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COMPOSITION, QUALITY CHARACTERISTICS AND MICROSTRUCTURE OF THE GRAIN *TRITICUM DICOCCUM*

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ABSTRACT
The compositional and quality characteristics of two wheat varieties *Triticum dicoccum* (*Triticum dicoccum* var. *dicoccum*, *Triticum dicoccum* var. *rufum*) produced in the Republic of Azerbaijan have been tested and are relatively useful in assessing their applicability to bread production. The wheat species studied, *Triticum dicoccum*, were found to have a higher protein and cell content, as well as essential proteins of lysine, phenylalanine, leucine and isoleucine, methionine and valine, relative to Gorbustan wheat varieties. The chromatographic method was used to determine the carbohydrate composition of the *Triticum dicoccum* grain. The following redistribution of low molecular weight carbohydrate fractions is noted: the maltose content is higher, and galactose, glucose and fructose are much lower than those of the modern wheat variety Gorbustan. Such a distribution of carbohydrates can reduce the formation of toxic products when baking bread. In addition, the wheat grain *Triticum dicoccum* is characterized by a higher content of sterols, in particular β-sitosterol. The antioxidant activity expressed as percentage inhibition of DPPG free radicals in the *Triticum dicoccum* grain is twice as high as this indicator for wheat of the commercial variety Gorbustan. By scanning electron microscopy, it has been established that the microstructure of the grain surface and the cross section has varietal characteristics. Grain *Triticum dicoccum* var. *rufum* has a thicker shell, tighter and tighter, unlike the grain of *Triticum dicoccum* var. *dicoccum*. With all the benefits of the wheat grain *Triticum dicoccum*, its technological properties were even worse. But the use of technological methods to boost gluten will ensure the production of high-quality healthy bread from old wheat grain.

Keywords: wheat; grain; composition; microstructure; antioxidant activity; technological properties

INTRODUCTION
Recent large-scale epidemiological studies have shown that regular consumption of whole-grain cereal products can reduce the risk of cardiovascular disease and certain types of cancer by 30%, protect against obesity and second type of diabetes **(Chatenoud et al., 1998; Montonen et al., 2003; Larsson et al., 2005; Duchoňová and Šturdík, 2010)**. Nutritionists recommend cereal-based products to ensure dietary fiber, protein, vitamin and mineral diets, primarily found in cereal hulls **(Buddrick et al., 2014; Vitaglione et al., 2008)**. Grain products are a good source of biologically active compounds that inhibit oxidation processes in human plasma. In whole grain products, there are compounds with antioxidant properties: ferulic acid, caffeine, p-coumaric, synapic and other phenolic acids. The highest proportion of total antioxidant potential is found in the bran fraction **(Verma, Hucl and Chibbar, 2009; Ivanišová et al., 2011)**.

Recently, people have become more and more interested in natural and organic foods. In this regard, the older wheat varieties *Triticum monococcum*, *Triticum dicoccum* and *Triticum spelta* have been discovered for use in food technology. The main value of these varieties is their ability to produce good yields on poor soils and to resist fungal diseases. Some populations are tolerant to drought and heat stress **(Zaharieva et al., 2010; Konvalina et al., 2011)**. The nutritional value of the grain of *Triticum dicoccum* is mainly due to the high protein content (18 – 23%), the total proportion of essential amino acids in the protein **(Stehno, 2007)** and the high degree of digestibility of the protein compounds **(Hanchinal et al., 2005)**. An increased concentration of antioxidants has been found in the grain of *Triticum dicoccum* **(Piergiovanni et al., 1996)**. Grain starch is mainly represented by persistent fractions, resulting in slower absorption of carbohydrates **(Mohan and Malleshi, 2006)**. Non-digestible resistant starch is one of the factors that increase the functionality of food products **(Duchoňová and Šturdík, 2010)**. The low glycemic index makes the grain of *Triticum dicoccum* particularly valuable for diabetic nutrition **(Buvaneshwari et al., 2003)**. However, *Triticum dicoccum* grain has been found to have lower β-carotene values than traditional wheat varieties **(Hailu and Merker, 2008)**. A higher concentration of phytic acid has been found in the grain of

Triticum dicoccum compared to traditional raw materials in the bakery industry **(Cubadda and Marconi, 1996)**. Ancient wheat species are known to produce low yields but contain more protein and minerals **(Lachman et al. 2012)**.

