

Influence of type of production on the contents of selected nutrients/phytochemicals in buckwheat grains

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A B S T R A C T

On a global level and over a number of years, many researchers have been studying the effects of organic and conventional production methods on the chemical composition of plants and their products. The aim of this study was to determine the potential difference in the contents of several nutrients/phytochemicals in organically and conventionally produced buckwheat grains. Buckwheat was sown in 2016 at a site in Nova Varoš. The experimental plot covered a surface area of 1 ha. The contents of the following nutrients and phytochemicals in buckwheat grains were determined: proteins, pigments (chlorophyll *a*, chlorophyll *b*, carotenoids), soluble sugars, starch, free and bound polyphenols and flavonoids, and phytosterols. In addition, the antioxidant properties of the grains were assessed using the ABTS⁺⁺ ((2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) test (ability to inhibit ABTS⁺⁺ radical cations). There was no statistically significant difference in the contents of proteins, chlorophyll *a* and free phenols or in regard to the % of antioxidant capacity. On the other hand, two different production methods caused differences in the other chemical parameters. In organic grains, higher contents of carotenoids (6.998 µg g⁻¹ dry matter (DM)), soluble sugar (6.48 mg 100 g⁻¹ DM), starch (2.46 mg 100 g⁻¹ DM), bound polyphenols (1034.3 mg (FAE) kg kg⁻¹ DM), free flavonoids (1094.7 mg (QE) kg⁻¹ DM) and bound flavonoids (1087.2 mg (QE) kg⁻¹ DM) were detected, while in conventional buckwheat grains, there were higher levels of chlorophyll *b* (24.16 µg g⁻¹ DM) and phytosterols (185.15 mg kg⁻¹ DM). The study showed no clear influence of the production method on the examined parameters.

Keywords: buckwheat, organic production, conventional production, grains, chemical parameters.

ИЗВОД

На глобалном нивоу, бројни истраживачи већ дуги низ година бавили су се испитивањем утицаја органског и конвенционалног начина производње на хемијски састав биљака и њихових производа. Циљ овог рада био је да се утврди потенцијална разлика у садржају неколико нутријената у органски и конвенционално произведеном семену хељде. Хељда је засејана 2016. године на локалитету Нове Вароши. Површина огледне парцеле износила је 1 ха. У семену хељде одређиван је садржај неколико нутријената/фитохемикалија: протеина, пигмената (хлорофил *a*, хлорофил *б*, каротеноиди), растворљивих шећера, скроба, слободних и везаних фенола и флавоноида, фитостерола. Поред тога, антиоксидативна својства семена процењена су применом АБТС++ ((2,2'-азино-бис(3-етилбензотиазолин-6-сулфонска киселина) теста (способност инхибиције АБТС++ радикал катјона). Није забележена статистички значајна разлика у садржају протеина, хлорофила *a*, слободних фенола, као ни када је у питању % антиоксидативности. Са друге стране, два различита начина производње условили су разлике за остале хемијске параметре. Код органског семена детектован је већи садржај каротеноида (6,998 µg g⁻¹ с.м.), растворљивих шећера (6,48 mg 100 g⁻¹ с.м.), и везаних фенола (1034,3 mg (FAE) kg⁻¹ с.м.), слободних флавоноида (1094,7 mg (QE) kg⁻¹ с.м.) и везаних фавоноида (1087,2 mg (QE) kg⁻¹ с.м.), док је код конвенционалног семена хељде забележен већи садржај хлорофила *б* (24.16 µg g⁻¹ с.м.). Може се закључити да не постоји јасан утицај начина производње на испитиван кељи закључити да не постоји јасан утицај начина производње на

Кључне речи: хељда, органска производња, конвенционална производња, семе, хемијски параметри.

1. Introduction

Buckwheat (*Fagopyrum esculentum* Moench; in Greek phagos – beech and pyros – grain) is an annual dicotyledonous plant of the Polygonaceae family, native

to China, which is considered a pseudo cereal due to its similarity to conventional cereals and, primarily, its high content of starch. It includes two genera of the family Polygonaceae: the Eurasian genus *Fagopyrum* and the North American genus *Eriogonum*. It is mostly grown in hilly and mountainous areas. It arrived in

