucans, pectins, gums, mucilages and some hemicelluloses<sup>4</sup>.

Minerals play an important role for the human body. They affect the utilization of dietary vitamins and are integral parts of bones, teeth, soft tissues, muscles, blood and nerve cells. At least 22 mineral elements are required for the well-being of humans and these can be supplied by a balanced diet<sup>33</sup>.

Pumpkin flour is currently the main processed product of

pumpkin fruit, because it can be easily stored for long time and conveniently used in manufacturing formulated foods. Adding pumpkin flour in the processing of noodles, breads and cakes, not only enhances the content of various nutrients, but also improves the flavour of products<sup>6</sup>.

The main aim of this study was to investigate the quality parameters of the pumpkin (*Cucurbita maxima* D.) fruit flesh flours of cultivars 'Justynka F<sub>1</sub>', 'Karowita' and 'Amazonka'.

### Materials and Methods

Three pumpkin (*Cucurbita maxima* D.) cultivars 'Justynka F<sub>1</sub>', 'Karowita' and 'Amazonka' were grown in the experimental field of ecological farm (Lithuania, Kaunas distr.). The field was not fertilized. Pumpkins were sown in the plastic cups in the end of April, 2012–2013 (2–3 seeds were put into one hole of 2–4 cm depth) and were considered in the glasshouse. Into the constant growing place of the field shoots were planted on the middle of May, 2012–2013. Plants were grown in four replications. Pumpkins were harvested at the end of September.

**Plant material and flour preparation:** The pumpkin fruits were washed, halved and the seeds were removed. The flesh and peel were sliced, and dried at 60°C in the thermostat (Termaks, Norway). Dried slices of pumpkin were grinded using ultra centrifugal mill (ZM 200, Retsch, Germany) to produce flours, which were kept chilled in an air-tight container at 12–18°C temperature, until the laboratory analysis. The samples were evaluated in triplicate for each analysis.

**Methods of sample preparation and chemical analyses:** The studies were carried out at the laboratories of Lithuanian Research Centre of Agriculture and Forestry, Agriculture and Food Sciences Institute of Agronomy Faculty of Aleksandras Stulginskis University and Faculty of Human Nutrition and Consumer Sciences, Warsaw University of Life Sciences (WULS-SGGW).

Dry matter (DM) content of the pumpkin flesh flours was determined by drying samples to the constant weight at 105°C<sup>20</sup>. Amounts of crude protein, crude fibre<sup>22</sup>, crude ash and crude fat were also determined<sup>22</sup>.

The amount of water-soluble carbohydrate (WSC) was determined using anthrone method<sup>35</sup>. Samples were subjected to the analyses of fiber components: neutral detergent fiber ((NDF) cellulose, hemicellulose and lignin) and modified acid-detergent fibre (MADF) using cell wall detergent fractionation method according to Faithfull<sup>5</sup> and Van Soest *et al.*<sup>34</sup>.

Carotenoids ( $\beta$ -carotene, lutein + zeaxanthin, lycopene) content were detected according to the methods Konings and Roomans<sup>15</sup> and Helsper *et al.*<sup>12</sup>. Analyses were performed with Shimadzu HPLC 10A system.

The experimental data was statistically processed by the analysis of variance (ANOVA), software STATISTICA 7.0 (StatSoft, USA). Arithmetical means and standard errors of means of the experimental data were calculated. Tukey test ( $p<0.05$ ) estimated statistical reliability of mean differences.

### Results and Discussion

One of the most important chemical content quality indicator is the amount of dry matter. It ensures the

quality and output of the recycled products. Depending on the type and cultivar, the amount of the above mentioned substances in pumpkin flesh can fluctuate from 4.15% to 23.1%<sup>16,27</sup>. The fruits of great pumpkins accumulate higher amounts of dry matter compared with the amount of the fruits of oil pumpkins. This is due to the relatively high sugar content in the flesh of *Cucurbita maxima* fruits<sup>1,26</sup>. Great pumpkins that are grown in Lithuania can accumulate 7.41–22.20% of dry matter. Plant fertilization with complex and humus fertilizers increases dry matter content in the fruits of pumpkins<sup>17</sup>.

