Research paper

Flavonoid concentration in milling fractions of Tartary and common buckwheat

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ABSTRACT

Common buckwheat (*Fagopyrum esculentum* Moench) and Tartary buckwheat (*F. tataricum* Gaertn.) samples were used in milling, sieving and analysing experiments. Flavonoids were analysed in buckwheat samples, in milling and sieving fractions and after the contact of flour particles with water, to simulate conditions in dough.

In Tartary buckwheat, there was even more than 100-times higher content of flavonoids flour in comparison to respective fractions of common buckwheat flour. The highest concentration of flavonoids in milling fractions of Tartary buckwheat flour (granulation over 100 μm up to including 1000 μm) was established as 3.5–4.5% flavonoids/DM.

Immediately after the direct contact of flour particles of common and Tartary buckwheat with water the apparent concentration of flavonoids rose (even for 100% or more) in the first 5–30 minutes of contact. After one hour, due to the degradation of flavonoids, their concentration decreased. Concentration of flavonoids are after 24 hours of contact of flavonoids with water in all milling fractions lower in comparison to the value after first 5 minutes of contact with water.

INTRODUCTION

Buckwheat is used as a food ingredient after husking, milled, prepared at different temperatures and in a diversity of media, predominantly in water. Research imitating real technological process producing foods and dishes from buckwheat are important for evaluating nutritional value of foods based on buckwheat. In buckwheat grain there are important polyphenolic substances, including flavonoids. Among consumers of buckwheat foods and dishes there is growing interest for the composition and nutritional value of products.

Among buckwheat species and cultivars there are differences in content of flavonoids, including rutin. The concentration of flavonoids may depend on genotype, development phases, weather, altitude, year of growing and harvest, storage and other factors. Different plant parts may contain different content of flavonoids. Several authors report higher content of rutin in Tartary buckwheat in comparison to common buckwheat (Suzuki et al., 2002; Fabjan et al., 2003; Lin, 2004; Asami et al., 2007; Fabjan, 2007). There could be as well differences among samples of Tartary buckwheat. Several authors (Fabjan et al., 2003; Briggs et al., 2004; Chai et al., 2004; Park B.J. et al., 2004; Suzuki et al., 2005; Jiang et al., 2007; Ghimeray et al., 2009, Kreft, 2013; Kreft et al., 2013; Kreft et al., 2016ab; Kreft 2016; Germ et al., 2019) report about diverse results on samples of buckwheat. According to Liu in Zhu (2007) the main flavonoid in Tartary buckwheat is rutin, along with quercetin and quercitrin (Fabjan, 2007; Morishita et al., 2007). In the grain of common buckwheat there are flavonoids rutin, epicatechin and epicatechingalat. Dietrych-Szostak and Oleszek (1999) isolated from common buckwheat 6 flavonoids, namely rutin, quercetin, orientin, vitexin, isovitexin and isoorientin. Rutin and isovitexin in dehusked buckwheat grain and all 6 of them in husk. Some literature data are presented in Table 1.

Crushing, milling and sieving are the main procedures to obtain buckwheat milling fractions. The gain of flour is in buckwheat normally about 40–50% of the total mass of grain. The rest are husks and peripheral parts of grain (testa, cotyledons). Peripheral parts of grain are crushed differently in comparison to endosperm, and they do not pass the fine sieves. Cotyledons are richer in rutin in comparison to endosperm, so flour may contain less rutin in comparison to the whole grain (Kreft, 1995). The methods of treatment of the grain, like husking, crushing, milling and sieving have an impact on the concentration of flavonoids and other polyphenolic substances. As well as the presence of husk and bran particles in darker flour milling fractions may also have impact on the flavonoids and other polyphenolic substances. Allocation of flavonoids in different parts of buckwheat grain have impact on the utilization value of milling fractions. Know-

