

Effect of germination and extrusion on the phenolic content and antioxidant activity of raw buckwheat (*Fagopyrum esculentum* Moench)

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Abstract. The aim of the research was to determine the total phenolic content, antioxidant activity and phenolic compounds in raw and germinated common buckwheat grain and their extruded products in order to estimate the effect of germination and extrusion on the total phenolic content, antioxidant activity and phenolic compounds in buckwheat. A total of 10 buckwheat samples were analysed, where the raw buckwheat grain was the control sample, four samples were germinated and then extruded. The total phenolic content was determined using the Folin–Ciocalteu assay. The antioxidant activity was determined using a micro plate assay and phenolic compounds with Liquid Chromatography –Time of Flight Mass Spectrometer method.

The results showed that germination of buckwheat significantly affected the total phenolic content, antioxidant activity, radical scavenging activity and content of phenolic compounds. The decrease of the total phenolic content, antioxidant activity, radical scavenging activity and the content of some phenolic compounds depended on germination time – 48 h of germination provided greater concentrations at the end of a 24 h germination period, whereas the content of some phenolic compounds like rutin, quercetin and vitexin increased substantially after germination, besides, the increase of phenolic compounds depended on the germination time. In total 26 different phenolic compounds were detected in raw and germinated buckwheat samples and only one compound with an m/z value 385.1282 was unidentified. The data of extrusion revealed a significant decrease of total phenolic content, antioxidant activity, radical scavenging activity and phenolic compounds content in buckwheat samples ($P < 0.05$). In the extruded buckwheat samples novel phenolic compounds like 4–hydroxybenzoic acid, homovanillic acid, catechin, ferulic acid, and hyperoside were detected.

Key words: buckwheat, germination, extrusion, phenolics, antioxidant activity.

INTRODUCTION

Buckwheat grain is a pseudo–cereal which is characterized as functional food due to its components with biological activity and healing properties like flavonoids, phenolic acids, phytosterols, etc. (Christa & Soral–Smietana, 2008; Filipcev et al., 2011; Torbica et al., 2012; Zhang et al., 2012). Rutin and quercetin compose the major part of bioactive compounds in buckwheat with antioxidant, antimicrobial and anti–

inflammatory activities (Halosava et al., 2002; Cai et al., 2004; Lin et al., 2009; Torbica et al., 2012; Wronkowska et al., 2015). The quantified phenolic compounds in buckwheat are caffeic acid, rutin, vitexin, quercitrin, quercetin and kaempferol (Peng et al., 2017). Zhang et al. (2015) identified 11 phenolic compounds in buckwheat: orientin, isoorientin, vitexin, isovitexin, rutin, kamperol-3-rutinoside, quercitrin, myricetin, luteolin, quercetin and kampferol. It is known that phenolic compounds like rutin, quercetin, etc. cannot be produced in human body, therefore it is important to take them with food. Wang et al. (2009) observed the reduction of chronic diseases risk by using buckwheat bran extract *in vivo*. Kawa et al. (2003) reported the reduction of serum glucose level by feeding buckwheat to rats. Lu et al. (2002) concluded that regular intake of buckwheat can reduce the blood glucose concentration and the risk of diabetes mellitus in humans.

Germination of seeds and grain is characterized as a process with added value because it allows one to improve the nutritional and biological value of product. Kim et al. (2004) observed an increase of phenolic compounds like rutin and quercitrin in buckwheat during germination, whereas Yiming et al. (2015) concluded that germination could be a good way of accumulating rutin in buckwheat grain. Some studies reported that an increase of phenolic compounds concentrations in buckwheat depended on germination time: the longer the time, the faster the increase (Yiming et al., 2015; Zhang et al., 2015).

