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Effects of α-amylase, endo-xylanase and exoprotease combination on dough properties and bread quality

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Abstract. The enzymes composition is an actual alternative to chemicals to improve functional properties of flours and to generate changes in the structure of the dough and bread quality. The objective of this study was to analyze the individual and synergistic effects of enzymes preparation (α-amylase, endo-xylanase and exoprotease), newly produced in Russia, on dough properties and bread quality made from wheat flour with different amylolytic activity. Reofermentometric results revealed decreases in gas-forming capacity of dough by 10.0–13.9% when single α-amylase preparates were used. The α-amylase addition had significant effect on gas retention coefficient in flour possessed low amylolytic activity. The effect of endo-xylanase and exoprotease on hydration and amount of wheat gluten was established. The fractional composition of gluten proteins in the dough made with combination of endo-xylanase and exoprotease was established using Lowry method immediately after kneading and after fermentation. It was found that mainly water-soluble, alcohol-soluble and alkaline-soluble proteins were undergone by transformation. The bread with enzymes had a higher specific volume, porosity and aldehyde content and lower shape stability indicator than the control bread made without enzymes. Bread with enzymes was characterized by tenderer and not crumbly crumb with developed thin-walled uniform porosity compared to the control. The crusts were more brightly colored. The combined usage of α-amylase and endo-xylanase and exoprotease retarded bread staling during 5-day storage period. New enzyme composition may be a potentially strong candidate for future applications in the bread-making industry.

Key words: alpha-amylase, bread quality, dough, endo-xylanase, enzymes, exopeptidase.

INTRODUCTION

Functional properties and enzymatic activity of flours greatly depend on diverse factors such as wheat variety and growing conditions. Enzymes preparation usage is alternative to chemical compounds which are used in bread making as a way of adjusting the variations in flour properties. Chemical improvers have been a common way of flour quality compensation for many years (Cauvain & Young, 2007). But last decades many

of chemicals are associated with health hazards, for example, azodicarbonamide may cause allergic reactions (Arts, & Kimber, 2017), semicarbazide produced from azodicarbomide and potassium bromate may cause some forms of cancer (Kornbrust et al., 2012; Ibrahim et al., 2019; Shanmugavel et al., 2019). The replacement of chemical dough improvers by enzymes allows production of safety products. The enzymes composition also allows generating changes in the structure of the dough and improving bread quality, but they are generally recognized as safe (GRAS) and do not remain active in the final product after baking. Therefore, enzymes do not have to mark on the label, which is an additional commercial advantage.

Nowadays a wide range of enzymes is available for bread-making industry. A variety of aims may be pursued by enzyme addition. Enzymes provides improved dough handling and technological process tolerance, increased bread specific volume, finer crumb structure, softer crumb, extended shelf life (Codina & Leahu, 2009; Goesaert et al., 2009; Ahmad et al., 2013; Kornbrustet al., 2012; Sanz-Penella et al., 2014; Ait Kaki El-Hadef El-Okki et al., 2017).

Amylases have been used in bread-making as standardizing and anti-staling agents of flour (Goesaert et al., 2007; Lagrain et al., 2008; Goesaert et al., 2009; He et al., 2017). The use of α-amylase in bread formulations significantly improves the product quality, including bread volume, texture, firmness, shelf life and flavor (Lagrain et al., 2008; Goesaert et al., 2009; Sahnoun et al., 2013; Sanz-Penella et al., 2014). α-Amylase functions are related to the reduction of dough viscosity during starch gelatinization as a result of the action of the enzyme (Goesaert et al., 2009; Sahnoun et al., 2013; Grewal et al, 2015; Bueno et al., 2016). Amylase decreases the molecular weight of starch polymers and causes the formation of maltodextrins, which contribute to increased crumb softness, improved crumb elasticity and decreased staling rate (Rojas et al., 2001; Hug-Iten et al., 2003; Goesaert et al., 2009; Calvin, O., 2016). Fungal α-amylase could be used to restore loaf volume losses caused by low protein content in flour (Goesaert et al., 2009; Sahnoun et al., 2013). α-Amylase increases the level of fermentable sugars in the dough, thus promoting the fermentation of yeast (Cauvain & Young, 2007) and the formation of Maillard reaction products, which, in turn, intensify bread flavor and crust color (Goesaert et al., 2009; Ait Kaki El-Hadef El-Okki et al., 2017).