The amount of dry matter in different great pumpkin fruit flesh flours ranged from 7.57 to 12.44% (Table 1). The significant higher quantities of the above mentioned substances have been found in 'Justynka F<sub>1</sub>' – 12.44%.

Protein is important for tissue repair and cell growth. They provide the building block for just about every tissue in human body (i.e. muscle, hair skin, blood, enzymes, etc.). They affect transport through the cell membranes of various vitamins and minerals. The content of crude protein in dry matter of tested pumpkin fruit flesh flours was in the range from 8.35 to 11.33% (Table 1). 'Amazonka' flesh accumulated the lowest amounts of crude protein (8.35%). This can be explained by the shortest vegetation period of this cultivar. In the pumpkin 'Karowita' flesh flours had twice higher amount of crude protein (Table 1).

Quantities of minerals in pumpkins are influenced by numerous complex factors including genotype, soil, environmental conditions and nutrition interactions<sup>32</sup>. It is very beneficial to consume food with sufficient amounts of these substances. The content of crude ash in the fruit flesh flours of tested pumpkins was in the range from 6.61 to 8.89% (Table 1). The highest amount of crude ash was accumulated in the flesh flours of 'Justynka'.

Cucurbits are among the most important plant families supplying with edible products and useful fibres<sup>2</sup>. The fibre is mainly present as cell-wall polysaccharides, which have cholesterol-lowering properties. Antioxidative effects of these pumpkin components have been also reported<sup>24</sup>. On the average, most of the crude fibre in dry matter was accumulated in 'Justynka F<sub>1</sub>' flesh flours (6.66%) (Table 1). The fruit flesh of analysed cvs. accumulated similar amounts of crude fibre.

Amount of crude fat in pumpkin fruit flesh flours ranged from 2.45 to 3.21%, between cultivars there were insignificant differences (Table 1). Pumpkin flesh flours have very low amount of crude fat.

Foods produced from plants abounds with natural biologically active compounds, such as polyphenols, vitamin C or  $\beta$ -carotene, have antioxidant properties and are the great value to human health<sup>3</sup>. The most notable positive effect of processing on the overall quality or health capacity of food is the increased bioavailability of  $\beta$ -carotene resulting in an increased antioxidant status. According to scientists, Pumpkin varieties with high lutein content and low carotene content show a bright yellow color in seeds<sup>19</sup>.

**Table 1.** Dry matter, crude protein, ash, fibre and fat contents (mean  $\pm$  s.d.) in the great pumpkin flesh flours.

Cultivars	Dry matter %	Crude protein % DM	Crude ash % DM	Crude fibre % DM	Crude fat % DM
'Justynka F <sub>1</sub> '	12.44 $\pm$ 1.89b*	9.91 $\pm$ 1.1ab	8.89 $\pm$ 0.62b	6.66 $\pm$ 0.82a	2.45 $\pm$ 0.40a
'Karowita'	8.35 $\pm$ 1.33a	11.33 $\pm$ 1.33b	6.85 $\pm$ 1.61a	6.09 $\pm$ 0.71a	3.21 $\pm$ 1.35a
'Amazonka'	7.57 $\pm$ 0.08a	8.35 $\pm$ 0.16a	6.61 $\pm$ 0.02a	5.61 $\pm$ 0.47a	2.90 $\pm$ 1.19a

\*Means in column with different letters are significantly different ( $p < 0.05$ ) for the different cultivars.

Our results show that great pumpkin flesh flours are rich in source of carotenoids, especially in lutein and zeaxanthin. Of course there are other carotenoids that are good precursors of vitamin A. It was established that the amount of lutein and zeaxanthin was significantly different in all cvs. pumpkin fruit flours (Table 2). According to our results significantly highest amount of lutein and zeaxanthin was in pumpkin flours of 'Karowita' 12.31 mg/100 g.

Murkovic *et al.*<sup>23</sup> reported that three species of pumpkin (*C. pepo*, *C. maxima* and *C. moschata*) consisted of beta-carotene (0.06–7.4 mg/100 g), alpha-carotene (0–7.5 mg/100 g) and lutein (0–17 mg/100 g).

Lycopene concentration is lower than that of other carotenoids. The fruit flesh of cv. accumulated lycopene amount varied from 0.72 to 0.81 mg/100 g, and the significant highest was identified in 'Justynka' F<sub>1</sub> flours (Table 2).

