INNOVATIVE TRENDS IN THE DEVELOPMENT OF ADVANCED TRITICALE GRAIN PROCESSING TECHNOLOGY

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Abstract: The study has been carried out at the All-Russian Research Institute of Grain and Its Processing Products. This paper describes the formation of new grades of triticale flour based on the cumulative ash curves the analysis of technological and biochemical indicators of which showed that flour of the grades T-60, T-70 and T-80 obtained from endosperm can be used directly in bakery, flour of the grades T-120 and T-220 obtained from peripheral parts and triticale bran can be limitedly used in bakery, and are mainly raw materials for further processing. On the basis of the study of the kinetics and efficiency of the effect of proteolytic and cellulolytic enzyme preparations (EP) and their compositions, optimal conditions for enzymatic modification (the EP dosage is 0.5–0.75 units of PA/g of flour, 0.3...0.4 units of CA/g of bran, the optimum temperature is 40–50°C, pH is 5.0 and 3.5, the duration of reactions is 1.5 and 2 hours) have been determined. It has been shown using the gel-chromatography method that the use of multienzyme compositions (MEC) of proteases allowed to hydrolyze triticale flour proteins completely and to use the obtained hydrolyzate as a component of hypoallergenic and gluten-free flour products. The use of cellulolytic EP allowed to increase the amount of reducing substances and soluble protein by 1.5–2.5 times in comparison with the control sample. The biomodified bran obtained using the MEC "Shearzyme 500 L" + "Neutrase 1.5 MG" and "Viscoferm L" + "Distizym Protacid Extra" has a high degree of hydrolysis of non-starch polysaccharides and proteins, is characterized by a certain ratio of high-, medium-, low-molecular peptides and amino acids, has different functional and technological properties. They can be used in the production of a wide range of general-purpose, functional and treatment-and-prophylactic food products.

Keywords: Triticale grain, flour, bran, grain processing technology, enzyme preparations, modified grain processing products, functional and technological properties

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INTRODUCTION

The relevant trends in the development of flour technology include both the improvement of traditional methods and the development of technologies of products with a high biological and nutritional value, the use of biotechnological methods in the technology of advanced processing products, the creation of technologies of new, non-traditional products, etc. The final objective of the technologies being developed is to obtain products with the specified composition and properties.

All-Russian Research Institute of Grain and Its Processing Products conducts fundamental and applied studies to develop the basic methods for managing technological processes of the preparation and grinding of grain of various crops in order to obtain products with the specified chemical composition and properties. Thus, using the example of processing of triticale grain into flour and cereals, principles of the formation of stable streams of flour from various anatomical parts of grains have been developed, which allows to form various types of flour with the specified properties. The application of the developed technologies allows to obtain such products from triticale grain as: graded baker's flour, cereals for children's and dietary nutrition and grits for pasta [1, 2, 3, 4].

Triticale is a new crop, this is the first grain crop obtained by crossing wheat (*Triticum*) with rye (*Secale*). The first report on the receipt of a wheat-rye hybrid was published in 1875 [5]. The main manufacturers of triticale in the world are Poland, Germany, France and Belarus, moreover, the cultivation area of this most promising culture expands both in the world and in Russia. The croppage in Russia was 624 thousand tons in 2017, according to Roskomstat. The average yield of triticale in Russia in 2016 is 27.8 c/ha, which is the largest value for the period of 2009-2016, and is also 4.7 c/ha more than in 2015 [6]. 75 grades of winter triticale and 14 grades of spring triticale have been added to the State Register of Selection Achievements approved for use in Russia (2017). All new grades are recommended for food purposes [7].

The biotopotential of triticale grain depends primarily on: varietal features and growing conditions. The nutritional value is related to a high protein content, essential amino acids and a balanced amino acid composition. The biological value of triticale grain depends on the predominance of water and salt-soluble

protein fractions and, as a consequence, a higher degree of assimilation of triticale proteins, as well as the presence of vitamins, macro- and micronutrients [4, 8, 9].

However, at present, in Russia, triticale is used mainly in the production of mixed fodder and alcohol. Perspective is the application of flour from triticale grain as a component of raw materials in the production of confectionery products: biscuits, cakes and crackers. It is possible to use triticale flour in the production of fast breakfasts or in the production of dietary bread, including multi-grain bread and that from whole grains [9, 10, 11]. There is no production of bread from graded triticale flour currently in Russia.

The use of methods for a biotechnological effect on various crops and their processing products with obtaining general-purpose, functional and treatment and prophylactic food products is a promising and relevant trend of scientific research for the technological development of the milling branch. At present, the use of enzymatic hydrolysis of biopolymers of food raw materials of both animal and vegetable origin is being actively and comprehensively studied and introduced into the practice of food and processing industries [12, 13, 14, 15, 16].

The use of modern biotechnological methods allows to develop methods for enzymatic modification of grain processing products (flour of various types, including that with a high content of peripheral parts, bran) using multienzyme compositions (MEC) based on proteolytic and cellulolytic enzyme preparations; to obtain modified products (protein hydrolyzate, structurally modified flour, biomodified bran) with various values of degree and depth of hydrolysis of proteins and non-starch polysaccharides with various functional and technological properties.

The study aims at developing a flexible technology based on the division of triticale grain into anatomical parts to obtain new general-purpose and special products with a high nutrition and biological value and to obtain components with specific functional and technological properties. The implementation of the taken aim will allow to design food products from grain with the specified composition and properties.

STUDY OBJECTS AND METHODS

The experimental studies have been carried out at the Federal State Budgetary Scientific Institution "All-Russian Research Institute of Grain and its Processing Products". In this paper, flour was used from of triticale grain of new grades formed on the basis of cumulative ash curves. Since the studied samples of triticale grain did not contain any foreign and grain impurities, the technological process of preparing triticale grain for milling included only hydrothermal treatment: the grain was moistened up to 14–15% and softened for 12 hours [3]. The technological process of grain grinding included 4 break, 6 reduction and 2 scratch systems. The parameters and grinding regimes corresponded to the recommended "Rules for the organization and conduct of a technological process at flour mills" for graded wheat milling according to a short process scheme. 6 samples of triticale grain of different grades were isolated for laboratory milling: Topaz (2011, 2012); Skolot (2012); Vocaliz (2012); Tribun (2012) and Donslav (2012). Thus, the range of values of the quality indicators of the studied samples was: glassiness is 55–72%, the natural weight is 715–737 g/l, the weight of 1000 grains is 40–44 g, the ash content is 1.85–1.89%, the crude gluten content is 17–24%, the gluten quality is 46–64 units of GDI, the falling number is 74–175 s and the protein content is 12–13% [1].

Figure 1 presents the process of grinding and forming the quality of flour in the form of cumulative ash curves. The presence of 3 stages of flour formation has been established, which is clearly seen from the graphs of cumulative curves (Fig. 1). In addition, the statistical analysis has shown the reliability of representation of cumulative curves in the form of three linear sections.

