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Research Article

Study on the impact of pumpkin seed flour on mineral content of wheat bread

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Abstract

The incorporation of various flours from seeds into wheat bread can improve its nutritional value. Pumpkin seeds flour can be a good alternative for nutritional enrichment of food products because of its high content of protein, oil, dietary fibers and minerals. The aim of the present study was to investigate the effect of pumpkin seed flour (PSF), added in the amount of 5% and 10% by the weight of flour, on the content of minerals in wheat bread. It was found that wheat bread with pumpkin seed flour had a significantly higher levels of minerals as compared to the control; except for sodium. Moreover, the minerals content of bread was found to increase markedly with increasing the substitutions ratio. The most pronounced was the effect of enrichment on the content of iron - at the addition of 10% PSF it was 8.98 mg/kg, which is 6.7 times higher than the control sample (1.34 mg/kg). A considerable increase in the content of magnesium and phosphorus was also found. Regarding the microelements, the amount of zinc increased most significantly – 5.09 mg/kg in the control sample; 9.27 mg/kg into the sample with 5% PSF; 12.7 mg/kg in the bread with 10% PSF added.

Keywords: pumpkin seed flour, wheat bread, mineral content, microelements, macroelements

Abbreviations: PSF – Pumpkin seed flour; CX STAN – Codex standard; ISO – International organization for standardization; ICP-AES – Inductively coupled plasma atomic emission spectrometry; BSS EN – Bulgarian state standard European norm; ICP – Inductively coupled plasma; ANOVA – Analysis of variance; EMEA – European medicines agency

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Introduction

Bread is the staple food of the population in many regions of the world. Nowadays in some Western European countries its consumption is declining due to factors such as changing eating patterns and an increasing choice of substitutes such as breakfast cereals (Prättälä et al. 2001; Siega-Riz et al. 2000). On the other hand, there is an increase in its consumption even in countries where bread is not a traditional food. Dewettinck et al. (2008) point out that in general, bread provides important amounts of most nutrients, but processing may decrease the levels of the bioactive components in grains. Many studies considered the incorporation of various flour from seeds such as sunflower seed, sesame seed, grape seed and others (Akusu et al. 2020; Kaur et al. 2013; Mironeasa et al. 2012; Skrbic and Filipcev 2008) into wheat flour in order to improve nutritional value.

Recently, the pumpkin seeds and the partially defatted pumpkin seed, a by-product of pumpkin seeds oil processing, become more and more interesting for bakery sector, because of its nutritional composition. It's well known that in some European countries, pumpkin seeds are used as raw material for the production of pumpkin seed oil (Ayyildiz et al. 2019; Vujasinovic et al. 2010), while in other countries they are regarded as an agro-industrial waste (Amin et al. 2019; Pham et al. 2017). Moreover, in some countries they are consumed (Patel 2013) raw or roasted, or used as an additive to bread, salads etc. (Xanthopoulou et al. 2009) due to their beneficial phytochemical composition (Tlili et al. 2020).

Pumpkin seeds can be a good alternative for the nutritional enrichment of food products (Gorgonio et al. 2011). They are recognized as a good source of nutrients for fortification of baked products, especially bread (Dhiman et al. 2009; El-Soukkary 2001). According to Mironeasa et al. (2016), Codină et al. (2017) and Nyam et al. (2013) pumpkin seeds could be used for food application, because of its high content of protein (37.80–45.40%), oil (25.20–37.00%), dietary fibres (16.84–24.02%) and minerals (4.59%). Minerals are important for enzymatic activities and normal physiological functions in the human body. In addition to the essential nutrients (protein, fat, and carbohydrates), the body requires inorganic nutrients such as sodium, calcium, potassium, and phosphorus in

available form (Chung et al. 2013; Gibney et al. 2018). Most baked products made from wheat and other cereals are poor sources of essential nutrients (Alobo 2001). Different efforts have been made to promote the use of composite flours in which pumpkin seeds flour replaces a portion of wheat flour for production of bakery products. Pumpkin seeds can be processed into flour raw or after roasting. Some authors studied how this affects the nutritional value and the properties of the flour, used as additive in bakery products. It has been found that roasting of pumpkin seeds significantly improves total and extractable minerals as well as physico-chemical properties of the seed flour (Hamed et al. 2008). Among mineral elements, pumpkin seed contain especially phosphorus, magnesium and potassium (Alfawaz 2004; El-Adawy and Taha 2001), while other authors emphasize that they are known as good source of elements such as zinc (Glew et al. 2006), iron and magnesium (Lim 2012). Apostol et al. (2020) studied the mineral composition of wheat flour (used as a control sample) and of mixtures containing wheat flour and pumpkin seed flour in a ratio of 95%:5%, 90%:10% and 85%:15%. They pointed out that pumpkin seeds flour is a valuable source of minerals, especially iron, potassium, calcium and magnesium.