western countries from China, where its wild form originates, while self-seeded species can still be found in the Himalayas, at about 4500 m above sea level (Glamočlija, 2004). In Serbia, it is grown mainly in hilly and mountainous areas and a famous locality for buckwheat production is the region around Zlatar, Nova Varoš with the Pešter plateau and the surroundings of Subotica (Dolijanović et al., 2014). It easily adapts to environmental conditions, and therefore it grows in places with low temperatures and precipitation. Buckwheat is profitable to grow as a subsequent or post-harvest crop. It is often used to prepare the soil for the organic production of other crops since it does not require pesticide treatment, and it improves soil quality because it acidifies the soil and contributes to the higher content of calcium and phosphorus when used as a fertilizer (Campbell, 1997). In recent years, buckwheat has recorded continuous growth in production on a global level, as the result of the growing interest of the human population in healthy, organic food, alternative crops, and old and traditional diets (Golian, 2016; Golian et al., 2017). The potential of buckwheat is tremendous, as evidenced by numerous studies on its nutritional and functional profile and its use not only in the production of pasta, but also in bakery and confectionery products (Škrobot, 2016). The health benefits of cereal grains are based on unique phytochemical composition. The their nutritional values of hulled buckwheat grain vary depending on the contents of individual components. which depend on the type of buckwheat, climatic conditions and production technology. Hulled buckwheat grain contains 55% of starch, 12% of protein, 4% of lipids, 2% of soluble carbohydrates, 7% of total dietary fibers, 2% of ash, and 18% of other components such as organic acids, polyphenolic compounds, tannins, nucleotides, and nucleic acids (Zhang et al., 2012). Among grains and alternative grains, buckwheat stands out as the species with the best source of polyphenols and high antioxidant capacity (Golijan and Kostić, 2016). Buckwheat contains three classes of flavonoids - flavonols, anthocyanins and C-glycosyl flavones, which are known to have antioxidant, hypocholesterolemic, and antidiabetic properties (Qin et al., 2013). In comparison to other types of cereals and from the nutritional point of view, buckwheat proteins are of high quality, and their high biological value is determined by a wellbalanced amino acid composition in relation to the biological value of proteins of true and pseudo cereals (Skrabanja et al., 2000). Numerous researchers around the world have been studying differences in the chemical composition between organically and conventionally produced plants and their products for many years. The results are very variable. Most researchers point out that organic foods contain more dry matter, total sugars, essential amino acids, vitamin C, macro-and microelements (especially Ca, Mg, Fe, P, Zn), and polyphenolic compounds, and significantly less nitrate than conventional ones (Woëse et al., 1997; Worthington, 1998; Bourn and Prescott, 2002; Heaton, 2001; Worthington, 2001; Rembiałkowska, 2007; Baranski et al., 2014; Pavlović et al., 2020). The majority of research studies compared the impact of organic and conventional fertilizers on the nutritional composition of products, but not the effect of the production system as a whole (Golijan and Sečanski, 2021). It is evident that differences in chemical

composition between products from organic and conventional production vary depending on the plant species, production season, morphological parts of the plant being analyzed and the tested nutrient, which makes it difficult to determine the clear impact of production methods on the nutritional composition of plants (Magkos et al., 2003). Therefore, the aim of this study was to examine potential differences in the composition of several nutrients in buckwheat grains obtained by organic and conventional methods of agricultural production. The results can provide an insight into a clearer picture of the impact of different production methods on the chemical composition.

2. Materials and methods

2.1. Grain production and collection

Organic and conventional buckwheat grains used in the study originated from a site in Nova Varoš (Southwest Serbia). Buckwheat (*Fagopyrum esculentum* Moench) was sown during May 2016 on a black mountain soil. Plot size was 1 ha. In conventional production, 200 kg ha⁻¹ NPK fertilizer was applied (16:16:16); in organic production, fertilization included 15 t ha⁻¹ manure and 7 L ha⁻¹ Slavol (foliar fertilizer). In both types of buckwheat production, weed control was performed manually. During the buckwheat growing season, the average air temperature was 15.7 °C and the average amount of precipitation was 563.2 mm. Mechanical harvesting was performed in September 2016 at the stage of full maturity of the grains.

Buckwheat grain samples were separated from damaged buckwheat grains and the grains of other weed species, and then ground into a fine powder. Thereafter, the samples were vacuumed in vacuum bags and stored in a dark and cool place until analysis.

2.2. Analytical methods

2.2.1. Extractions of nutrients/phytochemicals

Extraction of total proteins. Extraction was performed according to the standard AOAC method no. 960.52 (AOAC, 1997).

Extraction of soluble sugars and starch. A modified procedure according to Laware (2015) was used. A 0.5 g grain sample was weighed and covered with 10 mL of 80% methanol. The sample was then placed on a shaker for 5 minutes at room temperature. Thereafter, the cuvettes were centrifuged at 4000 g for 15 minutes. The resulting supernatant was collected and the process of extraction was repeated two times. The supernatants were collected and merged, and the volume in the cuvette was filled up to 50 mL with deionized water. The extract was then evaporated on a vacuum evaporator at 40°C to a volume of 1 mL. The residue was dissolved in deionized water to a volume of 10 mL and used to further determine the total soluble sugars. The residual sediment was covered with a digestion solution of 10 mL of 52% perchloric acid and 7.7 mL of deionized water, stirred intensely and stored in a container with ice for 1 h in the refrigerator at 0°C. The contents of the cuvettes were subsequently stirred vigorously and then centrifuged at 13.500 RPM (17000 g) for 15 minutes. The supernatant was set apart into a plastic cuvette, and the residue was refilled to determine the content of starch. *Extraction of polyphenols and flavonoids*. The extraction of free polyphenols in the grain samples was performed according to the previously described method (Kováčová and Malinová, 2007; Golijan et al., 2021). The obtained extracts were used to determine the contents of free and bound polyphenols and flavonoids, as well as to determine the antioxidant activity of the grain samples.

2.2.2. Spectrophotometric determination of nutrients/phytochemicals

Determination of total protein content. Protein content (\sum Pr) was determined according to AOAC – standard method no. 960.52 (AOAC, 1997). The results are expressed as % (mg 100 g⁻¹) dry matter (without moisture).

