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PHENOLICS CONTENT IN BUCKWHEAT FLOUR

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The objective of the current study was to analyse the total phenolic concentration (TPC), total flavonoid concentration (TFC), individual phenolic compound concentration and DPPH´ radical scavenging activity of four buckwheat (raw, roasted, white and dark) flour samples obtained from the Latvian market, in comparison to those of wheat flour, which is the most common ingredient in production of cereal products. TPC, TFC, and DPPH´ radical scavenging activity values were determined using spectrophotometric methods. Phenolic compounds were determined based on the high performance liquid chromatographic method (HPLC). All buckwheat flour samples had significantly higher TPC and TFC than wheat flour. The highest TPC (974.74 mg GAE·100 g^{-1} DW) and TFC (495.31 mg CE·100 g^{-1} DW) was found in raw buckwheat flour ($p < 0.05$). Buckwheat flour samples demonstrated similar DPPH´ radical scavenging activity, which ranged from 21.067 to 22.644 mM TE·100 g^{-1} dry matter, and was significantly higher ($p < 0.05$) than in wheat flour (0.731 mM TE·100 g^{-1} of dry matter). Dark buckwheat flour contained the highest level of rutin (4.613 mg·100 g^{-1}), whereas raw buckwheat flour displayed the highest level of 3.5-dihydroxybenzoic acid (6.356 mg·100 g^{-1}), sinapic acid (0.947 mg·100 g^{-1}) and epicatechin (2.608 mg·100 g^{-1}).

Key words: *buckwheat, phenolic compounds, flavonoids, DPPH´ radical scavenging activity.*

INTRODUCTION

Buckwheat's potential contribution to sustainable agriculture and to nutritional and health benefits of humans should not be underestimated (Izydorczyk *et al*., 2014). Buckwheat has high levels of flavonoids and other bioactive compounds (Krkoskova and Mrazova, 2005) with a potential to inhibit lipoprotein oxidation and to reduce the risk of cardiovascular diseases (Jiang *et al*., 2007). Epidemiological studies of Hertog *et al*. (1995) and Chao *et al*. (2002) have suggested a protective role of flavonoids in buckwheat seeds helping to prevent coronary heart diseases and possibly cancers. The content and composition of flavonoids in common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*Fagopyrum tataricum*) seeds vary widely. Li and Zhang (2001) reported that the total flavonoid content in common buckwheat was 10 mg·g^{-1}, whereas in tartary buckwheat it was 40 mg·g^{-1}. Buckwheat contains several flavonoids, like rutin, quercetin, kaempferol, orientin/isoorientin, and vitexin/isovitexin, which have demonstrated antioxidant, antimicrobial and anti-inflammatory properties (Cai *et al*., 2004). Compared to most grain crops, buckwheat contains more rutin, which is reported to be the most abundant flavonoid providing natural antioxidant, anti-inflammatory and anti-carcinogenic properties, i.e. rutin may inhibit lipid peroxidation within food (Oomah and Mazza, 1996; Lin *et al*., 2009; Choy *et al*., 2013; Wronkowska *et al*., 2015). Quercetin concentration in buckwheat is several times lower than that of rutin (Fabjan *et al*., 2003). Rutin and quercetin concentration changes depending on technological parameters applied in seeds processing (Bonafaccia *et al*., 2003). Holasova *et al*. (2002) pointed out that the antioxidant potential of buckwheat is determined mainly by phenolic compounds.

The objective of the current study was to analyse the total phenolic concentration (TPC), total flavonoid concentration (TFC), phenolic compound concentration and DPPH´ radical scavenging activity of four buckwheat (raw, roasted, white and dark) flour samples obtained from the Latvian market, in comparison to those of wheat flour, which is the most common ingredient in production of cereal products.

MATERIALS AND METHODS

The research was conducted using raw (Raw-BF), roasted (Roasted-BF), white (White-BF) and dark (Dark-BF) buckwheat flours obtained from the "Bebri" farmstead, Latvia. Fine wheat flour (WF) for control purposes was purchased from "Dobeles Dzirnavnieks", Latvia. Wheat flour was chosen as the control in this study due to its widespread and varied application in the food industry. Particle size of wheat and buckwheat flours was not bigger than 160 μm.