Pumpkin seed flour has been used for nutritional enrichment and for improving the rheological and sensory properties of different bakery products, including bread (Abdelghafor et al. 2011). Jukić et al. (2018) study the quality of biscuits produced from composite blends of pumpkin seed oil press cake and wheat flour. They concluded that pumpkin seed oil press cake flour can be successfully used as a functional and nutritionally valuable substitute for wheat flour, even in quantities up to 60%, without significant deterioration of the technological quality of biscuits. According to Jeevitha and Bhuvana (2019) the addition of pumpkin seed flour at 5%, 10% and 15% to the whole wheat flour improved bread properties. The authors found higher values of specific volume at 15% pumpkin seed flour addition. Proximate composition of bread samples showed higher crude protein content (12.22%).

Nowadays the enrichment of food products is an important tool to manage or prevent specific nutritional deficiencies. Thus, in order to meet the consumers' requirements, the enrichment of bread with different types of flour with higher content of minerals is an acceptable approach to increase daily

intake and to achieve consumption close to the recommended intake of minerals.

The aim of the present study was to investigate the effect of pumpkin seed flour (added in the amount of 5% and 10% by the weight of flour) on the content of minerals in wheat bread.

Materials and Methods

Materials

For the preparation of the bread samples, the following raw materials were used:

- commercial wheat flour (type 500) (average chemical composition: fat 0.90 g/100 g of which saturated 0.30 g; carbohydrates 70.30 g/100 g, of which sugars 3.40 g, fiber 4.00 g/100 g; protein 10.80 g/100 g);
- flour from cold-pressed pumpkin seed cake (average chemical composition: fat 10.00 g/100 g of which saturated 1.00 g; carbohydrates 34.00 g/100 g, of which sugars 0 g, fiber 16.00 g/100 g; protein 56.00 g/100 g). The manufacturer of the flour used is Balcho Agro Product, EOOD, Sofia;
- water – according to ISO 6107-1:2004;
- compressed yeast - supplied by Lesaffre Bulgaria Ltd;
- salt – according to Codex Standard for Food Grade Salt CX STAN 150-1985.

Methods

Preparation of dough and bread samples

Kneading was performed by a two-phase process of dough preparation to obtain a dough with a homogeneous mass and an initial temperature of 25 – 26 °C. First, knead the yeast (2.00 kg/ 100 kg flour), flour (control and experimental samples to obtain 100 g) and water (100 g) of dough in kneading machine (Labomix 1000, Hungary). The control sample was prepared only with wheat flour and the other bread samples tested were prepared with pumpkin seed (PS) flour replacing 5 % or 10 % of wheat flour. The dough thus prepared matured for 60 min at 33°C. Then the dough was mixed to obtain a homogeneous mass by adding the other ingredients (flour and water) according to the formulation and salt (1.33 kg/100 kg flour). The dough was left to rest for 20 min and was then divided into pieces of 440 g – pan bread. After

shaping, the dough was subjected to a final fermentation at 33 °C for 60 min in a fermenting chamber (Tecnopast CRN 45-12, Novacel Rovimpex Novaledo Trento, Italy). The dough was then baked in an electric floor oven Salva E-25 (Salva Industrial S.L.U., Lezo, Spain), preheated to a temperature of 220 – 230°C, for 22 – 24 min. After baking, the breads were allowed to cool for 3 h at room temperature (Chochkov et al. 2022).

The bread formulations are given in Table 1.

Table 1. Formulations of bread samples

Ingredients of bread recipe	Bread samples		
	Control sample of bread	Samples of bread with pumpkin seed flour (%)	
		5 %	10 %
Wheat flour, g	450.00	427.50	405.00
Water, ml	248.00	248.00	248.00
Yeast, g	9.00	9.00	9.00
Salt, g	6.00	6.00	6.00
Pumpkin seed flour, g	-	22.50	45.00

Mineral content determination

To determine the content of mineral elements a validated multistep analysis methodology was used using ICP-AES method. The atoms collide with energetically excited argon species and emit characteristic atomic and ionic spectra that are detected with a photomultiplier tube. Mineralization of the sample is performed according to BSS EN 13 805:2015 “Food products. Determination of trace elements. Digestion under pressure.” It includes ashing in a microwave system and dissolving the ash residue in HNO₃. The resulting solution is injected into the plasma. Wavelengths are given for each element as follows: Fe = 259.90 nm, K = 766.50 nm, Ca = 317.93 nm, Mg = 285.20 nm, Mn = 257.60 nm, Na = 589.60 nm, S = 181.00 nm, P = 213.82 nm, Zn = 213.86 nm, Cu = 327.40 nm, Ni = 232.00 nm. The concentration of the tested elements in the sample was determined by the calibration line method constructed using a standard solution Fluka 51844 Multielement standard solution 4 for ICP.