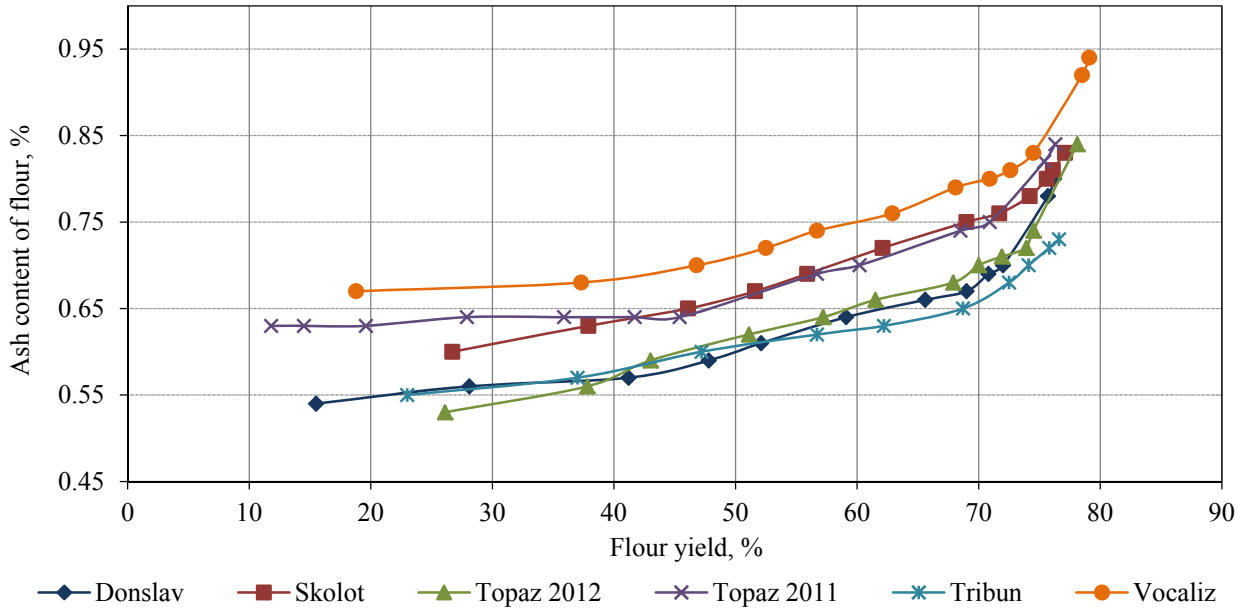

Fig. 1. Ash content cumulative curves.

The first stage of flour formation consisted in extracting the central part of endosperm with a flour yield of 40–45% and an ash content of 0.60% and included the 1st, 2nd, and 3rd reduction systems. The letter designation **A** has conditionally been assigned to the given flour stream. The second stage consisted of 5–7 technological systems and was characterized by a yield of triticale flour in the amount of 25–26% and ash content of 0.91%. The letter designation B has conditionally been assigned to the given flour stream. The third stage consisted of scraping with a flour yield of 5–7% and ash content of 2.20% and included the 6th reduction system and scratch systems. The conventional designation of flour stream is C. Further on, the flour of each of the stages was mixed to obtain individual flour grades, which resulted in obtaining 5 flour grades. The conventional designation of the grades includes the index T which stands for triticale, and a number which stands for the value of ash content × 100. Thus, flour T-60 was the stream A with an ash content of 0.60%, flour T-70 was a mixture of the streams A+B, flour T-80 was a mixture of the streams A+B+C, flour T-120 was a mixture of the streams B+C and flour T-220 was the stream C.

The soluble protein content was determined using the Lowry method [17] and the protease activity - using the modified Anson method [18], bovine serum albumin was used as the standard substrate, amine nitrogen - using the formol titration method, and reducing substances (RS) - using the Bertrand method [19]. Determination of the fractional composition of proteins according to Osborne: albumins were isolated using distilled water, globulins - using a 10% NaCl solution, prolamines - using 70% ethanol, and glutelins - using a 0.2% NaOH solution. The proteins and the products of proteolysis of triticale flour and bran were fractionated by molecular weight using the gel chromatography method with a column with Sephadex G-75 and Toyopearl gel HW-55F [19].

The following were used as proteolytic and cellulolytic enzymatic preparations: "Neutrase 1.5 MG" - a bacterial metalloproteinase (Zn) produced by *Bacillus amyloliquefaciens*, "Alcalase FG" - a bacterial proteinase produced by *Bacillus licheniformis* (Novozymes, Denmark); "Distizym Protacid Extra" - a fungal protease produced by *Aspergillus niger* (Döhler, Germany), "Protease GC-106" - a fungal protease produced by *Aspergillus oryzae* (Genencor, USA), "Shearzyme 500L" - a purified xylanase produced by *Aspergillus oryzae and Aspergillus aculeatus,* "Viscoferm L" - a balanced mixture of xylanase, β-glucanase, cellulase and α-amylase produced by *Aspergillus aculeatus* (Novozymes, Denmark). All the preparations are recommended for the hydrolysis of biopolymers of grain raw materials [20, 21].

The functional and technological properties were determined using the methods described in [22] and in [23, 24]. The water absorption capacity (WAC) was determined as the amount of water adsorbed by the modified triticale bran after centrifugation. To determine the fat emulsifying capacity (FEC), 50 ml of distilled water was added to the weighed amount of 1 g of modified triticale bran and suspended at 4000 rpm for 1 minute. Then 10 ml of refined sunflower oil was added to the mixture and emulsified for 5 minutes at a rate of 8000 rpm. The obtained emulsion was centrifuged for 5 minutes at 2000 rpm. FEC was calculated as a ratio of the emulsion volume and the overall system volume expressed as a percentage. The emulsion stability (ES) was determined by heating the emulsion for 30 min at 80°C, then cooled and centrifuged at 2000 rpm. ES was calculated as a ratio of the emulsion volume and the overall system volume expressed as a percentage. To determine the fat binding capacity (FBC), the weighed amount was put into a pre-weighed centrifuge tube, 5 ml of refined sunflower oil was added and mixed for 1 minute at 1000 rpm, then centrifuged for 15 minutes at 4000 rpm. The unadsorbed oil was drained, the tubes were weighed and the FBC was calculated as a ratio of the weight of the bound oil to the weighed amount. The foaming capacity (FC) was determined by mixing a weighed amount in 25 ml of distilled water in a graduated cylinder and thoroughly mixed, the volume was made up to 300 ml and shaken for 1 min. FC was expressed as a ratio of a foam height (mm) to a liquid height (%).

The analyses were performed in triplicate, presenting the results as average arithmetic ones. The discrepancy between parallel assays did not exceed 3% of the average arithmetic value with the confidence probability P = 0.95.

RESULTS AND DISCUSSION

Starting to develop methods for enzymatic modification of biopolymers of vegetable raw materials, it is necessary to consider the following main factors: first of all, these are the features of biopolymers of the given vegetable raw materials, the heterogeneity of a substrate, the presence of various kinds of effectors capable of activating or inhibiting both endogenous enzymes and enzymes in the composition of enzyme preparations, the presence concomitant enzymes in addition to the basic activity of enzymes, etc.; secondly, the conditions for enzymatic modification, the main kinetic parameters of enzymatic reactions involving the studied enzyme preparations, which may differ from the kinetic characteristics obtained in the studies of purified enzymes using standard substrates.

