The effect of buckwheat groats processing on the content of mycotoxins and phenolic compounds

Ilona Keriene, Audrone Mankeviciene, Saulius Bliznikas, Ruta Cesnuleviciene, Sigita Janaviciene, Danute Jablonskyte-Rasce and Stanislava Maiksteniene

ABSTRACT
Two buckwheat groats processing methods were used for production of final commercial product. The first one involved thermal processing (steamed) and then dehulling, and the second one dehulling without thermal treatment (raw). The research evidenced that the raw groats and hulls were several times more contaminated with aflatoxin B1 compared with steamed ones. High concentrations of aflatoxin B1 (75.8 µg kg⁻¹) and T-2 toxin (351.0 µg kg⁻¹) were detected in the raw hull samples. The total phenolics responded more sensitively to thermal treatment than phenolic acids. More than 20 times higher concentrations of quercetin (65.47 ± 6.3 µg g⁻¹) were determined in steamed hulls compared to other raw and steamed samples. Buckwheat groats and hulls, containing the highest concentrations of quercetin and hydroxybenzoic acids, were found to be 10-fold less contaminated with aflatoxin B1 and T-2 toxins; however, the correlations between the phenolics and mycotoxins were statistically insignificant.

1. Introduction
Buckwheat (Fagopyrum Esculentum Moench) is a popular plant on the global health market. Owing to its chemical composition, buckwheat grain is suitable for use in the production of functional foods and food additives (Matić, Mastilović, Cabarkapa, & Mandić, 2009). The colour of buckwheat groats depends on the dehulling method. Two methods of buckwheat dehulling are used – thermal and non-thermal. In the thermal treatment, buckwheat grains are saturated with moisture up to 22% of the dry weight and are steamed at 150–164°C temperature, sometimes from 130°C to 160°C under 5.5–6 bar pressure, then cooled, conditioned, separated by sieving into fractions and hulled. The result is a brown colour of grain (Antonov, Makevnina, Ivko, Iskusnov, & Korobov, 2013). According to the manufacturing company, during the non-thermal treatment raw grains are dehulled directly – buckwheat grains are moistened and dried until hulls become dry and kernels are moist and soft. Dry hulls break during the rolling process and moist kernels remain intact and of natural colour. Afterwards the kernels are dried. The final yield of kernels ranges from 50% to 60%.

Dehulling processes of buckwheat grain affect the content of biologically active compounds and nutritional value of groats. Research evidenced that thermal treatment of buckwheat flour is the cause of changes in the protein, crude lipid, fibre and ash contents (Pandey, Senthí, & Fatema, 2015), and that thermal processing methods caused a decrease in the total phenolics, total flavonoids and antioxidative activities of Tartary buckwheat flour extract (Dziedzic, Górecka, Marques, Rudzińska, & Podolska, 2015; Zhang, Chen, Li, Pei, & Liang, 2010). Another criterion associated with a decrease in the quality and quantity of food raw materials in food chain is the presence of mycotoxins. Unfortunately, buckwheat has been recently included in the list of cereals and cereal-derived products which may be susceptible to invasion by Aspergillus flavus, and consequently, aflatoxin contamination (EFSA,
Supporting Publications 2013: EN-406), especially, if buckwheat grain is stored for a long time. Aflatoxins remain essentially unaltered during food processing and, for this reason, could be present in processed foods – flour, pasta, baked goods and can reduce the quality and safety of products obtained from buckwheat (Chitarrini et al., 2014). Buckwheat hulls have found application in the manufacture of household goods, such as pillows, mattresses, toys. Therefore, it is vital that not only groats, which are a dietary product, but also hulls are high quality, free from toxin and mould fungi contamination, which can cause allergic reactions (Fritz & Gold, 2003). However, buckwheat is rich in phenolic compounds, which occur naturally in a range of food plants and display antimicrobial, antifungal activity, therefore it could be used for improving food safety as markers for tolerance against mycotoxicogen pathogens (Ansari, Anurag, Fatima, & Hameed, 2013; Lattanzio, Lattanzio, & Cardinali, 2006; Samapundo et al., 2007). The toxicity of phenolic compounds to pathogens is dependent on phenolic chemical structural features and is related to the presence of hydroxyl functions in the aromatic structure, which evidences that increased hydroxylation results in increased toxicity (Lattanzio et al., 2006; Teixeira, Gaspar, Garrido, Garrido, & Borges, 2013). Flavonoids account for a considerable part of chemical compounds found in buckwheat (Fabjan et al., 2003). Rutin, quercetin are included in the plant cellular structures and are characterized by a significant antioxidant activity (Dietrych-Szostak, 2004; Lattanzio et al., 2006). According to Chitarrini et al. (2014), quercetin, derived from rutin has strong antifungal inhibition against mould fungi. Lattanzio et al. (2006) suggest that free phenolic compounds, such as rutin and quercetin have stronger antifungal properties than bound forms of phenolics. Generally, found esterified or bound to cell wall in buckwheat are phenolic acids, and only a minor fraction exists as free compounds; however, phenolic acids may account for about one-third of the total phenolic compounds in our diet and can help to preserve stability of processed products in food industry (Lattanzio et al., 2006; Teixeira et al., 2013).

