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Flavonoids and total phenolic content in extruded buckwheat products with sweet and salty taste

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Abstract. The aim of research was to evaluate the effect of added ingredients used for taste improvement on flavonoids and total phenolic content in extruded buckwheat products. The added ingredients were – sugar, vanilla sugar, stevia, agave syrup, cinnamon, caraway, garlic powder, sweet pepper powder and salt. Six extruded buckwheat products were analysed, where an extruded buckwheat product without added ingredients was a control sample, two extruded buckwheat products were with sweet taste and three products with salty taste. Total phenolic content was determined using the modified Folin–Ciocalteu method but flavonoid content according to LC-TOF-MS method.
The highest total phenolic content ($p < 0.05$) was determined in extruded buckwheat product with vanilla sugar + stevia (91.17 ± 0.67 GAE mg $100g^{-1}$DW). It was almost three times higher than in the control sample. The significant differences were observed in extruded buckwheat products with caraway + salt + agave syrup (41.5 ± 0.12 GAE mg $100g^{-1}$DW) and sweet pepper powder + salt + agave syrup (42.39 ± 0.80 GAE mg $100g^{-1}$DW) comparing to other extruded products ($p < 0.05$). The highest content of rutin and quercetin ($p < 0.05$) was established in extruded buckwheat product with garlic powder + salt + agave syrup, whereas the highest content of catechin and epicatechin – in extruded buckwheat product with vanilla sugar + stevia. The extruded buckwheat product with caraway + salt + agave syrup in addition contained luteolin, kaempferol and isoquercitrin. The results of research showed that some added ingredients used for taste improvement can significantly influence the total phenolic content and flavonoid content.

Key words: buckwheat, extrusion, flavonoids, total phenolic content.

INTRODUCTION

Buckwheat is an alternative crop with high consumption in countries like China, Japan and Taiwan due to its functional food properties and biological value – great concentration of bio–active compounds such as phenols and flavonoids (Lin et al., 2009; Inglett et al., 2010; Qin et al., 2010; Zhang et al., 2012; Vollmannova et al., 2013). The diverse total phenolic content of buckwheat is reported in literature, the differences of results establish buckwheat species: common (*Fagopyrum esculentum* Moench) or tartary (*Fagopyrum tataricum* Gaertn); cultivars, growth conditions and technological production process. Mikulajeva et al. (2016) analysed 22 common buckwheat cultivars, which total phenolic content ranged between 0.897 and 4.226 mg GAE g^{-1} DW. Unal et al. (2017) reported about significant differences of total phenolic content between commercial buckwheat (produced in Kazakhstan) and Günes variety (produced in Turkey) – 207.12 ± 2.67 and 329.83 ± 3.88 mg GAE 100 g^{-1}, respectively. The diverse results about flavonoids content like rutin can be found in literature, too. Mikulajova et al. (2016) established rutin content in common buckwheat cultivars between 59.93 ± 2.16 and 304.45 ± 5.45 µg g^{-1} DW, whereas Vollmannova et al. (2013) – between 309.97 ± 10.84 and 507.78 ± 17.11 mg kg^{-1} DW.

The increase of buckwheat consumption in Latvia could be possible by production of new buckwheat products. In Asian countries and Italy there are buckwheat noodles, in Latvia there could be some buckwheat snacks using extrusion. There is not a single view about the effect of processing on phenolic and flavonoid content in buckwheat. Zielinska et al. (2007) and Wronkowska et al. (2015) concluded significant differences of phenolic compounds in raw and roasted buckwheat groats, whereas Hes et al. (2014) did not establish any negative effect on the nutritional properties in buckwheat groats after 30 min boiling in water. Sensoy et al. (2006) determined insignificant decrease of total phenolic content in dark buckwheat flour after roasting (200 °C, 10 min), whereas antioxidant activity was significantly lower in roasted dark buckwheat flour comparing to raw dark buckwheat flour. Our previous research (Beitane et al., 2018) showed significant decrease of total phenolic content, antiradical activity, radical scavenging activity and phenolic compounds concentration in buckwheat after extrusion.

