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ASSESSMENT OF A NEW ARTIFICIAL BUCKWHEAT SPECIES *FAGOPYRUM HYBRIDUM* AS A SOURCE OF PLANT RAW MATERIALS COMPARED TO *F. TATARICUM* AND *F. ESCULENTUM*

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ABSTRACT

A promising way to increase the use of buckwheat is the wider introduction of technologies for its processing, including grinding of non-hulled grain. It requires the search for new plant materials with more suitable characteristics. In this work, the possibilities to use the grain of a new artificial buckwheat species *Fagopyrum hybridum* for flour production are studied in comparison with two cultivated species *F. tataricum* and *F. esculentum*. Some chemical characteristics of *F. hybridum* flour were evaluated. According to the size of the kernel fragments in different modes of milling within each species the significant differences were identified within *F. esculentum* and *F. hybridum* (p <0.001 and p <0.05, respectively); there were no significant differences within *F. tataricum* (p >0.1). Fragments of the seed hulls of *F. tataricum* and *F. hybridum* compared to ones of *F. esculentum* were distinguished by the absence of pronounced acute angles. For the cultivated species, amino acid compositions of grain protein of the studied samples manifest no strong deviations from earlier published results. The new species *F. hybridum* has the amino acid composition similar to ones of the both cultivated species with slight superiority in the content of all essential amino acids. So, the content of Cysteine, Tryptophan, Arginine, Lysine, Methionine, Leucine + Isoleucine, Threonine, Histidine and Valine in seeds of *F. hybridum* was 5.2, 15.0, 25.8, 30.2, 31.2, 36.0, 38.4, 41.1 and 46.2% higher compared to *F. tataricum* and 11.1, 43.7, 39.2, 3.7, 31.2, 15.2, 14.8, 20.0, 18.9% higher compared to *F. esculentum*. Using DPPH it was assessed the antioxidant activity (AOA) of whole grain flour of three buckwheat species and decreasing of the AOA during heating up to 100 °C. After water extraction the AOA was maximal for *F. tataricum* flour; *F. hybridum* and *F. esculentum* manifested similar values with the same decline dynamics during heating. After ethanol extraction the flour of *F. hybridum* shown higher AOA compared to both cultivated species before temperature treatment (1.3 times) as well as after heating to 100 °C (1.2 times). The results of the analysis of the fractional composition of flour from the whole grain of the three buckwheats shown the fragments of the seed hulls of *F. tataricum* and *F. hybridum* compared to ones of *F. esculentum* were characterized by the absence of pronounced acute angles. Additional experiments are needed to optimize the technology of whole-grain buckwheat flour. But the grain of *F. tataricum* and *F. hybridum* looks like more suitable for these purposes than the non-hulled grain of *F. esculentum.*

Keywords: buckwheat; grain; flour; food industry

INTRODUCTION

Buckwheat, a pseudo-cereal crop belonging to the family Polygonaceae, genus *Fagopyrum*, is a popular health food in Asian and European countries **(Kreft et al., 2003)**. Together with well-balanced chemical composition **(Bonafaccia, Marocchini and Kreft, 2003; Zhu, 2016)** including the optimal amino acid composition of seed storage protein **(Prakash et al., 1987; Jiang et al., 2007)** buckwheat manifests a high level of antioxidant activity due to the content of flavonoids **(Holasova et al., 2002)**. In buckwheat grain it was identified rutin, quercetin, and flavone C-glycosides **(Zielińska et al., 2012)**. It has several pharmacological functions such as anti-inflammatory, anti-diabetic **(Lee et al., 2016**; **Kamalakkannan and Prince, 2006)**, blood capillary strengthening properties **(Chua, 2013)**, and lipid-lowering activity **(Tomotake et al., 2015)**. Also, rutin has cardioprotective effects **(He et al., 1995**; **Wojcicki et al., 1995**; **Annapurna et al., 2009)**. It allows considering buckwheat as a functional food. Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) contains approximately 100-fold higher amounts of rutin in its seeds compared to common buckwheat **(Fabjan et al., 2003)**. Sometimes buckwheat flour is used for the improvement of wheat-based products. So, wheat bread with buckwheat flour shown the level of antioxidant activity depends on the percentage of buckwheat flour, and the rutin content in such bread ranged from 7.76 to 26.90 mg kg^{-1} **(Lin et al., 2009**; **Bojňanská et al., 2009**; **Brindzová et al., 2009)**. Such products are recommended especially for people who live in conditions of oxidative stress **(Kuznetsova et al., 2018)**.

