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The effect of buckwheat adjunct on the technological profile of unhopped wort

Vplyv surogácie pohánkou na technologický profil sladiny

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ABSTRACT

Recently, there has been increasing interest of using nonconventional starch adjuncts, such as buckwheat, not only due to its distinctive taste but also to increase the polyphenol content of the beer, and thus, its antioxidant capacity. The objective of this study was to examine technological profile of sweet wort made with unmalted buckwheat adjunct substitute. Wort was prepared in five variants: 5%, 10%, 15%, 20% and 30% of buckwheat adjunct substitute. The results showed that partial replacement of barley malt with buckwheat adjunct in higher amount of 20% and 30% had a negative effect on the saccharification, filtration time and led to decrease of extract content of wort. The colour of the congress wort with higher addition of buckwheat increased, on the contrary, the turbidity decreased. On the other hand, substitute with buckwheat adjunct positively increased total polyphenols, flavonoids and phenolic acids content of examined wort. The highest content of total polyphenols (197.10 mg/g), flavonoids (71.96 mg/g) phenolic acids (46.97 mg/g) was found in samples with 30% buckwheat adjunct. The results demonstrate that the unmalted buckwheat as starchy adjunct in application up to 15% did not negatively affected wort technological profile and can be recommended in the production of the unhopped worth without adjustment of mashing process.

Keywords: wort, buckwheat, quality, adjunct

ABSTRAKT

V poslednej dobe sa zvyšuje záujem o používanie nekonvenčných škrobnatých surogátov, ako napr. pohánky, a to nielen kvôli jej výraznej chuti, ale aj kvôli zvýšeniu obsahu celkových polyfenolov v pive, a tým aj jeho antioxidačnej aktivity. Cieľom tejto práce bolo analyzovať technologický profil sladiny, ktorá bola vyrobená za použitia prídavku nesladovanej pohánky ako surogátu. Sladina bola pripravená v piatich variantoch v množstve: 5 %, 10 %, 15 %, 20 % a 30 % prídavok pohánky ako surogátu. Výsledky ukázali, že čiastočné nahradenie jačmenného sladu pohánkovým surogátom v množstve 20 % a 30 % malo negatívny vplyv na dobu scukrenia, rýchlosť filtrácie a viedlo k zníženiu obsahu extraktu v sladine. Farba kongresnej sladiny so stúpajúcim prídavkom pohánky sa zvýšila, naopak, zákal sa znížil. Na druhej strane so zvyšujúcim sa prídavkom pohánky sa pozitívne zvýšil obsah celkových polyfenolov, flavonoidov a fenolových kyselín v analyzovanej sladine. Najvyšší obsah celkových polyfenolov (197,10 mg/g), flavonoidov (71,96 mg/g) fenolových kyselín (46,97 mg/g) bol zistený vo vzorkách s 30 % prídavkom pohánky. Výsledky dokazujú, že nesladovaná pohánka ako škrobnatý surogát v aplikácii do 15 % negatívne neovplyvnila technologický profil sladiny a možno ju odporučiť pri výrobe sladiny bez úpravy procesu rmutovania.