The grain of *Triticum dicoccum* contains hard shells that attach closely to the weevil. *Triticum dicoccum* is used throughout the world for the production of traditional foods: roasted cereals, breakfast cereals, pancakes, cereals, baby foods, local pasta types. It has been established that wheat *Triticum dicoccum* can be used for bread making, but with a lower quality than traditional wheat varieties **(Hanchinal et al., 2005; Longin et al., 2016; Kissing et al., 2017)**.

Scientific hypothesis

The composition, quality characteristics and microstructure of the grain of *Triticum dicoccum* indicate the applicability of the varieties tested for the production of bread with yeast.

MATERIAL AND METHODOLOGY

We analysed the indicators of the technological qualities of the grain *Triticum dicoccum* var. *dicoccum*, *Triticum dicoccum* var. *rufum* and compared to Gobustan commercial wheat from *Triticum aestivum*. The tested crops grew in the Absheron peninsula in the Republic of Azerbaijan. The determination of crude protein, starch, cellulose and fat content was carried out according to the methods described by **Yermakov (1972)**. Free and protein bound amino acid content was determined after hydrolysis of the suspensions in sealed 6H HCl ampules for 24 hours using ion exchange chromatography using electrochemical detection on a BIOCHROM amino acid analyser (Biochrom Ltd., Great Britain). Antioxidant activity was determined by the spectrophotometric method in an alcohol extract described by **Silva et al. (2005)** based on percent inhibition of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. We have been determined by the optical density of the solutions in the interaction, Specord M40 (Carl Zeiss Industriel Messtechnik GmbH, Germany) at a wavelength of 515 nm. The concentration of sugars in the grain samples was determined by chromatography using electrochemical detection on an Agilent 1100 liquid chromatograph (Agilent Technologies, USA) with an ESA Coulochem III electrochemical detector. Separation of a mixture of sugars was performed on a grafted amino phase anion exchange column followed by electrochemical detection. The grain samples were milled in a laboratory mill and sieved through a sieve of 0.5 mm diameter. An acetate buffer (0.1 M, pH 5.0) was added to the weights of the resulting flour placed in conical flasks. The solids concentration in the suspension was 100 $g.L^{-1}$. The flasks were placed in a laboratory thermostatic shaker (40 °C, 250 rpm), where the water-soluble components were extracted for 2 hours. At the end of the process, the samples were centrifuged at 14,000 g for 20 minutes, the supernatant was collected and used to determine the sugar concentration with a 10-fold preliminary dilution. The lipid group composition was determined by thin layer chromatography on Silufol plates (Kavalier, Czech Republic) with a fixed layer of silica gel. The plate was previously moistened in a 2% solution of phosphomolybdic acid in acetone. About 10 µg of lipids in the ether solution were applied to the plate as a 10 mm long strip. The chromatogram was developed in the solvent system hexane (Merck, Germany):diethyl ether (Merck, Germany):acetic acid (Merck, Germany) – 80:30:1.5. The plate was air dried until the solvent odour disappeared and placed in a forced ventilation cabinet at a temperature of 80 °C until the appearance of blue spots on certain groups of lipids on the bottom yellow. The identification of individual groups of lipids was performed by comparing the Rf of standard substances with spots on the chromatogram. Quantitative determination of individual groups of lipids was performed by a densitometric method using a Chromoscan 200 instrument (Joyce Loebl & Co, USA). The calculation of the number of individual groups of lipids was performed by the internal standardization method using correction factors: polar lipids – 0.3; sterols – 0.1; free fatty acids – 0.5; triglycerides – 1.0; Sterol esters – 0.14. Microstructural studies were carried out using a JEOL JSM 6390 scanning electron microscope (JEOL, Japan). The pre-prepared samples were placed on a copper disk, a platinum layer was sprayed on a JEOL JEE 44E vacuum evaporator and scanned with a 15 kV accelerated voltage scanning microscope. Determination of the state of the carbohydrate-amylase complex was performed on the device "PChP-7" (LLC "Biophysical Apparatus", Russia) according to the method attached to the device. Gluten quality was determined on the IDK-1M ("Biofizpribor", Ukraine) device.

