CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

TOTAL PHENOLIC AND FLAVONOID CONTENT, AND ANTIOXIDANT ACTIVITY OF WHEAT BREAD ENRICHED WITH PUMPKIN, CHESTNUT AND ROSEHIP FLOUR

Petya Ivanova[1*], Rosen Chochkov[1], Denka Zlateva[2], Dana Stefanova[2]

[1] *University of Food Technologies, Plovdiv*
[2] *University of Economics, Varna*
**petia_ivanova_georgieva@abv.bg*

https://doi.org/10.34302/crpjfst/2023.15.2.4

Article history:
Received:
18 February 2022
Accepted
18 February 2023

Keywords:
Bread;
Phenols;
Flavonoids;
DPPH;
FRAP.

ABSTRACT

Pumpkin, chestnut, and rosehip are rich in fibres, vitamins, mineral and antioxidant substances, but their use in bread making is not widespread yet. The objective of this study was to evaluate the effect of pumpkin, chestnut, and rosehip flour enrichment on the antioxidant activity, total phenolic and flavonoids contents of bread. Breads were made by partially replacing of wheat flour with pumpkin, chestnut, and rosehip flour at the level of 5, 10, and 15%. Total phenolic and flavonoid content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and ferric reducing antioxidant power were examined to determine the content of ethanol extractable bioactive compounds. The results of all studies showed the highest activity and content of phenols and flavonoids in rosehip flour and bread obtained with it, and the lowest in the control sample (without additive). In all three studied supplements, with increasing their concentration, the amount of bioactive substances increases.

1.Introduction

In recent years, public attention has increasingly focused on the search for and consumption of healthy foods that reduce the incidence and spread of diseases such as diabetes, cardiovascular disease, obesity. Trends in the food industry are constantly changing to meet consumer demand. There is a tendency to use natural substances in the food as a source of antioxidant and functional ingredients (Meral and Köse, 2019).

Antioxidants eliminate free radicals in the cell and in food and are widely used in dietary formulations (Andrade *et al*., 2015). Polyphenols are natural phytochemicals with antioxidant properties. Flavonoids are phenolic substances isolated from a wide range of plants, where they act as antioxidants. When such substances are added to foods, they prolong their shelf-life. According to Wolfe *et al*. (2003), plant phenols prevent oxidation and maintain food product quality. Bread is one of the most popular foods in the world and is an important part of people's diets. The white bread is defined by a large part of the population as a good source of energy and irreplaceable food (Skrbic and Filipcev, 2008). However, people's increased awareness of the nutritional deficiencies of white bread is growing and this is the reason for looking for substitutes for commercial wheat flour. Besides white bread made from refined wheat flour is a food with a low antioxidant capacity (Meral and Köse, 2019) and low nutritional value (Ibidapo *et al*., 2020). The health properties of bread can be improved by enriching it with flour rich in bioactive components.

Pumpkin flour (PF) contains phenolic acids, flavonoids, vitamin C, vitamin E, bilirubin, albumin and β-carotene that can act as antioxidants in the human body (Wahyono *et al*., 2020). Generally, pumpkin flour is used to produce desserts as improved their yellow color and β-carotene content (Pongjanta *et al*., 2006). According to Dabash *et al*. (2017), pumpkin flour has a longer shelf-life and can be used in the manufacture of formulated foods. Pumpkin flour exhibited high levels of carbohydrate (79.57 %), starch (48.30 %), dietary fiber (12.1%) protein (7.81%) and total ash (5.29 %); low contents of lipid (3.60%) and crude fiber (3.65 %) (Saeleaw and Schleining, 2011).

Chestnut flour (CF) is a rich source of bioactive compounds that have high free radical scavenging properties being associated to protective effects against many diseases. The chemical composition of chestnut flour is similar to that of many other cereals (Aponte *et al*., 2014), which is why there is a growing interest in its use in the manufacture of bakery products. The composition of the flour includes: high quality proteins with essential amino acids (4–7%), relatively high amount of sugar (20–32%), starch (50–60%), dietary fiber (4–10%), and low amount of fat (2–4%) (Demirkesen *et al*., 2010). Other important components of flour are vitamin E, vitamin B group, potassium, phosphorous, and magnesium (Sacchetti *et al*., 2004; Chenlo *et al*., 2007). There is insufficient data in the literature on the use of CF in bread recipes.