Total phenolic content, content of phenolic compounds and antioxidant activity could be affected by the processing of buckwheat. A reduction of content of phenolic compounds was observed in buckwheat during heating at a temperature of 150 °C, the loss increased by the prolongation of time (Dietrych–Szostak & Oleszek, 1999). Sensoy et al. (2006) reported that the optimization of temperature and time during processing (roasting or extrusion) could provide the maintenance of phenolic compounds in buckwheat. Jozinovic et al. (2012) concluded that the extrusion of corn meal with buckwheat flour caused the decrease of total phenolic content and antioxidant activity. Zielinska et al. (2007) reported similar observation about the reduction of antioxidant capacity in buckwheat after roasting. In summary, the germination of buckwheat grain provided an improvement of biological value, but there is no information about the extrusion effect on phenolic content and antioxidant activity in germinated buckwheat grain. Therefore, the aim of the current research was to determine the total phenolic content, antioxidant activity and phenolic compounds in raw and germinated buckwheat grain and their extruded products in order to estimate the effect of germination and extrusion on the total phenolic content, antioxidant activity and phenolic compounds in buckwheat. The germination of buckwheat grain was carried out to increase the biological value, to improve the digestibility and the advanced grain will be used for production of flour or new functional products. The extrusion of raw and germinated buckwheat grains was used for production of new products like snacks, which could provide the consumption of buckwheat among the inhabitants of Latvia.

MATERIALS AND METHODS

Materials

Buckwheat (*Fagopyrum esculentum* Moench) grain was purchased from the organic farm 'Bebri' (Saldus area, Latvia).

Germination of buckwheat grain was performed in two time lengths (24 h or 48 h) in the climate control chamber (Memmert, ICH110, Germany). Subsequent drying took place at two different temperatures (40 °C or 60 °C) in the universal oven (Memmert, UF160, Germany). (Fig. 1).

Drying after germination was performed to provide the storage of buckwheat grain and to produce germinated buckwheat flour, which was used further for production of extruded germinated buckwheat products. The selected time of drying was 4 hours till the moisture content in germinated buckwheat grain reached 13–14%.

Extrusion of raw and germinated buckwheat grain was performed with the food extruder (PCE Extrusimeter L–Serie, Göttfert, Germany; Fig. 2). The extruded raw and germinated buckwheat grain were dried in the convective–rotary oven (SVEBA DAHLAN, Sweden).

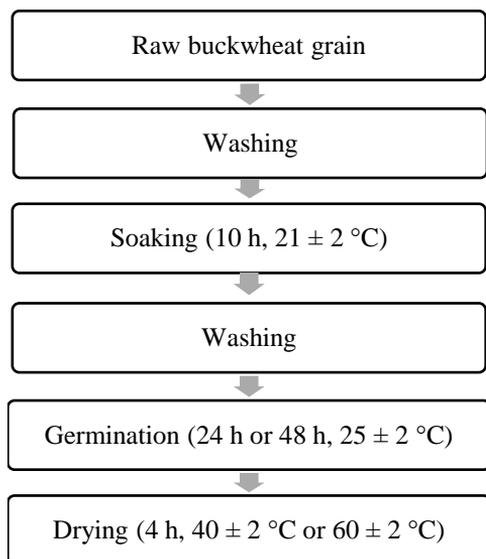


Figure 1. The process of obtaining germinated buckwheat grain.

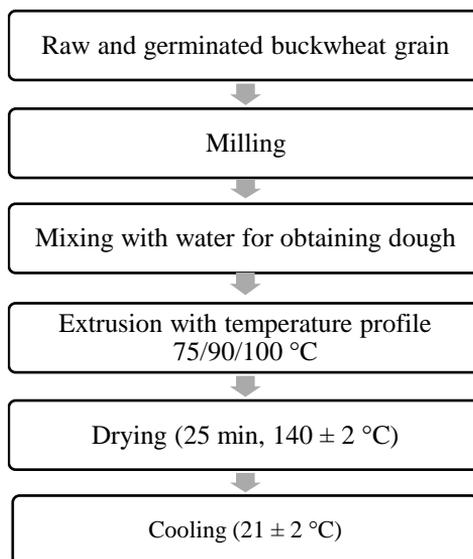


Figure 2. Technological process of obtaining extruded raw and germinated buckwheat products.