Statistical Analysis

All analyses were carried out in triplicate. The results are presented as an average mean ± standard

deviation. Statistical evaluation was performed by one-way analysis of variance (ANOVA) using Statgraphics Centurion statistical program (version XVI, 2009) (Stat Point Technologies, Ins., Warrenton, VA, USA). Mean differences were established by Fisher's least significant difference test for paired comparison with a significance level $\alpha = 0.05$.

Results and Discussion

In the present study, the content of seven mineral elements was analyzed: calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), iron (Fe), sulfur (S) and phosphorus (P), as well as an additional four trace elements: zinc (Zn), copper (Cu), manganese (Mn) and nickel (Ni).

The results concerning the effect of the pumpkin seed flour addition on the content of Fe, K, Ca, Mg, Na, S and P in bread are presented in Table 2.

Table 2. Mineral composition of wheat bread and bread containing PSF

Minerals	Mineral content of bread samples, mg/kg		
	Wheat bread (control sample)	Bread containing PSF in the amount of	
		5 %	10 %
Fe	1.34 ± 0.18^c	4.52 ± 0.64^b	8.98 ± 1.26^a
K	718.00 ± 50.77^b	1105.00 ± 78.14^a	1349.95 ± 95.39^a
Ca	108.00 ± 7.64^b	138.00 ± 9.76^{ab}	166.00 ± 11.74^a
Mg	158.00 ± 11.17^c	447.00 ± 31.61^b	652.00 ± 46.10^a
Na	3014.00 ± 213.12^a	2540.00 ± 179.61^{ab}	2203.00 ± 155.78^b
S	589.00 ± 41.64^b	934.00 ± 66.04^a	1037.00 ± 73.33^a
P	511.00 ± 36.13^c	1344.50 ± 94.40^b	1882.00 ± 133.08^a

PSF – pumpkin seed flour

* a-c Means in a row without a common letter differ significantly ($p < 0.05$).

The results in Table 2 show that bread prepared with pumpkin seed flour has a significantly higher levels of some minerals as compared to the control, such as Fe, K, Mg, S, P. Moreover, the minerals content of bread increased with increasing the substitutions ratio of flour.

The lowest iron content (1.34 mg/kg) was measured in the sample of bread prepared from wheat flour only. When 5% of PSF were added, iron content was 3.4 times higher. When the level of replacement of wheat flour with PSF was 10%, the iron content was about 9.00 mg/kg, which is 6.7 times higher than the control sample. Consumption of the usual amount of wheat bread per day satisfies only 3.05% of the recommended daily intake of iron (EFSA 2019), while the bread containing 10% PSF provides 20.41%. The importance of these results is connected with the fact, that iron deficiency adversely affects the physical growth of school-aged children. Iron deficiency anemia affects >1.2 billion individuals worldwide, and iron deficiency in the absence of anemia is even more frequent (Camaschella 2019). El-Ghany et al. (2010) reported that the iron content of pumpkin seeds is

9.76 mg/100 g. Increasing of iron content in biscuits supplemented with PSF in the amount of 5%, 10%, 15% and 20% on the basis of wheat flour is reported by Kanwal et al. (2015), although to a lesser extent. Probably it depends on how the PSF is obtained. Hamed et al. (2008) study the effect of roasting on the mineral composition of pumpkin seeds. Authors emphasize that Fe content significantly ($p < 0.05$) decreased when seeds are roasted – before roasting the iron content in pumpkin seeds is 23.97 mg/100g, and after roasting its amount decreases by 27.60%. Potassium in the control sample was 718.00 mg/kg. Supplementation of PSF (10%) causes almost twofold increase in its amount (1349.95 mg/kg). Our results are very close to the findings of El-Demery and Lotfy (2015). In bread containing 5% defatted PSF potassium content was 113.70 mg/100g, and at the addition of 10% PSF – 140.90 mg/100g. Other authors also pointed out the high content of potassium – Ike et al. (2020) found that pumpkin seed flour has almost 5 times higher potassium (994.10 mg/100g) than wheat flour (204.50 mg/100g).