Table 1: *Flavonoid content in buckwheat grain, husks and milling fractions*

Buckwheat species	Sample	Flavonoid concentration	Reference
Common buckwheat	Grain	24.4 µg/mg	Ghimeray et al. (2009)
Common buckwheat	Grain	0.04%	Jiang et al. (2007)
Common buckwheat	Grain	18.8 mg/100 g DM	Dietrych-Szostak in Oleszek (1999)
Tartary buckwheat	Grain	142.2 µg/mg	Ghimeray et al. (2009
Tartary buckwheat	Grain	2.04 %	Jiang et al. (2007)
Common buckwheat	16 milling fractions	2.35–135.4 mg/100 g	Hung in Morita (2008)
Common buckwheat	Flour from shop (Slovenia)	0.016	Avguštin (2009)
Common buckwheat	Flour	0.0098 %/DM	Quettier-Deleu et al. (2000)
Common buckwheat	Husk	0.0456 %/DM	Quettier-Deleu et al. (2000)
Common buckwheat (diverse cultivars)	Husk	102.1–151.5 mg/100 g	Dietrych-Szostak (2004)
Common buckwheat	Husk	74 mg/100 g DM	Dietrych-Szostak in Oleszek (1999)
Common buckwheat	Bread (mixed: wheat, buckwheat)	7.76–26.9 mg/kg	Bojňanská et al. (2009)
Tartary buckwheat (Korea)	Sprouts powder	24 g/kg	Gadžo et al. (2009)

ledge about the distribution of flavonoids in milling fractions, in the relation to the size of particles (granulation) is of importance for the simple, swift, and efficient way of obtaining flavonoid-rich milling fractions, especially in Tartary buckwheat.

MATERIAL IN METHODS

Material

Common buckwheat (*Fagopyrum esculentum* Moench) and Tartary buckwheat (*F. tataricum* Gaertn.) samples were used in milling, sieving and analysing experiments. Two samples (T1 and T2) of Tartary buckwheat were included, obtained from Luxemburg and a sample of common buckwheat (variety Darja, sample D), obtained from Biotechnical faculty, Ljubljana, Slovenia. By milling and sieving of Tartary buckwheat sample T1 and common buckwheat four fractions were obtained with different granulations. Each of them was mixed with water. Sample T2 was obtained as flour, which was sieved into two fractions with different granulation.

Methods

Samples T1 and D were milled by cereal mill Quadromat Junior Model No. 08 801 01 (Brabender Duisburg, Germany), to obtain two fractions by planary sieves (Table 2).

To the flour fractions, water was added and the dough was made. Amount of added water and contact time flour/water prior to freezing is reported in Table 3. Fractions over 1000 µm (T1 F_{22} and D F_{22}) contained mainly husk and some bran, so they were just rinsed in water (Table 3). After 30 days of storage below, the samples were freeze-dried. By spectrophotometric analyses (spectrophotometer TECAN Genios), using 5% $AlCl_3$ (reaction between flavonoids and $AlCl_3$), which results in yellow colour with maximum at 420 nm (Dutra, 2008; Zhang et al., 2005; Bohm, 1997), concentration of flavonoids was determined. Statistical analyses were performed using Microsoft Excel 2003 and program STAT G (Statgraphics 5.0, Statistical Graphics Corporation, ZDA), and by ANOVA, significance was accepted at $p<0.05$ (Ferligoj, 1997; Ferligoj and Lozar Manfreda, 2009). All measurements and analyses were performed in three independent samples.

RESULTS

Milling fractions of studied common and Tartary buckwheat samples contained very different amount of flavonoids (Table 4). Concentration of flavonoids was (sample D) much lower in common buckwheat in comparison to Tartary buckwheat (Table 4, Fig. 1.) Comparison of respective fractions of Tartary buckwheat T1 and common buckwheat D (Table 4, Fig. 1.) showed much higher (50 do 100-times higher) concentration of flavonoids in

Table 2: *Milling and sieving of common buckwheat (sample D) and Tartary buckwheat (samples T1 and T2) with characterization of fractions*

Sample	Process	Fractions	Further process	Subfractions	Granulations
Tartary buckwheat, grain (T1)	Milling	T1 F1	Sieving	T1 F11	≤ 100 µm
				T1 F12	100 µm < x ≤ 236 µm
		T1 F2	Sieving	T1 F21	236 µm < x ≤ 1000 µm
				T1 F22	> 1000 µm and bran, husk
Common buckwheat Darja, grain (D)	Milling	D F1	Sieving	D F11	≤ 100 µm
				D F12	100 µm < x ≤ 236 µm
		D F2	Sieving	D F21	236 µm < x ≤ 1000 µm
				D F22	> 1000 µm and bran, husk
Tartary buckwheat – flour (T2)	/	/	Sieving	T2 F11	≤ 100 µm
				T2 F12	>100 µm

T1 - *Tartary buckwheat, flour from entire grain*
D - *Common buckwheat Darja, flour from entire grain*
T2 - *Tartary buckwheat, obtained as flour*