The total phenolic concentration (TPC) of the buckwheat and wheat extracts was determined applying the Folin–Ciocalteu spectrophotometric method (Singleton *et al*., 1999) with some modifications. To 0.5 ml of extract 2.5 ml Folin–Ciocalteu reagent (diluted 10 times with water) was added and then, after 3 minutes, 2 ml sodium carbonate (Na_2CO_3) (75 g·l^{-1}) was added. The sample was mixed. The control sample contained all the reaction reagents except the extract. After 2 h of incubation at room temperature, the absorbance was measured at 765 nm. Total phenol concentration was expressed as gallic acid equivalents (GAE) 100 g^{-1} dry weight (DW) of the buckwheat or wheat flour.

The total flavonoid concentration (TFC) was measured by a colorimetric method (Kim *et al*., 2003) with minor modification. The extraction solution used was the same as TPC. To 0.5 ml of extract 2 ml double distilled H_2O was added and it was subsequently mixed with 0.15 ml 5% sodium nitrite ($NaNO_2$) (50 g·l^{-1}). After 5 min, 0.15 ml 10% aluminium chloride ($AlCl_3 \cdot 6H_2O$) solution was added. The mixture was allowed to stand for another 5 min and then 1 ml 1M sodium hydroxide (NaOH) was added. The reaction solution was thoroughly mixed. After 15 min of incubation at room temperature, the absorbance was measured at 415 nm. Total flavonoid concentration was expressed as catechin equivalents (CE) 100 g^{-1} DW of the buckwheat or wheat flour.

HPLC method was used for the analysis of phenolic compounds. The analysis was performed with a Shimadzu HPLC system LC–20 Prominence including Photo-diode Array detector SPD-M20A, Solvent Delivery Unit LC-20AD, Column Oven CTO-20A, Autosampler SIL-20A, System Controller CBM-20A and data system LC solution software (Fig. 1). Preparation of calibration solution was as follows: weighed in 100 ml volumetric flask with narrow neck 6.8 ± 0.1 mg gallic acid, 7.4 ± 0.1 mg 3.5-dihydroxybenzoic acid, 11.4 ± 0.1 mg 3.4-dihydroxybenzoic acid, 12.0 ± 0.1 mg catechin, 12.8 ± 0.1 mg 4-hydroxybenzoic acid, 13.1 ± 0.1 mg chlorogenic acid, 12.1 ± 0.1 mg homovanillic acid, 14.5 ± 0.1 mg vanillic acid, 13.8 ± 0.1 mg caffeic acid, 16.0 ± 0.1 mg epicatechin, 18.8 ± 0.1 mg syringic acid, 9.8 ± 0.1 mg vanillin, 12.1 ± 0.1 mg p-coumaric acid, 88.1 ± 0.1 mg sinapinic acid, 9.2 ± 0.1 mg ferulic acid, 11.2 ± 0.1 mg 2-hydroxycinnamic acid, 6.1 ± 0.1 mg rutin, 10.3 ± 0.1 mg trans-4-hydroxycinnamic acid, 4.3 ± 0.1 mg quercetin, 9.1 ± 0.1 mg luteolin and 9.6 ± 0.1 mg kaempherol and filled with HPLC grade CHROMASOLV® methanol till mark and mixed. Parameters of chromatography were as follows. An analytical column PerkinElmer C18, 4, 6 mm × 250 mm, 5 µm and temperature of column + 30 °C was used for separation of phenolic compounds at wavelength 278 nm. Injection volume of samples was 100 µL. Mobile phase was A (deionized water), B (HPLC grade CHROMASOLV® methanol) and C (Acetic acid solution for HPLC) in the gradient conditions (Table 1). Start flow rate was 1.0 ml·min^{-1}.