Rosehip flour (RF) is characterized by a high content of polyphenols, and has a high antioxidant activity (Ghendov-Mosanu *et al*., 2020). Common food obtained from rose hips are juice, wine, tea, jelly, marmalade, and syrup (Igual *et al*., 2021) and being rich in vitamin C, rosehip powder is successfully used as a health supplement (Larsen *et al*., 2003). There is no information in the literature on the use of RF in the production of bakery products. The only application that is detected is the use of the pomace resulting from juice production.

The objective of this study was to evaluate the antioxidant properties of wheat bread with the addition of pumpkin, chestnut, and rosehip flour (in the amount of 5%, 10% or 15% by the weight of flour).

2. Materials and methods

2.1. Materials

2.1.1. Raw materials

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

− commercial wheat flour (type 500) (average chemical composition: fat 0.9g/100 g of which saturated 0.3 g; carbohydrates 70.3 g/100 g, of which sugars 3.4 g, fiber 4.0 g/100 g; protein 10.8 g/100 g);

− pumpkin flour from defatted peeled pumpkin seeds (average chemical composition: fat 10g/100 g of which saturated 1 g; carbohydrates 34 g/100 g, of which sugars 0 g, fiber 16 g/100 g; protein 56 g/100 g);

− chestnut flour (average chemical composition: fat 3.7g/100 g of which saturated 0.7 g; carbohydrates 70.9 g/100 g, of which sugars 29.5 g, fiber 10.8 g/100 g; protein 6.4 g/100 g);

− rosehip flour (average chemical composition: fat 0 g/100 g of which saturated 0 g; carbohydrates 38 g/100 g, of which sugars 3 g, fiber 24 g/100 g; protein 2 g/ 100 g);

− water – according to ISO 6107-1:2004;

− commercial yeast (Lesafmaya);

− salt – according to Codex Standard for Food Grade Salt CX STAN 150-1985.

All chemicals used were of analytical grade and were obtained from Merck KGaA (Darmstadt, Germany) via Fillab (Plovdiv, Bulgaria).

2.2. Methods

2.2.1. Preparation of dough and bread samples

Bread was obtained by a two-phase method. Initially, knead the yeast, flour (control and experimental samples to obtain 100 g) and water of dough in a 1:1 ratio in kneading machine (Labomix 1000, Hungary). The dough thus prepared matured for 60 min at 33 °C and then was mixed to obtain a homogeneous mass by adding the remainder of the flour according to the formulation and salt (1.33 kg/100 kg flour). The bread dough was divided into pieces of a certain weight (440 g) and was formed, matured for 55 minutes at 36 °C (Tecnopast CRN 45–12, Novacel ROVIMPEX Novaledo, Italy). After the final fermentation, the pieces of dough were put into an electric oven (Salva E-25, Spain) pre-heated to 220–230 °C. The baking time was 24 min. After baking, the bread was allowed to cool down for 3 h at room temperature.

The details of bread formulations are given in Table 1.

2.2.2. Extraction of bioactive compounds

Before the extraction, breads were sliced (about 1.5 cm thick), dried (40 °C, 24 h), ground in a mill, and sieved (0.5 mm sieve). After that the extraction of bioactive compounds was carried out with 70% ethanol (solid to liquid ratio 1:20) in an ultrasonic bath (VWR, Malaysia; 45 kHz, 30 W) at 45 °C for 15 min according to Vasileva *et al.* (2018). Solid remnants of bread were removed by centrifugation at 1800xg for 15 min (MPW-251, Med. Instruments, Poland) and obtained supernatants were used for further studies.