The present study was aimed to determine the concentration of mycotoxins, establish the change of phenolic compound concentration in response to different treatments of buckwheat groats and hulls and evaluate the relationship between phenolic compounds and mycotoxins.

2. Materials and methods

Samples of buckwheat groats and hulls (n = 24) were collected from a Lithuanian manufacturing company and examined for total phenolics, rutin and quercetin contents. Seven phenolic acids (p-hydroxybenzoic, 3,4-dihydroxybenzoic, p-coumaric, ferulic, vanillic, syringic and sinapic) were identified and quantified. The fungal infection was estimated and identified and mycotoxins T-2 toxin (T-2), ochratoxin A (OCHA), aflatoxin B1 (AFLB1) concentrations were determined. The buckwheat groats samples were prepared from the grain grown organically in Lithuania. Analyses of mycotoxins and phenolic compounds (total and individual) were done in Akademija, Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry.

2.1. Preparation of extracts

All samples were milled in an IKA A11 Basic mill (Staufen, Germany) and stored at +4°C until analysis. Mycotoxin extraction was performed according to manufacturer’s instructions. AFLB1, T-2 and OCHA were extracted according to the R-Biopharm Ridascreen™ (Darmstadt, Germany) instruction.

Total phenolic content (TPC), rutin and quercetin were detected by the method reported by Mikašauskaitė, Ragužinskienė and Maruška (2013) with slight modification. Samples (2.500 ± 0.001 g) were extracted with 75% (v/v) aqueous methanol (25.0 ± 0.1 ml) at room (21 ± 1°C) temperature for 15 h in a shaker incubator Tu-400 (MRC, Holon, Israel) under constant shaking. The mixtures were centrifuged (Hermle, Wehingen, Germany) for 10 min at 4000 rpm and stored at −20°C until analysis.

Phenolic acids were extracted according to Kvasnička et al. (2008), One gram (±0.001 g) of ground sample was weighed and 25.0 ± 0.1 ml of 0.1 M NaOH added, shaken in a water bath Memmert WNB 14 (Memmert, Schwabach, Germany) at 40°C for 60 min, cooled to room temperature, acidified with 2 M HCl to pH 5–6 and supplemented with 20.0 ± 0.1 ml of methanol. The flask was placed in an ultrasonic bath (Bandelin Electronic, Berlin, Germany) for 30 min, cooled to room temperature and made up to volume with methanol. The filtrate after filtration by 0.22 µm membrane filter (Frisenette ApS, Knebel, Denmark) was analysed by high performance liquid chromatography (HPLC).

2.2. Chemicals and reagents

All the chemicals were of analytical grade and were used as received. For extraction, methanol HPLC LiChrosolv®, acetonitrile LiChrosolv® (MERCK, Darmstadt, Germany) were used. Deoxynivalenol standard, sodium carbonate, Folin–Ciocalteau reagent, acetic acid, sodium hydroxide, hydrochloric acid and mix of phenolic acids (p-hydroxybenzoic, 3,4-dihydroxybenzoic, p-coumaric, ferulic, vanillic, sinapic, syringic) standards were purchased from Sigma-Aldrich (Germany). Most of the reagents used for the determination of mycotoxins by enzyme-linked immunoassay (ELISA) method were contained in the Ridascreen™ test kit (Biopharma, Darmstadt (Germany). Deionized water with resistivity of 18.2 MΩ was generated by a Milli-Q plus system (Miliapore, Temecula, California, U.S.A.).