The application of xylanase has increased for the last few decades owing to its potential effectiveness in bread making. The xylanase may enhance dough rheological characteristics (extensibility, flexibility, stability), bread quality (crumb structure, texture, porosity), and shelf life (Driss et al., 2012; Cunha et al., 2018; Yegin et al., 2018; Sharma et al., 2020). Endo-xylanases increase dough viscosity and promote an improvement in the dough rheology by increasing the extensibility and malleability of the dough caused by hydrolysis of water-unextractable arabinoxylans (Ahmad et al., 2013). Xylanase acting on water-unextractable arabinoxylans provide more reduction in water absorption (Hardt, Boom & van der Goot, 2014; Bueno et al., 2016; Yegin et al., 2018). Xylanase can convert parts of insoluble polymers into soluble. It affects the water balance between components in the dough, therefore decreasing dough firmness, increasing volume and creating finer and more uniform crumbs. Enhanced water absorption is a desirable property as it leads to reduced dough stickiness and improved dough processing. Dough become more 'machine-friendly' and does not stick to the machinery parts (Autio 2009; Yegin et al., 2018; Both, 2020). Different xylanase preparations have different effects on arabinoxylans in terms of its scission point and

reaction products and therefore, they show different effects on bread making (Bueno et al., 2016; Yegin et al., 2018).

Proteases are used in the production of bread and other baked goods (Melim Miguel et al., 2013; Heredia-Sandoval et al., 2016). Proteases can be subdivided into two major groups according to their site of action: exopeptidases and endopeptidases. Exopeptidases are those proteases that cleave the peptide bond proximal to the amino or carboxy termini of the substrate (cleave N- or C-terminal peptide bonds of a polypeptide chain). Exopeptidase addition helps to achieve a partial gluten hydrolysis for improving rheological properties and machinability. These enzymes can be added to reduce mixing time, to decrease dough consistency, to assure dough uniformity, to regulate gluten strength in bread, to control bread texture and to improve flavor (Goesaert et al., 2009; Melim Miguel et al., 2013).

The wheat-milling and bread-making industries follow the dosages indicated by the enzyme suppliers. But obviously enzyme effects depend on the initial characteristics of the flour (Bueno et al., 2016; Kin & Yoo, 2020). For the best results, enzymes should be used at optimum levels, as the over or under dosage has adverse effects on the bread. Modern investigations have shown that it is a good strategy to use enzymes combination because of the synergistic effects which provide better results as compared to its sole use.

The objective of this study was to analyze the individual and synergistic effects of enzymes preparation (α-amylase, endo-xylanase and exo-protease), newly produced in Russia, on dough properties and quality of bread made from wheat flour with different amylolytic activity.

MATERIALS AND METHODS

Characteristic of ingredients

Wheat flour

Two wheat flour samples obtained from different milling companies located in Russia were used for this study. Flour had different amylolytic activit, i.e. different falling number indicator (FN). It is known, that flours with a high FN (more than 350 s) have low amylolytic activity and a reduced capacity to form fermentable sugars (Codina & Leahu, 2009; Struyf et al., 2016). In this study wheat flour with satisfactory amylolytic activity had FN 290 s (Every & Ross, 1996; Savkina et al., 2020) and wheat flour with low amylolytic activity had FN 440s (Codina & Leahu, 2009; Struyf et al., 2016).

The FN was determined according to ICC approved method 107/1 (1995). The wet gluten quantity and gluten quality were determined according to Russian Standard (State Standard of the Russian Federation, 2013), because this method commonly used all over the Russian Bread making Industry.

The amount of gluten was determined in the following way. Dough was mixed from 25 g of flour and 14 g ofwater, and then it was held for 20 min for hydration and the formation of intra- and intermolecular bonds in substances forming gluten (gliadin and glutenin). Crude gluten was washed by a working body of a mechanized device MOK-1M (Mototeh, Russia) using water to remove water-soluble substances, starch and brans from the dough. The resulting gluten was weighed and the percentage of crude gluten was calculated relative to the mass of the analyzed flour sample.

The gluten quality was determined as the deformation index of raw gluten under the influence of a load (120 g) for a 30 s on a special device `IDK-3M` (Plaun, Russian).

The results were expressed in units of the device IDK. According State Standard (State Standard of the Russian Federation GOST 27839-2013) the gluten quality is divided into the following groups: unsatisfactory strong (less than 32 units of the device), satisfactory strong (33–52 units of the device), good (53–77 units of the device), satisfactory weak (78–102 units of the device), unsatisfactory weak (more 103 units).

The basic quality parameters of wheat flour are presented in Table 1.

Table 1. Quality parameters of wheat flour

Indicators	Wheat flour	
	F1	F2
Moisture content, %	13.6 ± 0.3^{a}	14.8 ± 0.4^{b}
Falling number, s	290 ± 14^{a}	440 ± 18^{b}
Wet gluten,%	28.0 ± 0.9^{a}	24.2 ± 0.9^{b}
Gluten deformation index, units of device IDK	48 ± 3^{a}	51 ± 2^{a}

a–b = Means ± SD within the same row with different lowercase superscript letters are significantly different ($p \leq 0.05$).