Statistic analysis

The results were evaluated statistically using the Analysis of Variance. Procedure compares the data in six varieties. The results assays were expressed as mean ±*SD* of eight repeated samples. To evaluate the reliability of the test differences, *t*-statistics (a two-sample t-test for independent samples) were used. The *p*-value used to test the null hypothesis in order to quantify the idea of statistical significance have to be provided. The tests were conducted at a level of significance $p < 0.05$ using the Statistica 7.0 software (StatSoft Inc., USA).

RESULTS AND DISCUSSION

In some countries, traditional foods are made from wheat *Triticum dicoccum*. It is thought to be rich in biologically active compounds and its starch has a slow digestibility. However, the content and composition of biologically active compounds would vary according to geographic location, seasonal variations, varieties used, and methods of analysis used **(Dhanavath and Prasada Rao, 2017)**. The population of *Triticum dicoccum* growing in the Republic of Azerbaijan is poorly studied. Basically, it is used as a material for wheat breeding. However, interest in the production of organic food is growing in Azerbaijan. Table 1 presents the results of the determination of the main nutrient content in the studied species of *Triticum dicoccum*.

Table 1 Essential nutrient content of *Triticum dicoccum* grain.

Component	Content, % (*t/p*) *		
	Triticum dicoccum* var. *dicoccum	***Triticum dicoccum* var. *rufum***	***Triticum aestivum* Gorbustan**
Raw protein	14.8 ±0.21 (9.69/0.001)	16.2 ±0.19 (16.11/0.001)	12.3 ±0.15
Starch	60.1 ±0.56 (4.71/0.01)	59.4 ±0.38 (7.06/0.001)	63.4 ±0.42
Cellulose	6.4 ±0.04 (56.0/0.001)	6.8 ±0.06 (47.7/0.001)	3.6 ±0.03
Fat	1.8 ±0.02 (4.47/0.01)	1.8 ±0.02 (4.47/0.01)	1.9 ±0.01

Note: * (t - student's criterion, P - level of significance).

Table 2 Amino acid composition of wheat protein.

Amino acid	Content, %		
	Triticum dicoccum* var. *dicoccum	***Triticum dicoccun* var. *rufum***	***Triticum aestivum* Gorbustan**
Arginine	0.42	0.31	0.40
Lysine	0.40	0.39	0.34
Tyrosine	0.29	0.21	0.23
Phenylalanine	1.08	0.86	0.76
Histidine	0.34	0.21	0.21
Leucine + isoleucine	1.25	0.87	0.94
Methionine	0.21	0.17	0.15
Valin	0.82	0.38	0.60
Proline	1.63	1.24	1.15
Prolamin	0.54	0.47	0.42
Serine	0.59	0.52	0.47
Alanine	0.71	0.55	0.50
Glycine	0.55	0.36	0.40
Cysteine	0.18	0.15	0.17
Glutamic acid	5.02	3.56	3.30
Aspartic acid	0.84	0.59	0.53
Tryptophan	0.12	0.14	0.11

Table 3 The carbohydrate composition of the grain *Triticum dicoccum*.

