Article

Sustainable Utilization of Novosadska Variety Buckwheat as Cultivated Biodiversity-Friendly Crops

Boris Pisinov * , Radojica Rakić , Sveto Rakić , Zoran Ž Sekulić , Tijana Milićević , Gordana Kulić , Sanja Đurović

Posted Date: 2 July 2024

doi: 10.20944/preprints202407.0164.v1

Keywords: Novosadska variety buckwheat; storage; quality; nutritional properties; antioxidant activity; bioactive compound

Article

Sustainable Utilization of *Novosadska variety* Buckwheat as Cultivated Biodiversity-Friendly Crops

Boris Pisinov [1,*], Radojica Rakić [2], Sveto Rakić [2], Zoran Ž. Sekulić [3], Tijana Milićević [4], Gordana Kulić [2] and Sanja Đurović [1]

[1] Institute for Plant Protection and Environment, Teodora Drajzera 9, 11040 Belgrade, Serbia; stojakovicsm@yahoo.com (S.Đ.)
[2] Faculty of Agriculture, University of Belgrade, Nemanjina 8, 11080 Belgrade-Zemun, Serbia; radojicarakic@gmail.com (R.R.); sveto@agrif.bg.ac.rs (S.R.); gocaduvan@gmail.com (G.K.)
[3] Institute of Public Health of Belgrade, Bulevar Despota Stefana 54a, 11108 Belgrade, Serbia; zoran.sekulic@zdravlje.org.rs (Z.Ž.S.)
[4] Institute of Physics Belgrade, National Institute of the Republic of Serbia, University of Belgrade, Pregrevica 118, 11080 Belgrade, Serbia; tijana.milicevic@ipb.ac.rs (T.M.)
* Correspondence: boriss752002@yahoo.com

Abstract: Buckwheat is important not only as a crop that improves soil quality and reduces erosion, but its excellent nutritional profile for usage in functional foods. This study aimed to investigate how long-term storage (3, 6, and 9 months) affects *Novosadska variety* buckwheat's chemical, nutritional, and antioxidative properties, phenolic acids and bioflavonoids profiles. Standard traditional methods were used for quality determinations, and instrumental (spectrophotometry, reverse-phase high performance liquid chromatography) for antioxidant activity and bioactive compounds determination. One-way ANOVA, and Tukey's HSD post-hoc were performed for statistical data processing. Throughout the storage period, proximate composition and starch content significantly decreased (p<0.05), while total carbohydrates, β-glucan, and energy value significantly increased (p<0.05). Significant decrease in pH and alcoholic acidity of 0.55 pH units, and 0.33% DM, were observed. Total phenol content and antioxidant activity decrease to 5.57 mg GAE/g DM TPC, 22.20 μmol Fe2+/g DM FRAP and 8.12 μmol TE/g DM DPPH, during the storage (p<0.05). Of fifteen phytochemical compounds, gallic, p-coumaric, trans-cinnamic acids, and epicatechin were in high abundance, with a notable decrease in epicatechin (38%). To the authors' knowledge, dihydrocaffeic and phloretic acids, daidzein, naringin, and naringenin were quantified in buckwheat flour for the first time. Easy adaptability to the environment, ability to attract various insects, speedy short-season growing plant for food, many nutritional and health benefits, gives buckwheat potential to be sustainable and biodiversity-friendly crops.

Keywords: *Novosadska variety* buckwheat; storage; quality; nutritional properties; antioxidant activity; bioactive compound

1. Introduction

Buckwheat is a pseudocereal belonging to the genus *Fagopyrum* of the family Polygonaceae, and the two most commonly cultivated species are Common buckwheat (*F. esculentum*) and Tartary buckwheat (*F. tartaricum*). In agriculture, the importance of using buckwheat as an environmentally friendly crop that contributes biodiversity, is reflected in the improvement of the biological and physical conditions of the soil and the sustainability of the agricultural system directly throughout cover-crop, forecrops and crop rotations [1]. In addition, buckwheat mainly decreases soil acidification, increases available soil phosphorous and nitrogen as an efficient green fertilizer, plays a significant role in carbon cycle regulation, can be utilized in the phytoremediation of lead,

 doi:10.20944/preprints202407.0164.v1

aluminium, mercury and cadmium, prevents the spread of root and plant diseases, that provides effective and sustainable options to suppress pests, improve soil health and crop yield [1–4].

Buckwheat (*Fagoppyrum esculentum Moench*) is an ancient annual plant whose grains are used in human and animal nutrition. During flowering, it produces up to 2000 flowers per plant, which contain a large amount of nectar, that makes it a honey-bearing plant and contributes to the increased content of organic acids, phenolic and flavonoid compounds in honey [5]. Dealing with the conservation of biodiversity and yield of buckwheat, where the density of wild pollinators remained at the same level before and after the installation of bee hives, suggested that there was no competition for resources, due to the high density of flowers [6]. Buckwheat grains have a triangular shape, which size varies considerably. They are rich in carbohydrates, proteins, lipids, essential fatty acids, minerals, vitamins, essential amino acids, especially lysine (5.1 g/ 100 g protein) but it's gluten-free [7]. Grains have extremely favourable nutritional components, proteins contain all important amino acids necessary for the human body [8]. Buckwheat grains contain high levels of rutin formerly known as vitamin P, and the concentration of this bioflavonoid is considered to be key to quality assessment. Based on this property, buckwheat flour is used for food fortification [9]. The use of different levels of nitrogen fertilizer effect on an increase of the number and surface area of endosperm cells, as well as the content of amylose, amylopectin and starch [10]. Amylose content is a key factor in grain quality, while the fine structure of amylopectin defines the properties of buckwheat starch. Buckwheat starch has a smaller granule size, so due to this property, it is often used as a thickener, binder, film or foam in food and other industries [11]. Buckwheat bran is a by-product in its processing and contains various natural compounds, primarily fibres, proteins, flavonoids and phenols (about 40%) [12]. Investigations of bran and endosperm confirm the presence of natural antioxidants of phenolic type [13]. In buckwheat flour, some secondary metabolites (phenolic acids and bioflavonoids), which have functional properties, were the special subjects of many studies [14].

In these modern times, the search for nutritionally justifiable and health-beneficial food stands out in the foreground the functional food with bioactive ingredients, where buckwheat falls as the first choice, because it has major potential as a food ingredient, especially for the functional food industry. Consumer awareness and demands for health nutritional excellence, as well as good acceptability of end-products, represent well potential for innovative value-added buckwheat products contributing to sustainability. Buckwheat products, such as whole grains, husks, kernels or flour, are traditionally consumed in the forms of breakfast cereals and bakery products or added to enriched and fortified products such as bread, dough, pasta, pancakes, cookies and biscuits, noodles, sprout, extruded snack food or honey and tea [15–17]. Also, in the modern meat industry, the use of such ingredients in meat processed products gained importance and became one of directions of the development. Many researchers described technological procedures, where the addition of buckwheat raw materials up to a certain amount into recipes and meat product technologies effectively improved physical, chemical, functional, technological, structural, mechanical and organoleptic properties of combined meat products, where were included semi-smoked and frankfurter-type sausages, horsemeat and chicken patties, pork meatballs and more related products [18–21]. Likewise, buckwheat is recognized for its unique traits as a food with a good flavour, texture and colour [22]. As it contains flavonoids, which are pigments, it could give colour and initiate colour-sensory changes in many food end-products [19,23].

The importance of buckwheat and its products is also observed in various health-related benefits, such as hypoglycemic, anticancer, hypocholesterolemic, anti-hypertensive, and anti-inflammatory properties, which significantly raise their agricultural, industrial, and pharmaceutical utility value [17,20,24,25].

Also, the wide application of buckwheat in the daily lifestyle of consumers is reflected through the production of alcoholic beverages and products of fermentation such as vinegar [22].

As the world's need for food and the production of nutritionally justifiable and health-beneficial food is increasing, it is necessary to ensure long-term storage of raw cereals, including buckwheat and their later application in the food industry. The natural ageing process of grain causes

 doi:10.20944/preprints202407.0164.v1

physiological degradative changes in biological, physical and chemical properties. Storage conditions determine the preservation of nutritional characteristics for a longer period and may affect the aforementioned changes [26]. The lipid degradation has a negative effect on the taste and flavour, which reduces the utility value of the grain and the shelf life [27]. There are numerous studies dealing with this issue [28–31], but according to the best of our knowledge, the investigation of the *Novosadska variety* is not studied in this way.

In this sense, this experiment aimed to i) analyze and evaluate physico-chemical, chemical, nutritional and antioxidative properties, phenolic acids and bioflavonoids profiles of stored *Novosadska variety* buckwheat with investigating possible losses and changes during long-term storage (3, 6 and 9 months); ii) to propose the optimal storage time of *Novosadska variety* buckwheat grains to preserve the most nutritious components for obtaining more technologically justified and health-beneficial end-products. This study on *Novosadska variety* buckwheat, as a variety of alternative plant culture, was conducted in the perspective of favourable agricultural sustainable utilization and it's possible valuable extended cultivation on farming fields as biodiversity-friendly crops.

2. Materials and Methods

2.1. Materials and Storage Conditions

Buckwheat (*Novosadska variety*) used for this investigation, as a locally significant variety of alternative plant culture and pseudocereal, was harvested during 2022 at the technological maturity of the grain, and the obtained raw grains were cleaned of impurities and damaged grains. Sampling was performed according to ISO method [32]. A sample (about 4 kg) of freshly harvested buckwheat grains was brought to the laboratory. A detailed description of sample storage has been previously reported [33]. In short, the grain sample was equally distributed in eight closed plastic containers of the same volume. Six containers with samples were placed in a drying oven Digitheat-TFT (J.P. Selecta, Barcelona, Spain) at a temperature of (40 ± 2) °C in conditions with thermoregulation and common relative humidity for a period of 3, 6, and 9 months. Two containers of freshly harvested grains marked as 0 months and two containers of stored grains at the end of each tested period were taken, the contents of the same two containers were mixed, and a sub-sample (about 0.5 kg) was formed, then it was crushed and ground at a speed of 20000 rpm on a laboratory mill A10 (IKA Works Inc., NC, Wilmington, USA). For research, three samples of each sub-sample were separately prepared from flour material with a particle size of 1 mm, and analyzes were conducted in triplicate.

2.2. Chemicals, Reagents, and Standards

All chemicals, reagents, and standard solutions used in the experiments were analytical grade, and enzyme solutions were of the recommended enzyme activity. The Folin-Ciocalteu reagent was provided by Reagecon Diagnostics Ltd. (Shannon, Ireland), and 2,4,6-tripyridyl-s-triazine (TPTZ, ≥ 99%) by Fluka (Honeywell, Charlotte, NC, USA), while 1,1-diphenyl-2-picrylhydrazyl radical (DPPH, > 97.0%), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, 97%) were purchased from Sigma-Aldrich Chemie GmbH (Merck KGaA, Darmstadt, Germany). Analytical standards of phenolic acids (gallic acid, dihydrocaffeic acid, phloretic acid, trans-cinnamic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, hesperetic acid, ≥ 98.0%, w/w) and bioflavonoids (catechin, epicatechin, naringin, daidzein, naringenin, quercetin, ≥ 98.0%, w/w) were provided by Sigma-Aldrich Chemie GmbH (Merck KGaA, Darmstadt, Germany). For chromatographic analysis, gradient grade HPLC methanol CHROMASOLV™ (≥ 99.9%) and HPLC grade water CHROMASOLV Plus were purchased from Riedel-de Haën (Honeywell, Charlotte, NC, USA).

2.3. Determination of Chemical and Nutritional Properties

Physico-chemical, chemical and nutritional properties were performed through analysis of the proximate composition (moisture, total protein, total lipids, total ash and total carbohydrates content), and starch, β-glucan (dietary fibre), energy value, pH value, alcoholic acidity.

 doi:10.20944/preprints202407.0164.v1

Moisture content was determined by the gravimetric method according to ISO 712 [34] using a Digitheat-TFT electric oven (J.P. Selecta, Barcelona, Spain). Before adding samples, the metal dish with lid was dried at a temperature within the range (130 - 133) °C, the closed dish was cooled in a desiccator to room temperature, and then the weight of the closed dish was determined. The undried flour of 5 g was weighed into a metal dish, and then the mass of the sealed dish containing the sample was measured. An opened metal dish with flour and lid was dried for 90 min at a temperature between (130 - 133) °C, a closed dish with flour sample was cooled in a desiccator for 45 min and then the mass of the closed metal dish with flour was measured. The drying and cooling procedure was repeated until a constant mass was obtained. Moisture (in %) was calculated as the mass difference of the dish with lid plus undried flour and the dish with lid plus oven-dried flour divided by the mass difference of the dish with lid plus undried flour and the empty dish with lid.