Enzymes

The enzyme preparation 'Amiloryzin' (LLC 'Trading House 'Biopreparat', Moscow, Russia) was used in the experiments. Amilorizin is α-amylase from *Aspergillus oryzae* with amylolytic activity 2,500 FAU g^{-1}. The performance of the enzyme preparation from *Aspergillus oryzae* was tested in the bread-making process and compared to a currently commercialized α-amylase Fungamyl 2500 SG ® (Novozymes, Bagsvaerd, Denmark) from *Aspergillus oryzae.*

The enzyme preparation 'Protozym' (LLC 'Trading House 'Biopreparat', Moscow, Russia) was used in the experiment. Protozym is a complex enzyme preparation containing exopeptidase, endo-xylanase and β-glucanase, obtained from *Penicillium canescens*. The proteolytic activity of the preparation is 75,000–10,000 AU g^{-1}, xylanase - 800–1,000 FXU g^{-1}, β-glucanase - 250–270 AU g^{-1}.

Enzymes were used in optimum dosages. In preliminary analysis (data not shown), the best concentration of each enzyme was determined separately. The optimal dosages of Amyloryzin and Fungamyl were: 0.0002% for wheat flour with normal amylolytic activity (FN 290 s) and 0.0004% for wheat flour with low amylolytic activity (FN 440 s). The optimal dosages of Protozym were: 0.003% for wheat flour with normal amylolytic activity (FN 290 s) and 0.006% for wheat flour with low amylolytic activity.

Other ingredients

Potable grade water was used in the study, as well as edible sodium chloride (LLC 'Russol', Russia), pressed baker's yeast (Lesaffre, Russia), local sunflower oil (Bunge Ltd, Russia) and sugar (sucrose, RUSAGRO Group, Russia).

Individual effects of enzymes assessment

α-Amylase assessment

The influence of α-amylase preparation (Amylorysin and Fungamyl) on gas-forming capacity of the dough was determined using a Reofermentometer F3 (Chopin, France). Dough samples with α-amylase preparation were prepared in

accordance with the recipe in Table 2. Commercial enzyme Fungamyl (Novozyme) was used as positive control, and a trial with no enzyme was a negative control.

Dough samples weighing 315 g were placed on the bottom of the drum, preheated to 28.5 °C. Then it was installed on the dough piston and the system lid was tightly closed. The duration of the experiment was 300 minutes. The rise of the dough during the fermentation was estimated by the movement of the piston, which was mounted directly on the dough.

Assessment of enzyme preparations with endo-xylanase and exoprotease activities

The effect of enzyme preparations with combined endo-xylanase and exo-protease activities (Protozym) on hydration and amount of wet and dry gluten was studied in flour with normal amylolytic activity (FN 290 s). The gluten test method is described above. Dough formulation is in Table 2.

The conversion of gluten protein fractions was studied using Lowry method immediately after kneading the dough and after fermentation and proofing of the dough.

Table 2. Formulations of the dough with single enzyme preparation

Ingredients and parameters	Flour with FN 290 s			Flour with FN 440s	
	Control without enzymes	With α-amylase preparation (Amylorysin or Fungamyl)	With Protozym	Control without enzymes	With α-amylase preparation (Amylorysin or Fungamyl)
Wheat flour, g	100				
Pressed yeast, g	2.0				
Salt, g	1.5				
Enzyme, g	-	0.0002	0.003	-	0.0004
Water, g	56				
Process parameters					
Moisture content, %	41.5				

The fractional composition of flour and dough proteins was determined by successive dissolution of a sample in 0.05N NaCl, 70% ethanol and 0.05N NaOH (Puchkova, 2004). A weighed portion of a sample weighing 2–4 g was suspended in 20 cm^3 of 0.05 N NaCl for an hour on a mechanical shaker (PE-6410, Russia), and then left overnight in a refrigerator. Then the dispersion was centrifuged (centrifuge Hettich Rotofix 32A, German) for 15 min at 6,000 rpm and the supernatant was poured into a 100 cm^3 volumetric flask. The precipitate was again poured with the same amount of solvent, and then it was washed, centrifuged (centrifuge Hettich Rotofix 32A, German), and poured into the same volumetric flask. The solution was brought to the mark, and the precipitate was poured with another solvent, and all operations were repeated. The protein was determined in centrifugates according to Lowry (1951), the results were expressed as a percentage of the total content of alkali-soluble proteins in solution.