Carbohydrate	Content, $g.L^{-1}$ (*t/p*)*		
	Triticum dicoccum* var. *dicoccum	***Triticum dicoccum* var. *rufum***	***Triticum aestivum* Gorbustan**
Galactose	0.02 ±0.001	0.05 ±0.001	0.09 ±0.002
Glucose	0.21 ±0.014	0.24 ±0.012	0.32 ±0.018
Fructose	0.18 ±0.010	0.20 ±0.011	0.30 ±0.015
Maltose	1.85 ±0.028	1.78 ±0.018	1.16 ±0.020
Sucrose	0.14 ±0.003	0.12 ±0.001	0.11 ±0.002

Protein content is one of the most important characteristics of grain, which determines its nutritional value. Our data showed that both tested varieties of *Triticum dicoccum* had a higher protein content than commercial grade wheat. The highest value was obtained for *Triticum dicoccum* var. *rufum*. This is 3.9% higher than that of a modern commercial wheat variety. The high protein content of the grain of *Triticum dicoccum* is consistent with other data **(Dhanavath and Prasada Rao, 2017)**. Data from the literature on the fiber content of *Triticum dicoccum* in wheat grain is contradictory. Some researchers claim that *Triticum dicoccum* wheat is high in fiber **(Čurná and Lacko-Bartošová, 2017)**. According to **Shewry and Hey (2015)**, *Triticum dicoccum* may contain less dietary fiber than modern wheat varieties. According to our data, the fiber content of *Triticum dicoccum* wheat was 2.8 to 3.2% higher than in the grain of the modern variety. Our data on starch and fat content are consistent with those reported in the literature **(Giambanelli et al., 2013)**. Differences in the obtained values of the content of the main nutrients in the grain of *Triticum dicoccum* are significant, $p \leq 0.05$ in all studied parameters.

Table 4 Fractional composition of lipids in the grain *Triticum dicoccum*.

Fraction	Content groups of lipids in the grain, % (*t/p*)*		
	Triticum dicoccum* var. *dicoccum	***Triticum dicoccum* var. *rufum***	***Triticum aestivum* Gorbustan**
polar lipids	3.1 ±0.012 (8.9/0.001)	3.4 ±0.014 (4.5/0.01)	3.3 ±0.020
monoglycerides	0.3 ±0.002 (35.4/0.001)	0.5 ±0.001 (54.3/0.001)	0.4 ±0.002
diglycerides	0.27 ±0.01 (4.9/0.01)	0.31 ±0.03 (3.5/0.05)	0.2 ±0.01
sterols	1.3 ±0.010 (62.5/0.001)	1.5 ±0.010 (15.6/0.001)	0.5 ±0.008
β-sitosterol	2.8 ±0.018 (36.9/0.001)	2.9 ±0.014 (48.8/0.001)	2.0 ±0.012
free LCD	3.7 ±0.011 (66.9/0.001)	4.1 ±0.012 (85.0/0.001)	2.4 ±0.016
triglycerides	74.0 ±0.34 (2.86/0.05)	73.8 ±0.30 (2.6/0.05)	72.6 ±0.35
sterol esters	4.1 ±0.22 (11.1/0.001)	3.8 ±0.15 (14.4/0.001)	7.4 ±0.20

Note: * (*t* - student's criterion, *p* - level of significance).