At the first stage of the study, the main technological and biochemical characteristics of the study objects were studied, namely, the flour samples formed on the basis of cumulative ash curves and triticale bran (Table 1, 2 and 3).

The flour sample T-60, which is a fraction of the central part of endosperm, and is significantly different in whiteness, ash content, quantity and quality of gluten, had the best technological properties, as shown in Table 1. The obtained data allow to estimate the technological properties of new grades from triticale grain flour as high, with the prevalence of a wheat phenotype. It has been established that triticale grain is characterized by the absence of a significant dependence between the content of gluten and protein, both in grain and in single flour streams. The expected tendency of increasing the protein content in the systems of final grain grinding has been revealed.

Table 1. Quality of the formed triticale flour grades

Flour sample, grade	Moisture content, %	Whiteness, units of RZ-BPL device	Ash content, %	Amount of gluten,%		Quality of gluten, units of GDI
				Crude	Dry	
T-60	12.0	53.75	0.63	22.7	8.24	70 I – sufficient
T-70	12.1	49.75	0.72	21.0	7.96	66 I – sufficient
T-80	12.1	42.2	0.85	21.7	8.20	66 I – sufficient
T-120	11.7	29.95	1.14	15.8	6.10	57 I – sufficient
T-220	11.3	-8.675	1.99	0.4	0.08	89 II – satisfactory weak

Table 2. Chemical composition of new grades of triticale flour

Flour sample, grade	Protein (N×6.25), %	Starch, %	Fat, %
T-60	10.14	82.28	1.00
T-70	12.23	81.11	1.14
T-80	16.84	77.68	1.25
T-120	17.65	75.60	1.60
T-220	24.88	47.34	2.90

Table 2 presents the analysis of the total content of the main grain biopolymers in the formed grades of triticale flour.

The data presented in Table 2 show that the studied samples, especially the sample T-220, despite a high protein content, are characterized by low baking qualities, as evidenced by trial laboratory baking [1], but can be used as valuable food ingredients.

The study of the quantitative ratio and properties of various fractions of soluble grain albumins is, along with theoretical interest, of great practical interest for the technologies that use grain as the main raw material. Despite the fact that the separation of protein substances by solubility is rather relative, nevertheless, it is used quite widely at the present time. However, there are a lot of questions that remain unclear to this day. This is due, most often, to a difference in the methodological approach of different researchers.

The study of the fractional composition of the soluble proteins of the formed grades of triticale flour showed that the samples of T-60 and T-70 differ in the lowest content of albumins and globulins, but the highest content of prolamins and glutelins that are concentrated in endosperm and form gluten. The main part of albumins and globulins is found in the samples T-120 and T-220, this is apparently due to the presence of the refined germ and the aleuron layer in the flour samples. In the sample T-80 flour, the percentage of all fractions is approximately the same and is 20–25%, the given sample has been formed by mixing 3 main flour streams, which are characterized by a different composition of anatomical parts of the grains (Table 3).

Table 4. Proteolytic activity of the formed grades of triticale flour

Flour sample, grade	Protein, mg/ml	Proteolytic power (PP)	
		Acid proteinases, units of PP/mg of protein	Neutral proteinases, units of PP/mg of protein
T-60	0.080	0.60	0.85
T-70	0.080	0.80	1.20
T-80	0.100	1.40	1.80
T-120	0.160	1.40	2.10
T-220	0.400	0.80	1.00

It is known that proteolytic enzymes play an important part in the processes that proceed in grain when stored and processed. The flour obtained by effecting the grain, violating its integrity and, to a certain extent, by destroying the cellular structure, is a completely different object of study from a biochemical point of view. The object in which the oxidative and hydrolytic processes are primarily activated, including the processes related to the proteolysis of endogenous proteins.

The proteolytic enzymes of triticale grain and triticale flour have been studied poorly [25], much less than the parent proteases - that of wheat [26, 27] and rye [28]. The studies carried out at the Federal State Budgetary Scientific Institution "All-Russian Research Institute of Grain and Its Processing Products" on the p0roteolytic enzymes of triticale grain, revealed the presence of three types of proteinases that actively hydrolyze bovine serum albumin (a standard substrate) and self-proteins: acid proteinases with an optimum pH of 3.5; neutral proteinases with an optimum pH of 6.5 and alkaline proteinases with an optimum pH of 9.5 [29].

Table 4 presents data on the activity of acidic and neutral proteinases of the formed grades of triticale flour. The proteases were extracted as described in the paper [29]. Determination of protease activity using the modified Anson method.

Table 3. Fractional composition of soluble proteins of the formed grades of triticale flour

Flour sample, grade	Fractional composition of proteins,% of the total protein content				
	Albumins	Globulins	Prolamins	Glutelins	Insoluble residue
T-60	11.05	17.82	39.25	28.08	3.80
T-70	12.00	18.14	36.78	26.64	6.44
T-80	20.58	22.24	25.68	23.47	8.03
T-120	72.02	12.04	4.08	3.50	8.30
T-220	43.79	28.95	12.53	6.78	7.95

Table 5. Biochemical composition of triticale grain and triticale bran of different grades

Grade name	Protein (N×6.25), %		Starch, %		Reducing sugars, %		Fiber, %	
	grain	bran	grain	bran	grain	bran	grain	bran
Topaz	7.6	18.11	68.1	24.84	0.20	12.35	2.08	12.35
Skolot	14.5	18.81	62.6	25.25	0.20	14.85	2.40	14.85
Donslav	14.2	15.90	64.8	28.26	0.24	14.42	2.46	14.42
Vocaliz	12.5	17.06	66.4	32.72	0.28	14.68	2.40	14.68

Table 6. Fractional composition of triticale bran proteins, % of the total protein content

Grade name	Albumins	Globulins	Prolamins	Glutelins	Insoluble residue
Topaz	36.8	24.0	9.6	14.0	20.4
Skolot	38.6	22.2	10.2	14.6	19.8
Donslav	34.0	22.6	9.8	14.8	20.4
Vocaliz	38.0	22.4	10.0	14.6	20.0

The analysis of activity of acidic and neutral proteinases in the formed flour grades indirectly indicates that part of the proteolytic activity in triticale grain is related to gluten proteins, but still the highest activity was noted for the samples T-80 and T-120, that is, these are more likely the proteins of the germ and the subaleurone layer. At the same time, the activity of neutral proteases is 1.5–2.0 times higher than that of acid proteases. The value of proteolytic activity in the formed grades of triticale flour is, in addition to other biochemical indicators, of key importance, since proteinases are able to actively hydrolyze their own proteins, including glutens, which ultimately effects the technological process and the finished product. In addition, proteolytic enzymes participate in the regulation of the activity of other enzyme systems, for example, amylases.

The activity of amylolytic enzymes of grain and flour is another important technological and biochemical characteristic that determines along with other indicators the baking advantages of flour. It was estimated using the method for determining a falling number (FN). FN was 294 s for T-60; 266 s for T-70; 272 s for T-80; 245 s for T-120 and 174 s for T-220.