In Russia, at present, the traditions of buckwheat consumption mainly as a groats crop promote cultivating only common buckwheat which grain is more suitable for groats production. In China and several other Asian countries where buckwheat grain is used mainly for the production of flour the Tartary buckwheat is also cultivated. Grinding of non-hulled grain is one of most perspective approach for use of buckwheat since it is a simple method which allows producing various types of flour for making noodles, pasta, bread, confectionery, etc. **(Steadman et al., 2001)**. Grinding grain with hulls reduces the relative content of nutrients in the flour obtained but increases the content of dietary fiber **(Dziedzic et al., 2012)**. Buckwheat grains can be milled using any equipment designed for grinding cereals, for example, the millstones or roller mill **(Mazza and Oomah, 2005)**. Millstones are more often used to produce whole grain flour for making pancakes at home. White flour can be obtained from such flour by removing the bran by sieving. The fineness of grinding with millstones can be different and is adjusted by changing the gap between them. Grinding with millstones is a one-step process, unlike grinding with a roller mill, where the process can be divided into several stages with the release of several flour fractions **(Ohinata et al., 2001)**.

It seems promising to search, create, and evaluate new buckwheat samples which could be more suitable raw materials for the development of deeper processing products. Using hybridization *F. tataricum* (4x = 32) × *F. giganteum* together with selection in late generations (F_{10} and later) recently was created a new buckwheat species *F. hybridum* **(Fesenko and Fesenko, 2010)**. The artificial species manifests competitive yield ability and may be considered for registration as a cultivar **(Fesenko et al., 2017)**.

An objective of this paper was the evaluation of *F. hybridum* grain properties, including amino acid composition, antioxidant activity, and characteristics of fragments of kernels and hulls after milling by both roller mill and millstones, compared to ones of *F. tataricum* and *F. esculentum*.

Scientific hypothesis

The whole grain of *F. hybridum* (a new species obtained using hybridization of tetraploid *F. tataricum* with artificial amphidiploid *F. giganteum* Krotov) and *F. tataricum* (Tartary buckwheat) is more perspective row material for the production of functional food in terms of deep processing technology compared to a whole grain of *F. esculentum* (common buckwheat) which usually used for groats production.

MATERIAL AND METHODOLOGY

The work involved one sample of each species. *F. tataricum* – an accession k-17 from the Federal Research Center N.I. Vavilov All-Russian Institute of Plant Genetic Resourses (VIR) collection (St.Petersburg); *F. hybridum* is a new species of hybrid origin which has been created at the Federal Research Center of Grain Legumes and Groats Crops, Orel, Russia **(Fesenko and Fesenko, 2010)**; *F. esculentum* was represented by cv. Devyatka (Federal Research Center of grain legumes and groats crops).

The grinding of the grain was carried out both on a millstone with gaps of 0.01 and 0.3 mm, and on roller mill with two frequencies of rotation, $6.5x10^3$ and $10x10^3$ turns per minute. Micrographs of the grains fragments were taken with an AxioCam MRc5 camera (Axio Imager microscope. Al, Carl Zeiss). The measurements were made using the AxioVision program.

Antioxidant activity (AOA) was measured using spectrophotometry in both alcohol and water extracts based on inhibition of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical **(Silva et al., 2005)**. Flavonoids were extracted with both 96% ethanol and water. 1g of milled grain was mixed with 25 mL of ethanol or water. Extraction was carried out for 24 hours with constant stirring. The extracts were filtered. 0.025 DPPH was dissolved in 100 mL of 96% ethanol. 10 mL of the solution was mixed with 90 mL of 96% ethanol. The optical density of the solution was measured after 30 minutes on a spectrophotometer in a cuvette with a thickness of 1 cm, at a wavelength of 515 nm. To determine the antioxidant activity of the studied extract, 0.1 mL of the filtrate was added to 3.9 mL of the solution of DPPH, mixed, and placed in a dark for 5 – 10 minutes. Then the optical density of the solution was measured using a spectrophotometer at a wavelength of 515 nm.