2.3. Mycotoxin and phenols assay methods

Mould fungi infection level on groats and hulls were determined by the agar media method (Mathur & Kongsdal, 2003) on the potato dextrose agar medium (PDA). The fungal grain infection was estimated and identified according to the manual of Sutton, Fothergill and Rinaldi (2001). Analysis of T-2, OCHA, AFLB1 was carried out using an ELISA commercial kit Ridascreen™ (R-Biopharm AG, Darmstadt, Germany). The basis of the test is the antigen–antibody reaction. The optical densities of samples and controls from standard curve were estimated by a multichannel photometer Multiskan Ascent (Thermo Electron Corp., Vantaa, Finland), supplied with internal software, using a filter of 450 nm for T-2 (limit of detection (LOD) ~3.5 µg kg−1), OCHA (LOD 2.5 µg kg−1), AFLB1
Mycotoxin analyses of each treatment were repeated twice. A Shimadzu HPLC system was employed for determination of phenolic acids, rutin and quercetin content and consisted of the following modules: system controller SBM-20A, auto injector SIL-20A, UV-Vis detector SPD-20A, low-pressure gradient flow control valve LC-20AT, column oven CTO-20A, on-line degasser DGU-20As. Data collection and evaluation were performed by using operating system LCsolution Workstation (Shimadzu, Horiyamashita, Hadano, Kanagawa, Japan). Phenolic acids were separated by the method described by Amarowicz and Weidner (2001). Rutin and quercetin were separated by the method described by Fabjan et al. (2003) – Table 1.

HPLC conditions.

| Conditions | Rutin, quercetin | Phenolic acids |
|------------|----------------|---------------|
| Mobile phase, v/v | Methanol:water: Acetic acid 100:150:5 | A – methanol B – water:acetonitrile:acetic acid 88:10:2 |
| Elution | Isocratic | Gradient: 100% B (4 min), 0–100% A (15 min), holding 100% A 10 min, decrease to 0% A in 0.5 min; holding 100% B 6.5 min |
| Flow rate, ml/min | 1.0 | 1.0 |
| Column RP-18 | LiChrospher 100 250 × 4.6 mm, 5 µm | |
| Guard column | 7.5 × 4.6 mm |
| Column oven temp., °C | 30 | 30 |
| Injection volume, µl | 10 | 10 |
| Detection, nm | 360 | 260 and 320 |

Statistical analysis was calculated by using the packages a STAT ENG from software SELEKCIJA (Tarakanovas & Raudonius, 2003).

3. Results and discussion

3.1. Mycotoxin concentration in buckwheat groats and hulls

The concentrations of mycotoxins in buckwheat groats and hulls dehulled by thermal and non-thermal treatment are shown in Table 2. All the tested samples were found positive for the investigated toxins; however, the concentrations of OCHA and T-2 mycotoxins in groats in both treatments were low (Figure 1). The total contamination of groats and hulls with mould fungi was higher in the non-thermal treatment (raw) samples than in those in thermal treatment (steamed).

Aspergillus spp. infection level in steamed groats was four times as high as that in raw groats. Although AFLB1-producing species were detected in the groats samples of both

Table 2. The concentrations of mycotoxins in buckwheat groats and hulls.

| Test product | Mycotoxins, µg kg⁻¹ |
|--------------|---------------------|
| Groats, steamed | Aflatoxin B1 | Ochratoxin A | T-2 toxin |
| raw | 2.3 ± 0.6 | 0.7 ± 0.2 | 4.1 ± 0.5 |
| Hulls, steamed | 1.9 ± 0.3 | 3.8 ± 0.4 | 3.6 ± 0.2 |
| raw | 75.8 ± 1.6 | 3.8 ± 0.5 | 351.0 ± 15.8 |

Mean ± standard deviation (n = 6); LOD: limit of detection

Figure 1. Contamination of buckwheat groats and hulls with mould fungi. Predominant species: A. flavus, P. verrucosum, F. Sporotrichioides.

Figure 1. Contaminación de granos de trigo sarraceno y las cáscaras con moho. Especies predominantes: A. flavus, P. verrucosum, F. Sporotrichioides.
treatments, the AFLB1 concentrations in raw groats were approximately eight-fold higher (2.3 ± 0.6 µg kg⁻¹) compared with steamed groats (Table 2). The concentrations of AFLB1 toxins in baby foods exceeded the allowable limits (Regulation (EC) No.1881/2006, 0.1 µg kg⁻¹) several times and reached the highest allowable level for adults (2.0 µg kg⁻¹). Since buckwheat is widely used as a dietary product, such groats should not be used for consumption.