The falling number value for wheat flour at a level of 230–330 s characterizes the normal amylolytic activity of wheat flour, this value for rye flour is about 100 seconds less. The falling number values obtained in the study of triticale flour samples show that the activity of amylases (excluding the flour sample T-220) is similar to the activity of these enzymes in wheat flour, and along with other indicators confirms the predominance of the wheat phenotype in the triticale grain being studied.

Table 5 presents the biochemical composition of triticale bran. The comparative analysis of the main components of triticale grain and bran indicates a regular increase in the content of crude protein in bran - up to 15.90 ... 20.56%, of fiber - up to 10.68 ... 14.85% and a decrease in the starch content up to 32.72 ... 22.62%. The significant increase in sugars in bran fractions compared with whole grains is due, most likely, to the presence of a refined germ [1]. It should also be taken into account that the carbohydrate complex of triticale grain contains a significant amount of insoluble dietary fiber - hemicelluloses (up to 30%) [5].

The analysis of the fractional composition of soluble proteins (Table 6) showed that the proteins of bran from triticale grain differ in a relatively high total content of albumins and globulins, which is generally characteristic of triticale grain proteins, while the number of globulins is 3 to 3.5 times higher than in whole grain (7–8% of the total protein content). When in a dissolved state, they are actively hydrolyzed by endogenous proteolytic enzymes, giving a large number of hydrolysis products with different molecular weights. The content of prolamines is 2–2.5 times lower than in whole grain (23.6–25.0%).

The study of streams of flour from triticale grain allowed to reveal the most promising streams for obtaining advanced processing products [1, 4].

The scheme of advanced triticale grain processing (Fig. 2), which includes the stages of preparing grain for processing, namely: the selection of grain according to certain quality criteria, the formation of mill mixtures, cleaning and hydrothermal treatment and the division into anatomical parts.

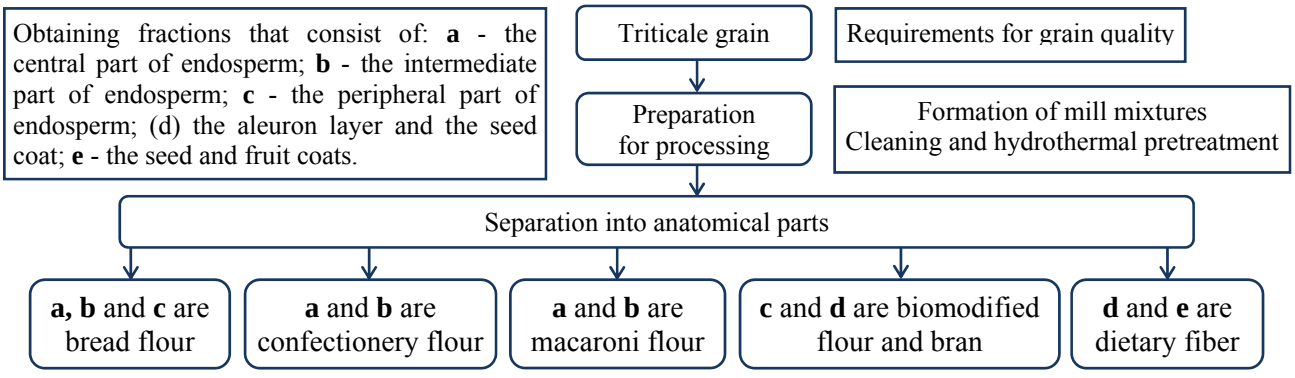

Fig. 2. Scheme of advanced triticale grain processing.

Table 7. Characteristics of the enzyme preparations of proteases during the hydrolysis of triticale flour proteins

Indicator	"Neutrase 1.5MG"	"Alcalase FG"	"Protease GC-106"	"Distizym Protacid Extra"
Initial velocity, V_0 (min)	30	30	30	30
Optimum temperature, °C	50	45	50	40
Optimum pH	5.5	6.5	5.5-6.0	3.5
Optimum amount of enzyme preparation, units of PA/g of flour	0.50	0.5	0.75	0.75
Saturated substrate concentration, mg/cm^3	100	100	100	100

The flour of the samples A, AB and ABC (T-60, T-70 and T-80) was obtained from endosperm and can be used directly in bakery, which was confirmed by trial baking [1], and also after enzymatic modification, as the components of special products that have specific functional and technological properties. The flour of the samples BC and C (T-120 and T-220) from the peripheral parts of endosperm, including the aleurone layer and the seed coat, may be limitedly used in baking, and is mainly a raw material for further processing.

At the second stage of the study, a study was carried out of the effectiveness of proteolytic and cellulolytic enzymatic preparations and the main kinetic parameters of enzymatic reactions in which different types of flour and triticale bran were used as a substrate. The enzymatic modification of proteins of vegetable raw materials, including proteins of grain crops, is an important stage in advanced technologies of advanced processing of grain raw materials. As a result of modification of the protein components of grain and flour with the use of proteolytic enzymatic preparations, hydrolysis products with a certain profile of peptides and a number of amino acids with specific properties can be obtained.

In case of the traditional characteristics of enzyme preparations, the optimum temperature and pH, as well as other kinetic parameters, is detected using a standard substrate [30]. At the same time, in production, in the conditions of a specific food production technology, a complex heterogeneous system acts as the latter, which leads to a change in the basic kinetic parameters of the enzymatic reaction. The composition of the grain substrate can effect the course of the proteolysis process and change the optimum values of temperature and pH [20].

Table 7 presents the main kinetic characteristics of the enzymatic reaction of hydrolysis of triticale flour proteins using bacterial and fungal proteolytic enzyme preparations. The hydrolysis was carried out at the optimum pH and temperature for 30 minutes. It has been previously established that the reaction is zero order for 30 min. The enzyme preparations were added in the amounts from 0.25 to 1.5 units of PA/g of flour, the substrate concentration varied from 20 to 120 mg/ml.

Taking into account the complex structure of the cell wall (the main component of bran), enzyme preparations with a whole complex of activities are required to degrade it and increase the degree of protein extraction: cellulase, hemicellulase and pectolytic activity [31].

Table 8. Characteristics of the enzymatic preparations "Shearzyme 500 L" and "Viscoferm L" when effecting the non-starch polysaccharides of triticale bran

Indicator	"Shearzyme 500 L"	"Viscoferm L"
Initial velocity, V_0 (min)	30	30
Optimum temperature, °C	50	50
Optimum pH	5.5	3.5
Optimal amount of enzyme preparation, units/g of bran	0.3 units of xylanase ability/g of bran	0.4 units of cellulolytic ability/g of bran

Table 8 presents the characteristics of the enzymatic reaction of hydrolysis of non-starch polysaccharides of triticale bran when effected by the enzymatic preparations "Shearzyme 500 L" and "Viscoferm L". The composition of the incubation mixture is the following: milled triticale bran and water (the hydromodule is 1 : 10), a phosphate-citrate buffer 0.1 M (20% of volume) and an enzyme preparation with the activity from 0.1 to 0.5 activity units/g of bran. It has been established that the reaction is zero order for 30 min. The optimum temperature and pH were revealed when studying the activity of the enzyme preparations under study in the range of 20–70°C and pH of 3.0–6.0. The hydrolysis efficiency was estimated by RS accumulation using the Bertrand method.