Methods

The total phenolic content (TPC) was determined by the Folin–Ciocalteu assay using the high–throughput 96–well plate method as described by Herald et al. (2012) with slight modifications. The measurement was conducted by mixing Folin–Ciocalteu solution (1:1 with water), sodium bicarbonate and ethanolic extract. The absorbance was measured after 90 min of incubation at 765 nm, along with the blank. TPC was expressed

as gallic acid equivalents (GAE mg 100 g⁻¹ DW), based on the gallic acid (GA) calibration curve (range 0.025–0.20 mg mL⁻¹, R² = 0.9997). Analyses were performed with the Infinite M200 PRO (Tecan Group Ltd., Männedorf, Switzerland) instrument in triplicate. The bandwidth was 9 nm and temperature 24 °C.

Characterisation of antioxidant activity using micro plate assay. The DPPH method is based on the ability to stabilize the free radical 2,2-diphenyl-picrylhydrazyl (DPPH) to react with hydrogen donors. Antiradical activity (ARA) was determined using the DPPH assay using the high-throughput 96-well plate method as described by Herald et al. (2012) with slight modifications. The measurements of buckwheat extracts were done by mixing a 150 µM DPPH solution in ethanol with an extract or standard samples. The absorbance was measured at 517 nm, along with the blank. ARA was expressed as ascorbic acid equivalents (AAE mg 100 g⁻¹ DW), based on the calibration curve (0.03–0.09 mg mL⁻¹, R² = 0.998). The analyses were performed on the Infinite M200 PRO (Tecan Group Ltd., Männedorf, Switzerland) instrument in triplicate. The bandwidth was 9 nm, temperature 26.4 °C. The percentage of radical scavenging activity of all extracts was calculated using the following formula (1):

$$\% \text{scavenging}[DPPH] = [(A_0 - A_1 / A_0)] \times 100 \quad (1)$$

where A₀ – the absorbance of the blank; A₁ – the absorbance in the presence of buckwheat extract.

The content of phenolic compounds was determined with the Liquid Chromatography –Time of Flight Mass Spectrometer (LC–TOF–MS) according to the description offered by Klavina et al. (2015).

During LC–MS analysis, each sample produces its own base peak chromatogram (BPC). In the positive ionization mode each compound can add a proton and can produce its own [M+H]⁺ mass spectra or stay in a positively charged state as M⁺ molecule. Each compound has its own chemical formula and molar mass. This chemical formula using *Mass Hunter Qualitative Analyses B.07.00* software can be used for the calculation of [M+H]⁺/[M]⁺ which is used for the extraction of compounds from the base peak chromatogram. Both values can be compared and the difference (Δ) between them should not be higher than 0.0030. HRMS experiments ensure accurate mass measurements resulting in the removal of background signals of complex matrix interferences. It is useful for non-targeted or retrospective post-targeted identification of unknown compounds by the processing of raw data obtained in different scan modes, including full scan in defined *m/z* windows. The experimental data were handled using the MassHunter version B07.00 software (Agilent Technologies).

All analyses were performed in triplicate.

Preparation of buckwheat extracts was carried as previously described (Carvalho et al. 2015).

Abbreviation of buckwheat samples

In total, ten buckwheat sample types were prepared and analysed, and they were encrypted with specific abbreviations (Table 1).

Table 1. Abbreviation of buckwheat samples

Abbreviations	Sample description
RB	Raw buckwheat
GB-24/40	Germinated buckwheat (germination time 24 h, drying temperature 40 °C)
GB-24/60	Germinated buckwheat (germination time 24 h, drying temperature 60 °C)
GB-48/40	Germinated buckwheat (germination time 48 h, drying temperature 40 °C)
GB-48/60	Germinated buckwheat (germination time 48 h, drying temperature 60 °C)
E-RB	Extruded raw buckwheat
E-GB-24/40	Extruded germinated buckwheat (germination time 24 h, drying temperature 40 °C)
E-GB-24/60	Extruded germinated buckwheat (germination time 24 h, drying temperature 60 °C)
E-GB-48/40	Extruded germinated buckwheat (germination time 48 h, drying temperature 40 °C)
E-GB-48/60	Extruded germinated buckwheat (germination time 48 h, drying temperature 60 °C)

Data processing

The data processing was performed using mathematical and statistical methods with statistical software Microsoft Office Excel 14.0. The results of the research were expressed as a mean \pm standard deviation and analysed using the analyses of variance (ANOVA). *T-test* was applied to compare the mean values and *P-value at 0.05* was used to determine the significant differences.