Table 3: *Dough samples being prepared for freezing*

Sample No.	Sample	Mass (g)	Water addition (mL)	Contact times (flour and water) prior to freezing	Freezing and storage
	Tartary buckwheat (T1) – milling fractions			*0.08 h, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h*	*0.5 h: –35 °C to –40 °C; 1 month: –15 °C to –20 °C*
1	T1 F11	250	200	SAME	SAME
2	T1 F12	125	100	SAME	SAME
3	T1 F21	100	130	SAME	SAME
4	T1 F22	125	200	SAME	SAME
	Common buckwheat (D) – milling fractions			*0.08 h, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h*	*0.5 h: –35 °C to –40 °C; 1 month: –15 °C do –20 °C*
5	D F11	250	200	SAME	SAME
6	D F12	250	200	SAME	SAME
7	D F21	250	235	SAME	SAME
8	D F22	250	400	SAME	SAME
	Tartary buckwheat flour (T2)			*0.08 h, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h*	*0.5 h: –35 °C to –40 °C; 1 month: –15 °C to –20 °C*
9	T2 F1	250	200	SAME	SAME
10	T2 F11	200	160	SAME	SAME
11	T2 F12	200	160	SAME	SAME

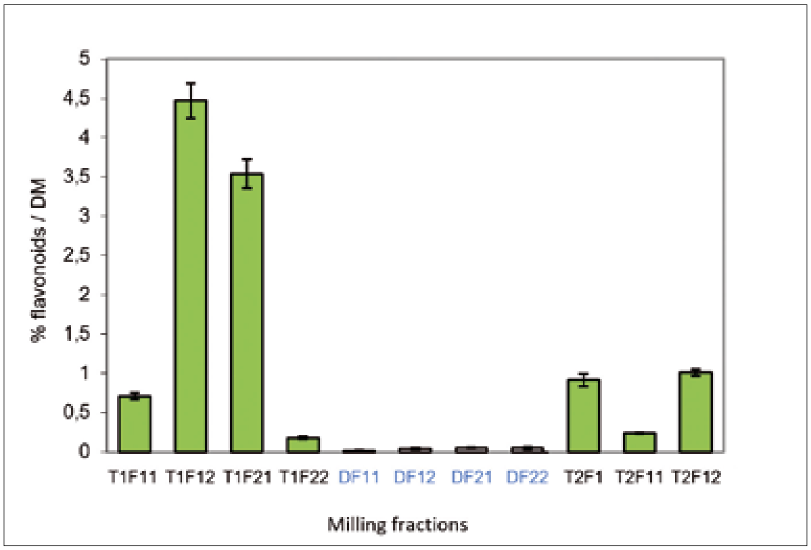
T1 - *Tartary buckwheat, whole grain flour*
D - *Common buckwheat, whole grain flour*
T2 F_1 - *Tartary buckwheat flour*

Table 4: *Comparison of flavonoid content in milling fractions of Tartary and common buckwheat (samples T1,T2, D) and in milling fractions with added water after 5 minutes and after 24 hours of flour-water contact*

Sample	Subfraction	Flavonoids		
		Milled sample	Dough (flour and water) 0.08 h (5 min)	Dough (flour and water) 24 h
		%/DM	*%/DM*	*%/DM*
Tartary buckwheat (T1)	T1 F_{11}	0.709	1.444	1.112
Tartary buckwheat (T1)	T1 F_{12}	4.470	4.766	4.311
Tartary buckwheat (T1)	T1 F_{21}	3.542	4.262	3.551
Tartary buckwheat (T1)	T1 F_{22}	0.178	0.178	0.062
Common buckwheat Darja (D)	D F_{11}	0.015	0,017	0.006
Common buckwheat Darja (D)	D F_{12}	0.043	0.085	0.042
Common buckwheat Darja (D)	D F_{21}	0.051	0,088	0.069
Common buckwheat Darja (D)	D F_{22}	0.055	0.071	0.055
Tartary buckwheat (T2)	T2	0.916	1.226	0.955
Tartary buckwheat (T2)	T2 F_{11}	0.243	0.363	0.199
Tartary buckwheat (T2)	T2 F_{12}	1.011	2.639	2.063

T1 - *Tartary buckwheat (from grain)*
D - *Common buckwheat Darja (from grain)*
T2 - *Tartary buckwheat (from flour)*
DM - *dry matter*