Calcium is an important bone related macro element (Soetan et al. 2010). Calcium content in bread increased with increasing substitution of the added PSF, but not as significantly as iron and potassium. The sample with 10% pumpkin seed flour gave the highest value compared to the control – the increase was 34.90%. A similar increase in the content of calcium in bread (i. e. 35.9%) with the addition of 10% PSF was reported by El-Demery and Lotfy (2015).

Enriching bread with pumpkin seed flour leads to a significant change in magnesium content – in the 100% wheat flour bread it was 158.00 mg/kg. The inclusion of only 5% pumpkin seed flour in the bread recipe increased its amount 2.8 times, and when the degree of substitution with PSF was 10%, the magnesium content was more than 4 times higher than in bread of wheat flour. Research conducted by other authors (Apostol et al. 2020) came to the same conclusion – the enrichment of bread with pumpkin seed flour is accompanied by an increase in magnesium content. High amount of Ca, K and Mg have been reported to reduce blood pressure, so the consumption of bread enriched with pumpkin seed flour may serve this purpose.

Sodium content is shown to decrease with increased substitution of PSF, this is probably due to the higher content of Na in wheat flour. The same trend was found by Apostol et al. (2020) and Oyet and Chibor (2020) – with the increase in the amount of pumpkin seed flour added, the sodium content decreases, although to a lesser extent. Other authors published results that contradict ours. According to El-Demery and Lotfy (2015), the sodium content of wheat bread increases with the addition of PSF. Probably in this case the variety of pumpkins from which the seeds are obtained, the composition of

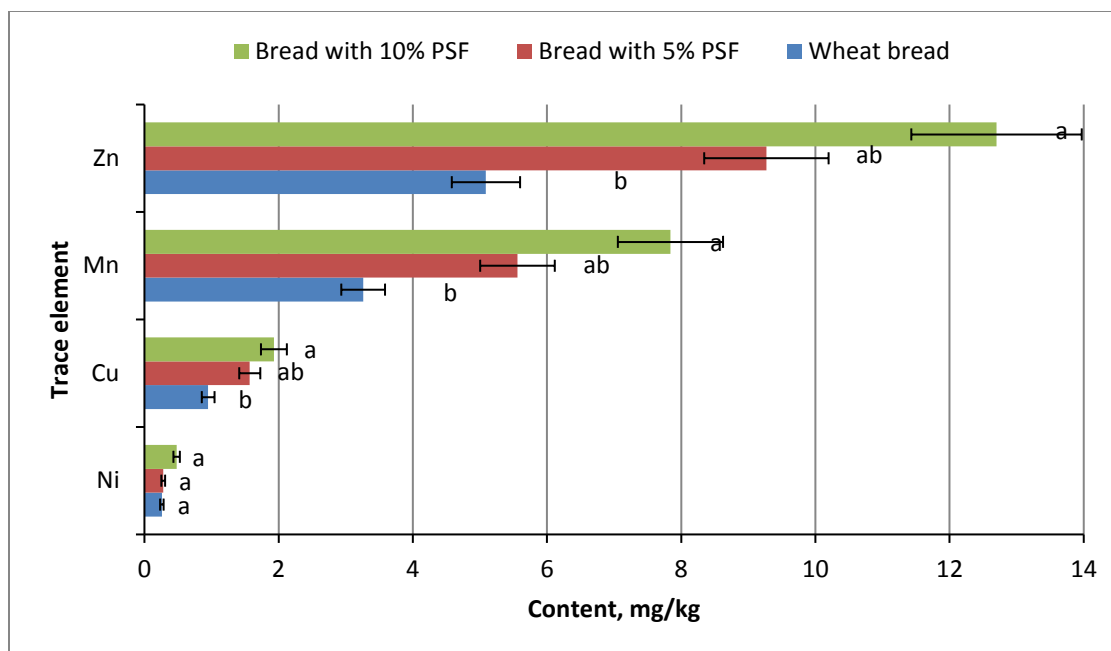
soil on which they are grown and other factors have an impact.

According to Samaha (2002), the seeds of pumpkin are rich in sulfur-containing amino acids. Therefore, the content of this element in wheat bread was significantly affected by PSF enrichment. With increased substitution of pumpkin seed flour sulphur content increased. The results range from 589.00 mg/kg to 1037.00 mg/kg, with sample with 10% PSF given the highest value followed by sample with 5% PSF and the control bread (made with wheat flour only).