Bread preparation

The dough was prepared using one-step method. For research, formulation of bread 'Nareznoi' traditional and commonly produced in Russia, Ukraine and Belarus was used. Doughs formulations presented in Table 3. Control doughs were made without enzymes.

Table 3. Formulations of the dough

Ingredients and parameters	Flour with FN 290 s		Flour with FN 440s	
	Control	Amylorysin+ Protosym	Control	Amylorysin+ Protosym
Wheat flour, g	100			
Pressed yeast, g	3.0			
Salt, g	1.5			
Sugar, g	4.0			
Sunflower oil, g	3.0			
Enzyme, g	-	0.0002 + 0.003	-	0.0004 + 0.006
Water, g	53.0			
Process parameters				
Moisture content, %	41.5			
Fermentation time, h	5	5	2.5	5

All ingredients were mixed for 5 minutes and the dough was then fermented for 1 h at 30 ± 1 °C. After fermentation all dough samples were shaped into 450-g round-shaped loaves, placed at aluminium pans, and leavened at 30 °C until the volume was twice the initial volume. The leavened dough was baked in an oven Miwe ideal (German) at 20 °C for 25 min.

Assessment of baked bread

The quality of bread was evaluated by following parameters.

Porosity was determined as the ratio between pore volume and the total volume of products (Puchkova, 2004), specific volume - as the ratio between product volume and mass of whole bread (cm g^{-1}) (State Standard of the Russian Federation GOST 27669-88. 2007).

The diameter D and the height H of the round pan bread was measured in millimeters (Puchkova, 2004). For hearth (pan) bread, the minimum and maximum diameters were measured. The shape stability indicator was counted as the ratio between the height and the diameter -H: D (Puchkova, 2004).

Aldehydes content assessment

There is a large list of volatile compounds reported in wheat bread, including alcohols, aldehydes, esters, ethers, ketones, acids, hydrocarbons, pyrazines, pyrrolines, furans, lactones or sulphur compounds. Volatile aldehydes found in wheat bread (crumb and/or crust) reported in literature their typical odours (Pico et al., 2015).

Method for the determination of aromatic substances in bakery products is based on the binding of aldehydes and ketones with sodium bisulfite (Koryachkina et al., 2010). Aldehydes and reactive ketones can be successfully transformed into charged bisulfite adducts that can then be separated from other organic components of a mixture by the introduction of an immiscible organic layer. 10 g of bread crumb was ground in a mortar with 0.4% sodium bisulfite solution and transferred to a 100 cm^3 flask. Water was added until 100 cm^3 and then flask was shaken for 10 minutes. After that the flask was left for 10 min for sedimentation, after that the precipitate was separated by filtration. 10 $cm^3$of the sediment was taken. Excess sodium bisulfite was first titrated with 0.1 mol dm^{-3} iodine solution until a weak violet-blue color was obtained. If iodine was overdose than necessary, the excess was titrated with 0.01 mol dm^{-3} hyposulfite

solution. The amount of iodine solution spent on the oxidation of excess sodium bisulfite was not taken into account. To destroy the aldehyde sulfite compound, a saturated solution of sodium hydrocarbonate was poured into the reaction liquid until pH ≥ 8. The sodium bisulfite пgenerating due to the sodium hydrocarbonate addition was immediately titrated with 0.01 mol dm^{-3} iodine solution. The titration was considered complete if the violet-blue color wasn`t disappear in a 15 s after stirring. The content of aldehydes is conventionally expressed in cm^3 of 0.1 mol dm^{-3} iodine solution used for titration of bisulfite bound to carbonyl compounds, accounted per 100 g dry matter.

Crumb firmness assessment

Crumb firmness was determined on the same loaves according to method created in Scientific Research Institute for the Baking Industry using a texture analyser, model `Structurometer ST-1M` (Scientific Research Institute for the Baking Industry, Russia). The method is based on determining the compression force value of 36-mm-diamete indenter (with a rounded edge) when it is inserted into a piece of loaf 25 mm thick to a depth of 6.25 mm (with a movement speed of 1 $mm \cdot s^{-1}$ with an initial contact force of 5 g) and on the establishment of the final loading force on the indenter. Then its reverse movement is carried out up to an effort of 5 g. The method for assessing the degree of staleness of baked products allows determining the structural and mechanical properties of the crumb of bread, which can act as a measure of the quality and freshness of products. The index of the final loading force on the indenter can be taken as an index of the softness of the crumb. Baked bread loaves were bagged after cooling for 180 min and kept at room temperature for 5 day. The crust and first two slices of bread were discarded. Eight readings were done for each loaf of bread (Chernykh & Maksimov, 2004).