Similar results were obtained using the flour samples T-120 and T-220 as a substrate. Thus, optimal conditions for the hydrolysis of non-starch polysaccharides of triticale bran and flour with a high content of peripheral parts of grains using the enzymatic preparations "Shearzyme 500 L" and "Viscoferm L" were selected.

The enzymatic hydrolysis of triticale bran proteins using enzyme protease preparations was carried out under the following conditions: the enzyme preparations "Neutrase 1.5 MG" and "Distizym Protacid Extra" were applied in the amounts from 0.25 to 1.5 units of PA/g of bran; the substrate concentration varied from 20 to 120 mg/ml (Table 9).

Table 9. Characteristics of the enzyme preparations "Neutrase" and "Distizym Protacid Extra" when effecting triticale bran proteins

Indicator	"Neutrase 1.5 MG"	"Distizym Protacid Extra"
Initial velocity, V_0 (min)	30	30
Optimum temperature, °C	50	40
Optimum pH	5.5	3.5
Optimal amount of enzyme preparation,units of PA/g of bran	0.50	0.75
Saturated substrate concentration, mg/cm^3	100	100

To estimate the efficiency of the studied enzyme preparations, the enzymatic hydrolysis was carried out under the optimal conditions, which were selected experimentally. The incubation mixture consisted of triticale bran, water (the hydromodule is 1 : 10), the appropriate buffer (20% of volume) and an enzyme preparation based on the final concentration of the corresponding optimum. Sampling was carried out every 30 minutes for 2 hours, the samples were transferred to centrifugal glasses and centrifuged at 6000 rpm for 10 minutes. The supernatant was used to determine the reducing sugars (reducing substances) using the Bertrand method and the amount of soluble protein using the Lowry method.

The hydrolysis efficiency was estimated by the accumulation of RS and soluble protein. The results are shown in Fig. 3 and 4. It has been shown that the enzymatic preparation "Shearzyme 500 L" increases the amount of RS and soluble protein by 2 times; and the preparation "Viscoferm L" increases the amount of RS by 1.5 times and the amount of soluble protein by 2.5 times. The obtained data indirectly indicate the possibility of a significant increase in the nutritional value of secondary products of grain triticale processing.

The flour, obtained from different parts of endosperm, was modified using the multienzyme compositions (MEC) based on bacterial and fungal microbial enzyme protease preparations.

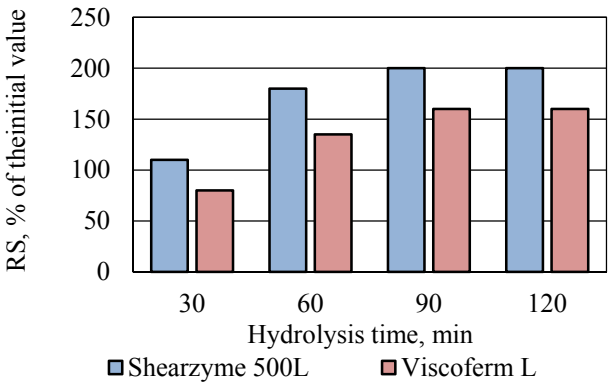

Fig. 3. Accumulation of RS during the hydrolysis of nonstarch polysaccharides of triticale bran using the preparations Shearzyme 500L and Viscoferm L.

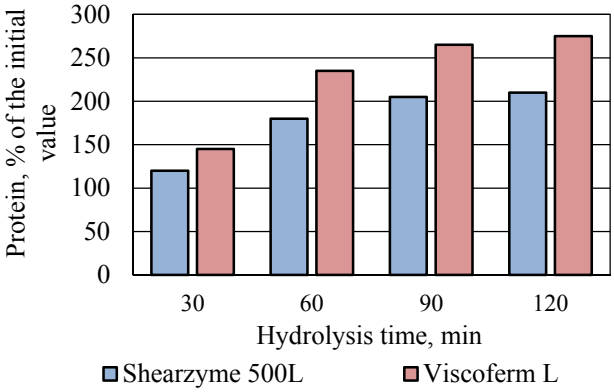

Fig. 4. Accumulation of soluble protein during the hydrolysis of non-starch polysaccharides of triticale bran using the preparations Shearzyme 500L and Viscoferm L.

The enzymatic hydrolysis of triticale flour when effected by the preparations "Neutrase 1.5 MG" and "Protease GC-106" was carried out for 2 hours. The suspension was then centrifuged at 6000 rpm for 15 minutes. 5 ml of supernatant was applied to a column filled with the gel Sephadex G-75. The elution was carried out using distilled water. The volume of aggregated fractions is 4 ml. The optical density of the eluate in the fractions was registered with a wavelength of 280 nm.

A water extract of triticale flour was used as a control sample. The elution profiles are shown in Figure 5.

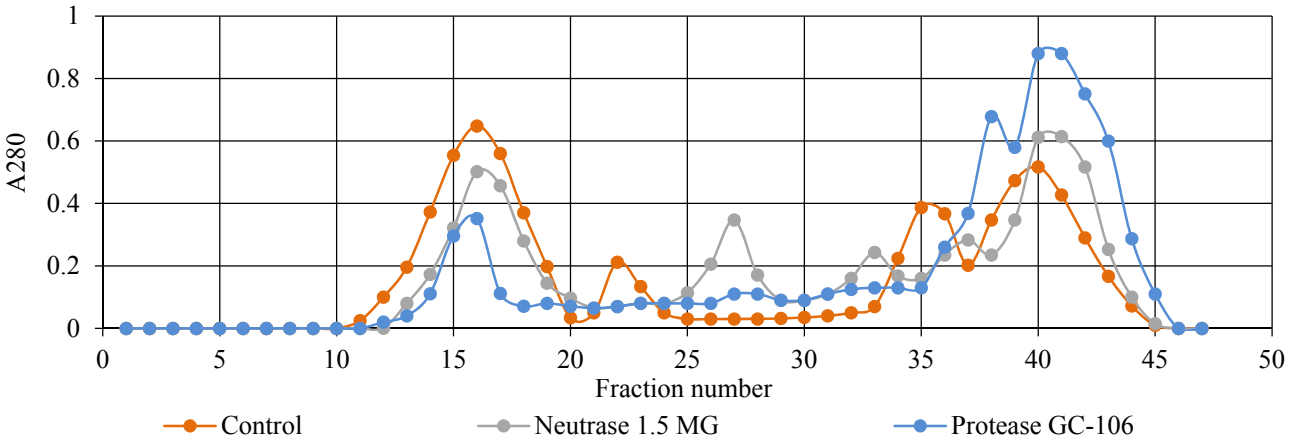

Fig. 5. Fractionation of the products of triticale flour proteolysis using preparations of microbial proteases on a column with Sephadex G-75.