Total lipids content was determined by the extraction-gravimetric method according to NMKL 160 [35] using a Soxhlet type extraction apparatus, DK-2000-IIIL water-bath (ChemLand, Stargard, Poland) and a Digitheat-TFT electric oven (J.P. Selecta, Barcelona, Spain). The flour sample (2 g) was placed in the Erlenmeyer flask, then it was boiled and hydrolyzed for 1 h with 50 ml of 4 mol/l hydrochloric acid in order to liberate bounded fats and transform fatty acids salts to free acids. The mixture was cooled to room temperature, filtered through filter-paper and washed three times with warm distilled water. The obtained residue on the filter-paper was dried in an electric oven for 90 min at (102 ± 2) °C. Dried filter-paper with residue was put into the extraction thimble and placed in the Soxhlet-type extraction apparatus, after which extraction was carried out for about 6 h. On the other hand, the weighed recipienting round-bottom flask was 2/3 fulfilled with the amount of extraction solvent (petroleum ether). About 10 ml of petroleum ether was condensed per minute during continuous extraction. After the extraction was completed, the solvent was distilled off, and recipienting round-bottom flask with fat residue was dried in the oven for 2 h at (102 ± 2) °C, then was cooled in a desiccator for 30 min and the mass was measured. The drying and cooling procedure was repeated until a constant mass was obtained. Total lipids (in %DM) were calculated by the mass difference of the recipienting round-bottom flask with fat residue after the extraction and empty recipienting round-bottom flask before the extraction, then recalculated on 100 g of the sample's dry matter.

Total ash content was determined by the gravimetric method according to ISO 2171 [36] using a ME 520 electrically heated muffle furnace (Prederi Vittorio & Figli s.n.c., Milan, Italy). Before adding the sample, the cleaned platinum ashing dish was placed in the furnace and heated to (900 ± 25) °C for 5 min, then cooled in a desiccator to room temperature and the mass of the empty ashing dish was measured. About 2 g of flour was placed in a platinum ashing dish and transferred to a muffle furnace heated (900 ± 25) °C, firstly for igniting, after which incineration was continued for at least 1 h, and then the platinum ashing dish with incineration residue was cooled for 20 min in a desiccator to room temperature. The mass of the ashing dish containing the ignited residue was then measured. The drying and cooling procedure was repeated until a constant mass was obtained. Total ash (in %DM) was calculated from the difference in mass determined before and after incineration, then recalculated on 100 g of the sample's dry matter.

ISO 20483 [37] was applied for the Kjeldahl volumetric determination of total nitrogen content, using the K-424 digestion system unit (Buchi, Flawil, Switzerland), K-350 distillation system unit (Buchi, Flawil, Switzerland) and Titrette® digital burette (Brand GmbH & Co KG, Wertheim, Germany). The flour sample (2.5 g) was placed in the digestion flask (Kjeldahl tube), and one Kjeldahl catalyst tablet (a mixture of 10 g of potassium sulfate, 0.3 g of copper sulfate pentahydrate, and 0.3 g of titanium dioxide), followed by 20 ml of sulfuric acid were added, then the flask was swirled until the content was completely wetted. The digestion flask with the mixture was placed in the digestion system unit heated at (420 ± 10) °C, and the digestion process was allowed to continue for at least 2 h, until the digestion mixture became clear, and foaming and vapouring stopped. Once the digestion phase was completed, the flask with the content was cooled, and 50 ml of water was added and stirred slowly. Then, the flask and its contents were transferred to the distillation system unit. At the same time, in the part below the condenser of the distillation system unit, a 250 ml Erlenmeyer flask

 doi:10.20944/preprints202407.0164.v1

filled with 50 ml of boric acid solution (40 g/l) was placed so that the end of the condenser hose was immersed in the solution. Then 10 drops of indicator (0.2 g bromocresol green and 0.2 g methyl red dissolved in 100 ml ethanol, and mixed in the ratio 5:1) were added. Steam distillation was started after 50 ml of sodium hydroxide (32%) was introduced into a distillation column. With distillation rate up to about 25 ml/min (distillation time 5 min), the process was stopped when 125 ml of distillate was obtained. The distillate was titrated with sulfuric acid standard volumetric solution (0.05 mol/l) until the colour change at the endpoint. The same procedure was performed with the blank sample. The consumption of sulfuric acid solution in the flour sample and the blank sample was notified, and total protein (in %DM) was calculated from nitrogen content multiplied by 6.25, then recalculated on 100 g of the sample's dry matter.

Total carbohydrates content was determined by difference, subtracting the sum of the mass percentage of constituents from the mass percentage of total solids according to AOAC 986.25 [38] using the following Equation (1):

$$\%\text{Total carbohydrates} = \%\text{total solids} - (\%\text{total protein} + \%\text{total lipids} + \%\text{total ash}) \quad (1)$$

Total carbohydrates (in %DM) were expressed as a percentage of mass, then recalculated on 100 g of the sample's dry matter.

Energy value was calculated according to the Codex Alimentarius Commission [39], using conversion factors (c_f 17 kJ/g for protein and carbohydrates, c_f 37 kJ/g for lipids) in the standard Equation (2):

$$\text{Energy} = (\%\text{total protein} \times c_f) + (\%\text{total lipids} \times c_f) + (\%\text{total carbohydrates} \times c_f) \quad (2)$$

The energy value was expressed in kJ/100g of sample and reported to the nearest whole number.

Starch content was determined by the Ewers polarimetric method according to ISO 10520 [40] using a Polartronic M TOUCH high-performance circle polarimeter equipped with 200 mm measuring tubes (Schmidt + Haensch GmbH & Co., Berlin, Germany). The method was performed by polarimetric determination in two steps. To determine the total optical rotation, about 2.5 g of flour was weighed into a 100 ml volumetric flask, after which 25 ml of dilute hydrochloric acid (0.309 mol/l) was added, shaken vigorously, then another 25 ml of same dilute hydrochloric acid was poured. The flask with its content was immersed in a boiling water bath and left to stand for 15 min ± 5 s, then removed, filled with 30 ml of cold water and cooled immediately to a temperature of (20 ± 2) °C. Further, 5 ml of Carrez solution I (10.6 g of potassium hexacyanoferrate(ll) trihydrate dissolved in 100 ml water) with 1 min shaking, and 5 ml of Carrez solution II (21.9 g of zinc acetate dihydrate and 3 g of glacial acetic acid dissolve and dilute to 100 ml with water) with 1 min shaking, were poured for clarification, then the solution was diluted to the mark with water, mixed and filtered. The optical rotation of the filtrate was measured at 20 °C by applying a polarimetric cell of 200 mm optical path length on a wavelength of 589.3 nm. To determine the optical rotation of substances soluble in 40% ethanol, about 5 g of flour was weighed into a 100 ml volumetric flask, after which 80 ml of ethanol solution (40%, v/v) was added, and the content was left to stand for 1 h at room temperature, with strong shaking six times within this period. The solution was diluted to the mark with the same ethanol solution, mixed and filtered. Then, 50 ml of the filtrate was pipetted into a 100 ml volumetric flask, 2.1 ml of dilute hydrochloric acid (7.7 mol/l) was added, and with a reflux condenser fitted to the flask, all was immersed in a boiling water bath for 15 min ± 5 s. After cooling to (20 ± 2) °C, the previous clarification procedure adding of 5 ml of Carrez solution I and II was repeated, and then the solution was diluted to the mark with water, mixed and filtered. The optical rotation was measured as in the previous determination. Starch (in %DM) was calculated by the difference between these two measurements multiplied by a factor, then recalculated on 100 g of the sample's dry matter.

β-glucan content was determined by the enzyme-spectrophotometric method according to AOAC 995.16 [41] using DK-2000-IIIL water-bath (ChemLand, Stargard, Poland), EBA 280 centrifuge (Hettich, Tuttlingen, Germany) and UV-VIS 2100 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The required reagents and solutions were prepared according to the procedures described in the Standard. About 0.1 g of homogenized flour sample was weighed into a centrifuge tube (17 ml

 doi:10.20944/preprints202407.0164.v1

capacity), after which 0.2 ml of ethanol solution (50%, v/v) and 4.0 ml of sodium phosphate buffer (20 mmol/l, pH 6.5) were poured. A centrifuge tube with content was incubated for 1 min on a boiling water bath, vortex mixed, and returned to incubate for a further 2 min. Then, the incubation was continued at 50 °C for 5 min, after which 0.2 ml of lichenase solution was added, and the caped centrifuge tube was incubated additional 1 h at 50 °C. Further, the procedure required the addition of 5.0 ml of sodium acetate buffer (200 mmol/l, pH 4.0) to the centrifuge tube and the equilibration at room temperature for 5 min, followed by a 10-min centrifugation at 2800 rpm. In each of three centrifuge tubes (12 ml capacity) 0.1 ml of supernatant was pipetted. To two of these tubes, 0.1 ml of β-glucosidase solution was added (reaction solution), while to the third tube 0.1 ml of sodium acetate buffer (50 mmol/l, pH 4.0) (reaction blank), then all tubes were incubated at 50 °C for 10 min. Then, the glucose oxidase-peroxidase-buffer mixture was added in a volume of 3 ml to each tube and the incubation in the water bath was continued at 50 °C for 20 min. With each set of determinations, reagent blank (0.2 ml of 50 mmol/l sodium acetate buffer and 3.0 ml glucose oxidase-peroxidase-buffer mixture) and D-glucose standard working solution for quality control (0.1 ml of 50 mmol/l sodium acetate buffer, 0.1 ml D-glucose standard of 1 mg/ml and 3.0 ml glucose oxidase-peroxidase-buffer mixture) were included. The absorbance measurements were performed on 510 nm against reagent blank within 1 h. β-glucan (in %DM) was calculated according to the equation specified in the Standard, and then recalculated on 100 g of sample's dry matter

Determination of pH was performed by the direct potentiometric method according to AOAC 943.02 [42] using HI2020 Edge pH-meter (HANNA Instruments Inc., Woonsocket, RI, USA) with a glass electrode. For calibration, standard buffer solutions of pH 4 and 9 were used. The sample of flour weighing about 10 g was placed in an Erlenmeyer flask and 100 ml of boiled water was added. The suspension was digested for 30 min, and left to stand for 10 min, after which supernatant was decanted into the 250 ml beaker, and pH was measured.

Alcoholic acidity was determined by the volumetric acid-alkaline titration method according to IS 12711 [43] using Titrette® digital burette (Brand GmbH & Co KG, Wertheim, Germany). About 5 g of flour was taken and transferred into a stoppered conical flask, and then 50 ml of neutral ethyl alcohol (90%, v/v) was added. The suspension was shaken and left to stand 24 h. The alcoholic extract was filtered, and the filtrate was titrated against a standard volumetric sodium hydroxide solution (0.05 mol/l) using phenolphthalein as an indicator until a permanent pink colour appeared. Alcoholic acidity (in %DM) was expressed as sulphuric acid and calculated according to the Equation (3), then recalculated on 100 g of the sample's dry matter.

$$\%\ \text{Alcoholic acidity (as H2SO4)} = \frac{24.52 \times A \times N}{M}, \quad (3)$$

where A is the volume of consumed standard volumetric sodium hydroxide solution, N is the normality of standard volumetric sodium hydroxide solution, and M is the weight of the sample.