The contamination of steamed hulls with mould fungi and mycotoxins was low; however, very high concentrations of mycotoxins were determined in the raw hulls samples that had a high mould fungi infection level too. Nearly 100% of raw hulls were contaminated with Penicillium spp. mould fungi (for groats about 10–15%); however, mycotoxin producers Penicillium viridicatum, Aspergillus ochraceus were not detected. Fusarium spp. contamination was minimal, F. sporotrichioides species, producing T-2 toxin (Yli-Mattila, 2006) were not detected. Kreft, Fabjan and Yasumoto (2006) found that the concentration of OCHA in buckwheat grain dust was significantly greater than in grain (p < 0.01) and deoxynivalenol was detected in 79.2% of grain samples and in 100% of grain dust samples. Buckwheat hulls are not used in food industry; however, attention should be drawn to their use as fillers, for example, pillows. Contaminated hulls are recommended to be used as feedstock for fuel production or mulch. Goldberg and Angle (1984) have reported that part of aflatoxins is adsorbed in the soil and biodegraded to water soluble products and CO₂ therefore the contamination of ground water is highly unlikely. Hulls, including underlying aleurone layer, hilum (navel) and a sizable portion of the germ, are usually more susceptible to mycotoxin contamination. It is likely that inadequate storage conditions of buckwheat raw material increase the likelihood of mould growth and synthesis of mycotoxins.

Krysińska-Traczyk, Perkowski and Dutkiewicz (2007) found that the concentration of OCHA in buckwheat grain dust was significantly greater than in grain (p < 0.01) and deoxynivalenol was detected in 79.2% of grain samples and in 100% of grain dust samples. Buckwheat hulls are not used in food industry; however, attention should be drawn to their use as fillers, for example, pillows. Contaminated hulls are recommended to be used as feedstock for fuel production or mulch. Goldberg and Angle (1984) have reported that part of aflatoxins is adsorbed in the soil and biodegraded to water soluble products and CO₂ therefore the contamination of ground water is highly unlikely. Hulls, including underlying aleurone layer, hilum (navel) and a sizable portion of the germ, are usually more susceptible to mycotoxin contamination compared to other grain parts (Mutungi, Lamuka, Arimi, Gathumbi, & Onyango, 2008). Little is known about the effectiveness of the dehulling process, that industrial manufacturers use, for the decontamination of the buckwheat grains and groats. Analysis of the spelt grain samples revealed that deoxynivalenol concentrations in glumes were five times as high as those in grain. Similar trends were identified for the concentrations of zearalenone and T-2/HT-2 mycotoxins (Jablonskyté-Račė et al., 2015). Based on the study by Mutungi et al. (2008), aflatoxin levels in whole-grain maize samples with a mean of 97.3 ng/g after dehulling process of the grains significantly decreased (p < 0.001) with a mean value of 57.3 ng/g. The maize by-products, comprising hulls and fines, had 2–7 times higher levels of aflatoxin than the whole-grain maize. The presence of an unidentified barrier(s) to endosperm contamination with mycotoxins was reported by Abbas and Shier (2009).

The observations of mycotoxins in rice hulls and bran show that fumonisins levels in dehulled and milled unpolished rice were very high in hulls (≥17 ppm), low in brown rice (0.9 ppm), moderate in bran (≤4 ppm), but were below the level of detection in polished rice. Llewellyn et al. (1988) indicated that buckwheat and rice hull media inoculated with Fusarium tricinctum yielded trichotheones (T-2 toxins) in the ppm range, with the buckwheat hull media producing approximately three times more T-2 toxins than the rice hull media. Buckwheat hull media yielded approximately twice the quantity of AFLB1 and AFLG1 than did rice hull media.

Mycotoxins are stable under heat treatment. As a result, treatment with boiling water, roasting or even autoclaving cannot adequately destroy them. Raters and Matisek (2008) have observed that OCHA seems to be stable up to 180°C; however, aflatoxin B1 was almost completely degraded at heating temperatures of 160°C and above. OCH and AFL thermal stability has been widely analysed by Bullerman and Bianchini (2007). According to the authors, OCH is stable during bread baking. Ordinary cooking of rice contaminated with aflatoxin B1 showed an average reduction of 34%. Even further reduction was obtained with pressure cooking (78–88%); boiling corn grits gave an average reduction of aflatoxins of 28%, while frying the boiled grits gave 34–53% total reduction.