Assessment of sensory characteristics

A panel of 10 non-specialists was used to evaluate the sensory characteristics of the bread produced. Then, they were asked to evaluate separately crust (shape, surface, colour) and crumb (colour, odour, taste, taste, chewiness and porosity). The ranking scale ranged from 1 to 5 (5–like extremely, 4.5–like very much, 4–like moderately, 3.5–like slightly, 3–neither like nor dislike, 2.5–dislike slightly, 2–dislike moderately, 1.5–dislike very much, 1–dislike extremely).

Statistical analysis of the data

All of the experiments were carried out a total of five times. Statistical analysis was performed using Excell software. Comparison of the influence of factors was carried out by the method with significance tested at the 95% confidence level and differences among means were determined using the least significant difference and Duncan's test of two-factor analysis of variance with one repetition (ANOVA). The confidence intervals shown in the histograms and in the table reflect the accuracy of the used methods.

RESULTS AND DISCUSSION

Individual effects of enzymes on wheat flour proprieties

Before evaluating the combined effect of new enzymes on the dough, the effect of each enzyme on flour quality was studied.

Influence of α-amylase on wheat flour

The effect of α-amylase on the gas-forming ability of wheat flour with different amylolytic activity was studied. Reofermentometric characteristics of dough samples are presented in Table 4.

The effect on the gas-forming capacity of dough was studied (Table 4). The total volume of released CO_2 was higher by 10.0–13.9% when the enzymes were added. It may be due to the fact, that fungal amylase affects the maltose generation from the starch (Van der Maarel et al., 2002; Goesaert et al., 2009; Codina & Leahu, 2009; Struyf et al., 2016). Yeast produces carbon dioxide from maltose because it is fermentable sugar (Cauvain & Young2007).

The gas retention coefficient was comparable in samples with Amylorysin and positive control with Fungamyl. And it was lower compared the negative control by 16–19.8% for flour with FN 290 and by 3.2–5.8% for flour with FN 440s. The decrease of gas retention coefficient also may be due to a fact that α-amylase reduces dough extensibility and stability because of the gluten protein alteration due to the high amounts of amylase-reducing polysaccharides (Sahnoun et al., 2016). In flour with different amylolytic activity the degree of the process was different.

Table 4. Influence of enzymes on the rheological characteristics of wheat flour

		Total volume of released CO_2, cm^3	Gas retention coefficient, %
Flour with FN 290 s+0.0002% of enzyme	Negative control	1,819.0 ± 88.0^a	85.2 ± 4,2^a
	Positive control (with Fungamyl)	2,029.0 ± 97.0^b	69.7 ± 3,2^b
	Amylorysin	1,987.0 ± 55.0^b	68.3 ± 3,2^b
Flour with FN 440 s +0.0004% of enzyme	Negative control	1,606.0 ± 71.0^c	80.7 ± 3,4^c
	Positive control (with Fungamyl)	1,797.0 ± 58.0^d	78.1 ± 3.6^d
	Amylorysin	1,829.0 ± 88.0^d	76.0 ± 1.3^d

a–d = Means ± SD within the same row with different lowercase superscript letters are significantly different ($P \leq 0.05$).

Influence of Protozyme and Pentopan on gluten

The combined effect of endo-xylanase and exo-peptidase on the on the amount and property of gluten and the content of the protein fraction was studied (Table 5).

After kneading the dough, the amount of wet gluten in the dough made with enzymes usage was similarly to the control without enzymes.

After fermentation, content of raw gluten was higher in the samples with enzymes than in the control. The water absorption capacity in the control sample was worser compared to the initial. In the sample with Protozym, on the contrary, it became better. It can be assumed that it was due to the action of exo-protease, which is part of Protozym. An increase in gluten content and an improvement in its hydration capacity when using endo-xylanases and exo-peptidases confirm the data obtained by other authors (Yegin et al., 2018; Both et al., 2020). Endo-xylanases cause the hydrolysis of insoluble arabinoxylans to form water-soluble arabinoxylans. At the same time, the water absorbed by insoluble arabinoxylans is released and redistributed between the structural components of the dough, mainly between gluten proteins and pentosans. Thus, endo-xylanases contribute to the creation of a continuous structure and strengthening of gluten (Autio, 2009; Filipcev et al., 2014; Yegin et al., 2018).