2.4. Extraction Process

To determine the antioxidant activity and profiles of phenolic acids and bioflavonoids, an extraction process was carried out. The extraction procedure with some modifications and a suitable extraction solvent system included a mixture of methanol and 10% hydrochloric acid in a ratio of 85:15 (v/v) were used according to the previously proposed methods [44,45]. The powdered samples (0.5 g) were extracted with 10 ml of solvent into conical-bottomed plastic centrifuge tubes (15 ml capacity), firstly allowed to stand for 15 min at room temperature, then extracted in a digital ultrasonic cleaner-bath model DU-45 (Argolab, Carpi, Italy) at ultrasound frequency of 40 kHz for 30 min. After extraction, samples were centrifuged at 6000 rpm for 15 min on EBA 280 centrifuge (Hettich, Tuttlingen, Germany). The residues were extracted again with 10 ml of solvent following the same steps. The supernatants were merged and evaporated to dryness at 45 °C under vacuum. Finally, the residues were diluted to 10 ml with solvent and filtered through 0.45 μm pore size PTFE membrane syringe filters to obtain the ready-to-use acidified methanol extracts, which were then stored for 1-2 weeks at 4 °C before analysis.

 doi:10.20944/preprints202407.0164.v1

2.5. Determination of Antioxidant Activity

Antioxidant activity was analyzed by conducting total phenol content (TPC), ferric reducing/antioxidant power (FRAP), and radical-scavenging activity (DPPH) assays.

The total phenol content (TPC) was determined spectrophotometrically using the Folin-Ciocalteu method according to the reported procedure by Dang et al., with some modifications [44]. Before use, Folin–Ciocalteu reagent was diluted with deionized water in a ratio of 1:1 (v/v). For analysis, 50 µl of sample extract was placed in the glass test tube, was mixed with 3 ml deionized water, 250 µl diluted Folin–Ciocalteu reagent, 750 µl 20% Na_2CO_3 solution, and left to react for 8 min at room temperature. Then, 950 µl of deionized water was added in each glass test tube, for a final volume of 5 ml. The obtained test sample solutions were kept away from light and incubated at room temperature for 2 h. Stock solution of gallic acid (1000 mg/l) was prepared in methanol, and working standard solutions (0, 25, 50, 75, 100, 150, and 200 mg/l) were prepared by its further dilution in methanol. Working standard solutions and blank samples (methanol instead of extract) were subjected to the same procedure steps as the test samples. The absorbances of working standard and test sample solutions were measured against the blank sample at a wavelength of 765 nm using a UV-VIS 2100 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). A calibration curve was constructed, and the equation of the regression line was performed. The total phenol content (in mg GAE/g DM) was calculated using gallic acid (GA) as the standard and then recalculated on 1 g of the sample's dry matter.

Ferric reducing/antioxidant power (FRAP) was determined spectrophotometrically using the previously described method by Benzie and Strain, with some modifications [46]. The FRAP reagent was prepared by mixing 2.5 ml of 10 mmol/l TPTZ in 40 mmol/l HCl solution, 2.5 ml of 20 mmol/l $FeCl_3 \times 6H_2O$ solution, and 25 ml of 300 mmol/l pH 3.6 sodium acetate buffer solution, in a simplified ratio 1:1:10 (v/v/v). The sample extracts were previously diluted 10-fold with methanol, and 150 µl of each was taken, placed in glass test tubes, then mixed with 4.5 ml of FRAP reagent, and thoroughly shaken. The obtained test sample solutions were incubated at 37 °C for 30 min, to react. Stock solution of $FeSO_4 \times 7H_2O$ (1 mmol/l) was prepared in deionized water, and working standard solutions (0, 25, 50, 100, 250, 500 and 750 µmol/l) were prepared by its further dilution in deionized water. Working standard solutions and blank samples (methanol instead of diluted extract) were subjected to the same procedure steps (except for 10-fold dilution) as the test samples. Based on the reduction of Fe^{+3} ion to Fe^{+2} ion, and the formation of the blue complex Fe^{+2}-TPTZ, the absorbances of working standard and test sample solutions were measured against the blank sample at the wavelength of 593 nm using a UV-VIS 2100 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). A calibration curve was constructed, and the equation of the regression line was performed. Ferric reducing/antioxidant power (in µmol Fe^{2+}/g DM) was calculated using $FeSO_4 \times 7H_2O$ as the standard and then recalculated on 1 g of the sample's dry matter.

Radical-scavenging activity (DPPH) was determined spectrophotometrically using the previously described method by Bakar et al., with some modifications [47]. The DPPH reagent was prepared in the form of a 0.1 mmol/l DPPH–methanol solution by dissolving 3.9 mg of DPPH into methanol up to the mark of 100 ml volumetric flask. Further, a volumetric flask was wrapped in aluminium foil and placed in a digital ultrasonic cleaner-bath model DU-45 (Argolab, Carpi, Italy) for 30 min. The sample extracts were previously diluted 10-fold with methanol, and 200 µl of each was taken, placed in glass test tubes, then mixed with 3.8 ml of DPPH–methanol solution, and thoroughly shaken. The obtained test sample solutions were kept in the dark and incubated at room temperature for 30 min, to develop colour. Stock solution of Trolox (1 mmol/l) was prepared in methanol, and working standard solutions (0, 20, 50, 100, 250, 500 and 750 µmol/l) were prepared by further dilution in methanol. Working standard solutions were subjected to the same procedure steps (except for 10-fold dilution) as the test samples. Based on the reduction of the free radical DPPH, the absorbances of working standard and test sample solutions were measured against blank sample (methanol) at the wavelength of 517 nm using a UV-VIS 2100 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). A calibration curve was constructed, and the equation of the regression

 doi:10.20944/preprints202407.0164.v1

line was performed. Radical-scavenging activity (in μmol TE/g DM) was calculated using Trolox (Trolox equivalents, TE) as the standard and then recalculated on 1 g of the sample's dry matter.

2.6. Determination of Phenolic Acids and Bioflavonoids Profiles - HPLC Analysis

The profiles of phenolic acids and bioflavonoids were determined by reverse-phase high performance liquid chromatography (RP-HPLC) according to the reported method, with some modifications [44]. The compounds were separated, detected, identified and quantified using a Nexera HPLC System (Shimadzu Corporation, Kyoto, Japan) consisting of the quaternary pump (Nexera XR LC-20AD XR), degassing unit (DGU-20A SR), auto-sampler (Nexera XR SIL-20AC XR), column oven (Prominence CTO-20AC), photodiode array detector (Prominence SPD-M20A), and it is equipped with reversed-phase column Zorbax SB C18 (250 x 4.6 mm, I.D. 5 μm; Agilent Technologies Inc., Santa Clara, CA, USA). The binary gradient elution system was consisted of two solvents as mobile phase: (A) water containing 0.1% formic acid and (B) methanol. Gradient programming of the solvent system (in v/v) was as follows: 0 min 5% B, 25 min 30% B, 35 min 40% B, 40 min 48% B, 50 min 70% B, 55 min 100% B, 65 min 5% B, re-equilibration time 10 min, with a flow rate of 1 ml/min. Therefore, the total running time for analysis was 75 min. From vials (1.5 ml capacity), a 10 μl of acidified methanol extract of sample and standard solution was injected and the column was thermostatically controlled at 25 °C. Dual wavelengths of 280 nm and 325 nm were used to detect the eluted phytochemicals. The obtained chromatograms of standards and samples were overlayed and the unknown phytometabolites from samples were identified by comparing the retention time of the peaks. Quantification was performed via a calibration curve made for each compound, based on the peak areas of a standard mixture of known concentration (0, 25, 50, 100, 150 and 200 mg/l). Stock solutions were prepared in methanol-water mixture (75:25, v/v) at a concentration of 1000 mg/l. The calibration standard mix solutions were prepared by combining the stock solutions and diluting them with the methanol-water mixture to appropriate concentrations. HPLC analysis at 280 nm was used to identify and quantify the peaks of gallic acid, dihydrocaffeic acid, phloretic acid, trans-cinnamic acid, catechin, epicatechin, naringin, daidzein, naringenin, while at 325 nm for chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, hesperetic acid, and quercetin (Figure 2). The content of phenolic acids and bioflavonoids (in μg/g DM) was calculated using a standard mixture and then recalculated on 1 g of the sample's dry matter.

2.7. Statistical Analysis

To interpret obtained results of the *Novosadska variety* buckwheat experiments, the data were presented as mean ± standard deviation (M±SD) and statistically processed by one-factor analysis of variance (one-way ANOVA, $p<0.05$) to evaluate the effect of storage time, and Tukey's HSD (Honestly Significant Difference, $p<0.05$) post-hoc test to determine differences between means, using Statistica 12.5 software (StatSoft, Inc., Tulsa, OK, USA).

3. Results

3.1. Physico-Chemical, Chemical and Nutritional Properties

The results of proximate composition, nutritional properties, pH values and alcoholic acidity of *Novosadska variety* buckwheat (flour) used in this research, during the 9-month storage, are presented in Table 1. Moisture, total protein, total lipids, total ash, and total carbohydrates contents were in the range of 6.02-10.21%, 11.57-13.46% DM, 2.64-3.44% DM, 1.94-2.01% DM, and 81.13-83.48% DM, respectively. During the storage period, trends of a significant ($p<0.05$) successive decrease in moisture, total protein and total lipids content were observed, in contrast to total carbohydrates content, where a trend of a significant ($p<0.05$) successive increase was recorded.

 doi:10.20944/preprints202407.0164.v1

Table 1. Influence of storage time (S) on proximate composition, nutritional properties, pH values and alcoholic acidity of *Novosadska variety* buckwheat flour.

n=9	Storage time (S)			
Parameters	**Freshly harvested grain**	**3 months**	**6 months**	**9 months**
Moisture, %	10.21±0.006^{d}	9.09±0.020^{c}	7.89±0.066^{b}	6.02±0.021^{a}
Total protein, % DM	13.46±0.344^{b}	12.62±0.590^{ab}	12.25±0.336^{a}	11.57±0.448^{a}
Total lipids, % DM	3.44±0.152^{b}	2.99±0.303^{ab}	2.64±0.235^{a}	2.99±0.050^{ab}
Total ash, % DM	1.97±0.020	2.01±0.020	1.94±0.010	1.96±0.050
Total carbohydrates, % DM	81.13±0.510^{a}	82.38±0.676^{ab}	83.17±0.465^{b}	83.48±0.370^{b}
Starch, % DM	62.55±0.105^{c}	49.12±0.315^{a}	47.67±0.737^{a}	52.12±1.342^{b}
β-glucan, % DM	0.050±0.0013^{b}	0.030±0.0012^{a}	0.100±0.0028^{c}	0.120±0.0027^{d}
Energy value, kJ/100 g	1558±2.5^{a}	1569±6.1^{a}	1584±5.1^{b}	1623±1.2^{c}
pH value	6.74±0.059^{c}	6.63±0.046^{b}	6.22±0.025^{a}	6.19±0.021^{a}
Alcoholic acidity, % DM (as H_2SO_4)	0.86±0.028^{c}	0.65±0.035^{b}	0.67±0.025^{b}	0.53±0.031^{a}

$^{a, b, c, d}$ Means within the same row with different superscripts differ significantly (p<0.05); DM – dry matter.

In the 9 months storage period, no significant changes (p>0.05) were noted in total ash content. There was a statistically significant difference (p<0.05) in moisture content between all treatments. However, for total protein, total lipids, and total carbohydrates contents, a statistically significant difference (p<0.05) was observed at 6 months, but later without significant (p>0.05) changes between treatments. Compared to freshly harvested grain, which had the highest moisture (10.21%), total protein (13.46% DM), and total lipids (3.44% DM) content, during storage the lowest moisture (6.02%) and total protein (11.57% DM) content was recorded at 9 months, and total lipids content (2.64% DM) at 6 months, resulting in a decrease of 41%, 14% and 23%, respectively. On the other hand, the highest total carbohydrates content (83.48% DM) was observed at 9 months, and the lowest amount (81.13% DM) in freshly harvested grain, which corresponded to an increase of 3%.