2.2.3. Analytical methods

Folin-Ciocalteu's reagent was used to determine of total polyphenols (Ainsworth and Gillespie, 2007) and the results were expressed as mg equivalents gallic acid (GAE) per gram dry weight (DW). Total flavonoids were determined using $Al(NO_3)_3$ reagent (Kivrak *et al.*, 2019) and the results were expressed as mg equivalents quercetin (QE) per gram DW.

2.2.4. In vitro determination of antioxidant activity

DPPH radical scavenging activity was estimated according to Dimov *et al.* (2018) with some modification – in a test tube were mixed 0.15 ml of ethanolic extract with 2.85 ml 0.06 mM DPPH (dissolved in 96% ethanol; freshly prepared). After 30 minutes in the dark at room temperature, the absorbance at 517 nm was read by spectrophotometer and the results were compared with the blank containing 70% ethanol (without sample addition). The results of DPPH analysis were expressed as mM Trolox equivalents (TE) per gram DW.

FRAP method – Ferric Reducing Antioxidant Power was measured according to the method of Dimov *et al.* (2018) with modifications – three ml FRAP reagent (freshly prepared) were mixed with 0.1 ml of the extracted sample. After incubation for 10 min at 37 °C (in the darkness), the absorbance was measured at 593 nm against blank prepared with 70% ethanol (without sample addition). A standard curve was built with $FeSO_4.7H_2O$. The results of FRAP analysis were expressed as µmol Fe^{2+} equivalents per gram DW.

Table 1. The formulations of breads (% on the flour basis)

Ingredients	**Bread samples**									
	Control	**with PF (%)**			**with CF (%)**			**with RF (%)**		
		5	10	15	5	10	15	5	10	15
WF, g	450	427.5	405.0	382.5	427.5	405.0	382.5	427.5	405.0	382.5
Water, ml	248	248	248	248	248	248	248	248	248	248
Yeast, g	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Salt, g	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
PF, g	-	22.5	45.0	67.5	-	-	-	-	-	-
CF, g	-	-	-	-	22.5	45.0	67.5	-	-	-
RF, g	-	-	-	-	-	-	-	22.5	45.0	67.5

WF – wheat flour, PF – pumpkin flour, CF – chestnut flour, RF – rosehip flour.

2.2.5. Data analysis

The tests were conducted with three replications. Data were analyzed by one-way analysis of variance (ANOVA) using Statgraphics Centurion statistical program (version XVI, 2009) (Stat Point Technologies, Ins., Warrenton, VA, USA). To compare the means, Fisher's least significant difference test was used for paired comparison with a significance level $\alpha = 0.05$.

3.Results and discussions

3.1. Determination of bioactive substances in different flours

The results of all analyzes of the flours are summarized in Table 2. Total flavonoid content ranged from 0.15 to 1.77 mg QE/g DW, as the highest value was registered at RF, while the lowest value, as expected, showed the control commercial wheat flour. From the studied 3 types of substitute flours the lowest content of total flavonoids showed PF (0.27 mg QE/g DW). These results show that it is likely that in the course of defatting of pumpkin seeds, treatment with organic solvents also affected the content of bioactive substances. The results obtained by us for the RF were similar to the data from the literature on the high content of common flavonoids. Igual *et al*. (2021) found 0.43 g/100 g DW total flavonoid content in rosehip pomace.

Table 2. Total flavonoids and phenols, and antioxidant activity of ethanolic extracts from flours

Flour sample	Total flavonoids, mg QE/g DW	Total phenols, mg GAE/g DW	DPPH, mM TE/g DW	FRAP, µmol Fe^{2+}/g DW
WF	0.15±0.00^{d}	0.98±0.03^{d}	0.35±0.03^{d}	2.38±0.05^{d}
CF	1.04±0.00^{b}	1.99±0.06^{b}	7.25±0.03^{b}	25.63±0.07^{b}
PF	0.27±0.01^{c}	1.25±0.05^{c}	2.41±0.23^{c}	10.43±0.20^{c}
RF	1.77±0.00^{a}	9.56±0.04^{a}	8.37±0.01^{a}	57.76±0.00^{a}

$^{a-d}$: Means in a column without a common letter differ significantly ($p < 0.05$).
Results were calculated on a dry matter basis (DW): 87.49% ± 0.04 for WF, 92.12% ± 0.17 for CF, 92.95% ± 0.02 for PF, and 88.59% ± 0.06 for RF.