Antioxidant activity of the buckwheat and wheat extracts was measured on the basis of the scavenging activities of the stable 2.2-diphenyl-1-picrylhydraziyl (DPPH´) radical as outlined by Yu *et al*. (2003). The antioxidant reaction

Table 1

GRADIENT CONDITIONS

Time	Mobile phase	
	B	C
Start	0	2.5
2 min	15	2.4
12 min	18	2.2
12 min	Flow rate = 0.8 mL·min^{-1}	
20 min	20	1.8
20 min	Flow rate = 0.6 mL·min^{-1}	
25 min	Flow rate = 0.5 mL·min^{-1}	
30 min	25	1.6
35 min	Flow rate = 0.4 mL·min^{-1}	
40 min	30	1.4
45 min	Flow rate = 0.8 mL·min^{-1}	
50 min	45	1
50 min	Flow rate = 0.7 mL·min^{-1}	
55 min	55	0.9
55 min	Flow rate = 0.8 mL·min^{-1}	
65 min	85	0.6
65 min	Flow rate = 0.85 mL·min^{-1}	
70 min	100	0
70 min	Flow rate = 1.0 mL·min^{-1}	
73 min	0	2.5
78 min	STOP	STOP

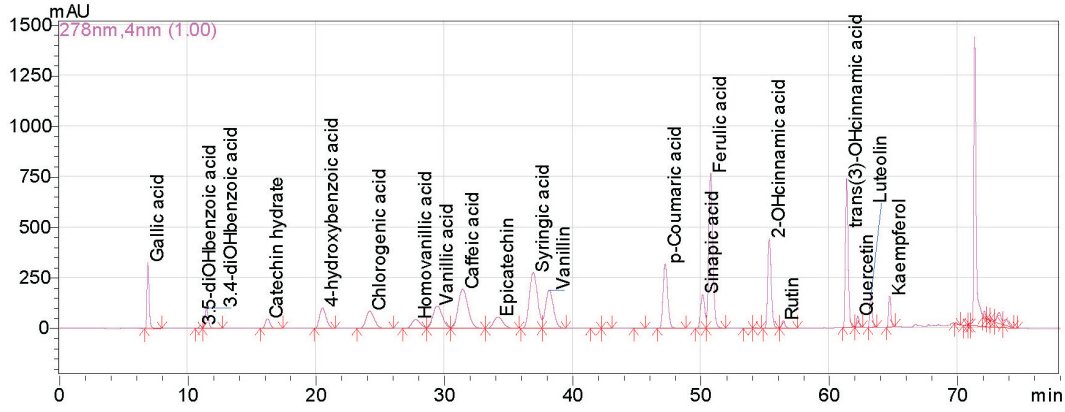

Fig. 1. Chromatogram of phenolic compounds.

was initiated by transferring 0.5 ml of buckwheat or wheat extract into a sample cavity containing 3.5 ml of freshly prepared DPPH´ methanol solution (0.004 g DPPH´ to 100 ml methanol). After 30 min of incubation in the dark at room temperature, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity (Zhao *et al*., 2008). The radical scavenging capacity (RSC) was expressed as Trolox mM equivalents (TE) 100 g^{-1} DW of the buckwheat and wheat flour.

Moisture content of wheat and buckwheat flour samples was determined according to the methods of LVS EN ISO 712:2010 A.

RESULTS

The total phenolic and total flavonoid concentrations of wheat and buckwheat flour samples are shown in Figure 2. All buckwheat flour samples had significantly ($p < 0.05$) higher total phenolic and flavonoid concentration than in wheat flour. The highest total phenolic (974.74 mg GAE·100 g^{-1} DW) and total flavonoid (495.31 mg CE·100g^{-1} DW) concentration was observed in the Raw-BF sample. Acquired results of Raw-BF were two (for Roasted-BF) or three times (for White-BF and Dark-BF) higher in comparison with other buckwheat flour samples. This finding is in line with previous studies on the total phenolic concentration in raw and roasted buckwheat groats (Wronkowska *et al*., 2015). Similar total phenolic and total flavonoid concentration was observed for White-BF and Dark-BF, whereas Roasted-BF showed higher total phenolic and total flavonoid concentration. The results of Analysis of Variance among the studied buckwheat flour samples revealed significant ($p < 0.05$) differences regarding the total phenolic and total flavonoid concentration between Raw-BF and other buckwheat flour samples; and between Roasted-BF and White- and Dark-BF.