Starch and β-glucan content, and energy value were in the range of 47.67-62.55% DM, 0.030-0.120% DM, and 1558-1623 kJ/100 g, respectively. Furthermore, progressive changes with increasing storage time were observed for all examined nutritional parameters of buckwheat flour. The trend of a significant (p<0.05) decrease in starch content was observed during storage, in contrast to β-glucan content and energy value, where trends of a significant (p<0.05) increase were recorded. There was a statistically significant difference (p<0.05) in β-glucan content between all treatments. However, for starch content and energy value, statistically significant differences (p<0.05) between treatments were observed at 3 months, i.e., at 6 months respectively, and beyond. Compared to freshly harvested grain, which had the highest starch content (62.55% DM), during storage, the lowest amount (47.67% DM) was recorded at 6 months, which corresponded to a decrease of 24%. On the other hand, the highest β-glucan content (0.120% DM), and energy value (1623 kJ/100 g) were observed at 9 months, while the lowest β-glucan content (0.030% DM) and energy value (1558 kJ/100g) were noted at 3 months, i.e., in freshly harvested grain, increasing by 300% (regarding freshly harvested grain was 140%) and 4%, respectively.

pH value and alcoholic acidity were in the range of 6.19-6.74, and 0.53-0.86% DM, respectively. During the storage period, trends of a significant (p<0.05) successive decrease in pH value and alcoholic acidity were observed. Statistically significant (p<0.05) alterations of pH value and alcoholic acidity were perceived between treatments at 3 months, and beyond, but without significant (p>0.05) changes in pH value among treatments at 6 months, and after. Compared to freshly harvested grain, which had the highest pH value (6.74), and alcoholic acidity (0.86% DM), during storage the lowest pH value (6.19) and alcoholic acidity (0.53% DM) were recorded at 9 months, resulting in a decrease of 0.55 pH units, and 0.33% DM, respectively.

 doi:10.20944/preprints202407.0164.v1

3.2. Potential of Antioxidant Activity and Capacity

The results of the antioxidant activity of *Novosadska variety* buckwheat (flour) used in this research, during the 9-month storage presented via total phenol content (TPC), ferric reducing/antioxidant power (FRAP), and radical-scavenging activity (DPPH) assays are shown in Table 2.

Total phenol content, FRAP, and DPPH were in the range of 5.57-7.28 mg GAE/g DM, 22.20-63.45 μmol Fe^{2+}/g DM, and 8.12-19.66 μmol TE/g DM, respectively. During the storage period, trends of a significant (p<0.05) successive decrease in total phenol content, FRAP, and DPPH were observed. There was a statistically significant difference (p<0.05) in FRAP value between all treatments. However, for total phenol content, and DPPH, a statistically significant difference (p<0.05) among treatments was observed at 6 months, and beyond. Compared to freshly harvested grain, which had the highest total phenol content (7.28 mg GAE/g DM), and FRAP (63.45 μmol Fe^{2+}/g DM), during storage, the lowest TPC (5.57 mg GAE/g DM) and FRAP (22.20 μmol Fe^{2+}/g DM) values were recorded at 9 months, resulting in a decrease of 1.3 and 2.9 times, respectively.

Table 2. Influence of storage time (S) on antioxidant activity and capacity of *Novosadska variety* buckwheat flour.

n=9	Storage time (S)			
Parameters	**Freshly harvested grain**	**3 months**	**6 months**	**9 months**
Total phenol, mg GAE/g DM	7.28±0.194^{c}	6.97±0.115^{c}	6.04±0.121^{b}	5.57±0.122^{a}
FRAP, μmol Fe^{2+}/g DM	63.45±1.520^{d}	28.68±0.476^{c}	25.32±0.445^{b}	22.20±0.440^{a}
DPPH, μmol TE/g DM	19.47±0.907^{c}	19.66±0.333^{c}	14.02±0.490^{b}	8.12±0.344^{a}

$^{a, b, c, d}$ Means within the same row with different superscripts differ significantly (p<0.05); DM – dry matter.

On the other hand, the highest DPPH (19.66 μmol TE/g DM) was observed at 3 months, and the lowest value (8.12 μmol TE/g DM) at 9 months, which corresponded to a decrease of 2.4 times.

3.3. Phenolic Acids and Bioflavonoids Profiles

In the present research, fifteen phytochemical compounds were studied, to identify and quantify them in *Novosadska variety* buckwheat (flour), during 9 months of storage. Nine of them were phenolic acids: gallic acid, dihydrocaffeic acid, phloretic acid, trans-cinnamic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, hesperetic acid, and six belonged to bioflavonoids: catechin, epicatechin, naringin, daidzein, naringenin, quercetin. The obtained results are presented in Table 3.

Table 3. Influence of storage time (S) on phenolic acids and bioflavonoids profile of *Novosadska variety* buckwheat flour.

n=9	Storage time (S)			
Parameters	**Freshly harvested grain**	**3 months**	**6 months**	**9 months**
Phenolic acids				
Gallic acid, μg/g DM	91.0±8.44	92.7±6.97	94.1±6.88	93.6±7.53
Dihydrocaffeic acid, μg/g DM	18.7±1.93^{b}	11.5±1.16^{a}	19.1±1.95^{b}	15.5±1.52^{ab}
Chlorogenic acid, μg/g DM	14.4±1.29^{c}	11.1±1.10^{b}	7.6±0.74^{a}	7.1±0.72^{a}
Caffeic acid, μg/g DM	38.2±3.83	37.6±3.97	39.2±3.78	38.3±3.87
Phloretic acid, μg/g DM	25.8±2.44^{a}	46.5±4.11^{b}	31.4±3.04^{a}	29.1±2.65^{a}
p-Coumaric acid, μg/g DM	102.4±7.52	101.9±7.57	97.8±6.14	95.9±6.90
Ferulic acid, μg/g DM	56.5±3.79	57.0±3.56	57.4±4.00	56.5±3.80
Hesperetic acid, μg/g DM	nd	nd	nd	nd

 doi:10.20944/preprints202407.0164.v1

trans-Cinnamic acid, µg/g DM	90.0±4.76	89.9±4.53	89.5±4.27	87.9±4.60
Bioflavonoids				
Catechin, µg/g DM	49.1±4.12	52.2±4.28	49.9±4.12	49.3±4.20
Epicatechin, µg/g DM	95.7±7.87^{c}	78.5±6.31^{b}	77.0±6.46^{b}	59.8±5.08^{a}
Daidzein, µg/g DM	57.0±4.25^{a}	59.3±4.25^{ab}	56.1±3.84^{a}	69.3±4.98^{b}
Quercetin, µg/g DM	9.8±0.93^{a}	12.5±1.27^{ab}	16.6±1.69^{b}	47.1±3.95^{c}
Naringin, µg/g DM	nd	42.7±2.91^{a}	54.9±4.22^{b}	51.9±3.70^{b}
Naringenin, µg/g DM	38.4±2.78	37.8±2.33	38.7±2.24	38.3±2.33

$^{a, b, c}$ Means within the same row with different superscripts differ significantly (p<0.05); nd - not detected; DM - dry matter.

Eight phenolic acids were ranged as follows: gallic acid (91.0-94.1 µg/g DM), dihydrocaffeic acid (11.5-19.1 µg/g DM), chlorogenic acid (7.1-14.4 µg/g DM), caffeic acid (37.6-39.2 µg/g DM), phloretic acid (25.8-46.5 µg/g DM), *p*-coumaric acid (95.9-102.4 µg/g DM), ferulic acid (56.5-57.4 µg/g DM), and trans-cinnamic acid (87.9-90.0 µg/g DM). Hesperetic acid, which belongs to the class of phenolic acids, was not detected in any section (0 - freshly harvested grain, 3, 6, and 9 months). Also, six bioflavonoids were ranged as follows: catechin (49.1-52.2 µg/g DM), epicatechin (59.8-95.7 µg/g DM), daidzein (56.1-69.3 µg/g DM), quercetin (9.8-47.1 µg/g DM), naringin (nd-54.9 µg/g DM), and naringenin (37.8-38.7 µg/g DM).

In regard to phenolic acids during storage, a trend of a significant (p<0.05) successive decrease in chlorogenic acid content was observed. Although the changes in the content of dihydrocaffeic and phloretic acid were also significant (p<0.05), irregular and mutually oppositely oriented patterns were found. During the storage period of 9 months, no significant changes (p>0.05) were recorded in the content of the following phenolic acids: gallic acid, caffeic acid, *p*-coumaric acid, ferulic acid, and trans-cinnamic acid. In the matter of bioflavonoids during storage, the trends of a significant (p<0.05) successive increase in daidzein, quercetin, and naringin content were observed, in contrast to epicatechin content, where a trend of a significant (p<0.05) successive decrease was recorded. During the observed storage period of 9 months, no significant changes (p>0.05) were recorded in the content of catechin, and naringenin.

Statistically significant (p<0.05) alterations in chlorogenic acid content were perceived between treatments at 3 months, and beyond, but without significant (p>0.05) changes among treatments at 6 and 9 months. The changes in the content of dihydrocaffeic and phloretic acid between treatments were significant (p<0.05) only at 3 months, but without significant (p>0.05) variations among other treatments, as it can be also seen on Figure 1a.

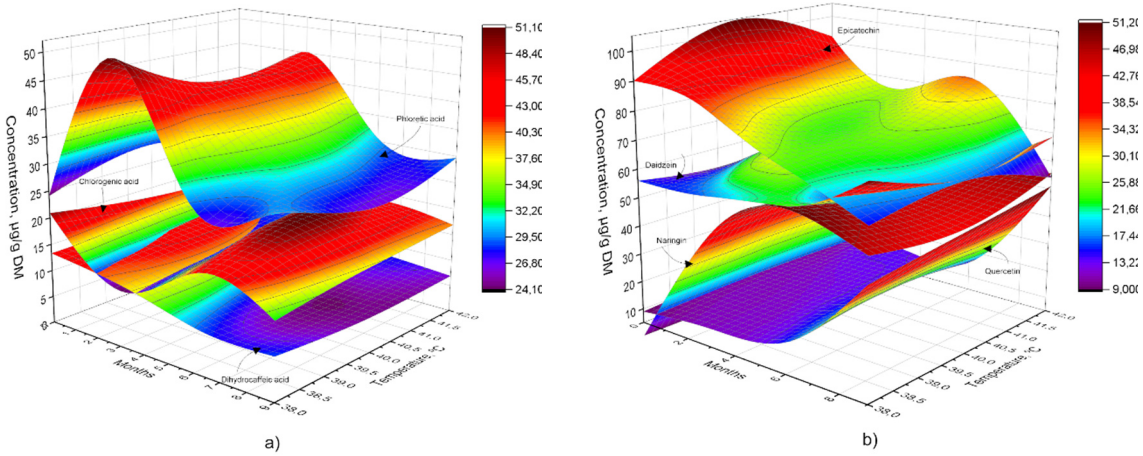


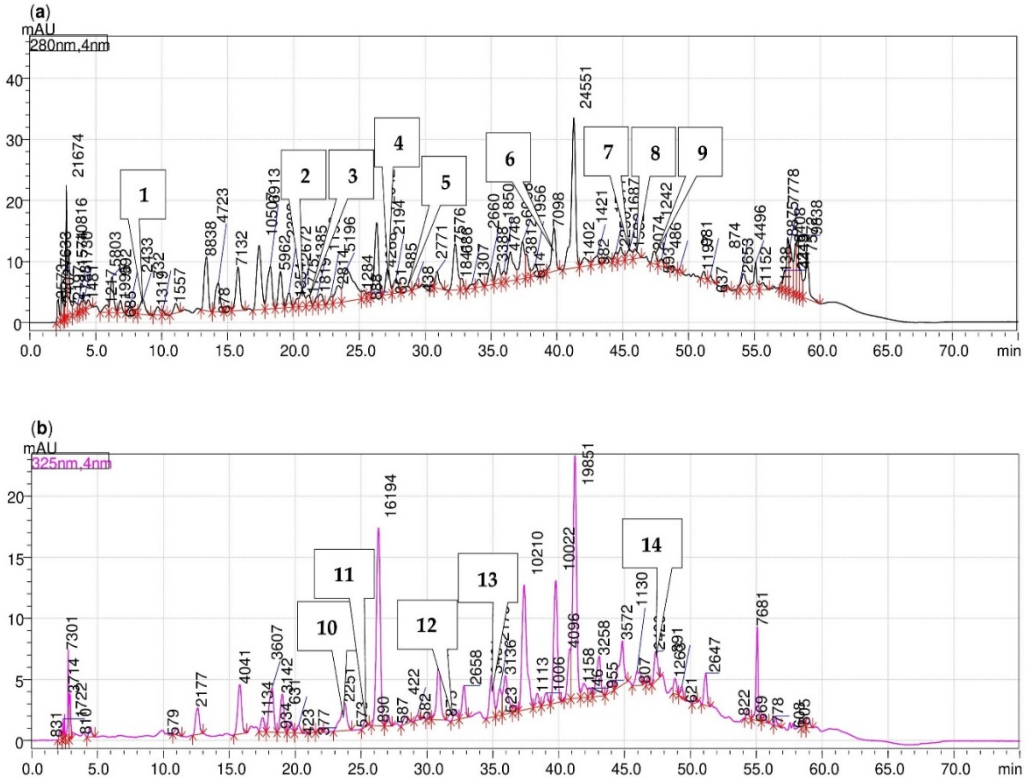
Figure 1. Surface plot of significant a) phenolic acids and b) bioflavonoids concentration in function with storage time (months) and temperature, °C.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 July 2024 doi:10.20944/preprints202407.0164.v1

Although, there were no statistically significant differences (p>0.05) in the contents of gallic acid, caffeic acid, ferulic acid, p-coumaric acid and trans-cinnamic acid between treatments (Table 3), it was an obvious tendency that reflected in a small increasing increment of gallic acid content, and a small decreasing increment of p-coumaric and trans-cinnamic acid content, or these changes in content between treatments were without a definite pattern as in caffeic and ferulic acid. In regard to bioflavonoids, during storage a statistically significant (p<0.05) alteration in epicatechin content among treatments was observed at 3 months and beyond, also seen in Figure 1b. However, for daidzein, quercetin, and naringin contents, statistically significant differences (p<0.05) between treatments were observed at 9 months, 6 months and beyond, as well as at 3 months and beyond, respectively, but for naringin content there was no significant (p>0.05) changes among treatments at 6 and 9 months. On the other hand, it was observed that along all treatments the individual contents of catechin, as well as naringenin, were at a very similar quantitative level, with invariability (p>0.05) and constancy in content.