In production of new products their taste has got important role, which could be improved with several ingredients. Furthermore the added ingredients used for taste improvement can affect the nutritional and biological value of the final product. In this research the added ingredients for taste improvement of extruded buckwheat products were sugar, vanilla sugar, stevia, agave syrup, cinnamon,-caraway, garlic powder, sweet pepper powder and salt. The brief description of used ingredients and their importance in food product production is given below.

Vanillin is the most used flavour in the food industry with antimicrobial and antioxidant properties (Zabkova et al., 2006).

Stevia rebaudiana or steviol glycosides are thermally stable and natural sweeteners with high sweetening potential, which are successfully used in cocoa and chocolate products, in chewing gums, flour or starch–based snacks etc. to reduce sugar consumption in the industrialized countries (Olsson et al., 2016; Shannon et al., 2016).

Agave syrup is a natural sweet product with functional properties due to biological active compounds, prebiotic molecules and antioxidant content, which is used as sugar replacement due to low glycaemic index (Foster–Powell et al., 2002; Phillips et al., 2009; Mellado–Mojica & Lopez, 2015, Muniz–Marquez et al., 2015).

Cinnamon is a culinary spice with anti–oxidant properties and free radical scavenging activity, which is used in traditional Chinese medicine and which has beneficial effects on serum glucose, triglyceride and total cholesterol level in diabetic patients (Parkash et al., 2007; Crawford, 2009; Otto, 2010; Adisakwattana et al., 2011).

Caraway is the most cultivated spice in Europe with wide use in folk medicine (Ghoneem et al., 2016; Kluz et al., 2016). Particular attention in scientific researches is paid to caraway essential oil with the main components – carvone and limonene (Ghoneem et al., 2016).

Garlic is a vegetable, which is known worldwide, with anti–bacterial and anti–fungal properties, high flavonoids content, especially quercetin, and antioxidant capacity (Griffiths et al., 2002; Beato et al., 2011; Onyeoziri et al., 2016).

Sweet pepper is a vegetable with high content of vitamins, carotenoids and phenols, which could be included in daily diet or used as additive in food production or in traditional medicine (Marti et al., 2011; Hernandez–Ortega et al., 2012; Sharma et al., 2016; Raybaudi–Massilia et al., 2017)

The aim of research was to evaluate the effect of added ingredients used for taste improvement on flavonoids and total phenolic content in extruded buckwheat products.

MATERIALS AND METHODS

Materials

Raw common buckwheat (Fagopyrum esculentum Moench) grown in Latvia in 2017, was produced by the organic farm 'Bebri'.

The added ingredients for taste improvement of extruded buckwheat products were purchased in grocery: sugar (Dan Sukker, Denmark), vanilla sugar produced from sugar and flavouring material vanillin (Mood, Lithuania), stevia (Canderel, The Czech Republic), agave syrup (Super Garden, Mexico), cinnamon (Santa Maria, Estonia), caraway (Santa Maria, Estonia), garlic powder (Kotanyi, Austria), sweet pepper powder (Santa Maria, Estonia) and salt (Artiomosol, Ukraine).

Dough preparation for extrusion

Raw buckwheat grain was milled using Mühle 2 (Hawo's, Germany) to obtain whole grain flour. Buckwheat flour with added ingredients and water was mixed with Sirman Planetary Mixers, model Pluton 10 (Minneapolis, Italy). The added ingredients for taste improvement of extruded buckwheat products were sugar, vanilla sugar, stevia, agave syrup, cinnamon, caraway, garlic powder, sweet pepper powder and salt. Six different doughs were prepared (Table 1), where buckwheat dough without added ingredients was prepared as control, two doughs were with sweet taste (vanilla sugar + stevia; sugar + cinnamon) and three – with salty taste (caraway + salt + agave syrup; garlic powder + salt + agave syrup; sweet pepper powder + salt + agave syrup).