3.2. Phenolic compounds concentration

Various antifungal compounds, such as phenolics, are used in the prevention of fungi and mycotoxin accumulation in different plant species (Ansari, Anurag, Fatima, & Hameed, 2013; Chitarrini et al., 2014; Samapundo et al., 2007). Buckwheat grain contains a wide range of phenolic compounds. Our previous studies were focused on the quantification of phenolic compounds in buckwheat grain (Keriëne et al., 2015). In the present study, we quantified three groups of phenolic compounds in buckwheat groats and hulls dehulled by thermal and non-thermal treatment: flavonoids (rutin, quercetin), hydroxybenzoic acids (p-hydroxybenzoic, 3,4-dihydroxybenzoic and vanillic) and hydroxycinnamic acids derivate (p-coumaric acid, sinapic and syringic) (Table 3). Analysis of the phenolic compounds showed that irrespective of the treatment method, phenolic compounds, except for p-hydroxybenzoic acid, were concentrated in hull samples. TPC in raw groats and hulls was 15% and 10% higher than in steamed ones. The effects of various thermal processing methods on the phenolics of Tartary buckwheat were studied by Zhang et al. (2010). The total phenolic and flavonoid content of Tartary buckwheat flour extracts significantly (p < 0.05) decreased by 9% after roasting at 120°C for 40 min and processing by pressured steam-heating at 0.2 MPa for 40 min. However, other authors did not find any statistically significant changes in the total phenolic compound contents after roasting (200°C, 10 min) and extrusion (170°C) of dark buckwheat flour (Sensoy, Rosen, Ho, & Karwe, 2006).

The distribution of individual phenolic compound concentrations in groats and hulls differed between the dehulling treatments. Hulls had twice as high rutin content as groats; however, treatment method did not have any effect on rutin concentration. Very different concentrations were established for quercetin (Table 3). More than 20 times higher concentrations of quercetin (65.47 ± 6.3 µg g⁻¹) were determined in steamed hulls compared to other raw and steamed samples. Other researchers reported that thermal treatment of buckwheat groats can affect flavonoid content. Hęś, Dziedzic, Górecka, Drożdżyńska and Gujska (2014) observed that boiled buckwheat groats contained significantly more catechins in comparison to raw buckwheat groats but no change in the rutin content was found. Kreft, Fabjan and Yasumoto (2006) determined that
Table 3. The concentration of phenolic compounds in buckwheat groats and hulls.

| Phenolic Compound                  | Steamed µg g⁻¹ d.w. | Raw µg g⁻¹ d.w. | Hulls µg g⁻¹ d.w. | Hulls µg g⁻¹ d.w. |
|-----------------------------------|---------------------|-----------------|------------------|-------------------|
| Total phenolic content            | 6789 ± 280          | 9263 ± 650      | 11126 ± 899      | 13781 ± 140       |
| Rutin                             | 150.4 ± 11.3        | 180.5 ± 44.4    | 384.3 ± 32.0     | 389.5 ± 32.0      |
| Quercetin                         | 2.5 ± 0.5           | 0.3 ± 0.2       | 65.47 ± 6.3      | 2.84 ± 0.3        |
| 3,4-Dihydroxybenzoic acid         | 23.0 ± 1.8          | 20.2 ± 1.9      | 57.5 ± 6.9       | 39.0 ± 6.3        |
| p-Hydroxybenzoic acid             | 14.6 ± 1.2          | 17.7 ± 1.1      | 6.7 ± 1.9        | 2.8 ± 0.8         |
| p-Coumaric acid                   | 1.0 ± 0.5           | 2.4 ± 0.8       | 2.2 ± 0.6        | 3.8 ± 1.3         |
| Vanillic acid                     | n.d.                | n.d.            | 2.2 ± 0.5        | 2.8 ± 0.1         |
| Ferulic, sinapic, syringic acids  | n.d.                | n.d.            | n.d.             | n.d.              |
| Total phenolic acid content       | 38.9 ± 1.9          | 40.0 ± 3.1      | 76.9 ± 6.5       | 67.9 ± 6.6        |

Mean ± standard deviation (n = 6); n.d.: not detected.
Promedio ± desviación estándar (n = 6); n.d.: no detectado.