Table 10. Fractionation of the products of proteolysis of triticale flour proteins

Fraction	Molecular weight, Da	% of the total		
		Control	"Neutrase 1.5 MG"	"Protease GC-106"
11–20	≥ 70000	42.71	24.76	19.10
21–25	40000 ÷ 30000	6.49	5.30	4.02
26–32	30000 ÷ 20000	3.36	20.50	7.68
33–36	20000 ÷ 10000	14.18	10.45	6.98
37–45	≤ 3000	34.12	38.91	62.14

Table 10 presents data on the molecular weight, the products of proteolysis of triticale flour proteins formed when applying the preparations of bacterial and fungal proteases, and the percentage of different fractions.

The comparative analysis of the elution profiles presented in Figure 5 and the data of Table 10 shows that the application of preparations of bacterial and fungal proteases does not only change the ratio of high, medium and low molecular weight proteolysis products, but also largely changes the pattern of elution: the nature of distribution of the proteolysis products formed as a result of the use of different preparations is completely different in fractions.

Thus, in case of the enzymatic hydrolysis of triticale flour proteins using the preparation "Neutrase 1.5 MG", there is a decrease in the high-molecular fraction (with a molecular weight of more than 70000 Da) by 42.03%, then, when effected by the preparation "Protease GC-106", - by 55.28%. The increase in the low molecular weight fraction (the molecular weight is less than 3000 Da) is 16.51% and 35.21%, respectively.

When using "Neutrase 1.5 MG", the amount of the formed medium molecular weight peptides with a molecular weight from 30000 to 20000 Da is approximately 2.5–3 times higher as compared to "Protease GC-106"; in turn, when effected by "Protease GC-106", the amount of low-molecular peptides (the molecular weight is 20000 ÷ 10000 Da) is 5.8 times higher than when effected by "Neutrase 1.5 MG".

Table 11. Fractionation of products of proteolysis of triticale flour proteins obtained using MEC

Fraction	Molecular weight, Da	% of the total	
		Control	MEC
11 – 20	≥ 70000	33.56	5.36
21 – 25	40000 ÷ 30000	8.54	4.82
26 – 32	30000 ÷ 20000	14.01	18.94
33 – 36	20000 ÷ 10000	4.57	30.92
37 – 45	≤ 3000	39.29	40.01

On the basis of the studies carried out, multitalzyme compositions have been compiled to obtain products of proteolysis of triticale flour proteins with a different degree of hydrolysis, and, consequently, with various functional and technological functions [32].

The use of MEC, which includes proteolytic enzymes with a different specific effect (the bacterial protease preparations "Neutrase 1.5 MG" and "Alcalase FG" and the fungal protease preparation "Protease GC-106"), allowed to hydrolyze proteins almost completely, as evidenced by this fractionation of products of triticale flour proteolysis using the gel chromatography method on a column with Sephadex G-75 (Fig. 6).

Thus, there are practically no high molecular weight fraction with a molecular weight of more than 70,000 and fraction with a molecular weight of 40,000–30,000 Da, while the amount of low molecular weight peptides and amino acids in the hydrolyzate has increased approximately by 2.5–3.0 times in comparison with the control sample.

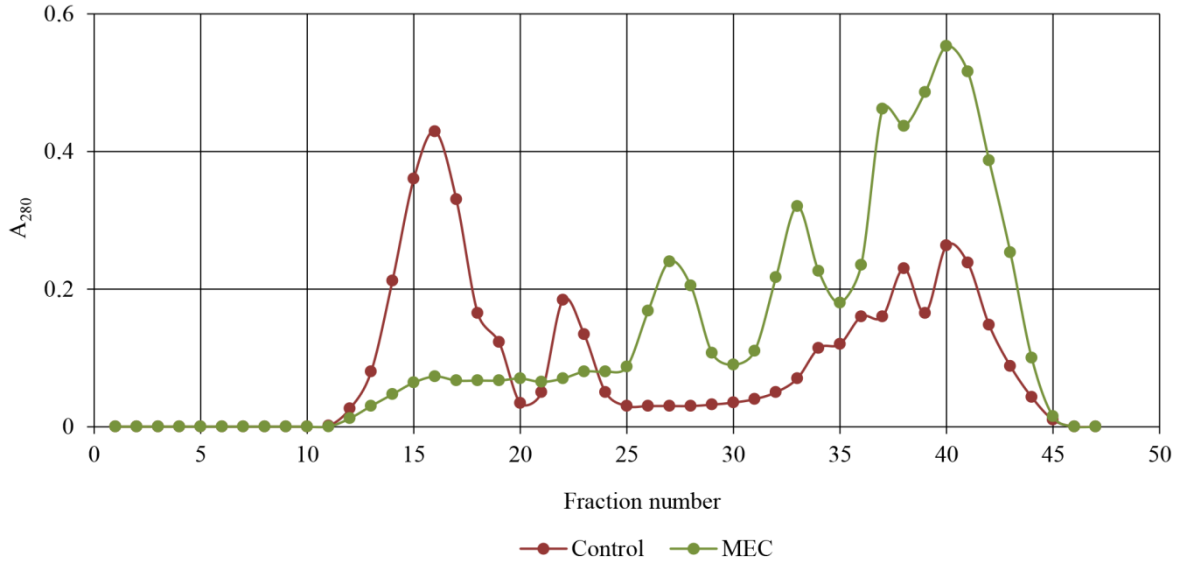

Fig. 6. Fractionation of products of proteolysis of triticale flour proteins obtained using MEC on a column with Sephadex G-75.

The obtained data allowed to position the hydrolyzate obtained with the use of MEC on the basis of enzyme protease preparations as a possible component of hypoallergenic and gluten-free products used for the therapeutic and prophylactic purpose.

Bran and flour with a high content of peripheral parts containing a large number of non-starch polysaccharides, in turn, were modified using MEC based on cellulolytic and proteolytic enzymatic preparations. As a result, products of enzymatic modification of flour and bran from triticale grain with a different degree of hydrolysis of proteins and non-starch polysaccharides and various functional and technological properties have been obtained [21, 31].

The composition of 2 multi-enzyme compositions used for the enzymatic modification of triticale bran and flour with a high content of peripheral parts included: "Shearzyme 500 L" + "Neutrase 1.5 MG" (MEC-1) and "Viscoferm L" + "Dystizym Protacid Extra" (MEC-2). The choice of enzyme preparations is caused by various specific effects and approximately the same effect optima: the optimum temperature is 50°C; pH is 5.5–6.0 for MEC-1 and 40°C; pH is 3.5 for MEC-2. The hydrolysis was carried out in 2 stages. At the first stage, a cellulolytic enzyme preparation was applied. At the second stage, a proteolytic enzyme preparation was applied. The dosage of enzyme preparations, the substrate concentration and the duration of each stage were selected experimentally [4]. Figures 7, 8 and Table 12 present the results of fractionation of the products of proteolysis using the gel chromatography method on a column with Toyopearl gel HW-55F.