Table 1. The formation of dough and abbreviations in buckwheat products

	Control	B+VS	B+SC	B+CSA	B+GSA	B+PSA
Buckwheat flour, g	100	100	100	100	100	100
Water, mL	75	75	75	75	75	75
Sugar, g	–	–	5	–	–	–
Vanilla sugar, g	–	5	–	–	–	–
Stevia, g	–	4	–	–	–	–
Agave syrup, g	–	–	–	5	5	5
Cinnamon, g	–	–	3	–	–	–
Caraway, g (milled)	–	–	–	5	–	–
Garlic powder, g	–	–	–	–	1.5	–
Sweet pepper powder, g	–	–	–	–	–	5
Salt, g	–	–	–	1.5	1.5	1.5

Extrusion

The prepared buckwheat doughs with different tastes were extruded with the food extruder PCE Extrusiometer L–Serie (Göttfert, Germany) by temperature profile 75/90/100 °C. After extrusion the products were cut in squares of 1 cm, dried in the convective–rotary oven (SVEBA DAHLAN, Sweden) at the temperature 140 ± 2 °C for 25 min and cooled to room temperature. Afterwards, six acquired extruded buckwheat products were used for further analysis.

Methods

Total phenolic content (TPC) of added ingredients for taste improvement and extruded buckwheat products was determined using the modified Folin–Ciocalteu method as described by Herald et al. (2012). The measurement was conducted by mixing Folin–Ciocalteu solution (1:1 with water), sodium bicarbonate and ethanolic extract. The absorbance was measured after 90 min of incubation at 765 nm againts a blank. TPC was expressed as gallic acid equivalents (GAE mg 100 g^{-1} DW), based on the gallic acid (GA) calibration curve (range 0.025–0.20 mg mL^{-1}, $R^2 = 0.9997$). Analyses were performed with the Infinite M200 PRO (Tecan Group Ltd., Männedorf, Switzerland) instrument in triplicate. The bandwidth was 9 mm and temperature 24 °C.

Determination of flavonoid content in extruded buckwheat products was made with the Liquid Chromatography – Time of Flight Mass Spectrometer (LC–TOF–MS) as described by Klavina et al. (2015). During LC–MS analysis, each sample produces its own base peak chromatogram (BPC). In the positive ionization mode each compound can add a proton and can produce its own $[M+H]^+$ mass spectra or stay in a positively charged state as M^+ molecule. Each compound has its own chemical formula and molar mass. This chemical formula using *Mass Hunter Qualitative Analyses B.07.00* software can be used for the calculation of $[M+H]^+/[M]^+$ which is used for the extraction of compounds from the base peak chromatogram. Both values can be compared and the difference (Δ) between them should not be higher than 0.0030. HRMS experiments ensure accurate mass measurements resulting in the removal of background signals of complex matrix interferences. It is useful for non–targeted or retrospective post–targeted identification of unknown compounds by the processing of raw data obtained in different scan modes, including full scan in defined *m/z* windows. The experimental data were handled using the MassHunter version B07.00 software (Agilent Technologies).

The extruded buckwheat products were analysed in three replications.

Data processing

The data of research were analysed using mathematical and statistical methods of Microsoft Office Excel 14.0. To determine the significant differences between the mean values were used the analyses of variance (ANOVA), *T–test* and *P–value* at 0.05.

RESULTS AND DISCUSSION

The total phenolic content (TPC) of ingredients used for taste improvement in extruded buckwheat products is given in Table 2.

The obtained data allows better to understand and explain the results of current research.

Table 2. Total phenolic content in extruded buckwheat products with different taste

Ingredients for taste improvement	TPC, GAE mg 100 g^{-1}
Sugar	n.d.
Vanilla sugar	125.86 ± 6.09
Stevia	n.d.
Agave syrup	26.68 ± 2.17
Cinnamon, g	1,534.07 ± 34.18
Caraway	202.62 ± 7.39
Garlic powder	51.60 ± 1.78
Sweet pepper powder	285.47 ± 8.01
Salt	n.d.

n.d. – not detected.

The extruded buckwheat products with sweet and salty taste displayed higher TPC as control sample (Fig. 1). It means that in generally the added ingredients for taste improvement of extruded buckwheat products had favourable effect on TPC. However statistically significant differences were observed between control and extruded buckwheat products with vanilla sugar + stevia; caraway + salt + agave syrup and sweet pepper powder + salt + agave syrup.