Table 5. Influence of Protozym on wheat gluten in the dough

Indicators	Indicators of gluten in dough			
	Immediately after kneading		After 2.5 h fermentation	
	Control	Protozym	Control	Protozym
Wheat gluten, %	27.90 ± 0,56[a]	28.10 ± 0.80[a]	23.3 ± 0.26[b]	24.0 ± 0.31[c]
Gluten deformation index, units of device IDK	47.0 ± 2.0[a]	47.0 ± 1.0[a]	30.0 ± 1.0[b]	24.0 ± 2.0[b]
Water absorption capacity, %	166.7 ± 0.8[a]	167.8 ± 1.3[a]	153.4 ± 1.2[b]	174.5 ± 1.2[c]
Dry gluten, %	8.8 ± 0.2[a]	9.0 ± 0.3[a]	7.2 ± 0.2[b]	7.7 ± 0.2[c]

a–c = Means ± SD within the same row with different lowercase superscript letters are significantly different ($P \leq 0.05$).

The Protozym affects the amount of protein fractions in the dough after kneading in different degrees (Table 6). After kneading, the amount of water-soluble, salt-soluble and alcohol-soluble proteins decreased in all samples in comparison with their amount in flour. The amount of alkali-soluble proteins, on the contrary, increased. The results obtained indicate that when the dough is kneaded, a part of water-soluble, salt-soluble, alcohol-soluble proteins aggregates with the alkali-soluble proteins formation. It confirms data that xylanases limit the aggregation of glutenin polymers as a result of pentosan breakdown which in turn strengthens the gluten network (Filipcev et al., 2014; Yegin et al., 2018; Both et al., 2020)

During the fermentation of the dough and proofing of the dough pieces, the amount of water-soluble proteins in the control sample was higher than in the experimental ones with enzymes. The content of salt-soluble proteins at all stages of dough preparation changed insignificantly (Table 6).

Table 6. Influence of enzymes on protein fraction of wheat flour

Indicators	Protein fractions, $mg \cdot g^{-1}$			
	Water-soluble	Salt-soluble (0.5 m NaCI)	Alcohol soluble (70% ethanol)	Alkali-soluble (0.05H NaOH)
Wheat flour	25.0 ± 1.0[a]	23.0 ± 1.0[a]	18.0 ± 1.0[a]	33.0 ± 1.0[a]
The control dough				
After kneading	19.5 ± 1.0[b]	11.5 ± 1.0[b]	8.3 ± 1.0[b]	51.7 ± 1.0[b]
After fermentation	21.2 ± 0.6[c]	11.0 ± 0.8[c]	9.2 ± 1.1[c]	49.0 ± 1.0[c]
After proofing the dough pieces	21.7 ± 1.0[c] x	11.2 ± 1.0[c] x	11.0 ± 1.0[d] x	46.0 ± 1.0[d] x
The dough with Protozym				
After kneading	16.8 ± 1.0[d]	11.7 ± 1.0[b]	10.5 ± 0.9[f]	53.3 ± 1.0[f]
After fermentation	17.6 ± 0.6[f]	11.7 ± 1.0[b]	8.8 ± 1.0[c]	52.7 ± 1.1[g]
After proofing the dough pieces	18.5 ± 1.2[g] y	12.0 ± 0.9[d] y	8.0 ± 0.9[b] y	50.1 ± 1.0[h] y

a–h = Means ± SD within the same column with different lowercase superscript letters denote significantly different among dough types ($P \leq 0.05$) while letters 'x-y' denote significantly different values among type of flours (Tukey's test, $p < 0.05$).

The amount of alcohol-soluble proteins gradually increased during the dough preparation process in the control samples, while in experimental samples with enzymes it decreased. An increase in the proportion of alcohol-soluble proteins during dough

preparation is consistent with the known data that during dough fermentation, protein macromolecules are depolymerized due to hydrolysis of peptide bonds under the action of flour proteolytic enzymes (Cauvain & Young, 2007). With the addition of endopeptidases and endo-xylanases, the degree of depolymerization is obviously less than the degree of aggregation of protein molecules. This conclusion is confirmed not only by an increase in the amount of alcohol-soluble and alkali-soluble proteins, but also by a decrease in the proportion of salt-soluble proteins in the dough preparation process, since the effect of baker's yeast on the assimilation of soluble peptides is present in all dough samples.

In all samples, during the dough preparation process, the amount of alcohol-soluble and alkali-soluble proteins decreased, which is consistent with the known fact of a decrease in the amount of gluten during dough fermentation (Cauvain & Young, 2007).

The strengthening of the gluten in the dough with enzymes compared to the control (noticed in Table 5) after fermentation and proofing may be associated with the content of the alcohol-soluble and alkaline-soluble fraction of gluten. In the dough with enzymes (Table 6) alkali-soluble fraction was higher by 8–9% than in control after fermentation and proofing. Alcohol-soluble fraction was comparable to the control after fermentation and was lower than in control by 37% after proofing. It is known that the higher alkali-soluble fraction (glutenin) levels make the dough more elastic thus giving dough its property of resistance to extension while higher alcohol-soluble fraction (gliadin) content increases the extensibility of the dough (Barak et al., 2013; Dhaka & Khatkar 2015).