Observing phenolic acids, compared to freshly harvested grain, which had the content of dihydrocaffeic acid and phloretic acid of 18.7 μg/g DM and 25.8 μg/g DM, during storage the lowest dihydrocaffeic acid content (11.5 μg/g DM) and the highest phloretic acid content (46.5 μg/g DM) were recorded at 3 months, resulting in a decrease of 1.6 times, and an increase of 1.8 times, respectively (also seen on Figure 1a). The highest content of chlorogenic acid (14.4 μg/g DM) was recorded at the beginning in the freshly harvested grain, but it decreased over time to the lowest value (7.1 μg/g DM) at 9 months, which corresponded to a decrease of 2 times. In the case of bioflavonoids, the highest content of epicatechin (95.7 μg/g DM) was in freshly harvested grain, which is 1.6 times higher than the lowest content (59.8 μg/g DM) at 9 months. On the contrary, after nine months of storage the amount of daidzein (69.3 μg/g DM) and quercetin (47.1 μg/g DM) was 1.2 and 4.8 times higher than in freshly harvested grain (57.0 μg/g DM, 9.8 μg/g DM). Similarly, at the beginning of storage in freshly harvested grain, naringin was not detected, but over time its content increased multiply to 54.9 μg/g DM (6 months) and 51.9 μg/g DM (9 months), also seen in Figure 1b.

The chromatographically determined profiles of phenolic acids and bioflavonoids, as an example of separation and detection on 3 months of storage, are introduced in Figure 2.



 doi:10.20944/preprints202407.0164.v1

Figure 2. Chromatogram of phenolic acids and bioflavonoids of one *Novosadska variety* buckwheat flour sample on 3 months of storage (the sequence of peaks (**a**) at 280 nm: 1 - Gallic acid, 2 - Catechin, 3 - Dihydrocaffeic acid, 4 - Epicatechin, 5 - Phloretic acid, 6 - Naringin, 7 – Daidzein, 8 - trans-Cinnamic acid, 9 – Naringenin; (**b**) at 325 nm: 10 - Chlorogenic acid, 11 - Caffeic acid, 12 - *p*-Coumaric acid, 13 - Ferulic acid, 14 - Quercetin, Hesperetic acid not detected).

4. Discussion

The results of these studies shown that the *Novosadska variety* buckwheat grains are a good source of energy, building and functional components necessary in the daily diet.

4.1. Physico-Chemical, Chemical and Nutritional Properties

Chemical changes caused by the natural ageing process, and especially under the conditions to which the samples in this study were exposed, led to changes that did not drastically affect the quality. Similar to our initial proximate composition findings on *Novosadska variety* buckwheat wholegrain flour (Table 1), in a study by Dapčević et al. [48] the moisture, total protein, total lipids, and total ash mass fractions of raw wholegrain buckwheat flour were 9.76, 13.40, 3.08 and 1.97% DM, respectively. In another study, the total carbohydrate mass fraction was slightly lower and reported to be 72.4% DM [49]. The decrease in moisture content during storage occurs due to thermal action at (40 ± 2) °C and gradual loss of water, since grain has a capillary porous structure [50]. Our results in the overall decreasing trends of total protein and lipid content remain within the ranges for buckwheat reported by Nalinkumar and Singh for protein 11%-14% DM and lipid 1.5%-3.7% DM [51]. However, a slight decrease in both content is due to biochemical processes occurring over time that are primarily manifested in protein and lipid hydrolysis processes attributed to the release of amino and fatty acids, and the process of lipid oxidation [50]. It is important to mention that the biological value of buckwheat protein is higher compared to wheat, oats, rye and barley, while buckwheat lipids mainly consist of 95% of saturated palmitic, and unsaturated oleic and linoleic fatty acids [51]. Although the increase in total carbohydrate contents over time is statistically significant, it could be attributed to the calculation methodology of the determination itself and the possible inhomogeneity of the sample. Nalinkumar and Singh [51] reported a similar value of total ash content (common buckwheat 2.1%, and Tartary buckwheat 1.8%) to the value we obtained at the beginning of the research. During long-term storage, there is little effect on the mineral content [52], which is consistent with our results.

Considering the starch content at the beginning of this research, results are slightly lower than the stated value in raw wholegrain buckwheat flour of 67.40% DM by Dapčević et al. [48]. Changes in starch content over time led to a partial overlap with the starch content of buckwheat ranging from 59% to 70% as stated by Nalinkumar and Singh [51]. The decrease content trend could be caused by enzymatic and chemical (acid) hydrolysis which efficiently converts starch into monosaccharides, assisted by pH (5.0–7.0) and thermal (45 °C) [50,53], which corresponds to the storage conditions in our experiment for pH range of 6.19-6.74 and temperature of (40 ± 2) °C. In addition, the differences in starch contents stated in the literature vary due to the different extraction methods applied and the variety of cultivars [51,54].

β-glucan content and structure in crops have implications for enhanced nutritional value. The findings reported by Singla et al. [55], somewise support our findings, as they stated that buckwheat is poor in β-glucan content compared to other cereals. However, our results were inconsistent with the findings of Hozová et al. [56], where they found a multipletimes higher amount of β-glucan in buckwheat cv. Spacinska (A and B) (more than 20%). A possible explanation for this huge difference in the β-glucan content is the influence of various factors, such as the species and cultivar, the quality of the agricultural soil where the plant was grown, and climatic conditions during the year of cultivation [56]. On the other hand, during storage, the growth trend could be explained by the gradual and easier release of this polysaccharide from the cell wall and the aleurone layer of the endosperm of the damaged grain, whereby it can be more easily determined [44].

 doi:10.20944/preprints202407.0164.v1

In this study, energy values were found, both at the beginning and after 9 months, to be very similar to the result of a previous research by Silav-Tuzlu and Tacer-Caba [49], where on this matter energy value was 320.0 kcal/100 g (1338 kJ/100 g). A minor increase in energy values during storage could be attributed to varying quantities of components due to measurement uncertainty, which enter in the calculation procedure of determination.

Our results of initial pH value and alcoholic acidity were similar to that of Jara et al. [57], who examined fine, wholegrain buckwheat flour, and found pH of 6.36, and an average titratable acidity value of 6.2% (recalculated to alcoholic acidity of 0.62%), and Mousavi et al. [58], who determined pH value of buckwheat flour as 6.25. In our research, both pH and acidity values were decreased with the progression of storage time, which corresponds to the declining pH trend found by Silav-Tuzlu and Tacer-Caba [49] of 5.57 to 5.27 in biscuits with buckwheat during 45 days of storage, and decreasing acidity found by Mgaya-Kilima et al. [59] due to acidic hydrolysis of polysaccharides where acid is utilized for converting non-reducing sugars into reducing sugars (mono- and disaccharides). These decreases in pH value may be due to the activity of some microorganisms or the release of fatty acids via lipid hydrolysis, and by sugar conversion to small amounts of alcohols and acids [50,60].

4.2. Potential of Antioxidant Activity and Capacity

Buckwheat grain has a higher antioxidative activity compared to cereal grains, and could protect the human body from oxidative damage caused by free radicals [22].

The amount of total phenolics (Table 2), due to the presence of polyphenols, significantly contributes to the overall antioxidant activity, which is reflected, among other things, in the inhibition of lipid peroxidation [61]. However, not all polyphenols have functional and nutritional benefits, for example, tannins could bind to proteins, carbohydrates, some vitamins, and minerals, reducing their intestinal absorption, which make them a group of polyphenols with antinutrient properties, ranged 15-41 mg/100 g [2]. Our results of initial total phenolic content were lower compared to findings by Djordjevic et al. [62], who determined TPC of 50.7 mg GAE/g dry extract of buckwheat (*Fagopyrum esculentum*) that had the highest content in relation to the other tested plant materials. It can be assumed that this apparently large difference in TPC values between the studies arose from the way the results were expressed, in our research per g dry matter of flour in contrast to g dry extract. Examining six buckwheat varieties Zhu et al. [63] found TPC in the range of 5.81- 14.40 mg GAE/g DM of which the content of 7.31 mg GAE/g DM in black common buckwheat was identical to the value in our research at 0. Month (freshly harvested grain). Similar to our research, in the obtained decreasing trend of TPC (about 23%) during 9-months storage, Starowicz and Zieliński [64] found a 24% decrease in the content of total phenolic compounds in rye-buckwheat cakes during 18-month storage, but for a period twice as long as ours and at a temperature almost twice low (23 °C). The decrease in TPC during storage could be explained precisely by the participation in strong antioxidative activity inhibiting lipid oxidation, but also partly by the compound degradation entering into various reactions forming Maillard reaction compounds or favorable polyphenol-sugar adducts which further rearranged to pigments [64].

Furthermore, antioxidant ability was assessed by ferric ion-reducing antioxidant power (FRAP), where wholegrain flour made from freshly harvested *Novosadska variety* buckwheat grain (0. month) shown 1.3 times higher activity of electron-donating substances compared to the findings of Djordjevic et al. [62], who discovered ferric reducing antioxidant power of 49.43 nmol Fe^{2+}/mg dry extract (recalculated on 49.43 μmol Fe^{2+}/g dry extract). Furthermore, our results showed a 1.8-fold higher antioxidant power capacity than that reported by Estivi et al. [65] which was 34.68 mmol TE/kg DM (recalculated on 34.68 μmol Fe^{2+}/g DM). Also, our results for FRAP activity were in agreement with the findings of Zhu et al. [63] who determined FRAP number of six buckwheat varieties in range 31.81-87.67 μmol Fe^{2+}/g DM, where compared to the five of them, *Novosadska variety* buckwheat had a higher antioxidant power capacity. The decrease in FRAP number during 9-months period could be explained by strong antioxidative activity which is based on a much less selective reduction, that resulted in consumption of electron-donating substances present in flour [62].

 doi:10.20944/preprints202407.0164.v1

Generally, buckwheat has very high DPPH· activity as the consequence of high polyphenolics content which OH-groups could donate H-atoms to free radicals or peroxide [61]. Zhu et al. [63] in their study found DPPH· antioxidant activities in range of 25.23-119.56 μmol TE/g DM which were higher 1.3-6.1 times compared to our initial results (0. month). Moreover, a possible explanation for these differences in DPPH· radical scavenging activity could be inequality in the phytochemical composition of grains that quantitatively and qualitatively depends on genotypes and environmental factors [63]. Observing a decreasing tendency in activity over time, Starowicz and Zieliński [64] found a reduction of 7% over 18 months, which was significantly less than the obtained reduction of 58% in DPPH· activity over 9 months in our study. The explanation could be in the different storage conditions, where Starowicz and Zieliński [64] conducted the experiment at a lower temperature of 23 °C, which did not lead to thermally aggressive degradation of the antioxidant compound, and thus in a decrease of scavenging ability, although the type and amount of used flour and the structure and interaction among the antioxidants are also important [2,62]. Reduction of DPPH· radicals reveals that examined materials possess radical inhibitors or scavengers with the possibility to act as primary antioxidants [61].