RESULTS AND DISCUSSION

In literature the given total phenolic content (TPC) differed depending on the buckwheat varieties and extractions method used (Inglett et al., 2010). In research of Vollmannova et al. (2013) TPC of five buckwheat varieties varied between 138.10–286.99 GAE mg 100 g⁻¹ DW, whereas Mikulajova et al. (2016) evaluated 22 cultivars of buckwheat, and TPC ranged from 89.7 to 145.6 GAE mg 100 g⁻¹ DW. Unal et al. (2017) reported significant differences between commercial buckwheat and Güneş variety (207.12 \pm 2.67 GAE mg 100 g⁻¹ DW and 329.83 \pm 3.88 GAE mg 100 g⁻¹ DW, respectively). Comparing the literature data with the results of this study about raw buckwheat (Table 2) similar or lower total phenolic content was determined where the differences could be explained by various determination methods used. Analysing the influence of germination on TPC, a significant decrease in TPC of buckwheat samples ($P < 0.05$) was observed. Furthermore, the decrease of TPC depended on the germination time (48 h of germination provided a greater concentration of TPC) and drying temperature after germination (a lower temperature provided a lower decrease of TPC). The greatest concentration of TPC among the germinated buckwheat samples was observed in GB-48/40. The rapid decrease of TPC during germination of first 24 h could be explained by a high demand of energy at the beginning of germination (Jia et al., 2015), whereas a greater TPC after 48 h of germination could be associated with single phenolic compounds increase during germination according to literature (Yiming et al., 2015; Zhang et al., 2015).

Table 2. Total phenolic content, antiradical activity and radical scavenging activity of raw and germinated buckwheat and their extruded products

Samples	TPC	ARA	DPPH
	GAE mg 100 g ⁻¹ DW	AAE mg 100g ⁻¹ DW	%
RB	132.85 ± 1.03 ^a	90.56 ± 0.51 ^a	94.76 ± 0.48 ^a
GB-24/40	74.13 ± 2.05 ^c	48.39 ± 0.06 ^c	55.03 ± 0.06 ^c
GB-24/60	66.44 ± 1.41 ^c	39.49 ± 0.54 ^d	44.43 ± 0.48 ^d
GB-48/40	96.92 ± 1.18 ^b	64.88 ± 0.59 ^b	71.81 ± 0.56 ^b
GB-48/60	90.81 ± 1.80 ^b	49.73 ± 1.31 ^c	54.79 ± 1.19 ^c
E-RB	34.14 ± 0.70 ^d	12.48 ± 0.09 ^e	21.20 ± 0.08 ^e
E-GB-24/40	28.13 ± 0.11 ^d	10.90 ± 0.60 ^e	19.85 ± 0.55 ^e
E-GB-24/60	30.90 ± 0.84 ^d	9.62 ± 0.05 ^e	18.66 ± 0.05 ^e
E-GB-48/40	31.62 ± 1.08 ^d	13.89 ± 0.01 ^e	22.70 ± 0.01 ^e
E-GB-48/60	28.99 ± 0.65 ^d	9.76 ± 0.09 ^e	18.66 ± 0.08 ^e

Different letters indicate statistically significant difference between buckwheat samples ($P < 0.05$).

After extrusion of raw and germinated buckwheat samples, a significant decrease of TPC ($P < 0.05$) was observed which was in concordance with literature (Jozinovic et al., 2012). Similar conclusions were reported by Hes et al. (2014) stating that TPC was affected by the boiling of buckwheat groats where the temperature was close to the maximum temperature of extrusion in this study and by Wronkowska et al. (2015) that after roasting buckwheat TPC was two times lower. In this research the losses of total phenolic content after extrusion composed 74.3% in E-RB and 53.49–68.08% in E-GB samples.