Determination of pigment content. The content of pigments (chlorophyll *a*, chlorophyll *b* and total carotenoids) was determined according to the Laware method (2015). About 0.1 g of finely ground grain samples was transferred into Eppendorf cuvettes and the pigments were extracted with 2 mL of 80% acetone, for 5 minutes, with stirring on a thermoshaker (600 RPM) (Thermomixer comfort, Hamburg) at room temperature. Centrifugation was then performed at 13.500 RPM (17000 g), for 15 minutes. The resulting supernatant 1 was transferred to a 10 mL centrifugal cuvette. The extraction procedure was repeated two times, the supernatants were combined and the total volume was adjusted to 10 mL with 80% acetone. To determine the pigments in the grain samples, the absorbance of the obtained extracts was read at 663 nm, 646 nm and 470 nm. The contents of chlorophyll *a*, chlorophyll b and total carotenoids were calculated according to the following patterns:

Chlorophyll *a* (μ g g⁻¹) = 12.21 · A₆₆₃ – 2.81 · A₆₄₆

Chlorophyll *b* (μ g g⁻¹) = 20.13 · A₆₄₆ – 5.03 · A₆₆₃

Carotenoids (µg g⁻¹) = $(1000 \cdot A_{470} - 3.27 \cdot chlorophyll a - 104 \cdot chlorophyll b)/227$

Determination of total soluble sugars and starch. Prepared and diluted methanol extracts were used by applying the standard Anthrone method according to Frølund et al. (1996). The results obtained are expressed as % of glucose (mg 100 g⁻¹ of glucose).

Determination of the contents of free and bound polyphenols. The standard Folin-Ciocalteau method was used (Singleton et al., 1999). The results obtained are expressed as mg ferulic acid equivalents (FAE) per kg of dry matter of the sample.

Determination of the contents of free and bound flavonoids. The method with aluminum chloride was applied, according to Lin and Harnley (2007). The obtained values of free and bound flavonoids are expressed as mg quercetin equivalents (QE) per kg of dry matter of the sample.

Determination of phytosterol content. The procedure according to Daksha et al. (2010) was

applied. A 1 g sample was weighed, dissolved in chloroform up to 10 mL and further diluted up to 10 times (10.000 ppm). Then, 3 mL of the diluted sample solution was mixed with 2 ml of Lieberman-Burchard reagent and 2 mL of chloroform. The test tubes were covered with black carbon paper and stored in a container with ice in a dark place for 15 minutes. The Lieberman-Burchard reagent reacted with sterol, which led to the appearance of a characteristic green color, the absorbance of which was determined on a spectrophotometer at 640 nm.

Determination of $ABTS^{*+}$ scavenging capacity. The ability of methanolic extracts of buckwheat grains to quench this radical was determined using the method developed by Serpen et al. (2007) and expressed as % of inhibition of $ABTS^{*+}$ (2,2'-azino-bis(3ethylbenzothiazoline-6-sulfonic acid) radical cation.

2.3. Statistical analysis

The results of all analyses were expressed as the mean of three replicates \pm standard deviation (SD). The significance of differences between the means (P < 0.05) was determined by using Tukey's test, software Statistica, version 8.0 (StatSoft Inc., USA).

3. Results and discussions

In this research, the chemical composition of *Fagopyrum esculentum* grains originating from organic and conventional production was determined. By analyzing the results shown in Table 1, due to the above-mentioned potential influences of production methods on the chemical composition of the grains, different contents of the tested chemical parameters in *F. esculentum* grains were determined.

Based on the presented table, statistically significant differences were observed in the following chemical parameters: chlorophyll *b* (μ g g⁻¹ DM), carotenoids, soluble sugars (mg 100 g⁻¹), starch (mg 100 g⁻¹), bound polyphenols (mg (FAE) kg⁻¹ DM), free flavonoids (mg (CE) kg⁻¹ DM), bound flavonoids (mg (QE) kg⁻¹ DM) and phytosterols (mg kg⁻¹ DM); while in the other examined chemical parameters there were no statistically significant differences.

According to the research conducted by Kim et al. (2004), proteins and lipids are the main group of macronutrients in buckwheat grains. A previous study (Golijan et al., 2019) reported significantly different total lipid contents for organic and conventional buckwheat grain: 4.32 $\,$ mg 100 g⁻¹ and 5.36 mg 100 g⁻¹ respectively. The results obtained in this research indicate no statistical difference in the total protein content between organically produced grains (9.81 mg 100 g⁻¹) and conventional ones (10.61 mg 100 g⁻¹). The obtained values of the total protein content are in agreement with the levels in the research of Guo et al. (2007) (8.81-18.71% i.e. mg 100 g⁻¹), showing that protein content primarily depends on the variety of F. esculentum. Most of the research related to the difference in protein content between organic and conventional crops refers to data regarding vegetables, while there is a small amount of data for cereals and legumes. Most authors agree that organically produced vegetables (such as spinach, beet, carrot, tomato and potato) and cereals (especially wheat, corn and rye) have lower contents of total proteins and free amino acids, but, on the other hand, they have higher contents of several essential amino acids (Woese et al., 1997; Ragasits and Kismanyoky, 2000; Kumpulainen, 2001; Bourn and Prescott, 2002; Magkos et al., 2003).

Table 1.

The results of the chemical composition of buckwheat grains with respect to selected nutrients/phytochemicals.