4.3. Phenolic Acids and Bioflavonoids Profiles

Buckwheat contains many bioactive compounds (about 180), which, in addition to essential nutrients, contribute to positive health benefits.

In addition to one phenyl group, phenolic acids have one carboxylic group and one or more hydroxyl groups. Further, Vieites-Álvarez et al. [66] stated that they are classified into two leading groups: hydroxycinnamic acids (derived from cinnamic acid) and hydroxybenzoic acids (derived from benzoic acid). The function of phenolic acids in plants is numerous and various, as these specialized compounds serve as signalling molecules in plant-microbe interactions, contribute to resistance responses to oxidative stress, attract pollinators and seed dispersers, provide UV protection or regulate enzyme activities, growth and development processes, survival and adaptation to environment [2,66]. Mostly, they influence or are involved in biological, biochemical, and chemical processes, such as the induction of oxidative stress in target plants, the modification of cell division and permeability or the alteration of photosynthesis, respiration, and transpiration, as well as the modulation of gene expression, protein biosynthesis, phytohormone activities, and enzyme functions [66]. Observed through the ability of antioxidant action, phenolic acids scavenge free radicals and other reactive oxygen species, helping in the protection of cells from oxidative damage. Likewise, phenolic acids contribute to sensory characteristics, primarily colour and flavour [66]. In our research, gallic, caffeic, *p*-coumaric, ferulic and trans-cinnamic acids were initially found in high quantity (0. month) (Table 3), which was in agreement with the findings of other researchers [14,66,67] who found a predominant content of these acids in buckwheat grains and flour. Compared to our findings, Zhu et al. [63] discovered in all examined buckwheat flours a similar average content of gallic acid, with two exceptions of lower and higher content (59.79 vs. 135.67 μg/g DM). In contrast, our results for *Novosadska variety* buckwheat were not in agreement with the findings of Beitāne et al. [14] and Škrobot et al. [68], who found 827 and 24 times, respectively, lower total content of gallic acid in buckwheat flour. Similarly, for ferulic acid content, the researchers [14,63,68] found significantly lower amounts 209, 13 and 12 times, respectively. For caffeic acid content, our results were comparable with findings of Škrobot et al. [68], who found slightly higher total value (recalculated for comparison to μg/g DM) of 48.08 μg/g DM (free 42.73 and bound 5.35 μg/g DM), but were not in agreement with the findings of Beitāne et al. [14] who determined 1.72 μg/g DM (recalculated) in buckwheat flour, which was 22 times lower. Our findings for *p*-coumaric acid content were in disagreement with the results of Beitāne et al. [14] and Škrobot et al. [68], who found 4.46 μg/g DM and a total of 2.34 μg/g DM (free 1.49 and bound 0.85 μg/g DM), respectively, which were 23 and 44 times lower contents. The content of trans-cinnamic acid found in our research was 391 times higher than that (0.23 μg/g DM) examined by Škrobot et al. [68], and 11.5 times higher than that (7.86 μg/g DM) determined by Zieliński et al. [67] in biscuits from whole buckwheat flour. The chlorogenic acid content examined in our research was multipletimes higher (22.5- and 8-fold) than those (0.64 μg/g

 doi:10.20944/preprints202407.0164.v1

DM and 1.86 μg/g DM) found by other researchers [14,68]. All differences in the phenolic acids content between studies could be explained by the different degree of release from the food matrix or pH-dependent transformations, the interactions between them and food components, as well as by the existence of differences in genotypes and species, the influence of environmental factors and climate changes, storage, etc [2,63,67]. Dihydrocaffeic and phloretic acids are products of reduction of hydroxycinnamic acids. Dihydrocaffeic acid is known as a metabolite of caffeic acid, it is based on catechol structure, and shows powerful antioxidant activity via an antiradical effect, even higher than that of α-tocopherol [69]. Previous studies were mainly focused on its monitoring in various plant species, especially in the flowers of rainforest tree *Polyscias murrayi* where the highest amount of 352.32 mg/kg was recorded, and on the observation of the presence in human plasma [70,71]. On the other hand, De Pasquale et al. [69] were conducted a study on semolina-pasta fortified with fermented black chickpea flour, but dihydrocaffeic acid was not detected in the unfermented black chickpea doughs. Considering that, this acid was found in our study of *Novosadska variety* buckwheat flour in the quantity of 18.7 μg/g DM, and as a new data it could be added to the list of well-studied phenolic acids that contribute to the overall antioxidant potential of buckwheat. Observing phloretic acid, it is known to belong to the class of organic compounds named phenylpropanoic acids. In the same study [69], phloretic acid also was not detected, which is in contrary to our findings of 25.8 μg/g DM. Also, it could be added to the list of phenolic acids that contribute to the overall antioxidant potential of buckwheat flour. Hesperetic acid is one of the hesperetin metabolites. Sytar et al. [72] found hesperetic acid in buckwheat leaves, in the amount of about 0.12 mg/g DM, in comparison with our study where was not detected. It could be assumed that hesperetic acid accumulated in leaves rather than in grains i.e., flour. During storage of 12 months, Škrobot et al. [68] found a significant increase in the total content of gallic, caffeic and *p*-coumaric acids, which was in contrast to our findings where were no significant changes during 9 months of storage, but noticeable higher values of gallic and *p*-coumaric acids about 6 and 26 times, respectively. Conversely, in our research, the content of caffeic acid was slightly lower by 1.4 times than in the findings of mentioned researchers [68]. Considering the trends of total content in a chlorogenic acid increase and a ferulic acid decrease, which were determined by Škrobot et al. [68] in buckwheat flour, there was a discrepancy with our findings, regarding the declining chlorogenic acid and unchanging ferulic acid contents, but both of higher quantities. The non-significant variability of the trans-cinnamic acid content observed in our study during storage was in agreement with the findings of the previous study [68] in which about 284 times less content was found. No appropriate comparative studies on buckwheat flour were found for the discussion on the content changes of dihydrocaffeic, phloretic and hesperetic acids during storage. All the compared content changes did not follow the same pattern and could be explained by enzyme activity, oxidation processes caused by storage conditions or conversion of one compound into another [68]. It is important to note that slight variations in the content of trans-cinnamic acid, *p*-coumaric acid, caffeic acid and chlorogenic acid in sections 0, 3, 6 and 9 months in buckwheat flour, as presented in Table 3, among others, may occur following the free phenolics inter-transformation pathway: trans-cinnamic acid via *p*-coumaric acid and caffeic acid to chlorogenic acid [73].

Buckwheat is particularly known for its high flavonoid content, which are the secondary metabolites and naturally occurring antioxidants with the main structure that includes two phenyl rings joined through a heterocyclic pyran ring [2,51,66]. Concerning the content of flavonoids in *Novosadska variety* buckwheat flour, the results showed that examined compounds (Table 3) were found in relatively high quantities in fresh and in stored samples, whereas naringin was not detected at the beginning. Quercetin is a precursor of rutin, and is characterized as a bitter compound [51,66]. The initial results for quercetin demonstrated a partial agreement with a quote of Zamaratskaia et al. [2] about Tartary buckwheat flour, due to the relatively high concentrations found, but disagreed with another study [68] investigated common buckwheat wholegrain flour where was determined in a 4 times lower amount (2.39 μg/g DM) or was not detected at all [74]. Catechin is also a very valuable buckwheat constituent, featuring a high antioxidant capacity. In the research of Škrobot et al. [68], the initial total catechin content of 76.76 μg/g DM (free 68.99 and bound 7.77 μg/g DM) was almost

 doi:10.20944/preprints202407.0164.v1

two times higher than in our investigation. *Novosadska variety* buckwheat flour contained 4 and 5 times higher epicatechin content than that found in previous studies [14,68], respectively, and reaffirmed by the mentioned quote of researchers [2] where was identified as dominant in three varieties of buckwheat (flour). There is limited data on daidzein, naringin, and naringenin, as bioactive compounds in buckwheat grains (flour) or generally in the buckwheat plant. To perform the promotion of *Novosadska variety* buckwheat in a certain way, some comparison of daidzein with other similar pseudocereal, such as quinoa, was made. Daidzein is the respective aglycone of glycoside daidzin. *Novosadska variety* buckwheat grains showed an initial high concentration of daidzein, almost 3 fold more than in quinoa seeds (*Chenopodium quinoa* Willd.) where it varied between 7.0 and 20.5 μg/g (recalculated for comparison to μg/g DM) [75]. Naringin and its aglycone form naringenin could be found in the leaves of buckwheat. From naringenin, dihydrokaempferol and dihydroquercetin are formed by enzymatic catalysis, from which kaempferol and quercetin are further produced [76]. In a previous work [44] the authors stated concentration ranges for naringin and naringenin of 2.37- 6.63 μg/g and 5.32- 9.08 μg/g in highland barley grains, which were generally speaking several times lower (least 6 and 4 times, respectively) than in *Novosadska variety* buckwheat grains, except for freshly harvested grain, where naringin was not detected. Concerning storage time, our results agreed with the findings of Škrobot et al. [68] in an increasing content trend of quercetin, but with 13 fold higher value in *Novosadska variety* flour at 9 months than those stated in their study, after one year of storage. The same researchers [68] found an increasing trend of total epicatechin and total catechin contents, which was in contrast to our findings of decreasing epicatechin and unchanging catechin contents. In the same study, the total epicatechin and total catechin content was 2 times lower and 2 times higher value, respectively, compared to our results at the 9-month storage section. Regarding the content of daidzein, naringin and naringenin during the nine-month storage in relation to the stated amounts in the papers [75,76], at least 3.4 times more daidzein was found than in quinoa grains, i.e., at least 7.8 and 4.2 times more naringin and naringenin than in highland barley grains. All differences and changes in the quantities of mentioned bioflavonoids, including disagreements with the results of other researchers, directly depend on factors such as the difference in growth factors of seed, size and shape of grains, the colour of the flower, and time of sowing, buckwheat varieties, soil quality and location, environmental fluctuations, climate changes, growth stages, area of collection, period and storage conditions, transformations and interactions between compounds, etc [2,51]. Also, it is important to note that to the best of our knowledge, this is the first report on the contents of daidzein, naringin, and naringenin in buckwheat seeds (flour).

5. Conclusions

This research provides insight and emphasizes the paramount importance of storage in maintaining sufficient grain in all seasons. Obtained results indicated that *Novosadska variety* buckwheat grains regarding proximate and nutritional composition are not inferior to grains of other commercial buckwheat varieties or cereals (wheat, barley), nor even after 9-months of storage at (40 ± 2) °C. In *Novosadska variety* buckwheat grains, a considerable amount of phenolic acids, such as gallic (91.0 μg/g DM), ferulic (56.5 μg/g DM), *p*-coumaric (102.4 μg/g DM), trans-cinnamic (90.0 μg/g DM), and chlorogenic (14.4 μg/g DM), conjugated polyphenols and the presence of bioflavonoids contribute to the high potential of antioxidant activity and capacity demonstrated via at least 1.8-fold higher ferric ion-reducing antioxidant power (FRAP), even after 9-months storage. Bioflavonoids quercetin (9.8 μg/g DM) and epicatechin (95.7 μg/g DM) were present in amounts 4-5 times higher than in the well-known common buckwheat wholegrain and other flours. However, concerning the content of dihydrocaffeic, chlorogenic and phloretic acids, as well as epicatechin, daidzein, quercetin and naringin, the storage period was shown to exert significant impacts. The important notes for *Novosadska variety* buckwheat grains (flour), to the authors' knowledge, dihydrocaffeic and phloretic acids have been quantified in buckwheat flour for the first time, same as the first report on the contents of daidzein, naringin, and naringenin.

Due to adaptability to extreme environmental and climate conditions, easy application of agroecological farming systems, ability to attract a variety of pollinators and provide shelter and food

 doi:10.20944/preprints202407.0164.v1

for beneficial insects and as speedy short-season plant which can give a relatively quick response to the growing demand for food of expanding population, it is necessary to promote and unlock the full potential of buckwheat (locally, *Novosadska variety*). Acceptance of value-added buckwheat end-products by consumers as an essential factor in the consumption process opens the possibility of improving sustainability capabilities. As pseudocereal with many nutritional and health benefits, and all the aforementioned properties, abilities and roles, buckwheat can be seen as sustainable and biodiversity-friendly crops.