Figure 1: *Comparison of flavonoid content in milling fractions of common and Tartary buckwheat*



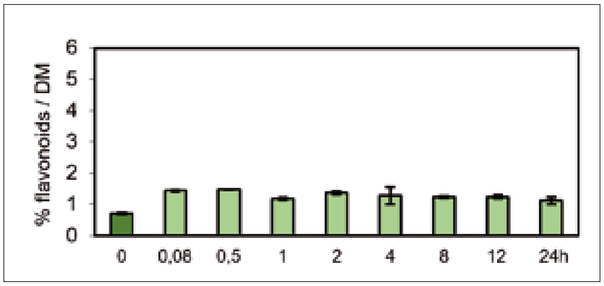
T1 F_{11} - *Tartary buckwheat flour, granulation ≤ 100 μm*
T1 F_{12} - *Tartary buckwheat flour, granulation 100 μm < x ≤ 236 μm*
T1 F_{21} - *Tartary buckwheat flour, granulation 236 μm < x ≤ 1000 μm*
T1 F_{22} - *Tartary buckwheat flour, granulation > 1000 μm, including bran and husk*
D F_{11} - *Common buckwheat flour, granulation ≤ 100 μm*
D F_{12} - *Common buckwheat flour, granulation 100 μm < x ≤ 236 μm*
D F_{22} - *Common buckwheat flour, granulation > 236 μm < x ≤ 1000 μm*
D F_{22} - *Common buckwheat flour, granulation >1000 μm, including bran and husk*
T2 F_{1} - *Tartary buckwheat flour, additional sample*
T2 F_{11} - *Tartary buckwheat flour, additional sample, granulation ≤ 100 μm*
T2 F_{12} - *Tartary buckwheat flour, additional sample Granulation >100 μm*

Tartary buckwheat milling fractions in comparison to respective common buckwheat milling fractions. However, among fractions, containing mainly husk and bran, in Tartary buckwheat it was only about 3 times more flavonoids at Tartary buckwheat in comparison to common buckwheat. In the investigated samples the highest concentration of flavonoids was in the range 3.5–4.5% in dry matter in milling fractions of Tartary buckwheat T1 (with granulation over 100 μm, including up to 1000 μm). These are milling fractions of dark coarse flours. Fraction of Tartary buckwheat husk had low content of flavonoids. Interestingly, husk fraction of common buckwheat had a high content of flavonoids, in comparison to other milling fractions of common buckwheat.

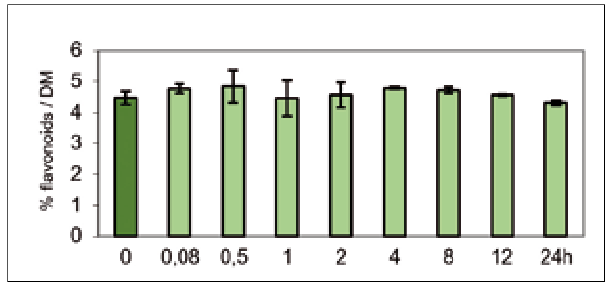
Concentration of flavonoids was different between two samples of Tartary buckwheat (Table 4, Fig. 1). In comparison of two fine milled light Tartary buckwheat flours (T1 in T2) with the same granulation (up to including 100 μm) we established different content of flavonoids (Table 4, Fig. 1), in both cases the concentration of flavonoids was very low. Comparison of Tartary buckwheat sample T1 and common buckwheat D showed different allocation of flavonoids among milling fractions (Fig. 1). In the Table 4 it was reported that in common buckwheat milling fractions with the granulation up to 100 μm it was much less flavonoids in comparison to fractions over 100 μm. Highest concentration was in the fraction F_{22} (husk and bran), and lowest in the fraction of light flour F_{11}.

It was studied the content of flavonoids in the dough, made from different milling fractions of Tartary and common buckwheat (samples T1, T2, D) after first 5 minutes, and up to 24 hours of contact of flour particles with added water (Table 5; Fig. 1).