Effects of α-amylase, endo-xylanase and exo-protease combination on bread quality

The performance of the combined action of new enzyme preparations (Amilorizin with alpha-amylase activity and Protozym with endo-xylanase and exo-peptidase activity) was investigated (Table 7).

Table 7. Bread quality indicators

Wheat flour	Bread formulation	Porosity, %	Specific volume, $cm^3\ g^{-1}$	Shape stability indicator, H/D	Aldehyde amount, mL 0.1 n iodine solution per 100 g dry matter
Flour (FN 290 s)	Control	84 ± 1^a	4.1 ± 0.3^a	0.31 ± 0.04^a	12.3 ± 0.3^a
	Amylorysin+Protosym	87 ± 2^b x	4.7 ± 0.3^b x	0.30 ± 0.04^b x	13.5 ± 0.5^b x
Flour (FN 440 s)	Control	78 ± 1^c	3.5 ± 0.3^c	0.39 ± 0.06^c	12.0 ± 0.4^a
	Amylorysin+Protosym	83 ± 1^d y	$4,2 \pm 0.3^d$ y	0.35 ± 0.04^d y	13.4 ± 0.5^b x

a–d = Means ± SD within the same column with different lowercase superscript letters denote significantly different among bread samples ($P \leq 0.05$) while letters 'x-y' denote significantly different values among type of flours (Tukey's test, $p < 0.05$).

The bread with enzymes had a higher specific volume, porosity and aldehyde content than the control made without enzymes. In comparison with the control, addition of enzymes improved bread specific volume significantly. It confirmed data obtained by other researchers (Hemalatha et al., 2010; Baratto et al., 2015; Kim & Yoo, 2020).

Shape stability indicator was lower for bread with enzymes. It is presumably related to the reduction of the dough viscosity during starch gelatinization as a result of the action of the enzyme (Goesaert et al., 2009; Fuentes et al., 2016) and reduction of dough extensibility and stability because of the gluten protein (Sahnoun et al., 2016).

According to the organoleptic assessment (Table 8), the experimental bread samples were characterized by tenderer and not crumbly crumb with developed thin-walled uniform porosity compared to the control. The crusts were more brightly colored (from yellow to light brown). It confirmed data obtained by other researchers that combination of amylases and xylanase improve sensory characteristics (Hemalatha et al., 2010; Barrato et al., 2015; Kim & Yoo, 2020). Better porosity may be due to the fact that xylanase improves the bread texture and volume (Moers et al., 2005). The pale or greyish crust colour of control samples, especially made using flour with low amylolytic activity, indicated a lack of residual sugars that might have resulted from a lean fermentation without added enzymes. Crumb colour became brighter when enzymes were used. This may be due to the α-amylase action (Sahnoun et al., 2016). The taste and smell were more pronounced. This is confirmed by the higher content of aldehydes in the samples with enzymes (Table 7). It consistent with data, obtained by other researcher, that α-amylase increases the amount of fermentable sugar and therefore enhances the yeast fermentation and the Maillard reaction products, which, in turn, strengthen the flavor and colour of bread (Sahnoun et al., 2013; Sahnoun et al., 2016). Other reason for taste and flour improvement is that the exopeptidases catalyze hydrolysis of peptide bonds and the free amino acids or small peptides formed. They may function in food as pleasant-tasting flavor compounds or as flavor precursors (Raksakulthai & Haard, 2003).

Table 8. Sensory characteristics of bread

Indicators	Flour (FN 290 s)		Flour (FN 440 s)	
	Bread formulation			
	Control	Amylorysin+ Protosym	Control	Amylorysin+ Protosym
Crust				
Shape	3.80 ± 0.18^{ax}	4.80 ± 0.13^{bx}	3.40 ± 0.11^{cy}	3.90 ± 0.13^{ay}
Surface	3.80 ± 0.18^{ax}	4.79 ± 0.13^{bx}	3.30 ± 0.12^{cy}	3.91 ± 0.11^{ay}
Colour	3.20 ± 0.16^{ax}	4.31 ± 0.19^{bx}	3.00 ± 0.15^{cy}	3.82 ± 0.14^{dy}
Crumb				
Colour	3.04 ± 0.10^{ax}	3.49 ± 0.38^{bx}	2.78 ± 0.12^{cy}	3.19 ± 0.18^{dy}
Odour	3.18 ± 0.18^{ax}	3.82 ± 0.28^{bx}	3.01 ± 0.14^{cy}	3.42 ± 0.12^{dy}
Taste	3.45 ± 0.08^{ax}	4.32 ± 0.18^{bx}	3.25 ± 0.14^{cy}	3.92 ± 0.11^{dy}
Chewiness	3.04 ± 0.09^{ax}	3.25 ± 0.15^{bx}	2.49 ± 0.14^{cy}	3.15 ± 0.17^{dy}
Porosity	3.59 ± 0.23^{ax}	4.63 ± 0.31^{bx}	3.59 ± 0.23^{ax}	4.13 ± 0.31^{cy}