The concentration of individual phenolic compounds in wheat and buckwheat flour samples is summarised in Table 2. There were nine phenolic compounds identified in wheat flour, fifteen — in Raw-BF, fourteen —– in Roasted-BF, sixteen — in White-BF, and eighteen — in Dark-BF. Wheat flour contained the highest concentration of catechin hydrate, quercetin, luteolin and trans(3)-hydroxycinnamic acid. A markedly higher concentration of 3.5-dihydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, epicatechin, p-Coumaric acid, sinapic acid, and 2-hydroxycinnamic acid was observed in Raw-BF. It was found that gallic acid and homovanillic acid concentration was higher in Roasted-BF, furthermore homovanillic acid was not present in other samples. White-BF contained the highest levels of 4-hydroxybenzoic acid, vanillin, and ferulic acid, whereas Dark-BF — 3.4-dihydroxybenzoic acid, rutin, and kaempferol. Rutin concentration, which plays a significant role in the antioxidant capacity of buckwheat seeds (Jiang *et al*., 2007), was several times higher in Dark-BF (4.613 mg·100 g^{-1}) in comparison with other flour. This might be explained by the presence of husks in Dark-BF, as buckwheat husks have been noted to have the highest total phenolic concentration and antioxidant activity (Kerienė *et al*., 2015).

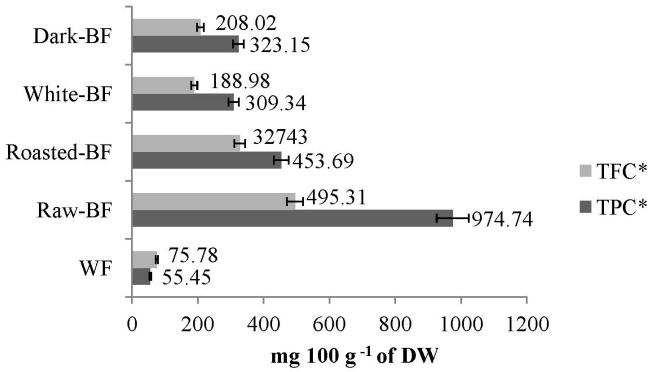

Fig. 2. The total phenolic and total flavonoid concentration of wheat (W) and buckwheat flour (BF) samples. TPC* (total phenolic concentration) was expressed as gallic acid equivalents (GAE)·100 g^{-1} dry weight (DW); TFC* (total flavonoid concentration) was expressed as catechin equivalents (CE)·100 g^{-1} DW.

Table 2

CONCENTRATION OF PHENOLIC COMPOUNDS IN WHEAT (W) AND BUCKWHEAT FLOUR (BF) SAMPLES, mg·100 g^{-1}

Phenolic compounds	WF	Raw-BF	Roasted-BF	White-BF	Dark-BF
Gallic acid	0	0.011	0.015	0.003	0.006
3.5-diOHbenzoic acid	0	6.356	2.016	1.470	2.901
3.4-diOHbenzoic acid	0	0	0	0.038	0.204
Catechin hydrate	3.014	0.641	0.145	0.340	0.268
4-hydroxybenzoic acid	0	0	0	0.018	0.015
Chlorogenic acid	0	0.064	0	0.051	0.020
Homovanillic acid	0	0	0.041	0	0
Vanillic acid	0	0.239	0	0.008	0.151
Caffeic acid	0	0.172	0.082	0.071	0.085
Epicatechin	0	2.608	0	0.428	0.603
Syringic acid	0	0	0	0	0
Vanillin	0.012	0.021	0.006	0.039	0.035
p-Coumaric acid	0	0.446	0.093	0.233	0.284
Sinapic acid	0.274	0.947	0.090	0	0.014
Ferulic acid	0.038	0.027	0.003	0.045	0.043
2-OHcinnamic acid	0.016	0.903	0.148	0.709	0.774
Rutin	0.238	0.852	0.321	0.480	4.613
trans(3)-OHcinnamic acid	0.210	0	0	0	0
Quercetin	0.230	0.017	0.017	0.002	0.104
Luteolin	0.072	0.012	0.005	0.005	0.034
Kaempferol	0	0	0.002	0	0.293