Similar trends were observed for antiradical activity (ARA) and radical scavenging activity (DPPH) of raw and germinated buckwheat grain and their extruded products. The highest ARA and DPPH were determined for raw buckwheat grain ($P < 0.05$). Germination and extrusion processes significantly affected the decrease of ARA and DPPH in buckwheat samples ($P < 0.05$). The decrease of ARA in buckwheat was 1.89 or 2.29 times by 24 h germination and 1.40 or 1.82 times by 48 h germination, whereas the extrusion decreased ARA 7.26 times in raw buckwheat and 4.10–5.09 times in germinated buckwheat samples. The decrease of DPPH was close to the results of ARA in germinated buckwheat samples but not so critical as the results of ARA in extruded buckwheat products. In literature there are reports about an increase of radical scavenging activity in buckwheat during germination, and there are indications that germination for 72 h showed the highest DPPH (Zhang et al., 2015). These conclusions have not been confirmed in the current research. The differences could be explained by different germination methods: in this research germinated buckwheat was dried, while in the research of Zhang et al. (2015) it was lyophilized.

In total, 26 different phenolic compounds were separated from raw and germinated buckwheat samples: catechin hydrate, chlorogenic acid, vanillic acid, caffeic acid, epicatechin, syringic acid, vanillin, p-coumaric acid, sinapic acid, 2-OHcinnamic acid, rutin, quercetin, 2-hydroxy-3-O-β-D-glucopyranosyl-benzoic acid, caffeic acid hexose, swertiamacroside, flavonol-glycosides, catechin-glucosides, epicatechin gallate, (epi)afzelchin-(epi) catechin isomer, vitexin, epiafzelchin-epicatechin-O-methylgallate, (-)epicatechin-O-3,4-dimethyl-gallate, epiafzelchin-epicatechin-O-dimethylgallate, procyanidin B2-dimethylgallate, and epiafzelchin-epiafzelchin-epicatechin. Only one compound with m/z value 385.1282 was unidentified.

The main phenolic compounds of buckwheat samples are given in Table 3. The research data confirmed the conclusions found in literature that buckwheat grain contains high level of flavonoids, especially rutin.

Table 3. Phenolic compounds of raw and germinated buckwheat grain and their extruded products, mg 100 g⁻¹ DW

Samples	Epicatechin C15H14O6	Rutin C27H30O16	Quercetin C15H10O7	Catechin- glucosides C21H24O11	Vitexin C21H20O10	Epiafzelchin- epicatechin-O- dimethylgallate C39H34O15
RB	39.13 ^a	16.38 ^b	3.84 ^b	165.93 ^a	1.44 ^{cd}	59.89 ^a
GB-24/40	19.89 ^b	19.59 ^b	4.96 ^b	95.88 ^b	2.25 ^c	20.64 ^b
GB-24/60	14.14 ^b	18.91 ^b	4.79 ^b	91.18 ^b	2.28 ^c	14.44 ^c
GB-48/40	38.34 ^a	32.44 ^a	9.71 ^a	81.84 ^c	63.26 ^a	18.74 ^b
GB-48/60	41.18 ^a	31.21 ^a	8.95 ^a	92.39 ^b	63.3 ^{4a}	15.50 ^c
E-RB	7.36 ^c	5.51 ^c	1.48 ^c	n.d.	2.44 ^c	5.50 ^d
E-GB-24/40	4.79 ^c	3.98 ^c	0.95 ^c	n.d.	1.13 ^{cd}	3.25 ^e
E-GB-24/60	4.38 ^c	3.59 ^c	0.96 ^c	n.d.	0.95 ^d	2.55 ^e
E-GB-48/40	4.37 ^c	3.48 ^c	0.82 ^c	n.d.	6.36 ^b	2.29 ^e
E-GB-48/60	4.35 ^c	3.91 ^c	1.04 ^c	n.d.	8.09 ^b	2.05 ^e

n.d. – not detected;

Different letters indicate statistically significant difference between buckwheat samples ($P < 0.05$).

Comparing the rutin content of raw buckwheat grain in this research with the data from literature, similar or lower levels of rutin were established. For example, Qin et al. (2010) reported that rutin content in 18 cultivars of common buckwheat ranged between 15.0 and 16.8 mg 100 g⁻¹ DW. In the study of Kiprovski et al. (2015) it was indicated that rutin content in buckwheat seeds was from 3.29 to 151.45 mg 100 g⁻¹ DW. Vollmannova et al. (2013) revealed that rutin content of 5 cultivars of buckwheat was between 30.99 and 50.77 mg 100 g⁻¹ DW. It confirmed the conclusions of literature that rutin content depends on the cultivars, species – common or tartary, and growth conditions (Qin et al., 2010; Mikulajova et al., 2016).

