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Effect of impact-activating-disintegration treatment on grain protein fraction of autumn rye

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Abstract. This paper studies the distribution of rye protein fractions according to their mass and amino acid composition while milling by machines with different work tools. The research was conducted on the autumn rye of harvest year 2017 with kernel moisture content of 8%. Cereals were milled in the machine of an impact-activating-disintegrating type DEZI-15 with three-row and five-row rotors which rotate at 120 s^{-1}, and in the laboratory mill with a knife-rotor work tool. The milling grain size was determined by a diffraction-type grain analyser Malvern Mastersizer 2000. The average grain size obtained from three-row disintegrating rotor was 167 µm, from the five-row rotor 158.1 µm, and from the laboratory knife-rotor mill 384 µm, respectively. The free amino acids composition in flour samples was investigated using the size exclusion chromatography method (SEC-method). The polypeptide composition of total grain protein has been determined by One-dimensional SDS-acrylamide gel electrophoresis. According to the electropherogram results obtained from all the the content of high-protein fraction of 200 kDa. The glutelin fraction with molecular weight of 116.25 kDa is definitely observed in the sample obtained from the three-row disintegrating rotor. Whereby the lowest glutelin content has been detected in the flour sample obtained from the five-row disintegrating rotor. Fractions with molecular weight of 60–75 kDa – globulin fractions – come up frequently in the sample obtained from the three-row disintegrating rotor. Prolamine fractions of 45–47 kDa are clearly observed in the flour sample obtained from the laboratory knife-rotor mill. The albumin fraction with molecular weight of 17–28 kDa are mostly observed in the samples obtained by three-row and five-row disintegrating rotors. Few LMW fractions (from 6.5 to 15 kDa) are found in samples obtained using the impact-activating-disintegrating technique, mostly in the sample milled in the laboratory knife-rotor mill. Based on the data of free amino acid content in sample investigated it can be concluded that the impact-activating-disintegrating techniques does not cause reduction in protein biological value. The albumin rich flour milled in the disintegrator can be used for production of functional food. Due to the low content of glutelin protein fraction the flour obtained from the five-row disintegrating rotor offers the greatest promise for production of gluten-free foods.

Key words: impact-activating-disintegrating technique, autumn rye, protein fractions, amino acid composition, biological value foods.

INTRODUCTION

Cereal products constitute an important part of everyday diet. They provide 21% of calories, 38% of carbohydrates and 20% of protein in human nutrition in protein being the second only to meat products (Bulgakov, 1976).

The promising raw material for food production is rye. Having enhanced root system this crop is one of the most cold-resistant with no demand of soil fertility. Moreover, rye protein is better balanced in composition of essential amino acids (8–16%) compared to wheat and barley protein because of high content of lysine (up to 0.619 g 100 g^{-1}), valine, threonine and methionine. Rye contains antioxidants, especially phenolic acid and polyphenols that protect body tissues from oxidative stress and cell death and prevent chronic diseases such as cardiovascular, nervous degenerative disorders and carcinomata. The merits of rye are rich vitamin composition, especially B group, and the content of macro- and microelements such as potassium, phosphorus, magnesium, manganese, iron, copper, zinc, selenium and others (Bakhitov et al. 2008).

The rye protein consists of the following fractions. Concentration of water-soluble proteins – albumins – is usually 5–15% of the total amount, but can be up to 30%. Salt-soluble proteins – globulins in the rye grain are about 15–25%, alcohol-soluble proteins – prolamines – account for 15–25%, alkali-soluble proteins – glutelins are about 30–40%.

The rye grain contains also other nitrogen compounds in small amounts: free amino acids and their amides, peptides, basic nitrogen and nucleotides, nucleic acids, etc. They account for 5–10% of the total amount of grain nitrogenous matter. Mostly they are found in the kernel and aleurone layer.

The rye protein fractions vary in amino acid composition as well as in the content of essential amino acids which determine the biological value of proteins. Albumins are of the greatest biological value, they contain the best ratio of all the essential amino acids, with some deficit in the content of methionine. Globulins are also characterized by a well balanced amino acid composition, although their content of some essential amino acids is lower compared to albumin (methionine, tryptophan, leucine).

Rye glutelins are characterized by a rather strong deficit of lysine, tryptophan and methionine. Prolamins have the lowest biological value. They are distinguished by a very low content of essential amino acids such as lysine, tryptophan, methionine, and a high concentration of glutamic acid and proline which accounts for up to 40–55% of the mass of these proteins (Rybakova & Glebova, 2012).