Parameters	OB	СВ
Total proteins (Nx6,25) (mg 100 g ⁻¹)	9.81±0.06 a	10.61±0.68 a
Chlorophyll <i>a</i> (μg g ⁻¹ DM)	6.32±0.13 a	5.43±0.92 a
Chlorophyll <i>b</i> (µg g ⁻¹ DM)	8.46±1.90 a	24.16±4.90 b
Carotenoids (µg g ⁻¹ DM)	6.998±1.48 a	3.17±1.79 b
Soluble sugars (mg 100 g ⁻¹)	6.48±0.11 a	2.57±0.11 b
Starch (mg 100 g ⁻¹)	2.46±0.13 a	1.62±0.06 b
Free polyphenols (mg (FAE) kg ⁻¹ DM)	6505.7±58.97 a	6656.1±113.62 a
Bound polyphenols (mg (FAE) kg ⁻¹ DM)	1034.3±39.99 a	161.87±10.43 b
Free flavonoids (mg (QE) kg ⁻¹ DM)	1094.7±79.14 a	879.19±6.76 b
Bound flavonoids (mg (QE) kg ⁻¹ DM)	1087.2±48.08 a	501.65±10.31 b
Phytosterols (mg kg ⁻¹ DM)	130.07±5.40 a	185.15±6.64 b
Antioxidant activity (% inhibition of ABTS•+)	92.82±3.49 a	97.18±1.94 a

*The table shows the mean values of three replicates±standard deviation. Different small letters in the same row show a statistically significant difference according to the t-test (*P*<0.05).

Woëse et al. (1997) reported that organically produced grains, especially wheat, contain less protein than the ones conventionally produced. The lower protein content in organic wheat may be a consequence of lower nitrogen availability (Starling and Richards, 1990), while other authors believe that organic wheat is nutritionally superior (Bourn and Prescott, 2002). The higher content of total proteins in conventional vegetables is probably due to the application of conventional fertilizers, which provide the plant with greater availability of nitrogen compared to organic fertilizers. It is known that the large use of nitrogen in plant production increases the concentration of total proteins, resulting in a lower nutritional value of protein and a lower content of essential amino acids (Worthington, 2001; Rembialkowska, 2007). In a threeyear research (2011; 2012; 2013) conducted by Gavrić et al. (2017), the percentage of protein (10.94%; 14.26%; 12.08%) was higher than the results obtained in this study. This can be explained by the fact that the average amount of precipitation during the research of Gavrić et al. (2017) (626 mm; 861 mm; 905 mm) was significantly higher than the amount of precipitation in this paper (563.2 mm m-2), due to which a higher protein content was achieved. Moreover, it can be concluded that the lower protein content is caused by external factors, which is in line with the statements by Bárta et al. (2004) and Gavrić et al. (2017).

The contents of photosynthetic active pigments depend on the influence of various stress factors, which arise as a result of environmental conditions (Carter, 1993). The results in this study indicate that the contents of photosynthetically active pigments of chlorophyll b and carotenoids were statistically different, while the content of chlorophyll a was not (6.32; 5.43 μ g g⁻¹ DM) in organic and conventional F. esculentum grain production. Namely, the results in this research show that the content of chlorophyll *a* was not affected by the method of production, indicating that organic and mineral fertilization, as well as organic and conventional production methods have the same effect on the content of chlorophyll *a*. Likewise, Elsayed et al. (2020) reported that the effects of mineral and organic fertilizers in dill crops during the first season

(2014/2015) had the same effect on the chlorophyll *a* content. In the same study, during the second year (2015/2016), minimum contents of chlorophyll *b* and carotenoids were obtained after treatment with organic fertilizers, which is similar to the chlorophyll *b* content in this study; that is, a higher chlorophyll bcontent was achieved in conventional grains (24.16 µg g⁻¹ DM) than in organic ones (8.46 µg g⁻¹ DM). However, in contrast to the above-mentioned results, other studies confirm the same (Hamlman, 2012), while in this study a higher grain carotenoid content was achieved in organic production (6.998 μ g g⁻¹ DM) than in conventional production (3.17 μ g g⁻¹ DM). The higher content of carotenoids is justified by the research of Gholami et al. (2018), who emphasized the positive effect of organic fertilizers on carotenoid content, as also achieved in the current research. By using synthetic fertilizers, large quantities of readily available nitrogen accelerate plant growth and development, which results in reduced production of plant secondary metabolites such as chlorophyll, polyphenols, and organic and amino acids (Winter and Davis, 2006).

The achieved positive impact of organic fertilizers and thus organic production methods improved the photosynthetic activity, which contributed to a positive increase in sugar and starch production. Namely, the soluble solids content of the grain in organic production (6.48 mg 100 g⁻¹) was statistically higher than in conventional production (2.57 mg 100 g⁻¹). A similar effect was achieved in the research conducted by Songhe et al. (2016), showing that an appropriate ratio of mineral to organic fertilizers resulted in an appropriate sugar content in F. esculentum grains. The total starch content of the grains was significantly higher in organic production (2.46 mg 100 g⁻¹) than in conventional production (1.62 mg 100 g⁻¹). This is explained by the research of Podolska et al. (2016), which shows different effects of production methods, i.e., cultural practices, on the starch content in buckwheat grains. Some authors reported more sugar in organically produced plants (Bøhn et al., 2014), while other studies showed no difference (Zörb et al., 2006; Langenkämper et al., 2006). Zörb et al. (2006) examined different types of metabolites and sugars in

organically and conventionally produced wheat, and found no significant influence of the production method on the amount of sugar. Konvalina et al. (2011) revealed an inverse correlation between the amount of starch and protein content in wheat grain. Namely, it has been proven that the increase in grain starch content is conditioned by unfavorable conditions for protein accumulation in the grain. One of the reasons for higher sugar levels in organic foods is the effect of nitrogen. As explained by Bourn and Prescott (2002), due to the increased use of nitrogen fertilizers, a larger amount of nitrogen reaches the plant from conventional production, causing reduced contents of dry matter, total sugars, methionine, numerous nutrients, and vitamin C.