To determine the thermal stability of bioflavonoids, the extracts were gradually heated in a water bath (t = 20 – 100 ºC), and AOA was measured at 20, 40, 60, 80, and 100 ºC. The antioxidant activity (AOA) of the extracts is calculated by the formula $AOA = \frac{A1-A2}{A1} \times 100\%$, where A1 is the optical density of the DPPH solution before adding the investigated extract; A2 – optical density of the DPPH solution after adding the investigated extract.

The amino acid composition was evaluated using amino acid analyzer BIOCHROM (Biochrom Ltd., Great Britain).

Statistical analysis

Standard statistical analysis was conducted using MS Excell in combination with XLSTAT. The significance of differences between compared variants was analyzed using t-statistics (a two-sample *t*-test for independent samples); p-value is indicated where it is necessary.

RESULTS AND DISCUSSION

Results of different milling approaches

Millstones milling

The results of coarse and fine grinding were not entirely unambiguous. By the size of the hulls fragments, the maximum values were significantly higher for coarse grinding, but significant differences between the mean values were only for *F. hybridum* ($t = 3.20$; $p = 0.02$) (Table 1). Also, in the case of *F. tataricum*, the mean value of this trait was higher for fine grinding (albeit the difference was not significant). The maximal fragments of the kernel were larger in all cases on coarse grinding, but the average values were significantly higher only for *F. tataricum* ($t = 6.14$; $p = 0.001$) and *F. hybridum* ($t = 2.04$; $p = 0.05$).