It should be noted that the nutritional value of the grain is determined not only by the total amount of protein, but to a greater extent by its amino acid composition. Table 2 presents the results of the determination of the amino acid composition of the *Triticum dicoccum* grain protein and the winter soft wheat grain traditionally used in food production. Despite the fact that the protein content is higher in wheat varieties, *Triticum dicoccum* var. *rufum*, protein *Triticum dicoccum* var. *dicoccum* has a great nutritional value. He found a higher content of essential amino acids lysine, phenylalanine, leucine and isoleucine, methionine, valine. The grain of *Triticum dicoccum* is richer in lysine than modern commercial wheat, which is consistent with data from other scientists **(Abdel-Aal and Hucl, 2002)**. Chromatographic method was used to determine the carbon composition of the *Triticum dicoccum* grain of two varieties and the modern wheat variety. The results obtained are presented in Table 3. The results indicate that the amount of monosaccharides in the grain of *Triticum dicoccum* var. *dicoccum* and *Triticum dicoccum* var. *rufum* is lower than in the grain of the modern wheat variety, but the maltose content is higher. It is a positive fact that is important for the safety of cereal products, in the technology for which there is a heat treatment. Acrylamide is one of the products of the Mayer's reaction. This toxic product that forms in food products during their processing due to the reaction between reducing sugar and asparagine at a temperature above 120 °C. maltose > lactose **(Wang and Xu, 2014)**. **Žilić et al. (2017)** observed a similar composition of reducing sugars in the wheat grain *Triticum dicoccum*. Table 4 presents the results of the study of the fractional composition of the lipids of wheat grain. In recent years, plant sterols have received increasing attention because of their health benefits. We found that in the wheat grain *Triticum dicoccum*, the sterol content was 2.6 to 3.0 times higher, the β-sitosterol was 0.8 to 0.9% higher and the fatty acids were 1.3 to 1.7% higher than modern grain wheat. According to **Iafelice et al. (2009)**, the sterol profile present in tetraploid and hexaploid wheat species is the same, but there are differences in relative amounts and distribution. **Grela (1996)** found that *Triticum dicoccum* had a high proportion of monounsaturated fatty acids in its fatty acid composition, averaging 21.5% compared to 12.1% for wheat. The ranges of variability of sterols in the grain of *Triticum dicoccum* are consistent with data from other authors **(Giambanelli et al., 2013)**. Although data on the content and composition of bioactive components of ancient wheat varieties are limited, published studies show that they differ little from modern wheat varieties in terms of the content of most compounds **(Shewry and Hey, 2015)**. Wheat is known to contribute significantly to the antioxidant status, having a beneficial effect on human health **(Hejtmánková et al., 2010)**. The results of the study showed that the overall antioxidant properties were different among the three wheat varieties tested. The grain of *Triticum dicoccum* var. *dicoccum* had an antioxidant activity of 15.5% inhibition of DPPH, *Triticum dicoccum* var. *rufum* – 18.3% inhibition of DPPH, Gobustan variety of *Triticum aestivum* – 7.9% inhibition of DPPH. The antioxidant activity of the grain of *Triticum dicoccum* is probably associated with a higher content of flavonoids and vitamins **(Serpen et al., 2008; Calzuola et al., 2013)**.

Figure 1 shows microphotographs of shell surface and grain cross-section of three wheat varieties studied. The native surface has a characteristic relief consisting of parallel formations of cellulose fibrils. In different varieties of wheat studied, the epidermal derivatives of the components of the polysaccharide matrix have a different coating thickness; therefore, on the microphotographs, the cords of cellulose are not clearly visible everywhere. In wheat, *Triticum dicoccum* var. *dicoccum* and *Triticum dicoccum* var. *rufum* is probably the superficial layer formed by hemicelluloses is more pronounced. Approval by **Hanchinal et al. (2005)**, that the *Triticum dicoccum* grain contains rigid shells that attach closely to the weevil and can not treat all varieties of this species. The microphotographs show that the grain of *Triticum dicoccum* var. *rufum* has a shell of 70 – 100 microns, closely related to the grain.