a–d = Means ± SD within the same line with different lowercase superscript letters denote significantly different among bread samples ($P \leq 0.05$) while letters 'x-y' denote significantly different values among type of flours (Tukey's test, $p < 0.05$).

Studies have shown that the new enzyme preparations improve the properties of the dough, physico-chemical and organoleptic indicators of the quality of bread made from wheat flour with normal and low autolytic activity.

Effects of α-amylase, endo-xylanase and exoprotease combination on bread staling

It was found (Fig. 1) that the compression force value after 24 h of storage of samples with enzymes was less compared to the control. Evaluation of indicators of the bread crumb staleness have showed that the crumb of bread with enzymes had a lower rate of staling after 120 h of storage compared to the control. The combined use of α-amylase and endo-xylanase and exo-peptidase retarded bread staling synergistically after a 5-day storage period. The findings confirm the findings of other researchers that alpha-amylases and endo-xylanase decreased crumb hardness and slow down staling (Barrato et al., 2015; Kin&Yoo, 2020).

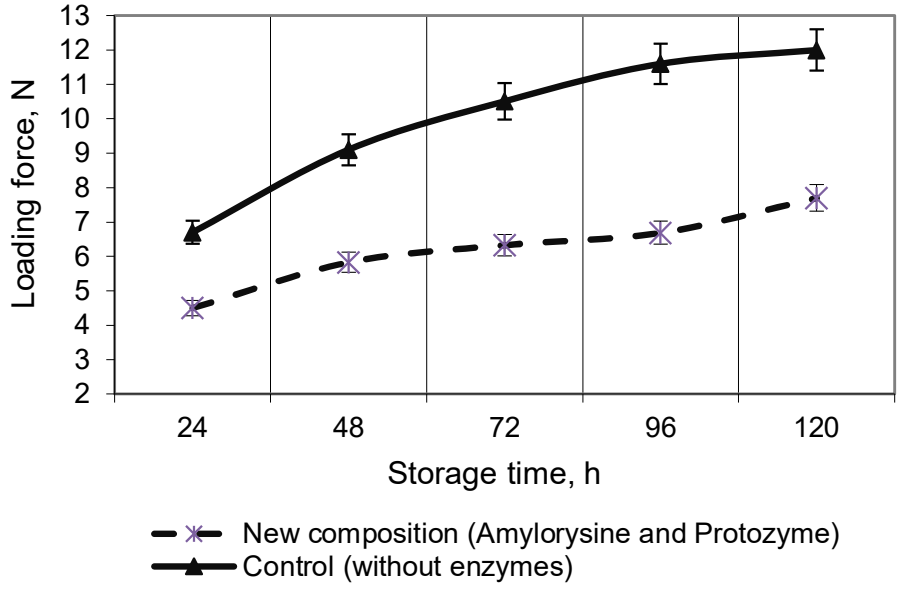

Figure 1. Dynamics of loading force indicators in the analysis of bread crumb.

CONCLUSIONS

Single addition of α-amylases preparation in the amount of 0.002 and 0.004% by weight of flour (0.5 and 1.0 units of AC / g flour) increased the gas-forming capacity of flour and reduced the gas retention coefficient. The technological properties of Russian α-amylase Amilorizin were no worse than those of the commercial preparation Fungamil 2500. It was found that single addition of new enzyme preparation with endo-xylanase and exoprotease activity (Protozym) increases the degree of hydration of gluten proteins and contributes to the strengthening of its structure. Endo-xylanase indirectly reduces the degree of peptization of gluten proteins during dough fermentation, which were confirmed by the higher amount of dry gluten in the experimental samples compared to the control. When used endo-xylanase and exoprotease, there was no significant change in the amount of salt-soluble proteins after kneading the dough, but water-soluble, alcohol-soluble and alkaline-soluble proteins was undergone transformation.

As a result of the addition of the formulated new composition of α-amylase, xylanase and exoprotease allowed the improvement of physico-chemical and organoleptic quality indicators and increasing shelf life of bakery products. New enzyme composition may be a potentially strong candidate for future applications in the bread-making industry.

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