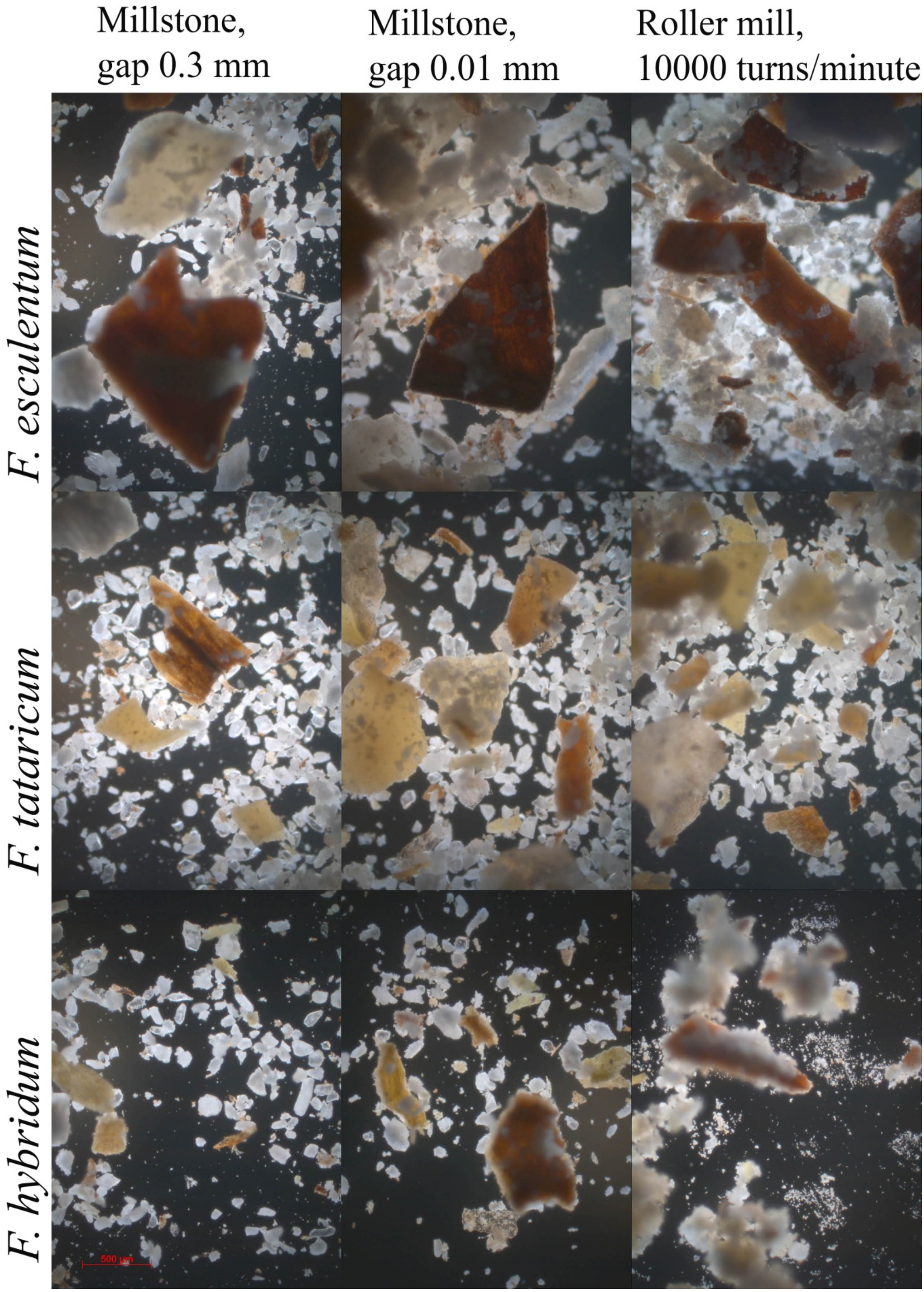

Figure 1 Kernels and hulls fragments of three buckwheat species after millstone (with gaps 0.3 and 0.01 mm) and rolling (10000 turns per minute) milling.

Table 1 Sizes (μm) of the kernels and hulls fragments of three buckwheat species after millstone milling.

Species	Fragments of	Gap = 0.01 mm		Gap = 0.3 mm	
		X ±m	Range	X ±m	Range
F. tataricum	**kernel**	66.1 ±1.7	21.4 – 158.9	90.0 ±3.5	46.7 – 298.8
	hulls	255.2 ±32.2	44.1 – 1429.0	207.7 ±33.1	14.6 – 2104.5
F. hybridum	**kernel**	90.9 ±4.1	27.4 – 641.7	108.5 ±7.6	46.2 – 851.0
	hulls	136.5 ±14.9	17.6 –675.9	207.2 ±16.3	20.7 – 1657.5
F. esculentum	**kernel**	128.6 ±7.6	29.6 – 936.9	96.0 ±7.1	24.3 – 1095.2
	hulls	271.9 ±78.2	22.4 – 1927.0	317.6 ±92.5	66.6 – 2527.0

Table 2 Sizes(μm) of the kernels and hulls fragments of three buckwheat species after milling by roller mill.

Species	Fragments of	Rotation frequency, turns per minute			
		$6.5x10^3$		$10x10^3$	
		X ±m	Range	X ±m	Range
F. tataricum	**kernel**	65.5 ±1.7	25.5 – 117.0	62.1 ±2.2	28.4 – 137.5
	hulls	172.8 ±11.3	41.9 – 625.5	233.9 ±55.3	48.3 – 1755.2
F. hybridum	**kernel**	70.1 ±4.6	32.3 – 154.7	59.0 ±3.1	13.2 – 208.6
	hulls	154.3 ±13.1	17.6 – 1066.8	116.6 ±16,3	10.7 – 557.7
F. esculentum	**kernel**	60.2 ±1.8	25.5 – 119.5	50.0 ±1.7	13.1 – 108.9
	hulls	347.8 ±84.4	43.2 – 1540.9	387.5 ±92.5	71.8 – 1397.1

In the case of *F. esculentum*, the fragments othe kernels were on average significantly larger on fine grinding (t = 3.13; p = 0.01). When comparing species on coarse grinding, in the size of the flour significant differences were found only between *F. tataricum* and *F. hybridum* (t = 2.21; p = 0.05). On fine grinding, no significant differences were found in the size of the hulls fragments between *F. esculentum* and the other two species, on average, although the maximal values were higher for *F. esculentum*. Fragments of the hulls of *F. tataricum* were significantly larger compared to *F. hybridum*. The fragments of the *F. tataricum* kernel were significantly smaller than in the other two species (p = 0.001). Fragments of the kernel of *F. esculentum* were larger than those of *F. hybridum* (t = 4.37; p = 0.001).

Roller milling

Grinding on a roller mill was carried out in two modes (Table 2). According to the size of the kernel fragments in different modes of milling within each species, the significant differences were identified within *F. esculentum* and *F. hybridum* (p <0.001 and p <0.05, respectively); there were no significant differences within *F. tataricum* (p >0.1). There were no significant differences in the size of hulls fragments in any case.