In food production technology the presence of different protein fractions of raw material plays an essential role. In the zymurgy the greater presence of free amino acids and LMW protein fractions intensify the output of ethyl alcohol due to consumption of α- amine nitrogen by yeasts (Rimareva et al.; 2008, Rimareva, 2010). In starch and grain syrup production and brewing reduction in content of HMW proteins facilitates the processes of filtration and clarification of wort, as well stabilizes the fobbing (Tretiak, 2009; Donkova & Donkov, 2014; Danina & Ivanchenko, 2015; Sergeeva, 2016). Thus, the main task of most food production technologies based on grain raw materials is to destruct the protein matrix into LMW components that increase their solubility (Maltseva, 1999; Sereda et al., 2010; Shakir et al., 2017). Additionally, the degradation of protein frame may lead to greater availability of other valuable components of grain raw materials such as starch (Stepanov et al., 2007; Alekseeva et al., 2011; Amelyakina et al., 2011).

Currently, the following machines can be employed for fine grain milling: mills – ball mills, vibration mills, ball electromagnetic mills, disintegrators and dismembrators, roll-oscillating mills, jet mills, ultrasonic mills, devices with velocity layer of ferromagnetic particle etc. (Kalinina et al., 2002: Sotnikov et al., 2002; Smirnova & Krechetnikova, 2005; Likhtenberg et al., 2007; Barakova & Ustinova, 2010; Oshkordin et al., 2011; O. Lomovsky & I. Lomovsky, 2011; Romanyuk et al., 2013).

The latest methods of grain handling also include the extrusion technology. The application of extrusion devices allows to produce new products from the vegetable feedstock as well as to enhance the current processes such as production of wort with high content of dry solids weight ratio up to 36 percent, usage of raw materials with high content of no starch polysaccharides, etc. (Nachetova & Barakova, 2013).

The deep grain destruction can be conducted by infrared grain handling (infrared radiation). Since the fluence rate of infrared radiation is high enough, the moisture concentrates in the grain which is heated up to 110–115 °C causing rapid increase of the vapour pressure and decrease of grain strength properties (Krikunova et al.; 2004, Andrienko et al., 2007).

However, we have the evidence that the employment of rough grain processing methods, such as extrusion and micronization (IR-treatment), reduces the biological value of processed grain. As a result the main amino acids of the grain milled are limited with the total amount of α-amine nitrogen reduction (Martirosyan & Malkina, 2010; Bikchantaev et al., 2016).

Another way of deep grain processing techniques of special interest is the impact devices such as disintegrators. One of the most important features of disintegrators is that the material processed is subjected to mechanical activation. The activation of particles applying high mechanical energy is the new advanced type of improvement of technological processes in various industries (Boldyrev, 2006; Sabirov et al., 2017a).

Disintegrators are the devices which function is based on the free impact principle (that's why the device name includes the term impact). In the 1980s, the specialists of the design and technology bureau 'isintegrator' (Tallinn, Estonia) developed the advanced design of disintegrators allowing to obtain fine milled mixtures. The material being milled is continuously fed into the mixing chamber in the middle between two high-speed counterrotating rotors. Impact tools are located on each rotor along the concentric circles. Rotors run into each other in such a way that the concentric circles with pins of one rotor come into the concentric circles with pins of another one.

The advantage of such disintegrators compared to other milling units is the rapidity of milling processes when the material processed therein within 10 s^{-1} receives 2 to 7 high-intensity impacts (Alimova et al., 2014; Sabirov et al., 2017b).

Research in the field provides the evidence of deep mechanochemical destruction of grain components on the example of winter wheat grains subjected to the impact-activating-disintegrating technique. As the electron microscopy method shows in the case of the impact-activating-disintegrating treatment the grain endosperm is deeply destructed. The starch grain of the endosperm takes an oval-round and lenticular shape, its size ranges from 25 to 40 μm. The protein matrices after the impact-activating-disintegrating treatment have more developed surface, the interspace protein can be separated much easier releasing more starch grains and partially leaving the starch grains in the protein matrix provided that the interspace protein of glassy endosperm is destructed during milling together with the firmly linked starch kernel.

Using the gas chromatography method it was found that during the impact-activating-disintegrating treatment of grain we observe the following: glucose (percent) – 1.41 (three-row rotor), 1.48 (five-row rotor), as well as maltose (percent) – 2.05 (three-row rotor), 1.84 (five-row rotor) while in the case of the rotor milling (close to treatment by rolls) the glucose content in the sample is 1.21 percent. In this case we observe the total absence of maltose. This fact indicates that during the impact-activating-disintegrating treatment of grain starch is destructed without an enzymatic process (Barakova & Tishin, 2010).