The polyphenol content of the flour follows the same order as the results for flavonoids, in increasing order, as follows: WF, PF, CF, RF (0.98-9.56 mg GAE/g DW). The results obtained by us for wheat flour were lower than those of Zhang *et al*. (2021) but probably the duration of storage of the flour and the extraction conditions are the reason for these differences. The values of total phenols in PF were again lower than those reported by another team of authors, according to which total polyphenols in pumpkin seed flour are in the range 2.44–3.82 mg GAE/g (Peng *et al*., 2021). The most significant source of phenols from the three substitute flours studied is RF (9.56 mg GAE/g DW) in which the amount of phenols was almost 10 times higher than the control sample of flour (0.98 mg GAE/g DW).

The data from DPPH and FRAP methods follow the same trend as those for total phenols and flavonoids. Again, the highest values of the studied 3 additives were measured for RF as in comparison with the control the increase was by 24 times in both methods – from 0.35 to 8.37 mM TE/g DW and from 2.38 to 57.76 µmol Fe^{2+}/g DW, respectively. Huang *et al*. (2015) also reported that the antioxidant activity is directly proportional to the total phenol. As a result, the higher was the phenolic content in a compound the higher was its antioxidant activity.

3.2. Determination of bioactive substances in breads

As can be seen from Table 3, an increase ($p < 0.05$) in total phenols and flavonoids, and DPPH and FRAP values was observed with the addition of substitute flours to the formulations. In our research, bread containing CF, PF and RF showed in most of the cases higher values in comparison with the control bread. However, in general, the amount of bioactive substances was significantly lower than the reported values in flour (Table 2). A similar decrease in *in vitro* antioxidant activity in the production of bread with the addition of herbs has been reported by another authors (Dimov *et al.*, 2018). Probably, the heat treatment during the baking of the bread damages or destroys the antioxidant compounds presented in different flours. According to Chlopicka *et al*. (2012), during thermal processes, the antioxidants are subject to modifications.

Table 3. Total flavonoid and phenol contents of ethanolic extract from bread samples

Bread sample	Total flavonoids, mg QE/g DW	Total phenols, mg GAE/g DW
Control	0.12 ± 0.00^{g}	0.55 ± 0.03^{h}
CF5	$0.14\pm0.00^{f,B}$	$0.96\pm0.03^{f,C}$
CF10	$0.16\pm0.00^{cd,A}$	$1.06\pm0.02^{e,B}$
CF15	$0.16\pm0.01^{bc,A}$	$1.28\pm0.04d^{,A}$
PF5	$0.15\pm0.00^{de,B}$	$0.32\pm0.01^{i,C}$
PF10	$0.16\pm0.00^{bc,A}$	$0.80\pm0.06^{g,B}$
PF15	$0.16\pm0.00^{b,A}$	$1.09\pm0.02^{e,A}$
RF5	$0.15\pm0.00^{e,C}$	$1.49\pm0.03^{c,C}$
RF10	$0.16\pm0.00^{bc,B}$	$1.95\pm0.02^{b,B}$
RF15	$0.17\pm0.00^{a,A}$	$2.86\pm0.03^{a,A}$

$^{a-i}$: Means in a column without a common letter differ significantly ($p < 0.05$).
$^{A-C}$: Means of the three samples (in a column) with various concentrations (5, 10 and 15 %) for a certain type of bread without a common letter differ significantly ($p < 0.05$).