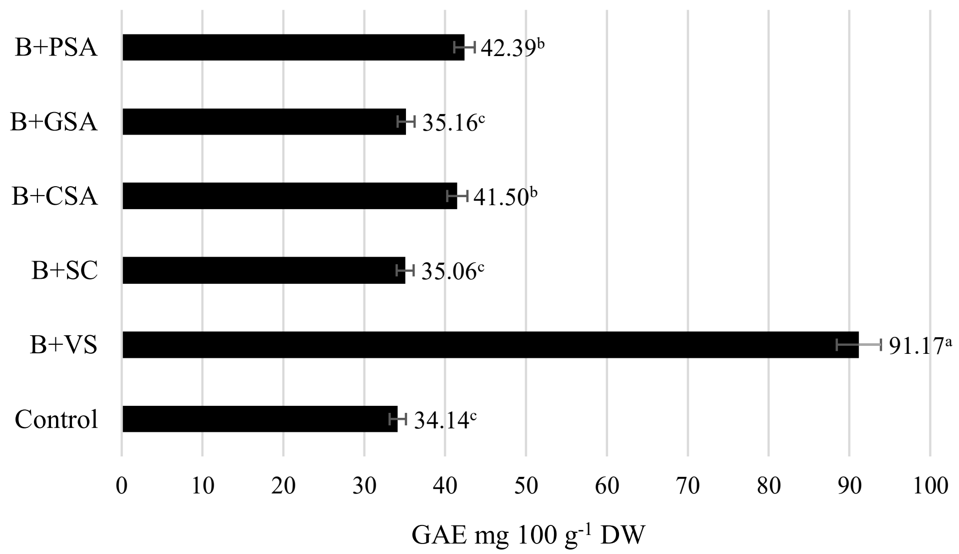

Figure 1. Total phenolic content in extruded buckwheat products with different taste.
Used letters (a, b, c) point to statistically significant difference between buckwheat products *(P* < 0.05*)*.

The highest TPC ($P < 0.05$) was determined in extruded buckwheat product with vanilla sugar + stevia (91.17 ± 0.67 GAE mg 100 g^{-1} DW). The impressive increase of TPC in extruded buckwheat product with vanilla sugar + stevia could be explained with added vanilla sugar. The results of current research (Table 2) showed, that vanilla sugar had a high total phenolic content (125.86 ± 6.09 GAE mg 100 g^{-1}), whereas in stevia it was not detected at all. There are not researches in literature about content of possible phenolic compounds in vanilla sugar. Only Burri et al. (1989) concluded that vanillin acts as an antioxidant in products of cereals. There are necessary profound researches about vanilla sugar, but it could be concluded, that the addition of vanilla sugar to extruded buckwheat product provided significant increase of TPC.

The great TPC ($P < 0.05$) in comparison to control was determined in extruded buckwheat products with caraway + salt + agave syrup and sweet pepper powder + salt + agave syrup, too. The increase of TPC in extruded buckwheat products could be based on addition of caraway and sweet pepper powder, because both presented great TPC (202.62 and 285.47 GAE mg 100 g^{-1}, respectively). Sharma et al. (2016) reported about high total phenolic content in red sweet pepper, which varied between 488.81 ± 8.18 and 977.63 ± 25.40 µg GAE g^{-1} FW (fresh weight). It is known that production process of sweet pepper powder can markedly affect the total phenolic and flavonoid content, however it is rich source of phenols. Shotorban et al. (2012) indicated that different processing techniques can significantly affect the content of bioactive compounds like polyphenols. Therefore the increase of TPC in extruded buckwheat product with sweet pepper powder + salt + agave syrup was not so marked. The other reason for increase of TPC in extruded buckwheat products with salty taste could be related with addition of agave syrup. Agave syrup is produced from agave plant and it rightly pointed to the presence of TPC in agave syrup (26.68 GAE mg 100 g^{-1}). Unfortunately, the few researches in literature about agave syrup chemical composition are focused on sugar composition.

The extruded buckwheat products with added sugar + cinnamon and garlic powder + salt + agave syrup did not provide expected increase of TPC, though cinnamon had the greatest total phenolic content among the added ingredients for taste improvement. It could be explained with the small amount of added cinnamon to dough, and extrusion, which had adverse effects on total phenolic content according to previous research (Beitane et al., 2018).

Extruded buckwheat product with garlic powder + salt + agave syrup had significantly greater concentration of rutin and quercetin ($P<0.05$) comparing to other products (Table 3).