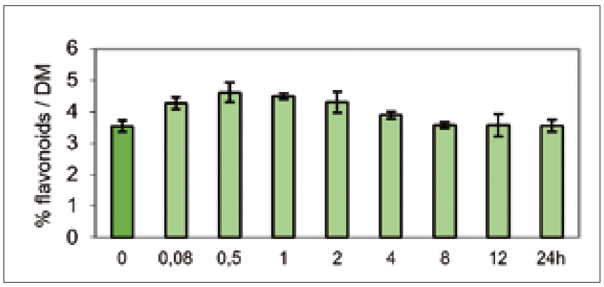
Figure 2: *Flavonoid concentrations in dough from different milling fractions of Tartary buckwheat (T1) over a 24-hour time period*



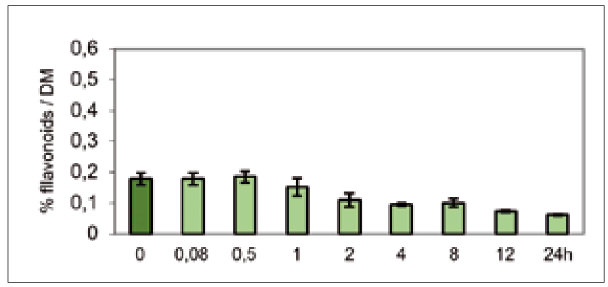
a Tartary buckwheat T1 F_{11}



b Tartary buckwheat T1 F_{12}



c Tartary buckwheat T1 F_{21}



d Tartary buckwheat T1 F_{22}

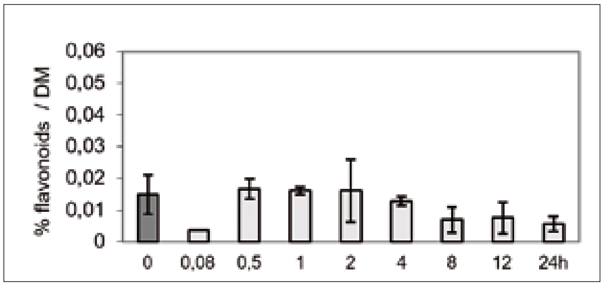
T1 F_{11} - *Tartary buckwheat, granulation ≤ 100 µm*
T1 F_{12} - *Tartary buckwheat, granulation 100 µm < x ≤ 236 µm*
T1 F_{21} - *Tartary buckwheat, granulation 236 µm < x ≤ 1000 µm*
T1 F_{22} - *Tartary buckwheat, granulation > 1000 µm including bran and husk*
0.08 - *5 minutes; 0.5 - 30 minutes, 1 – one hour; 2,4,8,12,24 – hours of contact with water*

Impact of water on the flavonoids concentration was similar for common and Tartary buckwheat (Figs. 2 and 3, Table 4). Apparent flavonoid concentration in most of milling fractions rose for about 2 times in the first five minutes after the addition of water, in comparison to untreated, dry samples. The highest elevation was in flavonoids concentration in coarse and fine flours (coarse and fine), and somewhat less in fractions with bran and husk. With few exceptions, it was gradually decreased during 24 hours of contact of flour particles with water. Gradual lowering of flavonoid concentration in the time 0.08 to 24 hours was different among samples and fractions, but lowering from apparent flavonoid concentration in the time 0.08 to 24 hours was a general appearance, it was a linear correlation among time and flavonoid concentration (r^2 = 0,9953; p<0,05; y = – 0,0733 + 0,8739x). Only in the fraction of bran and husk (F_{22}) flavonoids concentration was after 24-hours of contact of particles with water as low as 60 %, in comparison to starting concentration before the addition of water.

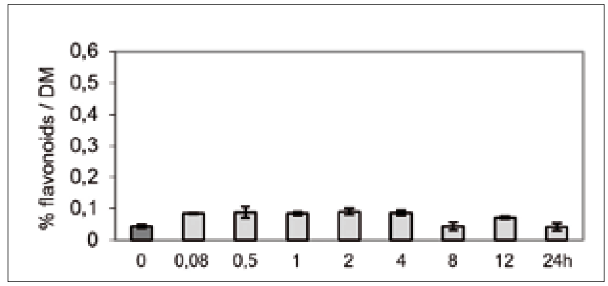
DISCUSSION

From the point of view of functionality most interesting are milling fractions with the granulation over 100 µm up to including 1000 µm (the milling gain of these fractions is about 30%); from point of view of nutritional functionality less interesting fractions are fine light flours with the granulation below 100 µm (in milling the gain of light flours is nearly about 50%), as they are poor in flavonoids, and also contain low concentration of proteins and minerals (Vombergar, 2010). Collection and mixing of fractions (with the granulation over 100 µm up to including 1000 µm), especially in Tartary buckwheat is the best possibility to obtain flour material rich in flavonoids, proteins and minerals.