High amounts of epicatechin, catechin-glucosides and epiafzelchin–epicatechin–O–dimethylgallate were determined in raw buckwheat grain. Quercetin content in raw buckwheat grain was lower compared to other phenolic compounds, except vitexin, but high enough compared to literature data where quercetin content in raw common buckwheat groats was reported to be 0.167 mg 100 g⁻¹ DW (Hes et al., 2014). Furthermore, in the study of Qin et al. (2010) quercetin was determined only in two cultivars among 18 common buckwheat samples.

After the germination of raw buckwheat grain a significant increase of rutin, quercetin and vitexin was observed. It confirmed the conclusions arrived at in literature that during germination the content of rutin and quercitrin increased gradually (Kim et al., 2004) or that the content of orientin, isoorientin, vitexin, isovitexin, rutin, kamperol–3–rutinoside, quercitrin and kaempferol increased during germination but quercetin was detected only in 24 h of germination (Zhang et al., 2015). Yiming et al. (2015) did not observe regular changes of rutin and quercetin content during germination, though the

total flavonoid content was greater in buckwheat after germination. Similar observation was with regard to epicatechin content of germinated buckwheat grain in this research. The content of catechin-glucosides and epiafzelchin–epicatechin–O–dimethylgallate in raw buckwheat grain decreased after germination. The increase or decrease of phenolic compounds was affected by the germination time – 48 h of germination provided a substantial increase of rutin (approximately 1.9 times), quercetin (approximately 2.5 times) and vitexin (approximately 44.0 times). The other phenolic compounds the content of which increased after 48 h of germination were catechin hydrate, vanillic acid, vanillin, p-coumaric acid, sinapic acid, caffeic acid hexose, and flavonol–glycosides. Similar results regarding the effect of germination time on the content of rutin, quercetin and vitexin had been obtained by Zhang et al. (2015).

The data of extrusion revealed a significant decrease of phenolic compounds in buckwheat samples ($P < 0.05$) which could be a consequence of high temperatures and heating time. The decrease of rutin concentration in E–RB was 66.36% and in E–GB samples – 79.68–89.27% comparing to RB and GB samples, respectively. The losses of quercetin were 2.59 times higher in E–RB than in RB samples and 4.99–11.84 times higher in E–GB samples compared to GB samples. Wronkowska et al. (2015) also reported a decrease of rutin in roasted buckwheat groats, but it was 23% and the losses of phenolic compounds were twofold, whereas Choy et al. (2013) reported about a substantial drop of rutin in instant noodles with buckwheat flour during the cooking process.

In addition, in certain extruded buckwheat samples novel phenolic compounds were detected, such as 4–hydroxybenzoic acid (1.51 mg 100 g⁻¹ DW in E–RB, 0.725–0.785 mg 100 g⁻¹ DW in E–GB samples), homovanillic acid (0.13 mg 100 g⁻¹ DW in E–RB, 0.277–0.962 mg 100 g⁻¹ DW in E–GB samples), catechin (3.97 mg 100 g⁻¹ DW in E–RB, 2.599–3.190 mg 100 g⁻¹ DW in E–GB samples), ferulic acid (7.42 mg 100 g⁻¹ DW in E–RB, 1.253–3.595 mg 100 g⁻¹ DW in E–GB samples), and hyperoside (0.900 mg 100 g⁻¹ DW in E–RB, 0.521–0.694 mg 100 g⁻¹ DW in E–GB samples), but vanillic acid, swertiamacroside, and catechin–glucosides were not detected. Consequently, they were not included in Table 3, because only some extruded products contained them.

CONCLUSIONS

By selecting the optimum germination time of raw buckwheat it is possible to increase the content of rutin, quercetin and vitexin. In further studies the germination time of buckwheat should be prolonged to 72 h in order to retain a higher content of phenolic compounds after extrusion. Extrusion had a negative effect on the content of phenolic compounds and their antioxidant activity. There is a need for more profound research to be carried out regarding the possibilities of decreasing the negative effect of extrusion on phenolic compounds in buckwheat by changing the temperature profile during extrusion.

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