The obtained experimental data on the kinetics of enzymatic reactions of hydrolysis of biopolymers of a grain substrate (different types of flour and triticale bran); the degrees of hydrolysis and the ratio of fractions with different molecular weights using the gel chromatography method on a column with Toyopearl gel HW-55F have formed the basis for the development of biotechnological methods for modifying the products of triticale grain processing.

The developed methods for modifying the products of triticale grain processing include the following stages:
– the preparation of a suspension - triticale flour, bran: water (the hydromodule is 1 : 4);
– the preparation of solutions of enzyme preparations; the creation of MEC;
– the enzymatic hydrolysis using MEC under the developed conditions (the substrate concentration, the dosage of enzyme preparations, the optimum temperature and pH);
– the inactivation of enzyme preparations; the product being obtained is hydrolyzed flour or bran (an unclarified hydrolyzate);
– centrifugation;
– the product being obtained is a hydrolyzate (a supernatant) and paste (a precipitate);
– drying;
– the product being obtained is a dry hydrolyzate and hydrolyzed flour and bran;

To estimate the possibility of using the products obtained in food branches, their functional and technological properties have been studied.

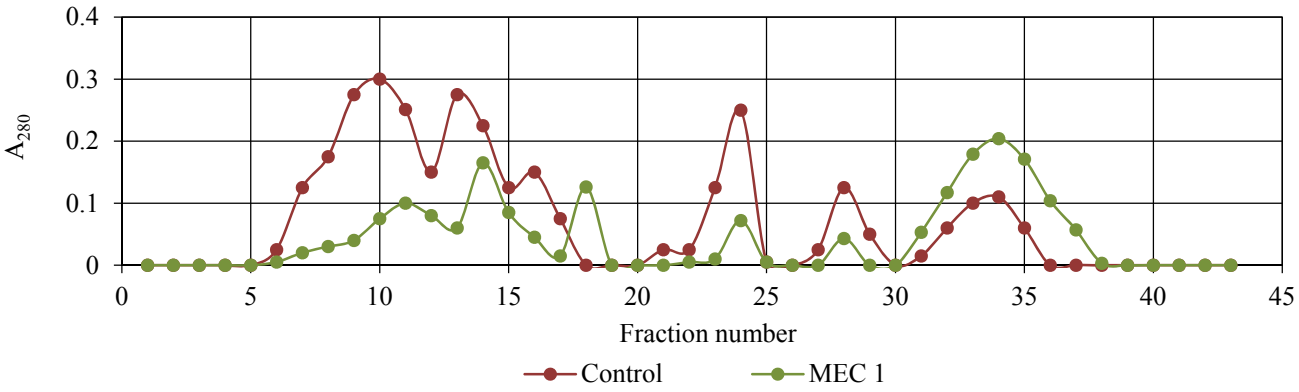

Fig. 7. Fractionation of the products of proteolysis of triticale bran proteins of MEC-1 using the gel chromatography method on a column with Toyopearl gel HW-55F.

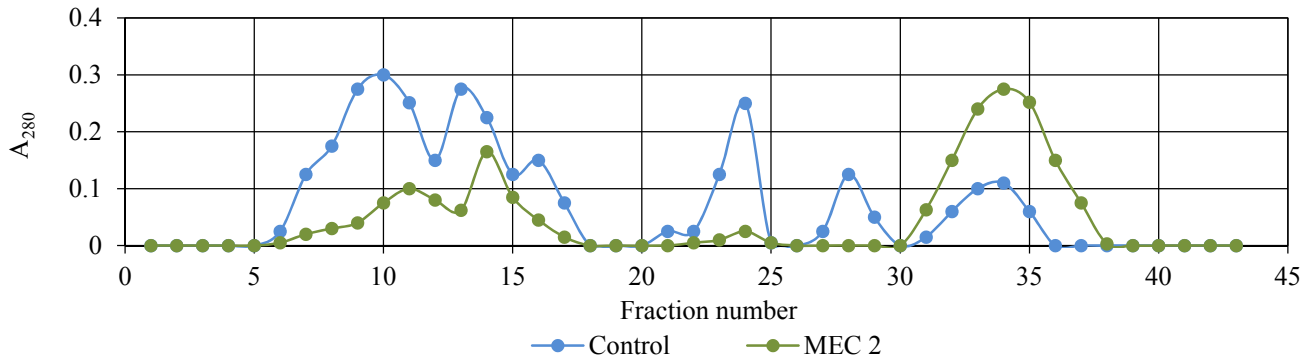

Fig. 8. Fractionation of the products of proteolysis of triticale bran proteins of MEC-2 using the gel chromatography method on a column with Toyopearl gel HW-55F.

Table 12. Fractionation of the products of proteolysis of triticale bran proteins using MEC

Fraction	Molecular weight, Da	% of the total		
		Control	MEC-1	MEC-2
Peak I 6 – 13	≥ 700000 (blue dextran yield)	35.81	2367	19.55
Peak II 14 – 15	450000 ÷ 350000	13.26	14.79	12.62
Peak III 16 – 19	300000 ÷ 100000	9.95	26.04	3.20
Peak IV 20 – 22	100000 ÷ 50000	13.26	0	0
Peak V 23 – 26	50000 ÷ 25000	10.08	5.02	1.77
Peak VI 27 – 30	25000 ÷ 1500	5.31	2.54	0
Peak VII 31 – 36	≤ 1000 (tyrosine yield)	12.33	51.06	62.63

Table 13. Functional properties of the modified triticale bran

Sample*	WBC, g/g	FBC, g/g	FAC , %	ES , %	FFC, %	FS, %
Control - C 1	1.56	1.32	52	58	50	32
Experiment 1 - E1	1.80	1.50	62	53	59	28
Experiment 2 - E2	1.20	1.40	56	46	42	24

Note. * Control C 1 - bran; Experiment 1 - bran + MEC1; Experiment 2 - bran + MEC2

A wide range of physico-chemical characteristics that determine the behavior in heterogeneous food systems during processing, storage and consumption, and also provide the desired structure, technological and consumer properties of food products are to be meant by the functional and technological properties of proteins and protein preparations. Vegetable proteins, as well as proteolysis products with various values of molecular weight, can act as the ingredients of general-purpose, treatment-and-prophylactic and special food products. This is due to the inherent unique functional properties [33]. Depending on the amino acid and fractional composition, molecular weights, the presence of charged and uncharged groups, hydrophilic and hydrophobic groups and other structural features, proteins can serve as gelling agents, foaming agents and form and stabilize suspensions and emulsions, etc. [34, 35].

The requirements for the functional properties of proteins are specific for a certain scope and type of product. For example, when making meat products, the most important are the water- and fat-retaining abilities, gelling, the emulsifying and adhesive properties; in bakery - the water-binding, emulsifying and foaming abilities; the main criterion for choosing a protein preparation in the production of beverages is solubility. To solve the problem of the applicability of specific proteins for obtaining various food products, it is necessary to know how their functional and technological properties change depending on a number of physico-chemical factors: the nature and concentration of proteins in the system, the temperature, pH, the presence and concentration of concomitant biopolymers and low molecular weight substances [33, 36].