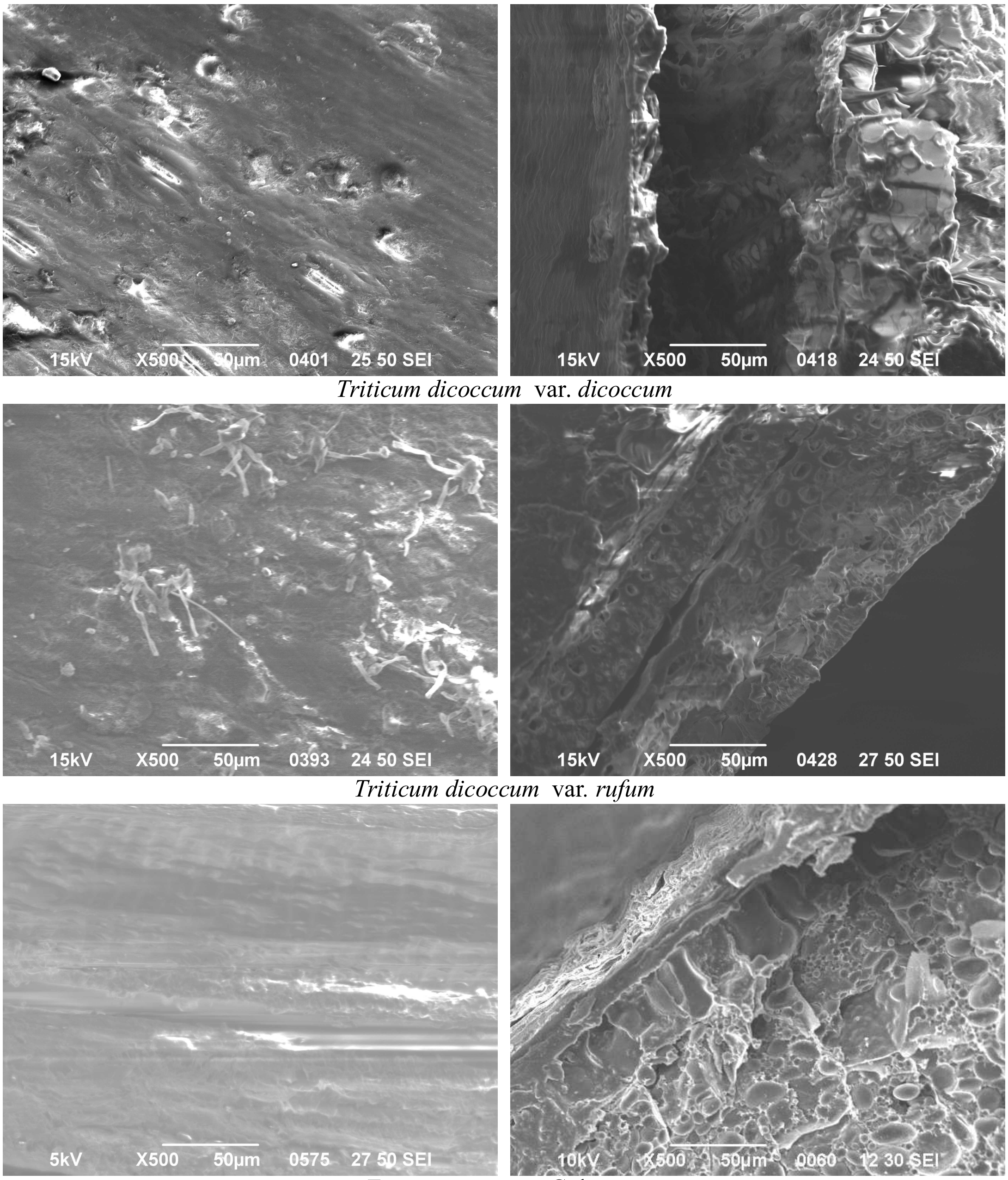

Figure 1 Microphotographs of superficial structures and cross-sections of the grain of three wheat varieties. Increase x500 zoom. Photo: Stolyarov (2019).

Table 5 Some technological properties of grain *Triticum dicoccum.*

Sample	Mass of raw gluten, g.100g^{-1} (*t/p*) *	Instrument reading IDK-1M, unit. (*t/p*) *	Value of the indicator PE, s (*t/p*) *
T*riticum dicoccum* var. *dicoccum*	14.8 ±0.21 (9.69/0.001)	16.2 ±0.19 (16.11/0.001)	12.3 ±0.15
Triticum dicoccum var. *rufum*	60.1 ±0.56 (4.71/0.01)	59.4 ±0.38 (7.06/0.001)	63.4 ±0.42
Triticum aestivum Gorbustan	6.4 ±0.04 (56.0/0.001)	6.8 ±0.06 (47.7/0.001)	3.6 ±0.03

Note: * (*t* - student's criterion, *p* - level of significance).