As is well known, buckwheat grains contain significant amounts of antioxidants, mostly polyphenolic compounds, in free and bound forms. Polyphenolic compounds present in bound form have the greatest significance in antioxidant activity (Andreasen et al., 2001). In the current study, the content of free phenols in organic grains (6505.7 mg (FAE) kg⁻¹ DM) and conventional grains (6656.1 mg (FAE) kg-1 DM) did not differ statistically, in contrast to the content of bound phenols in organic production (1034.3 mg (FAE) kg-1 DM) and conventional production (161.87 mg (FAE) kg⁻¹ DM) of *F. esculentum* grain. The same effects were achieved in the research conducted by Asami et al. (2003), where organic production methods had a greater effect on polyphenolic compounds in corn, strawberries and blackberries. A large number of authors have studied differences in the content of polyphenols and other antioxidants between organically and conventionally produced plants and their products. Baranski et al. (2014) conducted a meta-analysis of 342 publications that analyzed differences in the levels of numerous secondary metabolites, and macro- and micronutrients between organically and conventionally produced plants. They found that organically produced plants contained a significantly higher amount of antioxidants, primarily polyphenols, such as flavanones, flavones, flavonols, stilbenes and anthocyanins. As reported by Rembialkowska (2007), organic plant products have twice the amount of polyphenols in comparison to conventional ones. Langenkämper et al. (2006) did not find statistically significant differences in the content of total polyphenols between organic and conventional wheat. Elevated levels of polyphenols were determined in the grains of non-fertilized plants, which is why the authors claimed that elevated polyphenol levels can occur under nutrient deficiency stress to which plants are exposed. The research by Dixon and Paiva (1995) indicated that various biotic and abiotic stresses lead to the induction of polyphenolic compounds. Therefore, increased production of polyphenolic compounds in organic agriculture is expected because neither crop protection chemicals nor mineral fertilizers are used, whereby environmental stress that increases polyphenol production occurs. According to another claim, the content of polyphenols is explained by the influence of mineral nitrogen fertilizers. Specifically, mineral nitrogen fertilizers are used more in conventional and less in organic production, and thus the plant organism activates phenylpropanoid compounds that participate in the production of polyphenols, flavonoids, phytosterols, and other secondary metabolites (Winter and Davis, 2006).

The results for flavonoids obtained in this study showed that higher levels of free and bound flavonoids were achieved in organic production than by conventional methods. In organic buckwheat grains, the total contents of free and bound flavonoids (1094.7 mg (CE) kg-1 DM; 1087.2 mg (QE) kg-1 DM) differed statistically from their contents in conventional grains (879.19 mg (CE) kg-1 DM; 501.65 mg (QE) kg-1 DM, respectively). Similar levels of free and bound flavonoids in the conventional method of production were determined in the study of Inglett et al. (2011), which confirms that the organic method has a better effect on flavonoid production for the same reasons it has a better effect on the polyphenol content. As reported by Rembiałkowska (2007), there are two theories that explain differences in the metabolite content in plants. The first theory, the carbon/nitrogen balance theory, claims that, when nitrogen is readily available to plants, they will first synthesize nitrogenrich compounds such as growth proteins, and secondary metabolites that contain nitrogen, such as alkaloids, glucosinolates and amino acids that are not part of the protein. Under reduced nitrogen availability, metabolism is redirected to the synthesis of carbonrich compounds, such as starch, cellulose and nitrogenfree secondary metabolites such as polyphenols, and terpenoids.

The content of phytosterols in plants is especially important because they can help lower cholesterol levels in the intestinal tract (Leonova et al., 2008). The results of the research are statistically different, showing a higher content of phytosterols in conventional production (185.15 mg kg⁻¹ DM) than in the organic method (130.07 mg kg⁻¹ DM). Furthermore, in other crops, it is noteworthy that fertilization method can have different effects on the content of phytosterols, which is confirmed in the research conducted by Kirkhus et al. (2013).

As regards antioxidant capacity, the two methods of grain production had the same effect; that is, there was no statistically significant difference in antioxidant capacity between organic grain production (92.82%) and conventional grain production (97.18%). A similar effect was achieved in the research by Valavanidis et al. (2009), showing that the antioxidant potential largely depended on the polyphenolic content. In a paper published in 2005, Benbrook reported that organic foods had higher antioxidant capacity on average by 30% (Benbrook, 2005), and a paper published in 2008 reported about an 88% increase in total antioxidant capacity in organically produced foods (Benbrook et al., 2008). Tsao et al. (2005) discussed certain assumptions that the contents of some polyphenolic compounds exhibit different antioxidant capacities. This can be confirmed by the results of the current study considering that the contents of free polyphenolic compounds in organic and conventional production systems differed, unlike the levels of bound polyphenolic compounds.