Literature reports that antioxidant activity correlates significantly with the total phenolic concentration (Kerienė *et al*., 2015). Similar tendencies were observed in this study, where Raw-BF had the highest total phenolic concentration and the highest DPPH´ radical scavenging activity (27.17 mM TE·100 g^{-1} DM) among the flour samples. DPPH´ radical scavenging activity of wheat and buckwheat flour sam-

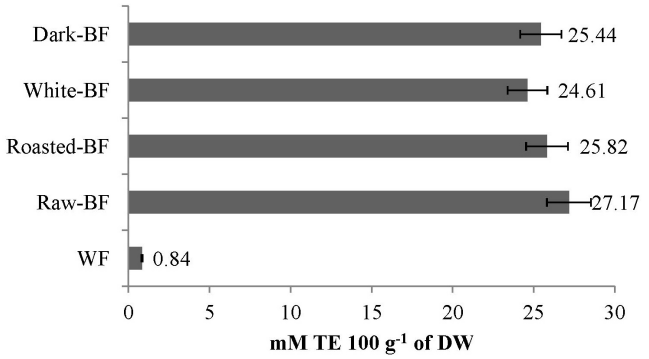

Fig. 3. DPPH´ radical scavenging activity of wheat (W) and buckwheat flour (BF) samples. DW, dry weight.

ples is presented in Figure 3. Buckwheat flour samples had a significantly ($p < 0.05$) higher DPPH´ radical scavenging activity than wheat flour, which is confirmed by literature data (Djordjevic *et al*., 2011). No significant ($p > 0.05$) differences were detected among the buckwheat flour samples, where DPPH´ radical scavenging activity ranged from 24.61 mM TE·100 g^{-1} DM for White-BF to 27.17 mM TE·100 g^{-1} DM for Raw-BF. The results showed that DPPH´ radical scavenging activity was not significant affected by the technological processes applied in seed processing (roasted buckwheat or raw) and the used raw material (buckwheat with husks or without). There is a need for further research.

DISCUSSION

The results of the current study confirm that buckwheat is an important source of bioactive substances, like phenols, flavonoids, and also has strong antioxidant activity. Djordjevic *et al*. (2011) indicated that buckwheat had the highest total phenolic concentration, with the highest DPPH´ radical scavenging activity among the four examined cereals. Similar conclusions were reported by Sedej *et al*. (2010), who reported that in buckwheat flour the concentration of phenolics and antioxidant activity were several times higher than those in winter wheat flour. However, the total phenolic concentration, total flavonoid concentration, concentration of phenolic compounds and DPPH´ radical scavenging activity of buckwheat was influenced the technological processes applied in seed processing (roasted buckwheat or raw) and the used raw material (buckwheat with husks or without). Roasting affects the functional properties and chemical composition of buckwheat groats: the reduction of parent antioxidants and the formation of Maillard reaction products (Wronkowska *et al*., 2015; Zielińska *et al*., 2007). Wronkowska *et al*. (2015) showed that raw buckwheat groats were almost two times richer in total phenolic and phenolic compounds than roasted groats. Similar conclusions were obtained in the current research. Roasted-BF had 62% lower rutin concentration than in Raw-BF which could be explained by the hydro-thermal processes used for roasting. However, the highest ($p < 0.05$) concentration of rutin was found in Dark-BF due to the presence of husks. This confirms the results of Li *et al*. (2013). Literature indicates that there is a positive correlation between the rutin and total phenolic concentration of buckwheat grain with husks (Kerienė *et al*., 2015). The current study did not confirm this, as Dark-BF had the highest rutin concentration, whereas Raw-BF had the highest total phenolic concentration among the buckwheat flour samples. Raw-BF contained significantly ($p < 0.05$) high amount of sinapic acid (0.947 mg·100 g^{-1}) among the buckwheat flour samples. Roy and Prince (2012) indicated that sinapic acid had a preventive effect against myocardial infarct, also a neuro-protective and anti-inflammatory effect.