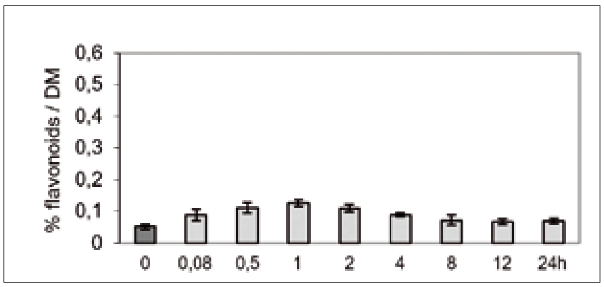
Figure 3: *Flavonoid concentrations in dough from different milling fractions of common buckwheat (D) over a 24-hour time period*



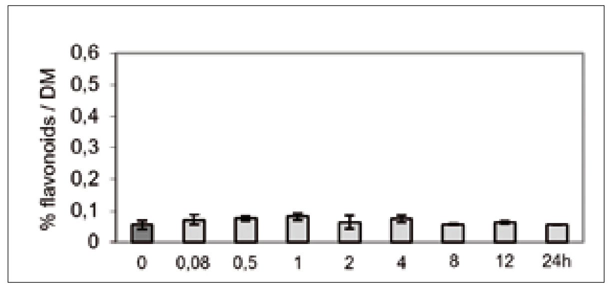
a Common buckwheat D F_{11}



b Common buckwheat D F_{12}



c Common buckwheat D F_{21}



d Common buckwheat D F_{22}

D F_{11} - *Common buckwheat, subfraction with granulation ≤ 100 µm*
D F_{12} - *Common buckwheat, subfraction with granulation 100 µm < x ≤ 236 µm*
D F_{22} - *Common buckwheat, subfraction with granulation > 236 µm < x ≤ 1000 µm*
D F_{22} - *Common buckwheat, subfraction with granulation >1000 µm and bran, husk*
0.08 - *5 minutes; 0.5 - 30 minutes, 1 – one hour; 2,4,8,12,24 – hours of contact with water*

Highest concentration of flavonoids was established in Tartary buckwheat T1 in milling fractions with the granulation over 100 up to 1000 µm (fractions F_{12} and F_{21}), namely 3.54–4.47% (Table 4). This is about 100-times more in comparison to the concentration of flavonoids in common buckwheat Darja with the same granulation groups (0.043–0.051%) (Table 4, Fig. 1). The results are in line with previous results about the difference in flavonoid concentration in common and Tartary buckwheat (Piao in Li, 2001; Škrabanja et al., 2004; Hung in Morita, 2008).

It was established that in common buckwheat it is not similar distribution of flavonoids among fractions as in the case of Tartary buckwheat (Table 4, Fig. 1). In common buckwheat it is the richest with flavonoids the fraction of bran and husk F_{22} with granulation over 1000 µm (D F_{22} 0.055 % flavonoids), what was not the case in Tartary buckwheat. This is the reason for the intensive research of the concentration of flavonoids, especially rutin, in the husk of common buckwheat (Oomah in Mazza, 1996; Watanabe et al., 1997; Dietrych-Szostak and Oleszek, 1999; Kreft et al., 1999; Quettier-Deleu et al., 2000; Steadman et al., 2001b; Dietrych-Szostak, 2004). We detected lower difference in the content of flavonoids between common and Tartary buckwheat in the fraction of husk, than between fractions of flours. So, we suggest the possibility for using of husk of common buckwheat as a source of flavonoids, especially in areas, where Tartary buckwheat is not a traditional crop, as they grow common buckwheat.

Milling affects the release of flavonoids during the extraction of buckwheat polyphenols. Size of particles is an important characteristic of flours. Smaller particles have relatively higher surface area, so the action of enzymes could be different in comparison to crude flour particles. Enzymes in fine milled flours with small particles could be more active. Polyphenols are included in many cell components. So, their extraction to the liquid phase could be different.

Suzuki et al. (2002) and Yasuda (2001, 2007) are reporting about the enzyme flavonol-3-glukosidase, important for the degradation of rutin in buckwheat under certain conditions. This enzyme is located in grain in the testa and cotyledons. Predominant amount of enzyme is in cotyledons, but more active is enzyme stored in testa (Suzuki et al., 2002). Rutin is degraded to quercetin. Suzuki et al. (2004) reported about the correlation of enzyme concentration in buckwheat flour with the concentration of water soluble acids. Mukasa et al. (2009) established that rutin in the husked round formed buckwheat grain is degraded quickly but it is not the case in soaked intact grain. It is supposed that this is due to structural isolation of rutin to the rutin degraded enzymes.