Table 3. Flavonoids content in extruded buckwheat products with different taste, mg 100 g^{-1} DW

	Rutin	Quercetin	Catechin	Epicatechin
Control	5.51 ± 0.72^{b}	1.48 ± 0.13^{b}	3.97 ± 0.23^{a}	7.36 ± 0.62^{a}
B+VS	6.75 ± 0.81^{b}	1.68 ± 0.12^{b}	4.45 ± 0.26^{a}	8.02 ± 0.69^{a}
B+SC	6.72 ± 0.77^{b}	1.59 ± 0.19^{b}	4.01 ± 0.20^{a}	6.24 ± 0.48^{b}
B+CSA	5.35 ± 0.90^{b}	1.28 ± 0.21^{b}	2.20 ± 0.18^{b}	4.20 ± 0.33^{c}
B+GSA	8.99 ± 0.69^{a}	2.27 ± 0.17^{a}	2.47 ± 0.17^{b}	7.26 ± 0.52^{a}
B+PSA	5.90 ± 0.72^{b}	1.52 ± 0.11^{b}	2.65 ± 0.22^{b}	5.07 ± 0.66^{c}

Mean values with different letters (a, b, c) within a column point to statistically significant difference between buckwheat products ($P < 0.05$).

The increase of quercetin concentration in extruded buckwheat product with garlic powder + salt + agave syrup could be based on literature, where Griffiths et al. (2002) reported about high content of flavonoids, especially, quercetin and its conjugates in garlic; Miean & Mohamed (2001) determined relatively high content of quercetin – 47.0 mg kg^{-1} DW, myricetin – 693.0 mg kg^{-1} DW and apigenin – 217.0 mg kg^{-1} DW in garlic. The extruded buckwheat products with garlic powder + salt + agave syrup contained great concentration of rutin in the present study. It could be due to the presence of added garlic powder and agave syrup, because both presented the content of phenols. Scientific reports about flavonoids composition in garlic did not provide any information about rutin concentration. However the conclusions of researches about flavonoids content in garlic are contrary, for example Beato et al. (2011) did not detect myricetin, quercetin, kaempferol and apigenin in garlic. Therefore it cannot exclude the possibility that the addition of garlic powder provided the increase of rutin content in extruded buckwheat product with garlic powder + salt + agave syrup. The aim of current research was not to evaluate the flavonoid content of added ingredients, therefore it could be investigate in the following research. It was positive that all products except extruded buckwheat product with caraway + salt + agave syrup had rutin and quercetin concentration higher than control sample.

The greatest concentration of catechin and epicatechin was observed in extruded buckwheat product with vanilla sugar + stevia. Product which had the highest TPC, too. Catechin concentration among extruded buckwheat product with vanilla sugar + stevia, control sample and extruded buckwheat product with sugar + cinnamon was insignificant ($P > 0.05$) as well as epicatechin concentration was insignificant among extruded buckwheat product with vanilla sugar + stevia, control sample and extruded buckwheat product with garlic powder + salt + agave syrup ($P > 0.05$).

The extruded buckwheat product with caraway + salt + agave syrup in addition contained luteolin – 0.24 mg 100 g^{-1} DW, kaempferol – 0.46 mg 100 g^{-1} DW and isoquercitrin – 1.55 mg 100 g^{-1} DW. Isoquercitrin was detected in extruded buckwheat products with garlic powder + salt + agave syrup and sweet pepper powder + salt + agave syrup (0.22 and 0.35 mg 100 g^{-1} DW, respectively).

The extruded buckwheat product with sugar + cinnamon showed insignificant results about TPC, rutin, quercetin and catechin content comparing to control sample, except epicatechin content, which was significantly lower. The addition of cinnamon did not give the expected result, though Adisakwattana et al. (2011) reported that in cinnamon extract the TPC varied between 0.17–0.21 g GAE g^{-1} and flavonoids content – between 48.85–65.52 mg QE g^{-1}.

CONCLUSIONS

The aim to produce new buckwheat product with increased nutritional value was achieved, because some added ingredients used for taste improvement can significantly influence the total phenolic content and flavonoid content. However there is a need for further researches to understand and explain how and why the added ingredients affected/not affected the total phenolic and flavonoid content in extruded buckwheat products.

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