Kľúčové slová: sladina, pohánka, kvalita, surogát

INTRODUCTION

Nowadays, the demand for new and interesting beers with added nutritional benefits is growing. Consumer interest in this beverage has increased due to assortment diversification and the reinvention of craft beer (Dulin´ski et al., 2020). Innovation in the beer industry often involves the use of new mixtures of cereals and pseudo-cereals or old cereals, new hop varieties or substitutes, some spices, and other flavouring compounds to improve the sensory characteristics of the finished product (Baiano, 2021; Iorizzo et al., 2021). Beer is alcoholic beverage and is one of the most popular internationally consumed. Beer is rich in nutrients such as carbohydrates, amino acids, vitamins, minerals, phenolic compounds, etc. (Piazzon et al., 2010). The main polyphenols in beer include phenolic acids, flavonoids, tannins, proanthocyanidins, and amino phenolic compounds (Zhao et al., 2010). Unmalted adjuncts are often employed in the brewing industry as an alternative cost-efficient source of extract, as well as for the individual functionality they bring to the brewing process and finished beers (Yorke et al., 2021). Buckwheat (Fagopyrum spp.) as a pseudo-cereal has the potential to be used as a raw material and adjunct in brewery industry for its gluten-free properties as recent studies have shown (De Meo et al., 2011; Deželak et al., 2014; Dabija et al., 2022). It is recognized for its nutritional composition especially in the production of gluten-free beers (Deng et al., 2019). Buckwheat malt is used in brewing to prepare beers suitable for people with celiac disease. As an unmalted adjunct, it is mainly used by craft breweries looking for beers with unique properties. Moreover, buckwheat is interesting raw material due to its high content of flavonoids, especially rutin and quercetin, which have a beneficial effect on the human body. Rutin is a flavonoid that has an antioxidant, anti-inflammatory and anticarcinogenic effects (Zhang et al., 2012). Additionally, buckwheat is good source of proteins with high biological value and balanced amino acid composition, lipid, dietary fibre, and minerals. Combined with other medically beneficial compounds, such as flavonoids, fagopyrin, and buckwheat sterols, it has recently attracted increasing attention as an alternative crop for organic cultivation and as an ingredient for health food products (Zhang et al., 2012; Dabija et al., 2022). Buckwheat grain has a higher antioxidant activity compared to other cereals. Various antioxidant compounds such as vitamins B1, B2 and E and phenolic compounds (including polyphenols such as catechins, rutin, quercetin and proanthocyanidins) have been identified in buckwheat hulls and grits. Different catechins (epicatechin and epicatechin gallate) mainly contribute to the antioxidant activity in buckwheat groats (Suzuki et al., 2020). The use of pseudocereals to produce beer may also contribute for the ingestion of naturally occurring antioxidant compounds, such as polyphenols. Therefore, a possible benefit from beer consumption may derive from its antioxidant properties (Ghiselli et al. 2000; Wei et al. 2001; Girotti et al. 2002). According to Dabija et al. (2022) the use of nonconventional starch adjuncts, such as buckwheat leads to an increase in the polyphenol content of the beer, and thus, its antioxidant capacity. Recent studies has shown that sensory properties of the obtained beer depend on the characteristics of each adjunct, but also on the forms in which the adjunct is added: whole grain, groats, malted grain, extruded grain or syrup (Cadenas et al., 2021). Law of the Ministry of Agriculture and Rural Development of the Slovak Republic no. 30/2014 on requirements for beverages and beer allow that barley malt can be replaced up to 30% by starchy or sugary adjuncts of the dry extract of the wort (Vyhláška MPRV SR, 2014).

Therefore, the aim of this study was to assess the impact of replacing 5%, 10%, 15%, 20%, 30% of barley malt with unmalted buckwheat adjunct on the technological profile of wort and its bioactive compound concentrations.

MATERIALS AND METHODS

Biological material

Pilsner type of malt to produce sweet wort was supplied by Malt house Heineken (Slovakia), common buckwheat was purchased from Marianna Company (Slovakia) and its origin was from the Ukraine. Buckwheat employed was without hulls and it was weighed and grinded to get powdered material as well as barley malt with a disk mill (DLFU, Bühler).

Wort preparation

The production process of wort was conducted in mini brewery of Research Institute AgroBioTech at Slovak Agricultural University in Nitra. Mash Tun (1-CUBE, the Czech Republic) was used for the production of wort. Congress worts were prepared using infusion mashing procedure according to Analytica EBC Method 4.5.1 using pilsner type of malt and buckwheat adjunct. Sweet non hopped wort was lautered using the grain bed as a filtration medium. Six variants of worts with partial replacement of barley malt with unmalted buckwheat were prepared in triplicate. Laboratory worts were prepared with 100% malted barley (reference) and with increasing proportions of unmalted buckwheat (5%, 10%, 15%, 20% and 30%; Table 1). The maximum percentage of barley malt to be replaced was chosen in compliance with the Slovakian legal definition of "beer" (Vyhláška MPRV SR, 2014).

Wort Analyses

All determinations were carried out according to European Brewery Convention recommended methods (EBC, 2010) and the Middle European Brewing Analysis Commission methods (MEBAK, 2011). Technological parameters such as extract content (EBC 4.5.1), wort saccharification rate and filtration time (EBC 4.5.1), wort colour (EBC 4.7.1), haze of wort at 90 ° (EBC 9.29), wort viscosity (EBC 4.8) were also analysed in the samples as well as wort clarity (MEBAK 3.1.4.2.6).