The phosphorus content in the tested samples varied from 511.00 mg/kg (in wheat bread) to 1882.00 mg/kg (in bread enriched with 10% PSF). This means that the addition of 10% pumpkin seed flour to the bread recipe causes almost 4 times higher phosphorus content. The so obtained results are in accordance with data published by other authors (Dhiman et al. 2009). It was observed by Akintade et al. (2019) and Ertop et al. (2020) that processing methods in the production of flour showed greatest retention with respect to other minerals, while increases were noticed in phosphorous content – in flour from raw pumpkin seed (*Curcubita maxima*) phosphorus content was 42.66 mg/100 g, from fermented seeds – 58.73 mg/100 g, while in flour from roasted pumpkin seed – 51.34 mg/100 g. Enrichment with PSF makes bread a much better source of phosphorus – while the control sample provides 23.23% of the recommended daily intake, bread with 10% PSF provides 85.55%.

Considering the macronutrients K, Ca, Mg and P, the results from the present study reveal that PSF is a substantial source of these minerals.

The results concerning the effect of the addition of PSF on the content of Ni, Cu, Mn and Zn in wheat bread are presented in Fig. 1.



PSF – pumpkin seed flour

a-b Means with different lowercase letters for a specific trace element differ significantly ($p < 0.05$)

Figure 1. Content of Ni, Cu, Mn and Zn in wheat bread and bread containing PSF (5% and 10%)

As it can be seen from the figure, addition of 5% PSF did not affect nickel content of wheat bread, at the level of substitution of 10% less than half milligram per kilogram bread was measured. This metal ion was classified as a “possibly essential element” for animals and humans as early as 1970-s (Nielsen and Ollerich 1974). The importance of nickel as a beneficial element for human health, especially being essential for microorganisms that colonize the human guts, is examined by Zambelli and Ciurli (2013). Nickel requirements by humans have been estimated between 5.00 and 50.00 μg per day (EMEA 2008).

According to Karim (2018), copper is an essential trace element. Its deficiency is involved in the etiology of many diseases of gastrointestinal and neurological systems as well as affects function of heart, pancreas etc. Wheat bread does not contain a high amount of copper. The bread with 10% PSF had twice higher copper content (1.93 mg/kg) than the control sample and 18.6% higher content than bread with 5% PSF. Higher copper content in mixtures of wheat flour and partially defatted pumpkin seeds flour than in the wheat flour was reported also by Apostol et al. (2020).

The manganese content of wheat bread was also affected by the enrichment with PSF, and in this case its amount had a clear effect. The lowest content was reported in the control sample (3.26 mg/kg). The addition of 5% PSF led to increase of manganese content almost twofold. The bread with 10% PSF had 1.4 times higher quantity of manganese than those with 5% PSF. Similar results were reported by Apostol et al. (2020) – a composite flour, containing 95% wheat flour and 5% partially defatted pumpkin seeds has 2.26 times higher contents of manganese than wheat flour. Wheat bread enriched with 10% PSF satisfies 65.33% of the recommended daily intake of manganese. This is 2.4 times higher than control sample and 1.4 time higher than sample with 5% PSF.

Of all the studied trace elements in wheat bread, zinc had the highest amount – 5.09 mg/kg. Replacing part of the wheat flour with PSF had a very pronounced effect on the zinc content of bread – it was 9.27 mg/kg into the sample with 5% PSF added, which is almost twice as high as the control. In this case, the influence of the amount of the additive on the content of the studied element was well visible. When 10 % of the wheat flour were replaced with PSF Zn content was 12.70 mg/kg.

Oyet and Chibor (2020) pointed out that inadequate intakes of micronutrients (including Zn) have been associated with increased disease conditions and mental impairment. Kaur and Sharma (2018) investigated the content of zinc in wheat cakes enriched with flour from raw and roasted pumpkin seeds, replacing 10%, 20% and 30% of wheat flour. It was observed that pumpkin seed flour supplementation whether in raw or roasted form in bakery products is highly acceptable. Zinc content increased in the samples supplemented with raw or roasted pumpkin seed. Other authors also found an increase in zinc content when PSF was added to wheat flour (Ike et al. 2020).

Conclusion

The results from the present study show that wheat bread prepared with the addition of pumpkin seed flour has a significantly higher level of all minerals as compared to the control; except for sodium. Moreover, the minerals content of bread was found to increase markedly with increasing the substitutions ratio. The most pronounced is the effect of enrichment on the content of iron, magnesium and phosphorus. Regarding the microelements, the amount of zinc increases most significantly.

The inclusion of pumpkin seed flour in supplemented bakery products should be encouraged so as the enriched bread samples would contribute substantially to the recommended dietary requirements for minerals.