4. Conclusions

For the final outcome of this research, it is important to point out that the production of buckwheat grains is successfully carried out in both conventional and organic production systems. This study shows that the production system can have a significant impact on the chemical composition of the grain produced.

The comparison of the chemical composition of organically and conventionally produced grains revealed statistically significant differences in the contents of chlorophyll *b*, carotenoids, soluble sugars, starch, bound polyphenols, free and bound flavonoids, and phytosterols resulting from production type. As regards antioxidant potential, protein content, chlorophyll *a* and free polyphenols, the same effect on the grain was achieved in both production systems.

Importantly, organic buckwheat grain produces about 45% higher carotenoid content, 40% higher sugar content and 66% higher starch content than conventional buckwheat grain. The levels of bound polyphenols, and free and bound flavonoids were up to several times higher (6.4, 1.2 and 2.2, respectively) in organic than in conventional buckwheat grains, while the content of phytosterols was 1.4 times higher in conventional than in organic buckwheat grain.

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Declaration of competing interest

The authors declare that they had no conflict of interests.

References

- Andreasen, M.F., Kroon, P.A., Williamson, G., Garcia-Conesa, M.T. (2001). Esterase Activity Able To Hydrolyze Dietary Antioxidant Hydroxycinnamates Is Distributed along the Intestine of Mammals. *Journal of Agricultural and Food Chemistry*, 49(11), 5679–5684.
- https://doi.org/10.1021/jf010668c AOAC (1997). Official methods of analysis 16th edition.
- Association of Official Analytical Chemists, Arlington, VA, USA.
- Asami, D.K., Hong, Y.J., Barrett, D.M., Mitchell, A.E. (2003). Comparison of the Total Phenolic and Ascorbic Acid Content of Freeze-Dried and Air-Dried Marionberry, Strawberry, and Corn Grown Using Conventional, Organic, and Sustainable Agricultural Practices. *Journal of* Agricultural and Food Chemistry, 51(5), 1237–1241. https://doi.org/10.1021/jf020635c
- Baranski, M., Srednicka-Tober, D., Volakakis, N., Seal, C., Sanderson, R., Stewart, G. B., Benbrook, C., Biavati, B., Markellou, E., Giotis, C., Gromadzka - Ostrowska, J., Rembialkowska, E., Skwarlo - Sonta, K., Tahvonen, R., Janovska, D., Niggli, U., Nicot, P., Leifert, C. (2014). Higher antioxidant and lower cadmium concentrations and lower incidence of pesticide residues in organically grown crops: a systematic literature review and metaanalyses. British Journal of Nutrition, 112, 794-811. https://doi.org/10.1017/S0007114514001366
- Bárta, J., Kalinová, J., Moudrý, J., Čurn, V. (2004). Effects of environmental factors on protein content and composition in buckwheat flour. *Cereal Research Communications*, 32, 541–548.
- Benbrook, C.M. (2005). Elevating Antioxidant Levels in Food through Organic Farming and Food Processing. An Organic Center of Science Review. Organic Center for Education and Promotion. (Available at https://organiccenter.org/reportfiles/AntioxidantReport.pdf)