There were significant differences between *F. esculentum* and two other species in the size of the kernel fragments in all cases (the fragments of the *F. esculentum* kernels are smaller). The fragments of the *F. esculentum* hulls were noticeably larger than those of the other two species: only in comparison with *F. tataricum* when grinding at 10,000 turns per minute the differences were not significant. The differences between *F. tataricum* and *F. hybridum* in the size of the fragments of both the kernels and hulls were significant only when grinding at 10,000 turns.

It should be noted that the fragments of the seed hulls of *F. tataricum* and *F. hybridum* compared to ones of *F. esculentum* were distinguished by the absence of pronounced acute angles (Figure 1). Additional experiments are needed to optimize the technology of whole-grain buckwheat flour. But the grain of *F. tataricum* and *F. hybridum* looks like more suitable for these purposes than the non-hulled grain of *F. esculentum*.

Antioxidant activity (AOA) of flour and decline dynamics of the AOA during heating

Although some antioxidant activity (AOA) is characteristic of many plants **(Bandyukova and Sergeeva, 1974**; **Chua, 2013)**, including some wheat species **(Kuznetsova et al., 2018; Kuznetsova et al., 2019)**, buckwheat, especially Tartary buckwheat grain contains an outstanding amount of antioxidants, and it is one of the main advantages of the crop **(Kitabayashi et al., 1995a; Kitabayashi et al., 1995b**; **Ohsawa and Tsutsumi, 1995; Kreft et al., 1999**; **Holasova et al., 2002; Fabjan et al., 2003; Jiang et al., 2007; Zielińska et al., 2012; Kreft, 2016; Lee et al., 2016)**. The processing of grain into bread and confectionery products is usually associated with heat treatment at some stages; therefore it is necessary to evaluate the resistance of antioxidants contained in flour to heat. Using DPPH it was assessed the AOA of flour from whole grain of three buckwheat species and decreasing of the AOA during heating up to 100 °C. After water extraction the AOA was maximal for *F. tataricum* flour; *F. hybridum* and *F. esculentum* manifested similar values with the same decline dynamics during heating (Table 3). After ethanol extraction, the flour of *F. hybridum* shown higher AOA compared to both cultivated species before temperature treatment (1.3 times) as well as after heating to 100 °C (1.2 times). Since alcohol extracts antioxidants more efficiently compared water, the results of alcohol extraction reflect the ratios of their contents in different types of flour. The method used does not give an accurate estimate of the ratio of flavonoids in seeds of different species.

Table 3 Dynamics of antioxidant activity (AOA) of buckwheat flour extracts during heating.

Species	Flavonoid extraction with	Antioxidant activity (% inhibition of DPPH) after temperature treatment at				
		20 °C	40 °C	60 °C	80 °C	100 °C
F. esculentum	water	6.0 – 6.1	5.0 – 5.8	2.8 – 2.9	1.9 – 2.5	1.3 – 2.0
	ethanol	23.6 – 24.8	16.2 – 20.2	14.9 – 16.1	14.9 – 15.0	13.5 – 13.7
F. tataricum	water	29.6 – 30.6	25.5 – 26.0	22.5 – 23.5	19.8 – 20.8	17.9 – 18.4
	ethanol	41.7 – 42.8	34.2 – 34.5	29.2 – 30.1	28.4 – 28.8	26.8 – 27.2
F. hybridum	water	9.4 – 9.6	5.0 – 5.3	4.4 – 4.7	4.0 – 4.3	3.9 – 4.1
	ethanol	54.9 – 55.3	49.6 – 50.1	39.9 – 40.3	35.4 – 36.1	32.0 – 33.0

Table 4 Amino acid composition of *Fagopyrum* sp. (g.100g^{-1} flour).