**Table 2.** Carotenoids content (mean ± s.d.) in great pumpkin flesh (mg/100 g).

Cultivars	Lutein+zeaxanthin	Lycopene	β- carotene
'Justynka F <sub>1</sub> '	7.96±0.07a*	0.81±0.01c	2.42±0.02b
'Karowita'	12.31±0.03c	0.79±0.01b	1.86±0.02a
'Amazonka'	7.96±0.02b	0.72±0.02a	2.44±0.02b

\*Means in column with different letters are significantly different (p < 0.05) for the different cultivars.

Our results show that the highest amount of b-carotene was in 'Justynka F<sub>1</sub>' and 'Amazonka' (accordingly 2.42 and 2.44 mg/100 g) and lowest in 'Karowita' (1.86 mg/100 g) pumpkin flesh flours.

A higher amount of lignin is undesirable in NDF fiber content, since it reduces the other fiber, hemicellulose and cellulose degradation<sup>21, 31</sup>. According to Nawirska *et al.*<sup>26</sup>, the NDF fiber content in *C. pepo* pumpkin flesh ranges 0.23–4.37% fresh matter, *C. maxima* pumpkin 1.20 – 4.37% fresh matter, ADF fiber, respectively, 0.22–0.47% fresh matter and 0.43–1.46% fresh matter. The NDF fiber content is more dependent on the cultivars than the genotype. The NDF content ranged between 18.80 and 21.37% DM in the flour of three pumpkin cultivars (Table 3). The NDF content is high in the pumpkin flour from 'Amazonka' and slightly lower in 'Justynka F<sub>1</sub>' and 'Karowita'.

**Table 3.** Fractional composition (mean ± s.d.) of dietary fiber and water-soluble carbohydrates in great pumpkin flesh flours% DM.

Cultivars	NDF	MADF	WSC
	amount, % DM		
'Justynka F <sub>1</sub> '	18.80±0.10a*	16.43±0.06a	44.73±0.12c
'Karowita'	18.87±0.25a	18.86±0.28b	36.40±0.26a
'Amazonka'	21.37±0.47b	20.13±0.15c	39.83±0.06b

\*Means in column with different letters are significantly different (p < 0.05) for the different cultivars.

The MADF content in the flour of all the pumpkin cultivars was different. The MADF content varied between 16.43 and 20.13% DM depending on cultivars (Table 3). The highest MADF content was in 'Amazonka' flour, the lowest content in 'Justynka F<sub>1</sub>'.

It was observed that 'Justynka F<sub>1</sub>' pumpkin flour contains maximum amounts of water-insoluble fiber. However, in 'Amazonka' pumpkins the highest number of NDF and MADF fiber was indicated.

WSC quickly digested energy-rich compounds are the primary products of photosynthesis, so their content is highly dependent on temperature, sunlight and other environmental factors<sup>9</sup>.

The content of WSC varied depending on cultivars. Pumpkin flours displayed high content of total WSC (44.73% DM) in 'Justynka F<sub>1</sub>' and lowest content in 'Karowita' (36.40% DM) (Table 3).

## Conclusions

The pumpkin fruit flours of 'Justynka F<sub>1</sub>' accumulated significantly highest content of dry matter, crude ash, crude fiber, water-soluble carbohydrates (WSC). The highest amount of crude protein, crude fat and lutein+zeaxanthin was in fruit flours of 'Karowita'. The maximum neutral detergent fiber (NDF) and modified acid-detergent fibre (MADF) was accumulated in 'Amazonka' pumpkin flour, respectively, 21.37, 20.13% DM, so the flour of this pumpkin variety could be most suitable to enrich manufactured food with dietary fiber.

## Acknowledgements

This publication is funded by European Social Fund and the Budget of the Republic of Lithuania (project "Eureka E! 6855 – ECORAW" "Higher functionality food products from organic vegetable raw materials").