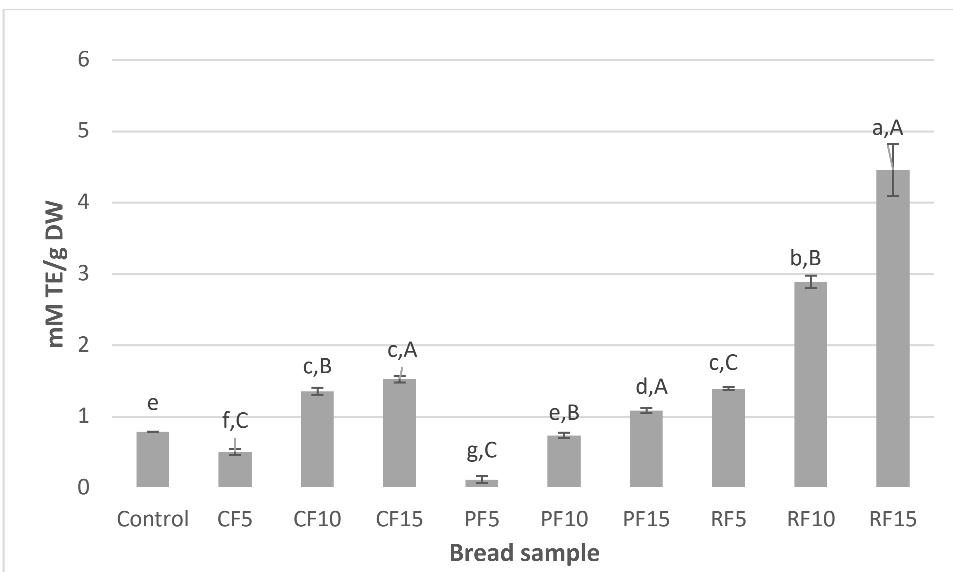

Figure 1. DPPH radical scavenging activity of ethanolic extracts from bread samples

$^{a-e}$: Means without a common letter differ significantly ($p < 0.05$).
$^{A-C}$: Means of the three samples with various concentrations (5, 10 and 15 %) for a certain type of bread without a common letter differ significantly ($p < 0.05$).

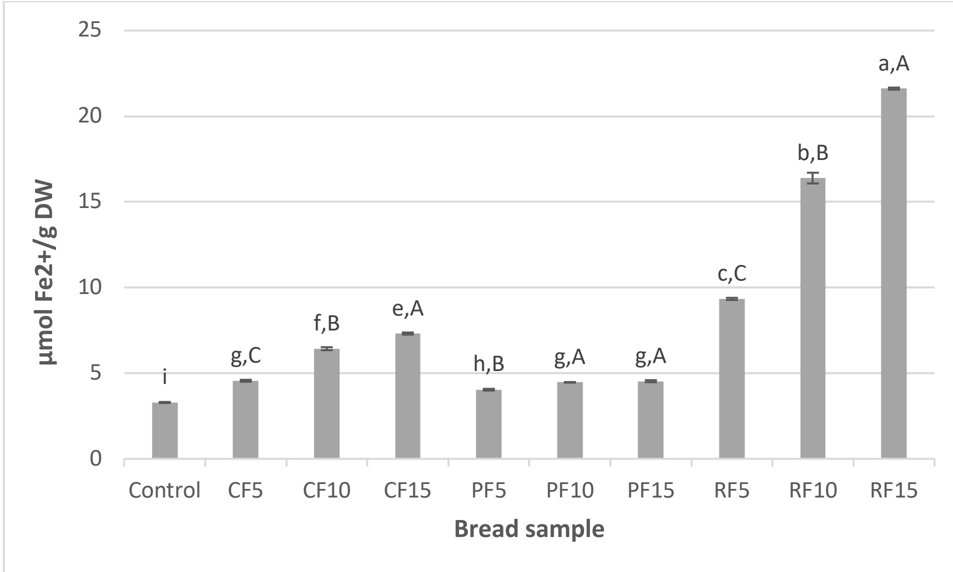

Figure 2. Ferric reducing antioxidant power of ethanolic extracts from bread samples

a-i: Means without a common letter differ significantly ($p < 0.05$).

A-C: Means of the three samples with various concentrations (5, 10 and 15 %) for a certain type of bread without a common letter differ significantly ($p < 0.05$).