It is worth mentioning that today scientific literature in the field provides no information on size distribution of grain proteins processed by a disintegrator. Thus, the paper studies the change in the size distribution of proteins as well as free amino acids of winter rye after the impact-activating-disintegrating treatment.

MATERIALS AND METHODS

This paper studies rye flour samples milled by machines with different work tools. The research was conducted on the first class rye of harvest year 2017. The moisture and starch content of test kernels were found to be 8 and 65% respectively. The rye grain moisture content was detected with Shimadzu MOC-120H. The amount of highly soluble carbohydrates was determined with the polarimeter AA-55 by Optical Activity.

For the experimental purposes cereals were milled in the machine of an impact-activating-disintegrating type DEZI-15 with three-row and five-row rotors (Central research institute for engineering materials 'Prometheus', Saint Petersburg). The reference sample was the grain milled in a laboratory mill with a knife-rotor work tool. The milling grain size was determined by diffraction-type grain analyzer MalvernMastersizer 2000. The average grain size obtained from three-row disintegrating rotor was 167 µm, from the five-row rotor – 158.1 µm, and from the laboratory knife-rotor mill – 384 µm, respectively.

The free amino acids composition in flour samples was investigated using the size exclusion chromatography method (SEC-method). In order to determine the quantitative composition of free amino acids in flour the sample was put into 1n hydrochloric acid, then trichloracetic acid was added for sedimentation of non-hydrolized proteins. The sample was kept during 30 minutes, then centrifuged. The supernatant (filter cake) was used for the analysis of free amino acids applying the SEC-method. The analytical tolerance is 0.5% or less.

The polypeptide composition of total grain protein was determined by One-dimensional SDS-acrylamide gel electrophoresis of acrylamide concentration gradient 10–20% in a separating gel (pH = 8.8) and 6% acrylamide in a stacking gel (pH 6.8). The running buffer was *tris*-glycine buffer (pH = 8.3) containing 0.1% SDS. Electrophoresis was conducted at constant current (25–30 mA) within 6–7 h at 4–60 °C.

RESULTS AND DISCUSSION

Analysis of protein fractions of rye flour samples studied is shown in Fig. 1. According to the electropherogram obtained all samples showed the content of high-protein fraction of 200 kDa. 116.25 kDa fraction is definitely observed in the sample obtained from the three-row disintegrating rotor – this is a glutelin fraction. The lowest content of glutelins can be found in the flour samples obtained from the five-row disintegrating rotor.

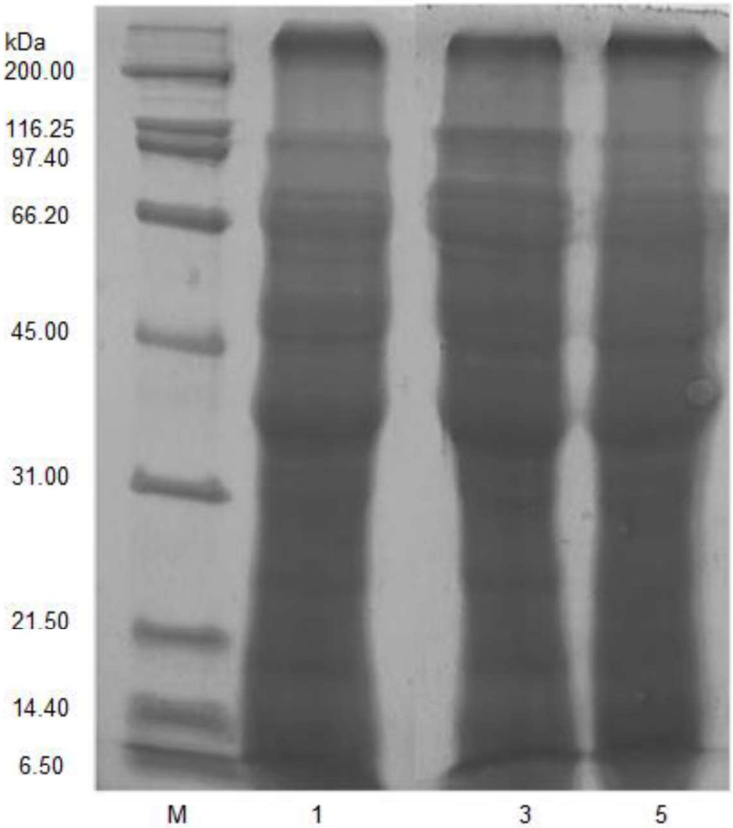

Figure 1. Electropherograms of protein fractions of rye flour samples. M – marker; 1 – electropherogram of the rye flour sample obtained in the laboratory mill with a knife-rotor work tool; 3 – electropherogram of the rye flour sample obtained from the three-row disintegrating rotor; 5 – electropherogram of the rye flour sample obtained from the five-row disintegrating rotor.