In some cases, to improve and regulate the functional properties in order to expand the scope of these or other protein preparations, they are modified using physical, chemical, enzymatic and other methods.

The enzymatic method for the modification of vegetable proteins is preferable to physico-chemical modification, since its advantage are soft reaction modes, the ability to regulate the degree of hydrolysis, its specific directivity and the retention of the biological value [32, 33, 37–40].

Tables 13 and 14 present the water binding capacity (WBC); the fat binding capacity (FBC); the fat emulsifying capacity (FEC); the emulsion stability (ES); the foam forming capacity (FFC) and the foam stability (FS) of the modified triticale bran.

The functional properties of bran from triticale grain and the hydrolyzed samples obtained using MEC1 and MEC2 differ from each other. Thus, the water-binding capacity of the hydrolysed bran in the first option increases by 16%, in option 2 - on the contrary, it decreases by 12.6% with respect to the unhydrolyzed triticale bran. The similar pattern can be seen with respect to the foam forming capacity (Experiment 1: an increase of 18.0%; Experiment 2: a decrease of 16.1%). The fat binding and fat emulsifying capacity increases in both experimental options by 13.6% and 6.1% and by 19.2% and 7.7% respectively.

The stability of the emulsion and foam of the modified triticale bran is reduced: ES - by 8.7%; FS - by 12.5% (Experiment 1) and ES - by 20.7%; FS - by 25.0% (Experiment 2).

Similar studies were carried out using flour samples with a high content of peripheral parts (Table 2).

There is a tendency for samples of the flour modified using MEC1 of an increase in WBC by 21.3 ... 26.0%; in FBC by 13.8 ... 16.0%; in FEC by 74 ... 9.0%. There is, on the contrary, a tendency for samples of the flour modified using MEC2 of a decrease in these functional characteristics: in WBC by 11.8 ... 18.3%; in FBC by 6.7 ... 22.3%; in FEC by 3.8 ... 4.0%.

The stability of the emulsion and foam of the modified flour from triticale grain is also reduced, as in the case of the modified triticale bran: ES - by 8.7%; FS - by 13.4% (Experiment 3) and ES - by 20.7%; FS - by 26.7% (Experiment 4); ES - by 9.1%; FS - by 27.3% (Experiment 5) and ES - by 8.0%; FS - by 30.2% (Experiment 6).

Table 14. Functional properties of the modified flour from triticale grain with a high content of peripheral parts

Sample	WBC, g/g	FBC, g/g	FAC, %	ES, %	FFC, %	FS, %
Control - C2	0.56	0.52	50	52	80	65
Experiment 3 - E3	0.67	0.59	54	50	83	55
Experiment 4 - E4	0.54	0.48	48	42	55	43
Control - C3	0.64	0.54	52	55	86	63
Experiment 5 - E5	0.80	0.62	57	50	98	58
Experiment 6 - E6	0.52	0.41	50	46	64	44

Note. * Control C2 - Flour T-120; Experiment 3 - T-120 + MEC 1; Experiment 4 - T-120 + MEC2; Control C3 - Flour T-220; Experiment 5 - T-220 + MEC 1; Experiment 6 - T-220 + MEC2

It is known that the functional properties of the products of enzymatic hydrolysis of protein raw materials depend on the physico-chemical properties of the initial protein, the specificity of the proteases used, the composition of MEC used, the conditions for hydrolysis, the degree of hydrolysis and the ratio of the fractions of proteolysis products with different molecular weights [36, 37].

The revealed differences in the functional properties in the initial and modified products of triticale grain processing are related, first of all, to the conditions for enzymatic modification (of the pH medium), the composition and specific effect of the enzymes that are part of the composition of MEC; obtaining products of various degrees of hydrolysis, and the number of high-, medium- and low-molecular compounds; an increase or decrease in free polar (charged) aggregations, hydrophilic and/or hydrophobic groups, providing interactions with different types of substances.

The obtained results indicate that the use of MEC on the basis of cellulolytic and proteolytic enzyme preparations allows for an advanced destruction of proteins of the products of triticale grain processing; to obtain products with various degrees of hydrolysis and the ratio of components by molecular weight, which leads to a change in the functional and technological properties of the initial flour and will allow to find its new scopes in food products. Thus, the samples with the pH values close to the neutral ones (modified using MEC1), taking into account the values of the foam forming and fat emulsifying capacities, can be used in foam-emulsion systems, bakery products, cakes and biscuits. The samples with low pH values (modified using MEC2), taking into account their functional properties, can be used to enrich fruit beverages, fermented milk products, salad dressings, sauces, etc. At the same time, it should be taken into account that with low pH values the rate of the Maillard reaction significantly decreases, which can have both negative and positive effects depending on the specific food technology, namely: the retention or reduction of the amount of amino acids and reducing sugars; the formation of melanoidins and a complex of aromatic compounds.

CONCLUSION

In general, the proposed technology allows to form various grades of triticale flour (bread, confectionery, macaroni flour, etc.) and cereals such as "semolina"; to carry out advanced processing of triticale bran and flour, including that with a high content of peripheral parts, using biotechnological methods (enzymatic modification); to receive valuable components for the enrichment and creation of new products with the given properties and composition, contributing thereby to the expansion of not only the raw material base, but also the range of the output products.

The studies carried out have shown that the functional and technological properties of the modified products of triticale grain processing finally depend on the specificity of enzyme preparations and the composition of MEC. The use of MEC on the basis of preparations of microbial proteases allows to hydrolyze triticale flour proteins almost completely, and to position the obtained hydrolyzate as a possible component of hypoallergenic and gluten-free flour products.

The use of cellulolytic and proteolytic enzyme preparations in the hydrolysis of biopolymers of triticale bran allowed to increase the amount of reducing substances (reducing sugars) by 1.5–2.0 times, soluble protein - by 2.0–2.5 times, and the use of MEC on their basis showed that the obtained hydrolysates have a high degree of hydrolysis of non-starch polysaccharides and proteins, a specific ratio of high-, medium- and low-molecular weight peptides and amino acids.

To solve the issue of the applicability of specific products whose proteins are modified, it is necessary to know in various food technologies not only a chemical composition, but also functional and technological properties. The obtained experimental data on the study of the water binding, fat binding, fat emulsifying and foam forming capacities, as well as the emulsion stability and foam stability of the modified triticale bran and flour with a high content of peripheral parts with the use of 2 multi-enzyme compositions showed that enzymatic modification leads to certain changes in the functional and technological properties of the initial flour and bran from triticale grain; and allow to find new and more rational scopes of the modified products as enrichers and as functional and technological components. Thus, the samples with the pH values close to the neutral ones (modified using MEC1), taking into account the values of foam forming and fat emulsifying capacities, can be used in the production of bakery products, cakes and biscuits. The samples with low pH values (modified using MEC2), taking into account their functional properties, can be used to enrich fruit beverages, fermented milk products, salad dressings, sauces, etc. The results of the studies have formed the basis for the development of methods for the enzymatic modification of triticale flour and bran. Hydrolysates, structurally modified flour and biomodified bran, which can be used in the production of a wide range of general-purpose, functional and treatment and prophylactic food products have been obtained.

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