Results in Table 3, and Figures 1 and 2 were calculated on a dry matter basis (DW): Control – 95.14% ± 0.57, CF5 – 95.63% ± 0.22, CF10 – 96.10% ± 0.13, CF15 – 96.54% ± 0.46, PF5 – 96.06% ± 0.25, PF10 – 95.63% ± 0.08, PF15 – 95.95% ± 0.25, RF5 – 95.77% ± 0.13, RF10 – 95.94% ± 0.14, RF15 – 96.20% ± 0.04.

The amount of total flavonoids in the breads was in the range 0.12-0.17 mg QE/g DW and that of total phenols was 0.55-2.86 mg GAE/g DW. The results demonstrated that these contents also raised with the increase in the level of substitute flours in breads. The highest total polyphenol content was found in a bread sample with the addition of 15% of RF, while the lowest one was seen in bread without additive. The percentage increase in bioactive substances in breads was not as noticeable as in flour. For example, the lowest results were observed for flavonoids, where the greatest increase compared to the control was less than once. In studies by other authors, flavonoids in bread were not even registered. For example, Petkova *et al*. (2018) found 45.6 mg QE/100 g total flavonoids in flour mixture, but any flavonoids were not detected in bread samples prepared from the mixture. This due to the greater sensitivity of flavonoids at high baking temperatures, because most bioactive compounds become unstable when exposed to heat. The extractability of the bioactive components after baking the bread at high temperature decreased, which was associated with the formation of complexes between polyphenols (e.g. flavonoids) with proteins and starch. According to Zhang *et al*. (2011), the starch interacts with quercetin, which reduces the digestibility of starch, as these interactions affect the bioactivity of antioxidants. Antioxidant activity of bread enriched with CF, PF and RF are shown in Figures 1 and 2. In Fig. 1, antioxidant activity of bread measured by the ability of the test sample to scavenge DPPH radical. From the three studied concentrations of added flours, the highest values were observed at 15% concentration. Rosehip flour addition significantly affected ($p < 0.05$) antioxidant activity (DPPH) of enriched bread (4.46 mM TE/g DW and 21.61 µmol Fe^{2+}/g DW). From the data in Fig. 1 interesting impression was made by the use of 5% supplement of CF and PF – DPPH values were lower compared to the control bread. Probably, that is due to the insufficient amount of imported substitute flour since wheat flour was dominant in the mixture. Wahyono *et al*. (2020) also established that increased pumpkin powder concurrently increased antioxidant activity of resulting bread. The highest values were measured when using the highest percentage of added flour (20%) and the control has the lowest antioxidant activity.

Of interest is the not so big difference in the increase of the value in the FRAP method, when 5, 10 and 15% PF were used as an additive. When adding this flour in a concentration of 5%, the value was 4.03 µmol Fe^{2+}/g DW, at 10% - 4.46 µmol Fe^{2+}/g DW and at 15% - 4.53 µmol Fe^{2+}/g DW, as between 10 and 15% there was no statistically significant difference in the results obtained by the FRAP method. When using CF, the increase in FRAP units with an increase in the concentration of the additive compared to the control was 1.39, 1.95 and 2.21 times, respectively. However, when RF is added in the same concentrations, the increasement was with 2.84, 4.81, and 6.57 times, respectively. Irakli *et al*. (2015) replaced wheat flour with rice bran in the bread recipe resulted in breads with a significantly increased total antioxidant activity. The authors found that with the increase of the level of rice bran from 10 to 30%, total antioxidant activity increased 2 to 5 times compared to the control. Increase in antioxidant activity within the same limits reported and Selimović *et al*. (2014) when replacing wheat flour with buckwheat flour. The inclusion of 15-40% buckwheat flour led to an increase in antioxidant activity from 2 to 5 times. According to our findings, chestnut flour, pumpkin flour, and especially rosehip flour are good sources of phenols and possessed good antioxidant activity.