## References

- Achinewhu, S., Ogbonna, C. C. and Hart, A. D. 1995. Chemical composition of indigenous wild herbs, spices, fruits, nuts and leafy vegetables used as food. *Plant Foods for Human Nutrition* **33**(4):226–261.
- Bisognin, D. 2002. Origin and evaluation of cultivated cucurbits. *Ciencia Rural* **32**(4):859–865.
- Danilčenko, H., Jarienė, E., Gajewski, M., Černiauskienė, J., Kulaitienė, J., Sawicka, B. and Aleknavičienė, P. 2011. Accumulation of elements in some organically grown alternative horticultural crops in Lithuania. *Acta Sci. Pol., Hortorum Cultus* **10**(2):23–31.
- Elleucha, M., Bedigan, D., Roiseux, O., Besbes, S., Blecker, Ch. and Attia, H. Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food Chem.* **124**:411–421.
- Faithfull, N. T. 2002. Methods in Agricultural Chemical Analysis: A Practical Handbook. Wallingford, USA, 266 p.
- Que, F., Mao, L., Fang, X. and Wu, T. 2008. Comparison of hot air-drying and freeze-drying on the physicochemical properties and antioxidant activities of pumpkin (*Cucurbita moschata* Duch.) flours. *International Journal of Food Science and Technology* **43**:1195–1201.
- Ferguson, L. R. 2005. Does a diet rich in dietary fibre really reduce risk of colon cancer? *Digest. Liver Dis.* **37**:139–141.
- Ferguson, L. R. and Harris, P. J. 2003. The dietary fibre debate: more food for thought. *The Lancet* **361**:1487–1488.
- Halling, M. A., Longland, A. C., Martens, S., Nesheim, L. and O'Kiely, P. 2004. Accumulation of water-soluble carbohydrates in two perennial ryegrass cultivars at nine European sites. *Grassland Science in Europe* **9**:954–956.
- Hamed, S. Y., El Hassan, N. M., Hassan, A. B., Eltayeb, M. M. and Babiker, E. E. 2008. Nutritional evaluation and physicochemical properties of processed pumpkin (*Telfairia occidentalis* Hook) seed flour. *Pakistan Journal of Nutrition* **7**:330–334.
- Han, R. M., Zhang, J. P. and Skibsted, L. H. 2012. Reaction dynamics of flavonoids and carotenoids as antioxidants. Review. *Open Access Molecules* **17**:2140–2160.
- Helsper, J. P. F. G., Vos, de C. H. R., Mass, F. M., Jonker, H. H., Broeck van der H. C., Jordi, W., Pot C. S., Keizer, L. C. P. and Schapendong, A. H. C. M. 2003. Response of selected antioxidants and pigments in tissues of *Rosa hybrida* and *Fuchsia hybrida* to supplemental UV – A exposure. *Physiol. Plant.* **117**:171–178.