- Benbrook, C., Zhao, X., Yáñez, J., Davies, N., Andrews, P. (2008). New Evidence Confirms the Nutritional Superiority of Plant - Based Organic Foods. The Organic Center. (Available at https://www.organic-center.org/newevidence-confirms-nutritional-superiority-plant-basedorganic-foods).
- Bøhn, T., Cuhra, M., Traavik, T., Sanden, M., Fagan, J., Primicerio, P. (2014). Compositional differences in soybeans on the market: Glyphosate accumulates in Roundup Ready GM soybeans. *Food Chemistry*, 153, 207– 215. https://doi.org/10.1016/j.foodchem.2013.12.054
- Bourn, D., Prescott, J. (2002). A Comparison of the Nutritional Value, Sensory Qualities, and Food Safety of Organically and Conventionally Produced Foods. *Critical Reviews in Food Science and Nutrition*, 42(1), 1–34. https://doi.org/10.1080/10408690290825439
- Campbell, C.G. (1997). Buckwheat. Fagopyrum esculentum Moench. Promoting the conservation and use of underutilized and neglected crops. 19. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy.
- Carter, G.A. (1993). Responses of leaf spectral reflectance to plant stress. *American Journal of Botany*, 80, 239–243.
- Daksha, A., Jaywant, P., Bhagyashree, C., Subodh, P. (2010). Estimation of sterols content in edible oil and ghee samples. *International Journal of Pharmaceutical Sciences Review and Research*, 5(1), 135–137.
- Dixon, R.A, Paiva, N.L. (1995). Stress-induced phenylpropanoid metabolism. *Plant Cell*, 7, 1085–1097.
- Dolijanović, Ž.K., Oljača, S.I., Kovačević, D.D., Šeremešić, S.I., Jovović, Z.M. (2014). The effect of growing regions, microbiological fertilizers and soil additives on productivity of buckwheat (*Fagopyrum esculentum* Moench). Journal of Agricultural Sciences, 59(1), 25–34. https://doi.org/10.2298/JAS1401025D
- Elsayed, S. I. M., Glala, A. A., Abdalla, A. M., El-Sayed, A. E. G. A., Darwish, M. A. (2020). Effect of biofertilizer and organic fertilization on growth, nutrient contents and fresh yield of dill (*Anethum graveolens*). Bulletin of the National Research Centre, 44(1). https://doi.org/10.1186/s42269-020-00375-z
- Frølund, B., Palmgren, R., Keiding, K., Nielsen, H. (1996). Extraction of extracellular polymers from activated sludge using a cation exchange resin. *Water Research*, 30(8), 1749–1758.
- Gavrić, T., Čadro, S., Gadžo, D., Đikić, M., Bezdrob, M., Jovović, Z., Jurković, J., Hamidović, S. (2018). Influence of meteorological parameters on the yield and chemical composition of common buckwheat (*Fagopyrum esculentum* Moench). *Agriculture and Forestry*, 64 (4), 113–120.
 - https://doi.org/10.17707/AgricultForest.64.4.13
- Gholami, H., Ghani, A., Raouf Fard, F., Saharkhiz, M. J., Hazrati, H. (2018). Changes in photosynthetic pigments and uptake of some soil elements by chicory supplied with organic fertilizers. *Acta Ecologica Sinica.* https://doi.org/10.1016/j.chnaes.2018.09.003
- Glamočlija, Đ. (2004). Posebno ratarstvo: žita i zrnene mahunarke. Draganić, pp. 301.
- Golijan, J. (2016). Motivi koji utiču na kupovinu organskih prehrambenih proizvoda. Agroekonomika, 45(72), 73–80.
- Golijan, J., Kostić, Ž.A. (2016). Značaj polifenola iz žitarica u ljudskoj ishrani. *Hrana i ishrana*, 57(2), 47–52.
- Golijan, J., Živanović, LJ, Kostić, Ž. A. (2017). Hemijski sastav heljde sa nutritivnog aspekta. *Hrana i ishrana*, 58(2), 9– 16.
- Golijan, J., Milinčić, D.D., Petronijević, R., Pešić, M.B., Barać, M.B., Sečanski, M., Lekić, S., Kostić, A.Ž. (2019). The fatty acid and triacylglycerol profiles of conventionally and organically produced grains of maize, spelt and buckwheat. *Journal of Cereal Science*, 90, 102845. https://doi.org/10.1016/j.jcs.2019.102845
- Golijan, J.M., Milinčić, D.D., Petronijević, R.B., Pešić, M.B., Stanojević, S.P., Barać, M.B., Lekić, S., Kostić, A.Ž. (2021). Comparison of sugars, lipids and polyphenols content in the grains of organically and conventionally grown

soybean in Serbia. *Zemdirbyste-Agriculture*, 108(1), 51–56. https://doi.org/10.13080/z-a.2021.108.007

- Guo, Y.-Z., Chen, Q.-F., Yang, L.-Y., Huang, Y.-H. (2007). Analyses of the seed protein contents on the cultivated and wild buckwheat Fagopyrum esculentum resources. *Genetic Resources and Crop Evolution*, 54(7), 1465–1472. https://doi.org/10.1007/s10722-006-9135-z
- Hallmann, E. (2012). The influence of organic and conventional cultivation systems on the nutritional value and content of bioactive compounds in selected tomato types. *Journal of the Science of Food and Agriculture*, 92(14), 2840–2848.
- Heaton, S. (2001). Organic Farming, Food Quality and Human Health: A Review of the Evidence. Bristol: Soil Association. https://www.soilassociation.org/media/4920/policy_re port_2001_organic_farming_food_quality_human_health.p df
- Inglett, G.E., Chen, D., Berhow, M., Lee, S. (2011). Antioxidant activity of commercial buckwheat flours and their free and bound phenolic compositions. *Food Chemistry*, 125(3), 923–929.

https://doi.org/10.1016/j.foodchem.2010.09.07

- Kim, S.L., Kim, S.K., Park, C.H. (2004). Introduction and nutritional evaluation of buckwheat sprouts as a new vegetable. *Food Research International*, 37, 319–327.
- Kirkhus, B., Lundon, A.R., Haugen, J.-E., Vogt, G., Borge, G.I.A., Henriksen, B.I.F. (2013). Effects of Environmental Factors on Edible Oil Quality of Organically Grown Camelina sativa. *Journal of Agricultural and Food Chemistry*, 61(13), 3179–3185. https://doi.org/10.1021/jf304532u
- Konvalina, P., Stehno, Z., Capouchová, I., Moudry, J. (2011). Wheat growing and quality in organic farming. *Research in organic farming*, 105-122.
- Kováčová, M., Malinová, E. (2007). Ferulic and coumaric acids, total phenolic compounds and their correlation in selected oat genotypes. *Czech Journal of Food Sciences*, 25, 325–332.
- Kumpulainen, J. (2001). Organic and conventional grown foodstuffs: Nutritional and toxicological quality comparisons. In Proceedings of International Fertiliser Society, 472, 1–20. https://agris.fao.org/agrissearch/search.do?recordID=US201300062841
- Laware, S.L. (2015). Sequential Extraction of Plant Metabolites. International Jurnal of Current Microbiology and Applied Sciences, 4 (2), 33–38.
- Langenkämper, G., Zörb, C., Seifert, M., Mäder, P., Fretzdorff, B., Betsche, T. (2006). Nutritional quality of organic and conventional wheat. *Journal of Applied Botany and Food Quality*, 80, 150–154.
- Lin L-Z, Harnly, J.M. (2007). A Screening Method for the Identification of Glycosylated Flavonoids and Other Phenolic Compounds Using a Standard Analytical Approach for All Plant Materials. *Journal of Agricultural and Food Chemistry*, 55 (4), 1084–1096.
- Magkos, F., Arvaniti, F., Zampelas, A. (2003). Organic food: nutritious food or food for thought? A review of the evidence. *International Journal of Food Sciences and Nutrition*, 54(5), 357–371.