3.3. Distribution of mycotoxin and phenolic compounds in buckwheat groats and hulls

Since phenolic compounds in buckwheat grain tend to inhibit pathogen growth and mycotoxin production, we compared the concentrations of mycotoxins and phenolic compounds in differently dehulled buckwheat groats and hulls. A trend was revealed that samples containing the highest concentrations of quercetin and hydroxybenzoic acids in groats and hulls were found to be 10-fold less contaminated with AFLB1 and T-2 toxins. A salient difference ratio between phenolic compounds and mycotoxin concentrations in the hull samples was determined. The difference ratio of quercetin and AFLB1 content in raw hulls was 1:27 and that in steamed hulls 34:1 (Figure 2(a)). The difference of the ratio between phenolic compound and mycotoxins concentrations in groats samples was lower; however, the same trend was revealed suggesting that the samples with higher quercetin, p-hydroxybenzoic acid and 3,4-dihydroxybenzoic acid concentrations had lower levels of AFLB1 and T-2 toxins (Figure 2(b)). However, no significant correlation between the investigated mycotoxins and phenolic compounds in raw or steamed groats and hulls was recorded. Thermal buckwheat grain treatment might have had a greater effect on the reduction of mycotoxin and mould contamination. However, protective effect of rutin and vanillic acid against pathogens and toxic metabolites produced by them has been proven (Beekrum, Govinden, Padayachee, & Odhav, 2003; Lattanzio et al., 2006). According to Chittarini et al. (2014), hulls of Fagopyrum esculentum not proportionally but are susceptible to A. flavus infection and rutin-derived quercetin appears to be more efficient in inhibiting aflatoxin biosynthesis than their parent compound rutin. Samapundo et al. (2007) determined that application of phenolic compounds did not have any effect on growth of the Aspergillus species in corn, but significantly reduced fumonisin B1 and AFLB1 production. As a result, high concentrations of mycotoxins in raw groats and hulls and utilization of phenolic compounds to reduce mycotoxin contamination in buckwheat groats and hulls warrant further research.
4. Conclusions

The research findings suggest that irrespective of the treatment method, phenolic compound contents were significantly higher in buckwheat groats compared with goats. The total phenolics responded more sensitively to thermal treatment than phenolic acids. The content of quercetin in steamed buckwheat groats and hulls was from 8 to 20 times as high as that in raw groats and hulls.

Steamed groats were characterized by lower mycological contamination and their hulls were either free from mycotoxins contamination or the contamination was at a trace level; however, the correlation between the studied mycotoxins and phenolics was statistically insignificant. In order to use raw buckwheat groats for baby food, the grains need to be thoroughly checked for AFLB1 presence (Regulation (EB) No.1881/2006). Because of the high concentrations of AFLB1 and T-2 mycotoxins and mycological contamination, we recommend that raw hulls should be used for human needs with great caution.

 Disclosure statement

No potential conflict of interest was reported by the authors.

 Funding

The study was supported by the Research Council of Lithuania, Project No. SVE-04/2014.

References

Abbas, H.K., & Shier, W.T. (2009). Mycotoxin contamination of agricultural products in the southern United States and approaches to reducing it from pre-harvest to final food products. In M. Appell, D. F. Kendra, & M.W. Truckess (Ed.), Mycotoxin prevention and control in agriculture. (Vol. 1031, Chapter 3, pp. 37–57). American Chemical Society. doi:10.1021/bk-2009-1031.ch003

Amarowicz, R., & Weidner, S. (2001). Content of phenolic acids in rye caryopses determined using DAD-HPLC method. Czech Journal of Food Sciences, 19, 201–205.

Ansari, A.M., Anurag, A.A., Fatima, Z., & Hameed, S. (2013). Natural phenolic compounds: A potential antifungal agent. In A. Méndez-Vilas (Ed.), Microbial pathogens and strategies for combating them: Science, technology and education (Vol. 2, pp. 1189–1195). Spain: Formatex reasercher center.