There are different ways of rutin degradation, for example the oxidation of rutin and some other biochemical reactions, transferring rutin to other metabolites. Enzymes, degrading rutin could be blocked in their function. Steaming, cooking and extruding preserve a part of rutin and may prevent the appearance of bitter taste (Paulíčková et al., 2004). Mukasa et al. (2009) confirmed that most of rutin remain in grain after cooking one hour. Thermal treatment may have impact on the degradation of flavonoids according to Dietrych-Szostak in Oleszek (1999). Şensoy et al. (2006) reported that roasting, treatment with dry hot air, has no impact on antioxidative properties of light or dark buckwheat flour.

Simulation of technological process of dough making (contact of flour with water) revealed the biochemical events, with impact to some dough constituents (mainly flavonoids – rutin and quercetin).

CONCLUSION

In regard to functional aspect and nutritional value are most interesting buckwheat milling fractions with granulation over 100 µm up to including 1000 µm (milling gain about 30%); less interesting are fractions of light fine flours with granulation less than 100 µm (milling gain nearly 50%), which does not contain much proteins, minerals and flavonoids. Collecting and mixing of fractions with granulation over 100 µm up to including 1000 µm), especially at Tartary buckwheat is the best possibility to get flour of high nutritional and functional value, because of flavonoids, proteins and minerals.

Tartary buckwheat has a much higher content of flavonoids in comparison to common buckwheat, even more than 100-times more in Tartary buckwheat flour in comparison to common buckwheat flour. The highest concentration of flavonoids in milling fractions of Tartary buckwheat flour T1 (granulation over 100 µm up to including 1000 µm) was established as 3.5–4.5% flavonoids/DM.

Flavonoids in milling fractions with different granulation are differently allocated. Allocation is different in Tartary buckwheat and common buckwheat.

Immediately after the direct contact of flour particles of common and Tartary buckwheat with water the apparent concentration of flavonoids rose (even for 100% or more) in the first 5–30 minutes of contact. After one hour, due to the degradation of flavonoids, their concentration became lower. Concentration of flavonoids are after 24 hours of contact of flavonoids with water in all milling fractions lower in comparison to the value after first 5 minutes of contact with water.

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IZVLEČEK

Z vidika funkcijskega dodatka ter hranilne in prehranske vrednosti so zanimive mlevske frakcije ajde z granulacijo nad 100 μm do vključno 1000 μm (teh je pri mletju okoli 30 %); nezanimive pa so frakcije finih belih mok z granulacijo pod 100 μm (pri mletju nastaja skoraj 50 % belih mok), saj so revne z beljakovinami, minerali in flavonoidi. Zbiranje in mešanje frakcij (z granulacijo nad 100 μm do vključno 1000 μm), predvsem pri tatarski ajdi, pomeni najboljšo izbiro glede vsebnosti beljakovin, mineralov in flavonoidov.

Tatarska ajda ima bistveno višjo vsebnost flavonoidov kot navadna ajda (tudi več kot 100-krat več flavonoidov v moki). Najvišja vsebnost flavonoidov je v mlevskih frakcijah tatarske ajde T1 (z granulacijo nad 100 μm do vključno 1000 μm) in sicer 3,5–4,5 % flavonoidov v sušini.

Flavonoidi, se po mlevskih frakcijah (z različno granulacijo) različno razporejeni. Razporeditev med mlevskimi frakcijami ni enaka pri tatarski in navadni ajdi.

Pri neposrednem stiku mlevskih frakcij tatarske in navadne ajde z vodo vsebnost flavonoidov v vseh mlevskih frakcijah naraste (tudi za 100 % in več) v prvih 5–30-ih minutah delovanja. Po eni uri začne koncentracija flavonoidov padati zaradi razpada flavonoidov, oksidacijsko redukcijskih procesov, encimatskih procesov in drugih biokemijskih reakcij. Koncentracija flavonoidov po 24-ih urah stika moke z vodo je vedno nižja v primerjavi z začetno vrednostjo flavonoidov v testu po 5-tih minutah stika z vodo.