Amino acid	*F.hybridum*	*F.tataricum*	*F.esculentum*
Arginine	0.78	0.62	0.56
Lisyne	0.56	0.43	0.54
Tyrosine	0.22	0.15	0.19
Phenylalanine	0.52	0.38	0.47
Histidine	0.24	0.17	0.20
Leucine+Isoleucine	0.83	0.61	0.72
Methionine	0.21	0.16	0.16
Valine	0.44	0.30	0.37
Proline	0.52	0.34	0.41
Threonine	0.54	0.39	0.47
Serine	0.55	0.44	0.47
Alanine	0.62	0.45	0.55
Glycine	0.52	0.37	0.46
Cysteine	0.20	0.19	0.18
Glutamicacid	2.26	1.67	2.02
Asparticacid	1.17	0.84	1.03
Tryptophan	0.23	0.20	0.16

It is known that differences in flavonoid content in seeds between *F. tataricum* and *F. esculentum* can be a hundredfold. However, these results correctly rank the test samples. *F. hybridum* in total AOA exceeds *F. esculentum* 2.2 – 2.3 times and *F. tataricum* 1.3 times.

The results of water extraction reflect the availability of antioxidants for enzymes that destroy them when using flour to make a dough **(Yasuda and Nakagawa, 1994; Suzuki et al., 2002, Suzuki et al., 2004, Suzuki et al., 2014)**. For *F. tataricum*, the maximum values of the efficiency of aqueous extraction were obtained: the AOA of an aqueous solution was 71% of the AOA of an alcohol solution. For *F. esculentum*, AOA of the aqueous solution was 25% of AOA alcohol. For *F. hybridum*, the AOA of the aqueous extract was only 17% of the AOA of the alcohol extract. Since the AOA of the alcohol extract of *F. hybridum* was maximal, this species is probably the most promising raw material for the production of products with high AOA. Lower AOA of aqueous extract of *F. hybridum* compared to *F. tataricum* can be elucidated either higher activity of antioxidants degradation enzymes or lower solubility of the antioxidants in water.

Amino acid composition

In spite of significant differences between the cultivated buckwheats, *F. esculentum* and *F. tataricum*, for the SDS PAGE spectra of seed storage proteins **(Rogl and Javornik, 1996; Lazareva and Fesenko, 2007; Lazareva et al., 2007; Li et al.,2008)**, both the species have a well-balanced amino acid composition of seed protein, with some variation among both species **(Prakash et al., 1987; Yang and Lu, 1992; Bonafaccia et al., 1994)**. *F. hybridum* has not been previously studied in this regard. We analyzed it for accessions studied in the work. The results are presented in Table 4.

The results manifest no strong deviations from earlier published results. The studied accession of new species *F. hybridum* has amino acid composition of seed protein similar to one of the cultivated species. In terms of the content of all essential amino acids, the sample *F. hybridum* is at least slightly superior to the samples *F. esculentum* and *F. tataricum* studied in our work. So, content of Cysteine, Tryptophan, Arginine, Lisyne, Methionine, Leucine + Isoleucine, Threonine, Histidine, and Valine in seeds of *F. hybridum* was 5.2, 15.0, 25.8, 30.2, 31.2, 36.0, 38.4, 41.1 and 46.2% higher compared to *F. tataricum* and 11.1, 43.7, 39.2, 3.7, 31.2, 15.2, 14.8, 20.0, 18.9% higher compared to *F. esculentum*.

CONCLUSION

So, the new species *F. hybridum* is better in some biochemical characteristics in comparison to cultivated buckwheats, *F. esculentum*, and *F. tataricum*. The total antioxidant activity of ethanol extract from *F. hybridum* flour was higher even compared to *F. tataricum*. Water extract from *F. hybridum* flour manifested only 17% AOA of ethanol extract. Probably, minimal efficiency of water extraction may indicate protection from dissolution by water and, accordingly, from the destruction of the flavonoids by enzymes, but the alternative explanation about the higher activity of antioxidants degradation enzymes is not yet rejected. In terms of the content of all essential amino acids, the studied sample of *F. hybridum* exceeds the studied sample of *F. tataricum* by 5.2% (Cysteine) – 46.2% (Valine), and the studied sample of *F. esculentum* by 3.7% (Lisyne) – 39.2% (Arginine). The milling fragments of *F. hybridum* seeds hulls, as well as *F. tataricum* ones, have no pronounced acute angles, probably due to less compact structure of the hulls compared to *F. esculentum*. The non-hulled grain of both *F. hybridum* and *F. tataricum* is more suitable for production of whole-grain flour than the non-hulled grain of *F. esculentum*. Using the whole grain flour allows making the products with a high share of dietary fibers.

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