Antonov, M. N., Makevina, E. I., Ivko, G. I., Iskusnov, J. V., & Korobov, I. A. (2013). Grain heat treatment method. Federa'noe gosudarstvennoe byudzhetnoe obrazovatel'noe uchrezhdenie vysshego professional'nogo obrazovanija Volgogradskaja gosudarstvennaja sel'skohoz- jajstvennaja akademija, Volgograd, Russian Federation. Retrieved from http://www.freepatent.ru/patents/2485790

Bullerman, L.B., & Bianchini, A. (2007). Stability of mycotoxins during food processing. International Journal of Food Microbiology, 119, 140–146. doi:10.1016/j.ijfoodmicro.2007.07.035

Chitarrini, G., Nobili, C., Pinzari, F., Antonini, A., De Rossi, P., Del Fiore, A., ... Reverberi, M. (2014). Buckwheat achenes antioxidant profile modulates Aspergillus flavus growth and aflatoxin production. International Journal of Food Microbiology, 189, 1–10. doi:10.1016/j.ijfoodmicro.2014.07.029

European Food Safety Authority (EFSA). (2013). Aflatoxins (sum of B1, B2, G1, G2) in cereals and cereal derived food products. Supporting Publications 2013:EN-406. Retrieved from http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/406e.pdf

F fabjan, N., Rode, J., Kosir, J.J., Wang, Z., Zhang, Z., & Kreft, I. (2003). Tertiary buckwheat (Fagopyrum tataricum Gaertn.) as a source of dietary rutin and quercitrin. Journal of Agricultural and Food Chemistry, 5, 6452–6455. doi:10.1021/jf034175s

Fritz, S.B., & Gold, B.L. (2003). Buckwheat pillow-induced asthma and allergic rhinitis. Annals of Allergy, Asthma & Immunology, 90, 355–358. doi:10.1016/S1081-1206(10)61807-8

Goldberg, B.S., & Angle, J.S. (1984). Aflatoxin Movement in Soil. JEQ, 14 (10), 224–228. doi:10.2134/jeq1985.00472422500140002014x

Hęś, M., Dziedzic, K., Górecka, D., Marques, A., Rudzińska, M., & Podolska, G. (2015). Content of phytosterols in raw and roasted buckwheat groats and by-products, food analysis, food quality and nutrition. Czech Journal Food Sciences, 33, 424–430. doi:10.17221/121/2015-CJFS

Jablonsky-Raščev, D., Mankevičienė, A., Supronienė, S., Kerienė, I., Maitienėniene, S., Bliznikas, S., & Cesnulevičienė, R. (2015). Grains of winter wheat spelt (Triticum Spelta L.) for save food production. International Scholarly and Scientific Research and Innovation, 17(7), Part II: 199–202.