- <sup>13</sup>Jenkins, D. J. A., Kendall, C. W. C. and Ransom, T. P. P. 1998. Dietary fiber, the evolution of the human diet and coronary heart disease. Nutr. Res. **18**:633-652.
- <sup>14</sup>Ježnach, M., Danilcenko, H., Jariene, E., Kulaitiene, J. and Černiauskienė, J. 2012. Accumulation of antioxidative vitamins and minerals in seeds of oil pumpkin (*Cucurbita pepo* L. var. *styriaca*) cultivars. Journal of Food, Agriculture & Environment **10**(1):245-247.
- <sup>15</sup>Konings, E. J. M. and Roomans, H. H. S. 1997. Evaluation and validation on an LC method for the analysis of carotenoids in vegetables and fruits. Food Chem. **59**(4):599–603.
- <sup>16</sup>Konopacka, D., Seroczyńska, A., Korzeniewska, A., Jesionkowska, K., Niemirowicz-Szczytt, K. and Płocharski, W. 2010. Studies on the usefulness of *Cucurbita maxima* for the production of ready-to-eat dried vegetable snacks with a high carotenoid content. Food Science and Technology **43**(2):302-309.
- <sup>17</sup>Kulaitienė, J. 2009. Influence of agrobiological factors on field pumpkin (*Cucurbita pepo* L.) fruit quality. Summary of doctoral dissertation, LUA, 16 p.
- <sup>18</sup>Lakshminarayana, R., Aruna, G., Sangeetha, R. K., Bhaskar, N., Divakar, S. and Baskaran, V., 2008. Possible degradation/biotransformation of lutein *in vitro* and *in vivo*: Isolation and structural elucidation of lutein metabolites by HPLC and LC-MS (APCI). Free Radical Biology and Medicine **45**:982-993.
- <sup>19</sup>Landrum, J. T. and Bone, R. A. 2001. Lutein, zeaxanthin, and the macular pigment. Arch. Biochem. Biophys. **385**:28–40.
- <sup>20</sup>LST EN 12145:2001 Fruit and vegetable juices - Determination of total dry matter - Gravimetric method with loss of mass on drying (Lithuanian Standard).
- <sup>21</sup>Mawamba, D. A., Gouado, I. and Leng, M. 2009. Steamed-dried squashes (*Cucurbita* sp.) can contribute to alleviate vitamin A deficiency. American Journal of Food Technology **4**(4):170-176.
- <sup>22</sup>Methodenbuch – VDLUFA. 1983–1999. Band III. Die chemische Untersuchung von Futtermitteln. - Verlag- Darmstadt (in German).
- <sup>23</sup>Murkovic, M., Mülleider, U. and Neunteufel, H. 2002. Carotenoid content in different varieties of pumpkins. Journal of Food Composition and Analysis **15**:633-638.
- <sup>24</sup>Nara, K., Yamaguchi, A., Maeda, N. and Koga, H. 2009. Antioxidative activity of water-soluble polysaccharide in pumpkin fruits (*Cucurbita maxima* Duchesne). Biosci. Biotechnol. Biochem. **73**(6):1416-1418.
- <sup>25</sup>Nawirska, A., Kwaśniewska, M. 2005. Dietary fibre fractions from fruit and vegetable processing waste. Food Chem. **91**:221-225.
- <sup>26</sup>Nawirska, A., Sokół-Łętowska, A., Kucharska, A. Z., Biesiada, A. and Bednarek, M. 2008. Porównanie zawartości frakcji włókna pokarmowego w dmianach dyni z gatunku *Cucurbita maxima* i *Cucurbita pepo* (Comparing the contents of dietary fiber fractions in some varieties of *Cucurbita maxima* and *Cucurbita pepo*). żywność: Nauka. Technologia. Jakość. **1**(56):65-73 (in Polish).
- <sup>27</sup>Nawirska-Olszańska, A. 2011. Przydatność owoców dyni jako surowca do przetwórstwa spożywczego. Monografie CXXXII. Wrocław 59 p. (in Polish).
- <sup>28</sup>Noor Aziah, A. A., Komathi, C. A. and Bhat, R. 2011. Evaluation of resistant starch in crackers incorporated with unpeeled and peeled pumpkin flour. Am. J. Food Technol. **6**(12):1054-1060.
- <sup>29</sup>Noor Aziah, A. A. and Komathi, C. A. 2009. Physicochemical and functional properties of peeled and unpeeled pumpkin flour. J. Food Scien. **74**(7):328-333.
- <sup>30</sup>Saha, P., Mazumder, U. K., Halder, P. K., Naskar, S., Kundu, S., Bala, A. and Kar, B. 2011. Anticancer activity of methanol extract of *Cucurbita maxima* against Ehrlich ascites carcinoma. Int. J. Res. Pharm. Sci. **2**(1):52-59.
- <sup>31</sup>Saha, B. C. 2003. Hemicellulose bioconversion. J. Ind. Mikrobiol. Biotechnol. **30**:279-291.
- <sup>32</sup>Šimić, D., Sudar, R., Ledencan, T., Jamnrovic, A., Zdunec, Z., Brkic, I. and Kovacevic, V. 2009. Genetic variation of bioavailable iron and zinc in grain of a maize population. Journal of Cereal Science **50**(3):392-397.
- <sup>33</sup>Subramanian, K., Ramsay, G., White, C., Hackett, A. and Broadley, M. 2010. Exploiting genetic variation for elevated mineral concentrations in potatoes. EUCARPIA, Wageningen pp. 89-92.
- <sup>34</sup>Van Soest, P. J., Robertson, J. B. and Lewis, B. A. 1991. Symposium: Carbohydrate methodology, metabolism, and nutritional implications in dairy cattle. J. Dairy Sci. **74**:3583-3597.
- <sup>35</sup>Yemm, E. W. and Willis, A. J. 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. **57**:508-514.