4. Conclusions

The addition of CF, PF and RF increased phenolic compounds, flavonoids and antioxidant activity when compared to the control bread. Their quantity increased with the increasing of the level of the substitute flour. The highest content of total phenols and flavonoids, and the highest antioxidant activity, measured by DPPH and FRAP methods, showed RF and the breads obtained from it, while the lowest were the results for PF and the breads obtained from its use. As the results were higher than those of the control, these flours have proven to be an effective ingredient to enrich bread, which can improve its taste, aroma and shelf-life.

5. References

Ainsworth, E.A. and Gillespie, K.M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nature Protocols*, 2, 875–877.

Andrade, S.C., Oliveira, S.F., Guiné, R.P.F., Correia, P.M.R. (2015). Determination of some chemical properties of uncommon flours. World Academy of Science, Engineering and Technology. *International Journal of Nutrition and Food Engineering*, 9(4).

Aponte, M., Boscaino, F., Sorrentino, A., Coppola, R., Masi, P., Romano, A. (2014). Effects of fermentation and rye flour on microstructure and volatile compounds of chestnut flour based sourdoughs. *LWT – Food Science and Technology*, 58(2), 387–395.

Chenlo, F., Moreira, R., Pereira, G., Silva, C.C. (2007). Evaluation of the rheological behavior of chestnut (*castanea sativa* mill) flour pastes as function of water content and temperature. *Electronic Journal of Environmental Agriculture and Food Chemistry*, 6(2), 1794–1802.

Chlopicka, J., Pasko, P., Gorinstein, Sh., Jedryas, A., Zagrodzkt, P. (2012). Total phenolic and total flavonoid content, antioxidant activity and sensory evaluation of pseudocereal breads. *LWT - Food Science and Technology*, 46, 548.

Dabash, V., Buresova, I., Tokar, M., Zacharova, M., Gal, R. (2017). The effect of added pumpkin flour on sensory and textural quality of rice bread. *Journal of Microbiology, Biotechnology and Food Sciences*, 6(6), 1269–1272.

Demirkesen, I., Mert, B., Sumnu, G., Sahin, S. (2010). Utilization of chestnut flour in gluten-free bread formulations. *Journal of Food Engineering*, 101, 329–336.

Dimov, I., Petkova, N., Nakov, G., Taneva, I., Ivanov, I., Stamatovska, V. (2018). Improvement of antioxidant potential of wheat flours and breads by addition of medicinal plants. *Ukrainian Food Journal*, 7(4), 671–681.

Ghendov-Mosanu, A., Cristea, E., Patras, A., Sturza, R. (2020). Rose Hips, a valuable source of antioxidants to improve Gingerbread characteristics and Marius Niculaua. *Molecules*, 25, 5659.

Huang, W., Niu, H., Ma, L., Li, K., Wang, N. (2015). Geranyl flavonoid from breadfruit regulate Dyslipidemia in Hypercholesterolemic rat. *Journal of Food and Nutrition Researc*h, 3(6), 399-404.

Ibidapo, O.P., Henshaw, F.O., Shittu, T.A., Afolabi, W.A. (2020). Quality evaluation of functional bread developed from wheat, malted millet (*Pennisetum Glaucum*) and 'Okara' flour blends. *Scientific African*, 10, e00622.

Igual, M., Chis, M.S., P˘aucean, A., Vodnar, D.C., Muste, S., Man, S., Martínez-Monzó, J., García-Segovia, P. (2021). Valorization of Rose Hip (*Rosa canina*) puree co-product in enriched corn extrudates. *Foods*, 10, 2787.

Irakli, M., Katsantonis, D., Kleisiaris, F. (2015). Evaluation of quality attributes, nutraceutical components and antioxidant potential of wheat bread substituted with rice bran. *Journal of Cereal Science*, 65, 74-80.

Kivrak, Ş, Göktürk, T., Kivrak, I., Kaya, E., Karababa, E. (2019). Investigation of phenolic profiles and antioxidant activities of some Salvia species commonly grown in Southwest Anatolia using UPLC-ESI-MS/MS. *Food Science and Technology (Campinas)*, 39(2), 423–431.