Grain *Triticum dicoccum* var. *dicoccum* has a pronounced detachment of the shell of the stone fruit. The thickness of the shell can be up to 35 microns, the cavity between the shell and the layer of aleuron has dimensions up to 90 microns. It can be assumed that the grain *Triticum dicoccum* var. *dicoccum* will be easier to peel during flour production. The composition of the seed coat of the grain *Triticum dicoccum* depending on the variety and area of growth will be the subject of our further research. Some technological properties of the grain of *Triticum dicoccum* have been studied in relation to a modern commercial wheat variety. The experimental data are presented in Table 5.

The quality of the flour for bread baking depends both on the protein content and the type of protein, in particular the composition of the gluten. In general, it should be noted that the two wheat varieties studied, *Triticum dicoccum*, had the worst technological properties for bread production compared to the modern commercial variety. This confirms the results of research by other scientists who have shown that, in addition to their protein content, the technological properties of ancient wheat varieties are considered worse than those of ordinary wheat **(Geisslitz et al., 2018; Petrenko et al., 2018)**. The grain of *Triticum dicoccum* has a gluten content and a high gluten stress index. This suggests that you can get high quality bread. However, the index of the gluten strain is within the limits of using yeast bread from *Triticum dicoccum* flour by applying special methods to enhance gluten. The preliminary laboratory preparation of bread from *Triticum dicoccum* flour resulted in bread with a porosity of 60% and a specific volume of 2.1 $g.cm^{-3}$ confirming the reception of bread. These products can be used for the manufacture of flour confectionery.

CONCLUSION

Thus, it has been established experimentally on the grain of the varieties of ancient wheat *Triticum dicoccum* var. *dicoccum* and *Triticum dicoccum* var. *rufum* has a nutritional relationship to the modern commercial wheat variety *Triticum aestivum* Gobustan. *Triticum dicoccum* was 2.5 and 3.9%, grain fats 2.8 and 3.2% more. Results of the distress of the composition. That is, the grain protein of ancient wheat has a higher nutritional value. The study of the grain carbohydrate composition showed that the amount of monosaccharides in the grain of *Triticum dicoccum* var. *dicoccum* and *Triticum dicoccum* var. *rufum* is lower than the modern wheat grain, but the maltose content is on average higher by 0.65 $g.L^{-1}$. This suggests that the rate of acrylamide formation when baking bread from the studied grain will be lower, which will ensure product safety for consumption. We also found that in the wheat grain *Triticum dicoccum*, the sterol content was 2.6 to 3.0 times higher, β-sitosterol was 0.8 to 0.9% higher and that the acids free fat was 1.3 to 1.7% higher than that of modern wheat grain. Grain *Triticum dicoccum* var. *dicoccum* had an antioxidant activity of 15.5%, the grain *Triticum dicoccum* var. *rufum* – 8.3%, variety of *Triticum aestivum* Gobustan – 7.9% inhibition of the radical DPPH. Microphotographs of shell surface and grain cross section of three varieties of wheat tested showed that there were varietal differences in coating thickness of epidermal derivatives of the polysaccharide matrix and the thickness of the grain envelope. The rigidity of the seed coat structure and the density of its weevil adhesion may also vary according to the varieties of *Triticum dicoccum*. The two wheat species studied, *Triticum dicoccum*, had the worst technological properties for bread production compared to the modern commercial variety. However, our preliminary bread baking laboratory with *Triticum dicoccum* flour has enabled us to obtain a good quality yeast bread. Thus, the use of *Triticum dicoccum* in breadmaking can broaden the range of healthy breads containing biologically active components and having preventive properties.

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