Antioxidant activity DPPH method

Free radical scavenging activity of samples was measured using the 2.2-diphenyl-1-picrylhydrazyl (DPPH) according to the procedures described by Sánchés-Moreno et al. (1998). The sample (0.4 mL) was reacted with 3.6 mL of DPPH solution (0.025 g DPPH in 100 mL methanol). Absorbance of the sample was determined using Jenway spectrophotometer (6405 UV/Vis, UK) at 515 nm. Free radical scavenging activity of the samples was expressed as mg/g DM Trolox equivalents (TE); (R^2 = 0.989). All analyses were performed in triplicate.

Total polyphenol content

Total polyphenol content (TPC) of samples was measured spectrophotometrically, using the Folin-Ciocalteu reagent as described by Singleton and Rossi (1965). 0.1 mL of sample was mixed with 0.1 mL of the Folin-Ciocalteau reagent, 1 mL of 20% sodium carbonate, and 8.8 mL of distilled water. The mixture was allowed to stand at room temperature for 30 min. in the dark. The absorbance was read at 700 nm using spectrophotometer Jenway (6405 UV/Vis, UK). The total phenolic content was expressed as mg/g DM gallic acid equivalent (GAE, R^2 = 0.998). All analyses were performed in triplicate.

Total flavonoid content

Total flavonoid content (TFC) was determined using the modified method by Shafii et al. (2017). 0.5 mL of sample was mixed with 0.1 mL of 10% ethanol solution of aluminum chloride, 0.1 mL of 1 M potassium acetate and 4.3 mL of distilled water. After 30 min in darkness, the absorbance at 415 nm was measured using the Jenway (6405 UV/Vis, UK) spectrophotometer. The total flavonoid content (R^2 = 0.9977) was expressed in mg/g DM quercetin equivalent (QE). All analyses were performed in triplicate.

Total phenolic acids content

For the analysis of phenolic acids (TPAC), (Farmakopea Polska, 1999), 0.5 mL of sample extract was mixed with 0.5 mL of 0.5 M hydrochloric acid, 0.5 mL Arnova reagent (10% $NaNO_2$ + 10% Na_2MoO_4), 0.5 mL of 1 M

Table 1. List of wort samples according to substitution of Pilsner type of malt with buckwheat adjunct

Wort samples	S1	S2	S3	S4	S5	S6
Percentage of buckwheat adjunct	0%	5%	10%	15%	20%	30%

sodium hydroxide and 0.5 mL of distilled water. The absorbance of the reaction mixture was measured spectrophotometrically (Jenway 6405 UV/Vis, UK) at 490 nm. The total phenolic acids content ($R^2 = 0.999$) was expressed in mg/g DM caffeic acid equivalents (CAE). All analyses were performed in triplicate.

Statistical Analyses

The experiment was performed in three replicates. To assess statistically significant differences among samples, the LSD multiple comparison test at $P < 0.05$ was used, using statistical software Statsoft Statistica 12.5 (Statsoft Inc., Tulsa, USA)

RESULTS AND DISCUSSION

Saccharification rate indicates the activity of amylolytic enzymes. When producing wort with the addition of unmalted adjuncts, it is necessary to take into account their reduced amylolytic activity (Deželak et al., 2014). Unmalted buckwheat contains a much smaller amount of amylolytic, proteolytic and cytolytic enzymes, because these complexes are synthesized during malting. The lack of these saccharification enzymes in the mash could cause a prolonged period of saccharification of the wort (Cadenas et al., 2021). From the point of view of saccharification rate (tested by the iodine solution), saccharification was done within 10 minutes only in the control sample. As the percentage of adjunct increased, the period of saccharification was prolonged (15-35 min.; Table 2). Samples with a high percentage of buckwheat adjuncts (20%, 30%) had an unsatisfactory length of saccharification rate (25-35 min.; Table 2) that could be attributed to lack of amylolytic enzymes in this unmalted adjunct. According to Kumar et al. (2013) good malt saccharifies in less than 10 min (when enzymes initiate hydrolysis of starch) a longer duration is caused by a bad disintegration of the starch.