Larsen, E., Kharazmi, A., Christensen, L.P., Christensen, S.B. (2003). An antiinflammatory galactolipid from rose hip (Rosa canina) that inhibits chemotaxis of human peripheral blood neutrophils in vitro. *Journal of Natural Products*, 66, 994–995.

Meral, R. and Köse, E.Y. (2019). The effect of bread-making process on the antioxidant activity and phenolic profile of enriched breads. *Quality Assurance and Safety of Crops & Foods*, 11(2), 171-181.

Peng, M., Lu, D., Liu, J., Jiang, B., Chen, J. (2021). Effect of roasting on the antioxidant activity, phenolic composition, and nutritional quality of pumpkin (*Cucurbita pepo* L.) seeds. *Frontiers in Nutrition*, 8:647354.

Petkova, N., Krustev, S., Denev, P. (2018). Enrichment of nutritional properties of wheat breads by addition of antioxidants and prebiotics. Conference: 14th *International Conference on Polysaccharides-Glycoscience*, Prague, Czech Republic.

Pongjanta, J., Naulbunrang, A., Kawngdang, S., Manon, T., Thepjaikat, T. (2006). Utilization of pumpkin powder in bakery products. *Songklanakarin Journal of Science and Technology*, 2006, 28(Suppl. 1), 71-79.

Sacchetti, G., Pinnavaia, G.G., Guidolin, E., Dalla-Rosa, M. (2004). Effects of extrusion temperature and feed composition on the functional, physical and sensory properties of chestnut and rice flour-based snack-like products. *Food Research International*, 37, 527–534.

Saeleaw, M. and Schleining, G. (2011). Composition, physicochemical and morphological characterization of Pumpkin flour. ICEF11- *11th International Congress on Engineering and Food* "FOOD PROCESS ENGINEERING IN A CHANGING WORLD", Athens, April 10-13, 2011.

Selimović, A., Miličević, D., Jašić, M., Selimović, A., Ačkar, D., Pešić, T. (2014). The effect of baking temperature and buckwheat flour addition on the selected properties of wheat bread. *Croatian Journal of Food Science and Technology*, 6(1), 43-50.

Skrbic, B. and Filipcev, B. (2008). Nutritional and sensory evaluation of wheat breads supplemented with oleic-rich sunflower seed. *Food Chemistry*, 108, 119-129.

Vasileva, I., Denkova, R., Chochkov, R., Teneva, D., Denkova, Z., Dessev, Tz., Denev, P., Slavov, A. (2018). Effect of lavender (*Lavandula angustifolia*) and melissa (*Melissa Officinalis*) waste on quality and shelf life of bread. *Food Chemistry*, 253, 13–21.

Wahyono, A., Dewi1, A.C., Oktavia, S., Jamilah, S., Kang, W.W. (2020). Antioxidant activity and total phenolic contents of bread enriched with Pumpkin flour. *IOP Conf. Series: Earth and Environmental Science*, 411, 012049.

Wolfe, K., Xianzhong, W., Riu, H.L. (2003). Antioxidant activity of apple peels. *Journal of Agricultural and Food Chemistry*, 51(3), 609–614.

Zhang, Y., Truzzi, F., D'Amen, E., Dinelli, G. (2021). Effect of storage conditions and time on the polyphenol content of wheat flours. *Processes*, 9, 248.

Zhang, L., Yang, X., Li, S., Gao, W. (2011). Preparation, physicochemical characterization and in vitro digestibility on solid complex of maize starches with quercetin. *LWT-Food Science and Technology*, 44, 787-792.

Acknowledgment

Authors would like to thank to the Ministry of Education and Science of Bulgaria about the subsidy in accordance with the Ordinance on the Terms and Procedure for the Evaluation, Planning, Allocation and Expenses of the State Budget Funds for the Financing of the Inherent Research Activities of the State Higher Education Institutions. The acknowledgements are also to the academic management of University of Economics – Varna for the allocations in project NPI-55/2021 "Improving the quality and usefulness of food – trends and innovative practices (on the example of bread)".