Buckwheat is recognised as a good source of flavonoids with a potential to inhibit lipoprotein oxidation and to reduce the risk of cardiovascular diseases (Izydorczyk *et al*., 2014; Jiang *et al*., 2007). However, the current research data showed significant differences in the total flavonoid content across the buckwheat flour samples. Izydorczyk *et al*. (2014) indicated that the groats of tartary buckwheat contained a 40–60 times higher concentration of total flavonoids than the other buckwheat species. Similar conclusions were reported by Uddin *et al*. (2013), who found that the flavonoid concentration depended on the cultivar. The current study showed that the total flavonoid concentration was affected by the technological processes applied in seed processing and the used raw material.

The highest DPPH´ radical scavenging activity was observed in Raw-BF which confirmed previous observations (Hęś *et al*., 2014). Guo *et al*. (2011) suggested that tartary buckwheat may serve as an excellent dietary source of free radical scavengers.

CONCLUSIONS

Buckwheat flours are products that are rich in bioactive substances, the concentration of which is affected by the used raw material (buckwheat with husks or without) and the technological processes applied in seed processing (roasted buckwheat or raw). Raw-BF showed the highest total phenolic and total flavonoid concentration as well the highest DPPH´ radical scavenging activity. Raw-BF had a high potential as a raw material for functional food development. Due to the high level of rutin, Dark-BF can be used as a functional food.

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FENOLU SATURS GRIĶU MILTOS

Griķi ir pseido graudaugs ar augstu uzturvērtību un ir būtisks bioloģiski aktīvu vielu avots cilvēku uzturā. Tomēr kopējo fenolu, kopējo flavonoīdu un fenolu savienojumu saturu, kā arī antiradikālo aktivitāti griķu miltos būtiski ietekmē sēklas apstrādes tehnoloģiskais process (vai sēklas tiek tikai attīrītas un kaltētas, iegūstot zaļos griķus, vai papildus tiek veikta termiskā apstrāde — grauzdēšana vai tvaicēšana pirms kaltēšanas, tādējādi iegūstot grauzdētos vai kaltētos griķus) un izmantotās sēklu sastāvdaļas (ar vai bez sēnalām). Pētījuma rezultāti parādīja, ka visos griķu miltos (zaļajos, grauzdētajos, baltajos un tumšajos) ir būtiski augstāks kopējo fenolu un kopējo flavonoīdu saturs, kā arī DPPH´ antiradikālā aktivitāte, salīdzinot ar kviešu miltiem. Savukārt būtiski augstākais kopējo fenolu (974,74 mg GAE·100 g^{-1} sausnas) un kopējo flavonoīdu (495,31 mg CE·100 g^{-1} sausnas) saturs starp griķu miltu paraugiem tika konstatēts zaļajiem griķiem. Rutīna saturs, kuram ir būtiska nozīme griķu antiradikālajā aktivitātē, būtiski variē starp griķu miltu paraugiem. Visaugtākais rutīna saturs tika noteikts tumšajos griķu miltos (4,613 mg·100 g^{-1}), salīdzinot ar baltajiem (0,480 mg·100 g^{-1}), zaļajiem (0,852 mg·100 g^{-1}) un grauzdētajiem (0,321 mg·100 g^{-1}) griķu miltiem, ko varētu skaidrot ar sēnalu klātbūtni tumšajos griķu miltos. Izvērtējot DPPH´ antiradikālo aktivitāti, visi griķu miltu paraugi uzrādīja būtiski augstāku aktivitāti, salīdzinot ar kviešu miltiem. Tomēr starp pašiem griķu miltu paraugiem būtiskas atšķirības netika konstatētas. DPPH´ antiradikālā aktivitāte bija robežās no 24,61 mM TE·100 g^{-1} sausnas līdz 27,17 mM TE·100 g^{-1} sausnas. Pētījuma dati apstiprina, ka griķu milti ir nozīmīgs fenolu, flavonoīdu un polifenolu avots uzturā un tie varētu tikt izmantoti funkcionālu pārtikas produktu izstrādē.