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References

Abdelghafor R., Mustafa A., Ibrahim A., Krishnan P. Quality of bread from composite Flour of Sorghum and Hard White Winter Wheat. *Advance Journal of Food Science and Technology*, 2011, 3(1): 9-15.
Akintade A., Awolu O., Ifesan B. Nutritional evaluation of fermented, germinated and roasted pumpkin

(Cucurbita maxima) seed flour. *Acta Universitatis Cibiniensis Series E: Food Technology*, 2019, 23(2): 179-186. <https://doi.org/10.2478/auft-2019-0021>
Akusu O., Kiin-Kabari D., Isah E. Anti-nutrients, Bioaccessibility and Mineral Balance of Cookies Produced from Processed Sesame Seed Flour Blends. *International Journal of Food Science and Nutrition Engineering*, 2020, 10(1):1-11 <https://doi.org/10.5923/j.food.20201001.01>
Alfawaz M. Chemical Composition and Oil Characteristics of Pumpkin (Cucurbita Maxima) Seed Kernels. *Food Science and Agriculture Research*, 2004, 129: 5–18.
Alobo A. Effect of sesame seed on the millet biscuit characteristics. *Plants Food Human Nutrition*, 2001, 56(2): 195–202. <https://doi.org/10.1023/A:1011168724195>
Amin M., Islam T., Uddin M., Uddin M., Rahman M., Satter M. Comparative study on nutrient contents in the different parts of indigenous and hybrid varieties of pumpkin (Cucurbita maxima Linn.). *Heliyon*, 2019, 5(9): e02462. <https://doi.org/10.1016/j.heliyon.2019.e02462>
Apostol L., Moşoiu C., Iorga S., Sanmartin A. Effect of the addition of pumpkin powder on the physicochemical qualities and rheological properties of wheat flour. *Romanian Biotechnological Letters*, 2020, 25(3): 1594-1600. <https://doi.org/10.25083/rbl/25.3/1594.1600>
Ayyildiz H., Topkafa M., Kara, H. Pumpkin (Cucurbita pepo L.) Seed Oil. In: Ramadan, M. (eds) *Fruit Oils: Chemistry and Functionality*. Springer, Cham. 2019. https://doi.org/10.1007/978-3-030-12473-1_41
Camaschella C. Iron deficiency. *Blood*, 2019, 133(1): 30–39. <https://doi.org/10.1182/blood-2018-05-815944>
Chochkov R., Savova-Stoyanova D., Papageorgiou M., Rocha JM., Gotcheva V., Angelov A. Effects of Teff-Based Sourdoughs on Dough Rheology and Gluten-Free Bread Quality. *Foods*. 2022;11(7):1012. <https://doi.org/10.3390/foods11071012>
Chung K., Shin K., Hwang H., Choi K. Chemical composition of nuts and seeds sold in Korea. *Nutrition Resource Practice*, 2013, 7(2): 82-88. <https://doi.org/10.4162/nrp.2013.7.2.82>
Codină G., Atudorei D., Cimpoi A., Mironeasa S., Todosi-Sănduleac E. Quality evaluation of wheat-pumpkin-golden flaxseed composite bread. *Food and Environment Safety Journal*, 2017, 16(2): 61-70
Dewettinck K., Van Bockstaele F., Kühne B., Van de Walle D., Courtens T., Gellynck X. Nutritional value of bread: Influence of processing, food interaction and consumer perception, *Journal of Cereal Science*, 2008, 48(2): 243-257. <https://doi.org/10.1016/j.jcs.2008.01.003>