Filtration time is affected by the composition of the wort, and the addition of unmalted adjuncts can extend the wort filtration time. The optimal length of wort filtration is less than hour according to Basařová et al. (2015). From the point of view of filtration time all samples were filtrated within 60 minutes (Table 2). In the variant with 30% buckwheat adjunct, the filtration time was the longest (45 min.; Table 2). The length of the filtration time is also affected by the viscosity of the wort. The density of the wort can be negatively affected by compounds such as arabinoxylans and β-glucans (Kunze, 2019). Much attention, according to Basařová et al. (2015) is also addressed to wort clarity (i.e. wort turbidity). Furthermore, all samples provided clear wort in all cases.

The viscosity of the congress wort is an excellent indicator of the activity of cytolytic and amylolytic enzymes in the unhopped wort (Kunze, 2019). It points to the degree of malt modification, the rate of degradation of β-glucans and also to the length of filtration. The higher the viscosity is the longer the filtration time will be. The viscosity of the wort is a parameter characterizing the potential filtration rate of the mash and the clarity of the wort (Kunze, 2019). Basařová et al. (2015) state that the optimal viscosity value of congress wort should range between 1.5-1.6 mPa/s. This study showed that with the increasing percentage of buckwheat adjunct, the viscosity values of the wort increased from 1.51 mPa/s (10% buckwheat adjunct) up to 1.63 mPa/s (30% buckwheat adjunct, Table 2) that is not believed to cause brewing problems when using the buckwheat as an adjunct. The viscosity value of the control sample was 1.48 mPa/s that according to Basařová et al. (2015) points to highly modified malt. Similar conclusions were reached by Podeszwa et al. (2016) who observed changes in the quality parameters of congress wort after the addition of buckwheat malt. Authors found that the addition of buckwheat malt increased the viscosity of the wort (2.07 mPa/s). Worts produced with amaranth, buckwheat or maize were reported with high viscosities of 2.0-13.3 mPa/s (Zarnkow et al., 2005). Other authors assumed that the viscosity of wort within 1-2 mPa/s for mashed raw material other than barley malt does not cause any problems during mash filtration (Zarnkow et al., 2005; Klose et al., 2011).

Table 2. Technological parameters of analysed wort samples based on multiple comparisons from the LSD test

Sample	S1	S2	S3	S4	S5	S6
Viscosity (mPa/s)	1.48^{ab}	1.47^{a}	1.51^{bc}	1.53^{cd}	1.55^{d}	1.63^{e}
Colour (EBC units)	6.80^{a}	6.90^{b}	7.21^{c}	7.49^{d}	7.56^{e}	7.66^{f}
Haze at 90° (EBC units)	4.48^{e}	3.82^{d}	3.66^{c}	3.50^{b}	3.51^{b}	2.96^{a}
Extract content in malt (%)	84.29^{e}	81.17^{d}	81.54^{d}	79.95^{c}	78.65^{b}	77.44^{a}
Saccharification rate (min.)	10	15	20	20	25	35
Filtration time (min.)	30	35	35	35	35	45
Wort clarity	clear	clear	clear	clear	clear	clear

Legend: S1-S6 wort samples, different letters at mean represent statistically significant differences among varieties ($P < 0.05$)

Extract is one of the most important indicators of malt quality because it affects fermentation process. Moreover, it also affects the chemical composition of beer and its organoleptic properties (Ogbonna, 2013). Its value indicates the amount of extract that can be obtained during mashing, which translates into the volume of beer that can be produced with a given amount of malt (Kunze, 2019).

Basařová et al. (2015) reported an ideal range of extract values in dry matter for light pilsner-type malts of 79-83%. In this study the extract yield has decreased with the increase of buckwheat contribution. Statistically significant ($P < 0.05$) the highest extract content was confirmed in control sample (84.29%; Table 2). The lowest yield of extract was confirmed in sample with 30% buckwheat adjunct (77.44%; Table 2). A decrease in extract yield was observed in samples with malt substitution by buckwheat. Samples with a high percentage of buckwheat adjuncts 20% and 30% reached low extract values, below 80%. These results were predictable due to the lower enzyme potential in these samples. Similar results have been described in work by Cela et al. (2022).