Kerienė, I., Mankevičienė, A., Bliznikas, S., Jablonsky-Raščev, D., Maitienėniene, S., & Cesnulevičienė, R. (2015). Biologically active phenolic
compounds in buckwheat, oats and winter spelt wheat. Zemdirbyste-Agriculture, 102(3), 289–296. doi:10.13080/z-a.2015.102.037
Kraft, I., Fabjan, N., & Yasumoto, K. (2006). Rutin content in buckwheat (Fagopyrum esculentum Moench) food materials and products. Food Chemistry, 98, 508–512. doi:10.1016/j.foodchem.2005.05.081
Kryśinska-Traczyk, E., Perkowski, J., & Dutkiewicz, J. (2007). Levels of fungi and mycotoxins in the samples of grain dust collected from five various cereal crops in eastern Polan. Annals of Agricultural and Environmental Medicine, 14, 159–167.
Kvasnička, F., Čopíková, J., Ševčik, R., Krátká, J., Syntytsia, A., & Voldlích, M. (2008). Determination of phenolic acids by capillary zone electrophoresis and HPLC. Central European Journal of Chemistry (CEJ), 6, 410–418.
Lattanzio, V., Lattanzio, V.M., & Cardinali, A. (2006). Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. In F. Imperato (Eds.), Phytochemistry: Advances in Research (pp. 23–67). Kerala: Research Signpost.
Llewellyn, G.C., Sherertz, P.C., Armstrong, K.W., Jr., Reynolds, J.D., Kimbrough, T.D. . . . Dashek, W.V. (1988). Mycotoxicogenic isolates and toxin production on buckwheat and rice hulls used as bedding materials. Journal of Industrial Microbiology, 3, 351–356. doi:10.1007/BF01569556
Mathur, S. B., & Kongdsal, O. 2003. Common laboratory seed health methods for detecting fungi (p. 425). Copenhagen: International Seed Testing Association.
Matić, J.J., Matilović, J., Cábarkapa, I.S., & Mandić, A.J. (2009). Mycotoxins as a risk in the grain food. Zbornik Matice Srpske Za Prirodne Nauke /Proc Natural Sci, Matica Srpska Novi Sad, 117, 79–86. doi:10.2298/ZMSPN0917079M
Mikašauskaitė, J., Ragažinskienė, O., & Maruška, A. (2013). Variation of total amount of phenolic compounds, radical scavenging activity and volatile compounds of Liriodendron tulipifera L. and Ginkgo Biloba L. leaves extracts during different vegetation periods. Biologija, 59, 175–186. doi:10.6001/biologija.v59i2.2750
Mutungi, C., Lamuka, P., Arimi, S., Gathumbi, J., & Onyango, C. (2008). The fate of aflatoxins during processing of maize into mutokai – A traditional Kenyan food. Food Control, 19, 714–721. doi:10.1016/j.foodcont.2007.07.011
Pandey, S., Senthí, A., & Fatema, K. (2015). Effect of hydrothermal treatment on the nutritional and functional properties of husked and dehusked buckwheat. Journal of Food Processing & Technology (JFPT), 6, 461. doi:10.4172/2157-7110.1000461
Raters, M., & Matisek, R. (2008). Thermal stability of aflatoxin B1 and ochratoxin A. Mycotoxin Research, 24(3), 130–134. doi:10.1007/BF03032339
Samapundo, S., De Meulenaer, B., Osei-Nimoh, D., Lamboni, Y., Debevere, J., & Devlieghere, F. (2007). Can phenolic compounds be used for the protection of corn from fungal invasion and mycotoxin contamination during storage? Food Microbiology, 24, 465–473. doi:10.1016/j.fm.2006.10.003
Sedej, L.J., Sakač, M.B., Mišan, A.C., & Mandić, A.J. (2010). Antioxidant activity of wheat and buckwheat flours. Zbornik Matice Srpske Za Prirodne Nauke /Proc Natural Sci, Matica Srpska Novi Sad, 118, 59–68. doi:10.2298/ZMSPN1018059S
Senson, I., Rosen, R.T., Ho, C., & Karwe, M.V. (2006). Effect of processing on buckwheat phenolics and antioxidant activity. Food Chemistry, 99, 388–393. doi:10.1016/j.foodchem.2005.08.007
Sutton, D.A., Fothergill, M.A., & Rinaldi, M.G. (2001). Guide to clinically significant fungi. Moskva: Mir.
Tarakanovas, P., & Raudonius, S. (2003). Statistical analysis of agronomical research data with computer programs ANOVA, STAT, SPLIT-PILOT from packet SELEKCIJA and IRRISTAT (pp. 58). Kaunas: Lithuanian University of Agriculture. (in Lithuanian).
Teixeira, J., Gaspar, A., Garrido, E.M., Garrido, J., & Borges, F. (2013). Hydroxycinnamnic acid antioxidants: An electrochemical overview. BioMed Research International, 2013, 1–11. doi:10.1155/2013/251754
Yang, L., Zhang, H., Cheng, L., Gu, Z., Hua, D., Qi, X. . . . Wang, L. (2014). Effect of extrusion on the hydrophilic antioxidant capacity of four whole grains. Journal of Food and Nutrition Research, 2(2), 80–87. doi:10.12691/jfr-2-2-4
Yli-Mattila, T. (2010). Ecology and evolution 353 of toxigenic Fusarium species in cereals in Northern Europe and Asia. Journal of Plant Pathology, 92(1), 7–18.
Yoo, J., Kim, Y., Yoo, S.-H., Inglett, G.E., & Lee, S. (2012). Reduction of rutin loss in buckwheat noodles and their physicochemical characterisation. Food Chemistry, 132, 2107–2111. doi:10.1016/j.foodchem.2011.12.065
Zhang, M., Chen, H., Li, J. Pei, Y., & Liang, Y. (2010). Antioxidant properties of tartary buckwheat extracts as affected by different thermal processing methods. LWT - Food Science and Technology, 43, 181–185. doi:10.1016/j.lwt.2009.06.020
Zielinski, H., Michalska, A., Piskula, M.K., & Kozlowska, H. (2006). Antioxidants in thermally treated buckwheat groats. Molecular Nutrition & Food Research, 50(9), 824–832. doi:10.1002/mnfr.200500258