https://doi.org/10.1080/09637480120092071

- Pavlović, N., Zdravković, M., Mladenović, J., Štrbanović, R., Zdravković, J. (2020). Analysis of fresh and processed carrots and beets from organic and conventional production for the content of nutrients and antioxidant activity. Acta Agriculturae Serbica, 25(50), 171–177. https://doi.org/10.5937/AASer2050171P
- Podolska, G. (2016). The Effect of Habitat Conditions and Agrotechnical Factors on the Nutritional Value of Buckwheat. *Molecular Breeding and Nutritional Aspects of Buckwheat*, 283–297. https://doi.org/10.1016/b978-0-12-803692-1.00022-5
- Qin, P., Wu, L., Yao, Y., Ren, G. (2013). Changes in phytochemical compositions, antioxidant and α -

glucosidase inhibitory activities during the processing of tartary buckwheat tea. *Food Research International*, 50(2), 562–567.

https://doi.org/10.1016/j.foodres.2011.03.028

Ragasits, I., Kismanyoky, T. (2000). Effects of organic and inorganic fertilization on wheat quality. *Novenytermeles*, 49, 527–532.

https://www.cabdirect.org/cabdirect/abstract/2001314 4236

- Rembiałkowska, E. (2007). Quality of plant products from organic agriculture. Journal of the Science of Food and Agriculture, 87, 2757–2762. https://doi.org/10.1002/jsfa.3000
- Serpen, A., Capuano, E., Fogliano, V., Gökmen, V. (2007). A new procedure to measure the antioxidant activity of insoluble food components. *Journal of Agricultural and Food Chemistry*, 55, 7676–7681.
- Singleton, V.L., Orthofer, R., Lamuela Raventós, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *In Methods in Enzimology*, 299, 152–178.
- Skrabanja, V., Lærke, H.N., Kreft, I. (2000). Protein-polyphenol interactions and in vivo digestibility of buckwheat groat proteins. *Pflügers Archiv European Journal of Physiology*, 440(7), R129–R131.

https://doi.org/10.1007/s004240000033

- Škrobot, D. (2016). Senzorski, nutritivni i funkcionalni profil integralne testenine sa dodatkom heljdinog brašna. Doktorska disertacija. Univerzitet u Novom Sadu, Tehnološki fakultet.
- Starling, W., Richards, M.C. (1990). Quality of organically grown wheat and barley. Aspects of Applied Biology, 25, 193–198. https://www.cabdirect.org/cabdirect/abstract/1992231

3943

- Tsao, R., Yang, R., Xie, S., Sockovie, E., Khanizadeh, S. (2005). Which polyphenolic compounds contribute to the total antioxidant activities of apple? *Journal of Agricultural and Food Chemistry*, 53, 4989–4995.
- Valavanidis, A., Vlachogianni, T., Psomas, A., Zovoili, A., Siatis, V. (2009). Polyphenolic profile and antioxidant activity of five apple cultivars grown under organic and conventional agricultural practices. *International Journal* of Food Science & Technology, 44(6), 1167–1175. https://doi.org/10.1111/j.1365-2621.2009.01937.x
- Winter, C.K., Davis, S.F. (2006). Organic foods. Journal of Food Science, 71, R117-R124.

0010(199707)74:3<281::AID-JSFA794>3.0.CO;2-Z

Worthington, V. (1998). Effect of agricultural methods on nutritional quality: a comparison of organic with conventional crops. Alternative Therapies in Health and Medicine, 4, 58–69.

https://pubmed.ncbi.nlm.nih.gov/9439021/ Worthington, V. (2001). Nutritional Quality of Organic Versus Conventional Fruits, Vegetables, and Grains. *Journal of*

- Alternative and Complimentary Medicine, 7(2), 161–173. https://doi.org/10.1089/107555301750164244 Zhang, Z.L., Zhou, M.L., Tang, Y., Li, F.L., Tang, Y.X., Shao, J.R.,
- Zhang, Z.L., Zhou, M.L., Fang, F., Li, F.L., Fang, F.X., Shao, J.K., Xue, W.-T., Wu, Y.M. (2012). Bioactive compounds in functional buckwheat food. *Food research international*, 49(1), 389–395.

https://doi.org/10.1016/j.foodres.2012.07.035

Zörb, C., Langenkämper, G., Betsche, T., Niehaus, K., Barsch, A. (2006). Metabolite profiling of wheat grains (*Triticum aestivum* L.) from organic and conventional agriculture. *Journal of Agriculture and Food Chemistry*, 54, 8301–8306. https://doi.org/10.1021/jf0615451