Fractions with molecular weight of 60–75 kDa – globulin fractions – are frequently found in the sample obtained from the three-row disintegrating rotor. Prolamine fractions of 45–47 kDa are clearly observed in the flour sample obtained from the laboratory knife-rotor mill. The albumin fraction with molecular weight of 17–28 kDa are mostly observed in the samples obtained by the three-row and five-row disintegrating rotors.

Few LMW fractions (from 6.5 to 15 kDa) are found in samples obtained using the impact-activating-disintegrating technique, mostly – in the sample milled in the laboratory knife-rotor mill.

Thus, the flour milled by the three-row disintegrating rotor is richer in the glutelin protein fraction and hence is more suitable for bakery product production. The glutelin fraction present in flour comprises of two proteins gliadine and glutenin plays role for the dough making process and its qualitative properties. The flour obtained from the five-row disintegrating rotor contains the highest amount of the albumin fraction which is mostly rich in essential amino acids and is suitable for production of functional foods. The low content of glutelin fraction can make such foods with low glutelin content fit for a gluten-free diet (Kolpakova et al., 2001).

In order to evaluate the influence of grain handling by a disintegrator on other nitrogen compounds we have measured the content of free amino acids in the flour samples. The results obtained are shown in Table 1.

Table 1. Free amino acids of rye flour samples investigated

Amino acid	Content of free amino acids, mg g^{-1}		
	Flour obtained by the knife-rotor mill	Flour obtained by three-row disintegrating rotor	Flour obtained by five-row disintegrating rotor
Aspartic acid	0.2752	0.3426	0.3720
Serine	0.0379	0.0414	0.0469
Threonine	0.6093	0.5083	0.6019
Glutamic acid	0.2529	0.2865	0.2934
Proline	0.0676	0.1639	0.2487
Glycine	0.0223	0.0397	0.0328
Alanine	0.1177	0.1847	0.2132
Cysteine	0.0	0.0	0.0
Valine	0.0414	0.0449	0.0467
Methionine	0.0	0.0	0.0
Isoleucine	0.0164	0.0285	0.0285
Leucine	0.0207	0.0224	0.0276
Tyrosine	0.0	0.0216	0.0250
Phenylalanine	0.0129	0.0181	0.0319
Histidine	0.3918	0.3529	0.3789
Lysine	0.0440	0.0397	0.0449
Tryptophane	0.5320	0.5057	0.5782
Arginine	0.1562	0.1812	0.1648
Total amount	2.5993	2.7822	3.1354
Total content of essential amino acids	1.2767	1.1676	1.3594

* The error in the content of amino acids in the test samples is not more than 0.0001%; $p = 0.95$.

As seen from Table 1 the total content of free amino acids increases according to the intensification of the grain milling process. Thus, the content of free amino acids in the flour milled by the three-row disintegrating rotor is higher by 6.5 percent compared to the flour obtained from the knife-rotor mill. And the flour milled by the five-row disintegrating rotor shows the content of free amino acids higher by 17 percent than the sample obtained from the knife-rotor mill and by 11.3 percent compared to the flour milled by the three-row disintegrating rotor.

Concerning the total content of essential free amino acids we have obtained mixed result. We observe the highest content of these amino acids in the sample obtained from the five-row disintegrating rotor. The sample shows the content of essential amino acids higher by 6 percent than the sample obtained from the knife-rotor mill and by 14.1 percent than the sample from the three-row disintegrating rotor. This proves that the three-row disintegrating rotor has less mechanochemical effect on the raw material milled. Amino acids such as arginine and histidine, which are essential for a child's body, are totally the same in all three flour samples investigated (Soboleva et al., 2015).

Based on the data proven we can assume that grain milling by the disintegrator shall changes the mechanochemical structure of the raw materials handled causing the increase of the diffusion properties of LMW components. Moreover, the impact-activating-disintegrating technique does not reduce the biological value of proteins compared to such methods as the extrusion or micronization.

CONCLUSIONS

The paper shows that the impact-activating-disintegrating technique for rye milling allows to obtain a fine milled grain with the high biological value compared to the techniques and devices employed today because:

– the total content of water-soluble protein particles is higher in flour samples obtained by disintegrator;
– the total content of free amino acids is also higher in flour samples obtained from rye treated by the impact-activating-disintegrating technique.

The albumin rich flour milled in the disintegrator can be used for the production of functional food. Due to the low content of glutelin protein fraction the flour obtained from the five-row disintegrating rotor is likely to be the most promising for the production of gluten-free foods.

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