Author Contributions: Conceptualization, B.P. and R.R.; methodology, B.P., S.Đ., Z.Ž.S. and G.K.; software, Z.Ž.S. and T.M.; validation, B.P., S.Đ. and G.K..; formal analysis, B.P., S.Đ., R.R. and G.K.; investigation, R.R. and B.P.; resources, B.P. and S.R.; data curation, B.P.; writing—original draft preparation, B.P.; writing—review and editing, S.Đ., Z.Ž.S. and S.R.; visualization, T.M.; supervision, B.P. and T.M.; project administration, T.M.; funding acquisition, all authors. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Science, Technological Development and Innovations, Republic of Serbia, for Faculty of Agriculture grant numbers: 451-03-65/2024-03/200116, for Institute for Plant Protection and Environment grant numbers: 451-03-66/2024-03/200010, for Institute of Physics Belgrade grant numbers: 0801-116/1.

Data Availability Statement: All relevant data are included in the manuscript. All other data are available from the corresponding author upon reasonable request.

Acknowledgements: No.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Šteinberga, V.; Muter, O.; Jansone, I.; Alsina, I.; Dubova, L. Effect of buckwheat and potato as forecrops on soil microbial properties in crop rotation. *Proc. Latvian Acad. Sci., Section B* **2012**, *66 (4/5)*, 185-191. https://doi.org/10.2478/v10046-012-0027-7
2. Zamaratskaia, G.; Gerhardt, K.; Knicky, M.; Wendin, K. Buckwheat: an underutilized crop with attractive sensory qualities and health benefits. *Crit. Rev. Food Sci. Nutr.* **2023**, 1-16. https://doi.org/10.1080/10408398.2023.2249112
3. Pinski, A.; Zhou, M.; Betekhtin, A. Editorial: Advances in buckwheat research. *Front. Plant Sci.* **2023**, *14*, 1190090. https://doi.org/10.3389/fpls.2023.1190090
4. Farooq, S.; Rehman, R.Ul.; Pirzadah, T.B.; Malik, B.; Ahmad Dar, F.; Tahir, I. Cultivation, agronomic practices, and growth performance of buckwheat. In *Molecular breeding and nutritional aspects of buckwheat*; Zhou, M., Kreft, I., Woo, S.H., Chrungoo, N., Wieslander, G., Eds.; Academic Press: Cambridge, Massachusetts, 2016, 23, pp. 299-319. https://doi.org/10.1016/B978-0-12-803692-1.00023-7
5. Ongalbek, D.; Tokul-Olmez, O.; Sahin, B.; Küçükaydın, S.; Aydogmus-Oztürk, F.; Sıcak, Y.; Yeskaliyeva, B.; Oztürk, M. Classification of buckwheat honey produced in Kazakhstan according to their biochemical ingredients and bioactivities by chemometric approach. *Food Chem.* **2024**, *451*, 139409. https://doi.org/10.1016/j.foodchem.2024.139409
6. Fijen, T.P.M.; Bodegraven, V.; Lucassen, F. Limited honeybee hive placement balances the trade-off between biodiversity conservation and crop yield of buckwheat cultivation. *Basic Appl. Ecol.* **2022**, *65*, 28-38. https://doi.org/10.1016/j.baae.2022.09.003
7. Liu, F.; He, C.; Wang, L.; Wang, M. Effect of milling method on the chemical composition and antioxidant capacity of Tartary buckwheat flour. *Int. J. Food Sci. Tech.* **2018**, *53 (11)*, 2457-2464. https://doi.org/10.1111/ijfs.13837
8. Mir, S.A.; Farooq, S.; Shah, M.A.; Sofi, S.A.; Dar, B.N.; Sunooj, K.V.; Khaneghah, A.M. Recent advancements in the development of multigrain bread. *Cereal Chem.* **2023**, *100 (1)*, 72–82. https://doi.org/10.1002/cche.10578
9. Wang, L.; Zhao, J.; Mao, Y.; Liu, L.; Li, C.; Wu, H.; Zhao, H.; Wu, Q. Tartary buckwheat rutin: Accumulation, metabolic pathways, regulation mechanisms, and biofortification strategies. *Plant Physiol. Biochem.* **2024**, *208*, 108503. https://doi.org/10.1016/j.plaphy.2024.108503
10. Ma, Q.; Yu, Y.; Zhou, Z.; Wang, L.; Cao, R. Effects of different treatments on composition, physicochemical and biological properties of soluble dietary fiber in buckwheat bran. *Food Biosci.* **2023**, *53*, 102517. https://doi.org/10.1016/j.fbio.2023.102517

11. Li, W.; Cao, F.; Fan, J.; Ouyang, S.; Luo, Q.; Zheng, J.; Zhang, G. Physically modified common buckwheat starch and their physicochemical and structural properties. *Food Hydrocoll.* **2014**, *40*, 237–244. https://doi.org/10.1016/J.FOODHYD.2014.03.012
12. Rachman, A.; Chen, L.J.; Brennan, M.; Brennan, C. Effects of addition of buckwheat bran on physicochemical, pasting properties and starch digestion of buckwheat gels. *Eur. Food Res. Technol.* **2020**, *246 (10)*, 2111–2117. https://doi.org/10.1007/s00217-020-03560-6
13. Zhang, W.; Zhu, Y.; Liu, Q.; Bao, J.; Liu, Q. Identification and quantification of polyphenols in hull, bran and endosperm of common buckwheat (*Fagopyrum esculentum*) seeds. *J. Funct. Foods Part A* **2017**, *38*, 363–369. https://doi.org/10.1016/j.jff.2017.09.024
14. Beitāne, I.; Krūmiņa-Zemture, G.; Krūma, Z.; Cinkmanis, I. Phenolics content in buckwheat flour. *Proc. Latv. Acad. Sci. Sect. B: Nat. Exact Appl. Sci.* **2018**, *72 (2)*, 75-79. https://doi.org/10.2478/prolas-2018-0012
15. Devrajan, N.; Prakash, P.; Jindal, N. Effect of extrusion cooking on colour (L*, a*, b*) of germinated buckwheat-corn based snacks. *Int. J. Curr. Microbiol. App. Sci.* **2018**, Special Issue 7, 3413-3424
16. Małgorzata, S.; Georgios, K.; Henryk, Z. Sensory analysis and aroma compounds of buckwheat containing products: A review. *Crit. Rev. Food Sci. Nutr.* **2018**, *58 (11)*, 1767-1779. https://doi.org/10.1080/10408398.2017.1284742
17. Sofi, S.A.; Ahmed, N.; Farooq, A.; Rafiq, S.; Zargar, S.M.; Kamran, F.; Dar, T.A.; Mir, S.A.; Dar, B.N.; Khaneghah, M.A. Nutritional and bioactive characteristics of buckwheat, and its potential for developing gluten-free products: An updated overview. Food Sci. Nutr. 2023, 11 (5), 2256-2276. https://doi.org/10.1002/fsn3.3166
18. Hęś, M.; Szwengiel, A.; Dziedzic, K.; Le Thanh-Blicharz, J.; Kmiecik, D.; Górecka, D. The effect of buckwheat hull extract on lipid oxidation in frozen-stored meat products. *J. Food Sci.* **2017**, *82 (4)*, 882-889. https://doi.org/10.1111/1750-3841.13682
19. Atambayeva, Z.; Nurgazezova, A.; Assirzhanova, Z.; Urazbayev, Z.; Kambarova, A.; Dautova, A.; Idyryshev, B.; Sviderskaya, D.; Kaygusuz, M. Nutritional, physicochemical, textural and sensory characterization of horsemeat patties as affected by whole germinated green buckwheat and Its flour. *Int. J. Food Prop.* **2023**, *26 (1)*, 600-613. https://doi.org/10.1080/10942912.2023.2174552
20. Salejda, A.M.; Olender, K.; Zielińska-Dawidziak, M.; Mazur, M.; Szperlik, J.; Miedzianka, J.; Zawiślak, I.; Kolniak-Ostek, J.; Szmaja, A. Frankfurter-type sausage enriched with buckwheat by-product as a source of bioactive compounds. *Foods* **2022**, *11 (5)*, 674. https://doi.org/10.3390/foods11050674
21. Yessengaziyeva, A.; Uzakov, Y.; Chernukha, I.; Kaimbayeva, L.; Kalashinova, L.; Zhantleuov, D. The use of buckwheat flour in the technology of semi-smoked sausage. *Potr. S. J. F. Sci.* **2023**, *17*, 311-323. https://doi.org/10.5219/1861
22. Suzuki, T.; Noda, T.; Morishita, T.; Ishiguro, K.; Otsuka, S.; Brunori, A. Present status and future perspectives of breeding for buckwheat quality. *Breed. Sci.* **2020**, *70 (1)*, 48-66. https://doi.org/10.1270/jsbbs.19018
23. Wang, X.; Wang, Z.; Zhuang, H.; Nasiru, M.M.; Yuan, Y.; Zhang, J.; Yan, W. Changes in color, myoglobin, and lipid oxidation in beef patties treated by dielectric barrier discharge cold plasma during storage. *Meat Sci.* **2021**, *176*, 108456. https://doi.org/10.1016/j.meatsci.2021.108456
24. Fotschki, B.; Juśkiewicz, J.; Jurgoński, A.; Amarowicz, R.; Opyd, P.; Bez, J.; Muranyi, I.; Petersen, I.L.; Laparra-Llopis, M. Protein-rich flours from quinoa and buckwheat favourably affect the growth parameters, intestinal microbial activity and plasma lipid profile of rats. *Nutrients* **2020**, *12 (9)*, 2781. https://doi.org/10.3390/nu12092781
25. Mondal, S.; Ashfaquddin, M.D.; Bhar, K.; Pradhan, N.K.; Anjum, M.D.; Molla, S. Silver hull buckwheat (*Fagopyrum esculentum* Moench) is a part of nature that offers best health and honour. *Discov. Phytomed.* **2021**, *8 (4)*, 137-159. https://doi.org/10.15562/phytomedicine.2021.180
26. Fleurat-Lessard, F. Qualitative reasoning and integrated management of the quality of stored grain: A promising new approach. *J. Stored Prod. Res.* **2002**, *38 (3)*, 191-218. https://doi.org/10.1016/S0022-474X(01)00022-4
27. McGorrin, R.J. Key aroma compounds in oats and oat cereals. *J. Agric. Food Chem.* **2019**, *67 (50)*, 13778-13789. https://doi.org/10.1021/acs.jafc.9b00994
28. Huyghebaert, G.; Schoner, F.J. Influence of storage and addition of enzyme on metabolisable energy concentration of wheat 1. Impact of storage and enzyme addition. *Arch. Geflügelkd.* **1999**, *63 (1)*, 13-20.
29. Rakić, S.; Janković, S.; Demin, M.; Bucalo, D.; Maslovarić, M. Quality and condition of wheat grain (*Triticum* spp.) during storage. *Biotechnol. Anim. Husb.* **2012**, *28 (3)*, 595-602. https://doi.org/10.2298/BAH1203595R
30. Rakić, S.; Janković, S.; Krivokapić, M.; Jovanović, R.; Ikanović, J. Grain quality and status of oats (*Avena sativa L.*) during storage. *Biotechnol. Anim. Husb.* **2012**, *28 (4)*, 863-871. https://doi.org/10.2298/BAH1204863R
31. Lingua, M.S.; Gies, M.; Descalzo, A.M.; Servent, A.; Páez, R.B.; Baroni, M.V.; Blajman, J.E.; Dhuique-Mayer, C. Impact of storage on the functional characteristics of a fermented cereal product with probiotic potential, containing fruits and phytosterols. *Food Chem.* **2022**, *370*, 130993. https://doi.org/10.1016/j.foodchem.2021.130993