Authors state that unmalted adjuncts, due to the missed malting process, give the wort a low sugars and soluble nitrogen compounds content leading to low extract efficiency. Podeszwa et al. (2016) in their study monitored the effect of replacing barley malt with buckwheat malt. The authors found that the addition of buckwheat malt reduced the resulting wort extract. The extract of the control wort of the study was 80.5%, and after the addition of 20% buckwheat malt, the extract value dropped to 69.8%. Similarly, Deželak et al. (2014) compared the quality parameters of wort prepared from 100% barley malt and 100% buckwheat malt. The study showed that the extract value of wort made with buckwheat malt was 62.8%.

The assessment of congress wort colour reflects the type of malt used for manufacturing. It is one of the basic sensory attributes of beer. Colour substances in unhoped and hopped wort are formed by thermal action, oxidation of polyphenols, Maillard reaction and caramelization (Mikyška and Psota, 2019). For pale malts wort colour should not go over 4 EBC units, and for medium-coloured malts should range from 5 to 8 EBC units (Basařová et al., 2015; Kunze, 2019). The congress wort colour was in the range from 6.8 to 7.6 EBC units (Table 2). By adding the buckwheat adjunct, the colour values of the wort gradually slightly increased. Kunze (2019) state that use of adjuncts can also increase the colour during the caramelization process when mashing the wort.

The haze of the wort is closely related to the quality of the final product. It is influenced by the content of turbid substances, such as β-glucans, originating from the input raw materials, as well as by the composition of the water and the method of mashing (Kunze, 2019). Basařová et al. (2015) characterize an acceptable unhoped wort haze value in the range of 3.0-4.2 EBC units. In this experiment the wort haze values ranged from 2.96 to 4.48 EBC units. Statistically significant (P < 0.05) the highest wort haze was confirmed in control sample (4.48 EBC; Table 2). From Table 2 it is clear that with the increasing addition of buckwheat adjunct the value of wort haze decreased. Similar conclusions were reached by Cela et al. (2022). Authors state that when unmalted grains are used in brewing as a partial replacement of barley malt, there is a reduction in protein content and low-molecular nitrogen compounds, thereby preventing haze formation. Psota et al. (2018) also examined congress wort, where authors determined the wort haze in the range of 0.72 to 1.35 EBC units.

The scavenging effect of samples on DPPH radical expressed as mg TEAC/g is presented in Table 3. Polyphenols, melanoidins and vitamins are considered to be the main antioxidants of wort. During kilning and mashing, melanoidins, ketones and other substances with antioxidant properties are formed by the Maillard reaction (Yang and Gao, 2021). Buckwheat is an excellent source of antioxidants, especially due to its high rutin content. Vollmannová et al. (2013) compared the antioxidant activity in five different varieties of buckwheat. In their study, the authors found that the antioxidant activity of buckwheat, depending on the variety, ranged from 2.32 to 4.64 µmol TE/g and similarly authors Unal et al. (2017) found 1.4 to 2.86 µmol TE/g. From obtained results the highest antioxidant activity was measured in sample with 30% buckwheat substitute (72.46 mg TEAC/g); (Table 3). Control sample reached 67.55 mg TEAC/g (Table 3). Similar results regarding control sample were presented by Mikyška et al. (2010) who found the average antioxidant activity of sweet wort prepared from barley malt 52.6 mg TEAC/g.

In Table 3, TPC, TFC and TPAC of all the evaluated samples are shown. Malt is the main source of polyphenolic substances in wort. Malt polyphenols help to delay the aging of beer and prevent the formation of non-biological turbidity mainly thanks to their antioxidant activity. They also have the ability to bind with polypeptides and thus help to eliminate turbid substances during cooling of the wort (Basařová et al., 2015). Significantly the highest content of TPC was obtained in sample with 30% buckwheat adjunct (197.10 mg GAE/g; Table 3) and the lowest TPC values were found in control sample (172.34 mg GAE/g). Selecký and Šmogrovičová (2006) state, that the average content of total polyphenols in the malt wort represents 153.1 mg GAE/g. According to Mikyška and Psota (2019) polyphenols in malt are bound in cell structures along with polysaccharides and proteins and thus their concentration in the wort depends on the intensity of mashing and the sparging of spent grains. Authors measured TPC of 188 mg GAE/g in sweet malt