- Dhiman A., Sharma K., Attri S. Functional constituents and processing of pumpkin: A review. *Journal of Food Science and Technology*, 2009, 46(5): 411-417.
- EFSA. Dietary Reference Values for Nutrients Summary report, 2019. Available at: <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/sp.efsa.2017.e15121>
- El-Adawy T., Taha K. Characteristics and composition of different seeds oils and flours. *Food Chemistry*, 2001, 74(1): 47-54. [https://doi.org/10.1016/S0308-8146\(00\)00337-X](https://doi.org/10.1016/S0308-8146(00)00337-X)
- El-Demery M., Lotfy L. Quality characteristics of pan bread enriched with defatted germinated pumpkin seed flour. *Alexandria Journal of Food Science and Technology*, 2015, 12(1): 29-37. <https://doi.org/10.12816/0025354>
- El-Ghany A., Dalia A., Soha M. Biological study on the effect of pumpkin seeds and zinc on reproductive potential of male rats. The 5th Arab and 2nd International Annual Scientific Conference on: Recent trends of developing institutional and academic performance in higher specific education institutions in Egypt and Arab world, Mansoura University, Egypt, 2010, 2384-2404.
- El-Soukkary F. Evaluation of pumpkin seed products for bread fortification. *Plant Foods for Human Nutrition*, 2001, 56(4): 365-384. <https://doi.org/10.1023/A:1011802014770>
- EMEA. Guideline on the Specification Limits for Residues of Metal Catalysts or Metal Reagents. European Medicines Agency, 2008. Available at: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-specification-limits-residues-metal-catalysts-metal-reagents_en.pdf
- Ertop M., Bektaş M., Atasoy R. Effect of cereals milling on the contents of phytic acid and digestibility of minerals and protein. *Ukrainian Food Journal*, 2020, 9(1): 136-147. <https://doi.org/10.24263/2304-974X-2020-9-1-12>
- Gibney M., Barr S., Bellisle F., Drewnowski A., Fagt S., Livingstone B., Masset G., Varela Moreiras G., Moreno L., Smith J., Vieux F., Thielecke F., Hopkins S. Breakfast in Human Nutrition: The International Breakfast Research Initiative. *Nutrients*. 2018-1;10 (5):559. <https://doi.org/10.3390/nu10050559>
- Glew R., Glew R., Chuang L., Huang Y., Millson M., Constans D., Vanderjagt D. Amino acid, mineral and fatty acid content of pumpkin seeds (*Cucurbita* spp) and *Cyperus esculentus* nuts in the Republic of Niger. *Plant Foods Human Nutrition*, 2006, 61(2): 51-56. <https://doi.org/10.1007/s11130-006-0010-z>
- Gorgonio C., Pumar M., Mothe C. Macroscopic and physiochemical characterization of a sugarless and gluten-free cake enriched with fibers made from pumpkin seed (*Cucurbita maxima*. L.) flour and cornstarch. *Food Science and Technology* (Campinas), 2011, 31(1): 109-118. <https://doi.org/10.1590/S0101-20612011000100015>
- Hamed S., El Hassan N., Hassan A., Eltayeb M., Babiker E. Nutritional evaluation and physiochemical properties of processed pumpkin (*Telfairia occidentalis* hook) seed flour. *Pakistan Journal of Nutrition*, 2008, 7(2): 330-334. <https://doi.org/10.3923/pjn.2008.330.334>
- Ike C., Emeka-Ike P., Ogwuegbu H. Nutritional and microbiological qualities of pumpkin (*Cucurbita pepo*) seed composite flours. *GSC Biological and Pharmaceutical Sciences*, 2020, 12(03): 051-060.
- Jeevitha G., Bhuvana S. Study on the impact of pumpkin seed flour on whole wheat bread characteristics. *International Journal of Chemical Studies*, 2019, 7(4): 273-277.
- Jukić M., Lukinac J., Čuljak J., Pavlović M., Šubarić D., Koceva Komlenić D. Quality evaluation of biscuits produced from composite blends of pumpkin seed oil press cake and wheat flour. *International journal of food science & technology*, 2019, 54(3): 602-609. <https://doi.org/10.1111/ijfs.13838>
- Kanwal S., Raza S., Naseem K., Amjad M., Bibi N., Gillani M. Development, physico-chemical and sensory properties of biscuits supplemented with pumpkin seeds to combat childhood malnutrition in Pakistan. *Pakistan Journal of Agricultural Research*, 2015, 28(4): 400-405.
- Karim N. Copper and Human Health - A Review. *Journal of Bahria University Medical and Dental College*, 2018, 8(2): 117-122. <https://doi.org/10.51985/JBUMDC2018046>
- Kaur M., Sharma S. Development and nutritional evaluation of cake supplemented with pumpkin seed flour. *Asian Journal of Dairy and Food Research*, 2018, 37(3): 232-236. <https://doi.org/10.18805/ajdr.DR-1310>
- Kaur A., Sandhu V., Sandhu K. Effects of flaxseed addition on sensory and baking quality of whole wheat bread. *International Journal of Food Nutrition and Safety*, 2013, 4(1): 43-54.
- Lim T. K. *Edible Medicinal and Non-Medicinal Plants*. Springer Dordrecht Heidelberg, New York, 2012, pp. 272-76. ISBN 978-94-007-1763-3, e-ISBN 978-94-007-1764-0. Available at: http://eprints.stiperdharmawacana.ac.id/120/1/%5BT_K_Lim%5D_Edible_medicinal_and_non-medicinal_pla%28BookFi%29.pdf. DOI 10.1007/978-94-007-1764-0
- Mironeasa S., Codină G., Mironeasa C. Effects of the pumpkin seed addition on bread quality of wheat flour with a very good quality for bread making. In: *Modern Technologies in the Food Industry. Proceeding of the International Conference*, 2016