32. ISO 24333:2009. Cereals and cereal products – Sampling. Geneva: International Organization for Standardization.
33. Ping-Ping, T.; Yang-Yong, L.; Wen-Jing, Y.; Shuai-Bing, Z.; Yuan-Sen, H. Effect of artificial aging on wheat quality deterioration during storage. *J. Stored Prod. Res.* **2019**, *80*, 50-56. https://doi.org/10.1016/j.jspr.2018.11.009
34. ISO 712:2009. Cereals and cereal products - Determination of moisture content - Reference method. International Organization for Standardization, Geneva, Switzerland.
35. NMKL 160:1998. Fat - Determination in foods. Nordic Committee on food analysis, Oslo, Norway.
36. ISO 2171:2023. Cereals, pulses and by-products - Determination of ash yield by incineration. International Organization for Standardization, Geneva, Switzerland.
37. ISO 20483:2013. Cereals and pulses - Determination of the nitrogen content and calculation of the crude protein content - Kjeldahl method. International Organization for Standardization, Geneva, Switzerland.
38. AOAC 986.25:1988. Proximate analysis of milk-based infant formula. AOAC Official Methods of Analysis, 17th ed., 2000; The Association of Official Analytical Chemists, Arlington, VA, USA.
39. CAC/GL 2-1985:2017. Guidelines on Nutrition Labelling. Codex Alimentarius Commission, Food and Agriculture Organization of the United Nations and World Health Organization, Rome, Geneva.
40. ISO 10520:1997. Native starch - Determination of starch content - Ewers polarimetric method. International Organization for Standardization, Geneva, Switzerland.
41. AOAC 995.16:1995. β-D-Glucan in Barley and Oats - Streamlined Enzymatic Method. AOAC Official Methods of Analysis, 17th ed., 2000; The Association of Official Analytical Chemists, Arlington, VA, USA.
42. AOAC 943.02:1943. PH of flour - Potentiometric method. AOAC Official Methods of Analysis, 17th ed., 2000; The Association of Official Analytical Chemists, Arlington, VA, USA.
43. IS 12711:1989 (R2005). Bakery products - Methods of analysis: 14. Determination of alcoholic acidity. Bureau of Indian Standards, New Delhi, India.
44. Dang, B.; Zhang, W.G.; Zhang, J.; Yang, X.J.; Xu, H.D. Evaluation of nutritional components, phenolic composition, and antioxidant capacity of highland barley with different grain colors on the Qinghai Tibet plateau. *Foods* **2022**, *11*, 2025. https://doi.org/10.3390/foods11142025
45. Đurović, S.; Nikolić, B.; Luković, N.; Jovanović, J.; Stefanović, A.; Šekuljica, N.; Mijin, D.; Knežević-Jugović, Z. The impact of high-power ultrasound and microwave on the phenolic acid profile and antioxidant activity of the extract from yellow soybean seeds. *Ind. Crop. Prod.* **2018**, *122*, 223-231. https://doi.org/10.1016/j.indcrop.2018.05.078
46. Benzie, F.F.I.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.* **1996**, *239 (1)*, 70-76. https://doi.org/10.1006/abio.1996.0292
47. Bakar, M.F.; Mohamed, M.; Rahmat, A.B.; Fry, J.R. Phytochemicals and antioxidant activity of different parts of bambangan (Mangifera pajang) and tarap (Artocarpus odoratissimus). *Food Chem.* **2009**, *113 (2)*, 479-483. https://doi.org/10.1016/j.foodchem.2008.07.081
48. Dapčević, H.T.; Torbica, A.; Hadnađev, M. Rheological properties of wheat flour substitutes/alternative crops assessed by Mixolab. *Procedia Food Sci.* **2011**, *1*, 328-334. https://doi.org/10.1016/j.profoo.2011.09.051
49. Silav-Tuzlu, G.; Tacer-Caba, Z. Influence of chia seed, buckwheat and chestnut flour addition on the overall quality and shelf life of the gluten-free biscuits. *Food Technol. Biotechnol.* **2021**, *59 (4)*, 463–474. https://doi.org/10.17113/ftb.59.04.21.7204
50. Mar'in, V.A.; Vereshchagin, A.L. Effects of humidity and the content of sprouted and spoiled buckwheat grains on the changes of acid number of fat and grain acidity. *Foods Raw Materi.* **2014**, *2 (1)*, 31-35. https://doi.org/10.12737/4126
51. Nalinkumar, A.; Singh, P. An overview of buckwheat (*Fagopyrum* spp)-An underutilized crop in India-nutritional value and health benefits. *Int. J. Med. Res. Health Sci.* **2020**, *9 (7)*, 39-44.
52. Kramer, A. Effect of storage on nutritive value of food. *J. Food Qual.* **2007**, *1 (1)*, 23 – 55. https://doi.org/10.1111/j.1745-4557.1977.tb00998.x
53. De Souza, I.A.; Orsi, D.C.; Gomes, A.J.; Lunardi, C.N. Enzymatic hydrolysis of starch into sugars is influenced by microgel assembly. *Biotechnol. Rep.* **2019**, *22*, e00342. https://doi.org/10.1016/j.btre.2019.e00342
54. Ahmad, M.; Ahmad, F.; Dar, E.A.; Bhat, R.A.; Mushtaq, T.; Shah, F. Buck wheat (*Fagopyrum esculentum*)-A neglected crop of high altitude cold arid regions of Ladakh: Biology and nutritive value. *Int. J. Pure App. Biosci.* **2018**, *6 (1)*, 395-406. http://dx.doi.org/10.18782/2320-7051.6001
55. Singla, A.; Gupta, O.P.; Sagwal, V.; Kumar, A.; Patwa, N.; Mohan, N.; Ankush; Kumar, D.; Vir, O.; Singh, J.; Kumar, L.; Lal, C.; Singh, G. Beta-glucan as a soluble dietary fiber source: origins, biosynthesis, extraction, purification, structural characteristics, bioavailability, biofunctional attributes, industrial utilization, and global trade. *Nutrients* **2024**, *16*, 900. https://doi.org/10.3390/nu16060900
56. Hozová, B.; Kuniak, Ľ.; Moravčíková, P.; Gajdošová, A. Determination of water-insoluble β-d-glucan in the whole-grain cereals and pseudocereals. *Czech J. Food Sci.* **2007**, *25 (6)*, 316–324. https://doi.org/10.17221/747-CJFS

57. Jara, P.A.; Schoeninger, V.; Dias, L.M.; Siqueira, V.C.; Lourente, E.R.P. Physicochemical quality characteristics of buckwheat flour. *Eng. Agríc.* **2022**, *42 (4)*, e20210026. http://dx.doi.org/10.1590/1809-4430-Eng.Agric.v42n4e20210026/2022
58. Mousavi, M.H.; Gharekhani, M.; Alirezalu, K.; Roufegarinejad, L.; Azadmard-Damirchi, S. Production and characterization of nondairy gluten-free fermented beverage based on buckwheat and lentil. *Food Sci. Nutr.* **2023**, *11*, 2197–2210. https://doi.org/10.1002/fsn3.3095
59. Mgaya-Kilima, B.; Remberg, S.F.; Chove, B.E.; Wicklund, T. Influence of storage temperature and time on the physicochemical and bioactive properties of roselle-fruit juice blends in plastic bottle. *Food Sci. Nutr.* **2014**, *2 (2)*, 181–191. https://doi.org/10.1002/fsn3.97
60. Tabikha, M.M.M.; El-Shehawy, Sh.M.M.; Helal, D.M.A. Changes in chemical and nutritional quality during cold storage of some fruit and vegetable juice blends. *J. Food Dairy Sci.* **2010**, *1 (4)*, 181-191.
61. Sedej, I.J.; Sakač, M.B.; Mišan, A.Č.; Mandić, A.I. Antioxidant activity of wheat and buckwheat flours. *Proc. Nat. Sci.* **2010**, *118*, 59-68. https://doi.org/10.2298/ZMSPN1018059S
62. Djordjevic, T.M.; Šiler-Marinkovic, S.S.; Dimitrijevic-Brankovic, S.I. Antioxidant activity and total phenolic content in some cereals and legumes. *Int. J. Food Prop.* **2011**, *14 (1)*, 175-184. https://doi.org/10.1080/10942910903160364
63. Zhu, H.; Liu, S.; Yao, L.; Wang, L.; Li, C. Free and bound phenolics of buckwheat varieties: HPLC characterization, antioxidant activity, and inhibitory potency towards α-glucosidase with molecular docking analysis. *Antioxidants* **2019**, *8 (12)*, 606. https://doi.org/10.3390/antiox8120606
64. Starowicz, M.; Zieliński, H. Changes in the antioxidant capacity and polyphenols content of rye--buckwheat cakes fortified with spices during their long-term Storage. *Ital. J. Food Sci.* **2019**, *31 (2)*, 253-263. https://doi.org/10.14674/IJFS-1343
65. Estivi, L.; Pellegrino, L.; Hogenboom, J.A.; Brandolini, A.; Hidalgo, A. Antioxidants of amaranth, quinoa and buckwheat wholemeals and heat-damage development in pseudocereal-enriched einkorn water biscuits. *Molecules* **2022**, *27 (21)*, 7541. https://doi.org/10.3390/molecules27217541
66. Vieites-Álvarez, Y.; Reigosa, M.J.; Sánchez-Moreiras, A.M. A decade of advances in the study of buckwheat for organic farming and agroecology (2013-2023). *Front. Plant Sci.* **2024**, *15*, 1354672. https://doi.org/10.3389/fpls.2024.1354672
67. Zieliński, H.; Wiczkowski, W.; Topolska, J.; Piskuła, M.K.; Wronkowska, M. Bioaccessibility of phenolic acids and flavonoids from buckwheat biscuits prepared from flours fermented by lactic acid bacteria. *Molecules* **2022**, *27 (19)*, 6628. https://doi.org/10.3390/molecules27196628
68. Škrobot, D.; Mišan, A.; Sakač, M.; Mandić, A.; Pestorić, M.; Belović, M. Preliminary results of the effect of storage on functional properties of buckwheat. *J. Proc. Energ. Agric.* **2017**, *21 (4)*, 201-203. https://doi.org/10.5937/JPEA1704201S
69. De Pasquale, I.; Verni, M.; Verardo, V.; Gómez-Caravaca, A.M.; Rizzello, C.G. Nutritional and functional advantages of the use of fermented black chickpea flour for semolina-pasta fortification. *Foods* **2021**, *10*, 182. https://doi.org/10.3390/foods10010182
70. Buchanan, M.S.; Carroll, A.R.; Edser, A.; Parisot, J.; Addepalli, R.; Quinn, R.J. Tyrosine kinase inhibitors from the rainforest tree *Polyscias murrayi*. *Phytochemistry* **2005**, *66 (4)*, 481–485. https://doi.org/10.1016/j.phytochem.2004.12.022
71. Van Rensburg, C.J.; Erasmus, E.; Loots, D.T.; Oosthuizen, W.; Jerling, J.C.; Kruger, H.S.; Louw, R.; Brits, M.; van der Westhuizen, F.H. Rosa roxburghii supplementation in a controlled feeding study increases plasma antioxidant capacity and glutathione redox state. *Eur. J. Nutr.* **2005**, *44*, 452–457. https://doi.org/10.1007/s00394-005-0555-x
72. Sytar, O.; Cai, Z.; Brestic, M.; Kumar, A.; Prasad, M.N.V.; Taran, N.; Smetanska, I. Foliar applied nickel on buckwheat (*Fagopyrum esculentum*) induced phenolic compounds as potential antioxidants. *CLEAN* **2013**, *41 (11)*, 1129-1137. https://doi.org/10.1002/clen.201200512
73. Horbowicz, M.; Chrzanowski, G.; Koczkodaj, D.; Mitrus, J. The effect of methyl jasmonate vapors on content of phenolic compounds in seedlings of common buckwheat (*Fagopyrum esculentum* Moench). *Acta Soc. Bot. Pol.* **2011**, *80 (1)*, 5-9. https://doi.org/10.5586/asbp.2011.001
74. Kalinova, J.; Vrchotova, N. The influence of organic and conventional crop management, variety and year on the yield and flavonoid level in common buckwheat groats. *Food Chem.* **2011**, *127 (2)*, 602–608. doi: 10.1016/j.foodchem.2011.01.050

 doi:10.20944/preprints202407.0164.v1

75. Lutz, M.; Martínez, A.; Martínez,E.A. Daidzein and Genistein contents in seeds of quinoa (*Chenopodium quinoa* Willd.) from local ecotypes grown in arid Chile. *Ind. Crops Prod.* **2013**, *49*, 117-121. https://doi.org/10.1016/j.indcrop.2013.04.023
76. Huda, M.N.; Lu, S.; Jahan, T.; Ding, M.; Jha, R.; Zhang, K.; Zhang, W.; Georgiev, M.I.; Park, S.U.; Zhou, M. Treasure from garden: Bioactive compounds of buckwheat. *Food Chem.* **2021**, *335*, 127653. https://doi.org/10.1016/j.foodchem.2020.127653