Table 3. Antioxidant activity, total phenolic, flavonoid and phenolic acid contents in the samples based on multiple comparisons from the LSD test

Sample	TAC (mg/g)	TPC (mg/g)	TFC (mg/g)	TPAC (mg/g)
S1	67.55^{a}	172.34^{a}	22.18^{a}	35.82^{a}
S2	69.68^{b}	173.78^{b}	27.95^{b}	38.38^{b}
S3	70.20^{b}	178.66^{d}	30.52^{c}	38.86^{c}
S4	71.71^{c}	177.35^{c}	40.10^{d}	43.04^{e}
S5	72.43^{cd}	183.71^{e}	58.01^{e}	41.23^{d}
S6	72.46^{d}	197.10^{f}	71.96^{f}	46.97^{f}

Legend: S1-S6 wort samples, different letters at mean represent statistically significant differences among varieties (P < 0.05), TAC – total antioxidant capacity, TPC – total phenolic content expressed as gallic acid equivalent, TFC – total flavonoid content expressed as quercetin equivalent, TPAC – total phenolic acids content expressed as caffeic acid equivalent.

wort. On the other hand, Cela et al. (2022) found TPC of 599 mg GAE/g and Unal et al. (2017) 207.12 mg GAE/100 g in pure unmalted buckwheat.

Flavonoids are the most studied phytochemicals due to their beneficial effects on the human body. The bioactive properties of flavonoids, which include their antioxidant activity, depend not only on their natural structure but also on their metabolites (Deng et al., 2019). Total content of flavonoids (TFC) in wort samples varied significantly from 22.18 mg QE/g (control sample) to 71.96 mg QE/g (sample with 30% buckwheat; Table 3). With an increasing substitution of buckwheat adjunct, the flavonoid content of wort increased. The concentration of flavonoids increased between control wort and wort with buckwheat adjunct by about 31%. Deng et al. (2019) compared the total content of flavonoids and the content of rutin in wort prepared from barley malt with buckwheat malt substitution. The study indicates the value of the total content of flavonoids in 100% barley wort at the level of 294.75 mg QE/g. By adding 20% of buckwheat malt, the content of total flavonoids increased to the value of 516.75 mg QE/g, and with 40% replacement with buckwheat malt up to the level of 876.75 mg QE/g. Buckwheat contains a high amount of flavonoids, mainly rutin and quercetin. Based on the determined content of flavonoids, the following order of individual variants was determined: variant standard > variant 5% > variant 10% > variant 15% > variant 20% > variant 30%.

The most frequently found phenolic acids in malt are p-coumaric acid, caffeic acid and ferulic acid (Vollmannová et al., 2018). The results showed that significantly ($P < 0.05$) the highest content of total phenolic acids (TPAC) 46.97 mg CAE/g was measured in sample with 30% buckwheat (Table 3). With the addition of the buckwheat adjunct, the content of total phenolic acids in the wort samples increased from 35.82 mg CAE/g (control sample) to 46.97 mg CAE/g (sample with 30% buckwheat; Table 3).

CONCLUSION

To conclude, the obtained results clearly indicate that the addition of buckwheat increased the values of nutritionally beneficial substances such as total polyphenols, flavonoids and phenolic acids in the wort samples and thus gives it a unique nutritional composition. Buckwheat as a pseudocereal is excellent source of highly appreciated bioactive compounds with benefits for the human health. Partial replacement of barley malt with unmalted buckwheat grains in higher amount of 20% and 30% resulted in a significant reduction in extract content and had a negative effect on the saccharification and filtration time. On the other hand, application of the unmalted buckwheat as starchy adjunct up to 15% did not negatively affected wort technological profile and can be recommended to use in the production of the worth without adjustment of mashing process. Findings from this study suggested that brewing with partial replacement of barley malt with unmalted buckwheat can be attractive for small craft breweries that are looking for new interesting raw materials to create unique beers that will attract new consumers. However, some technological issues must be taken into account when mashing with using buckwheat. The wort produced with the buckwheat substituted (more than 20%) had a reduced amylolytic activity and gave a lower extract. Therefore, it is recommended to use additional enzymes during mashing. In fact, brewing with unmalted grains allows increasing the sustainability of the brewing process by reducing costs for malting process.

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