- October 20-22, Chişinău. pp. 229-237. ISBN 978-9975-87-138-9
- Mironeasa S., Codina G., Mironeasa C. The effects of wheat flour substitution with grape seed flour on the rheological parameters of the dough assessed by Mixolab. *Journal of Texture Studies*, 2012, 43(1): 40-48. <https://doi.org/10.1111/j.1745-4603.2011.00315.x>
- Nielsen F., Ollerich D. Proceedings: Nickel: a new essential trace element. *Federation Proceeding*, 1974, 33(6): 1767-1772.
- Nyam K., Lau M., Tan C. Fibre from pumpkin (*Cucurbita pepo* L.) seeds and rinds: physicochemical properties, antioxidant capacity and application as bakery product ingredients. *Malaysian Journal of Nutrition*, 2013, 19(1): 99-109.
- Oyet G., Chibor B. Amino Acid Profile, Mineral Bioavailability, and Sensory Properties of Biscuits Produced from Composite Blends of Wheat, Coconut and Defatted Fluted Pumpkin Seed Flour. *European Journal of Agriculture and Food Sciences*, 2020, 2(6): 1-7. <https://doi.org/10.24018/ejfood.2020.2.6.169>
- Patel S. Pumpkin (*Cucurbita* sp.) seeds as nutraceutic: A review on status quo and scopes. *Mediterranean Journal of Nutrition and Metabolism*, 2013, 6: 183–189. <https://doi.org/10.1007/s12349-013-0131-5>
- Pham T., Tran T., Ton N., Le V. Effects of pH and salt concentration on functional properties of pumpkin seed protein fractions. *Journal of Food Processing and Preservation*, 2017, 41 (4): e13073. <https://doi.org/10.1111/jfpp.13073>
- Prättälä R., Helasoja V., Mykkanen H. The consumption of rye bread and white bread as dimensions of health lifestyles in Finland. *Public Health Nutrition*, 2001, 4(3): 813-819. <https://doi.org/10.1079/PHN2000120>
- Samaha O. Evaluation of pumpkin fruits as promising crop in food processing. *Alexandria J Agric Res*, 2002, 47:117-125.
- Siege-Riz A., Popkin B., Carson T. Differences in food patterns at breakfast by sociodemographic characteristics among a nationally representative sample of adults in the United States. *Preventive Medicine*, 2000, 30(5): 415-424. <https://doi.org/10.1006/pmed.2000.0651>
- Skrbic B., Filipcev B. Nutritional and sensory evaluation of wheat breads supplemented with oleic-rich sunflower seed. *Food Chemistry*, 2008, 108(1): 119-129. <https://doi.org/10.1016/j.foodchem.2007.10.052>
- Soetan K., Olaiya C., Oyewole O. The importance of mineral elements for humans, domestic animals and plants: A review. *African Journal of Food Science*, 2010, 4(5): 200-222. <https://doi.org/10.5897/AJFS.9000287>
- Tlili, I. et al. Pumpkin. In: Nayik, G.A., Gull, A. (eds) *Antioxidants in Vegetables and Nuts - Properties and Health Benefits*. Springer, Singapore. 2020. https://doi.org/10.1007/978-981-15-7470-2_5
- Vujasinovic V., Djilas S., Dimic E., Romanic R., Takaci A. Shelf life of cold-pressed pumpkin (*Cucurbita pepo* L.) seed oil obtained with a screw press. *Journal of the American Oil Chemists Society*, 2010, 87(12): 1497–1505. <https://doi.org/10.1007/s11746-010-1630-x>
- Xanthopoulou M., Nomikos T., Fragopoulou E., Antonopoulou S. Antioxidant and lipoxigenase inhibitory activities of pumpkin seed extracts. *Food Research International*, 2009, 42(5-6): 641–646. <https://doi.org/10.1016/j.foodres.2009.02.003>
- Zambelli B., Ciurli S. Nickel and Human Health. In: *Interrelations between Essential Metal Ions and Human Diseases. Metal Ions in Life Sciences* (A. Sigel, H. Sigel, R. Sigel Eds). Springer, Dordrecht, 2013, 13, 573 pages. Print ISBN: 978-94-024-0271-1, eBook ISBN: 978-94-007-7500-8, <https://doi.org/10.1007/978-94-007-7500-8>