# The influence of the flour amylolytic enzymes activity, dosage of ingredients and bread making method on the sugar content and the bread quality

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Abstract. The aim of this study was to study the effect of the sugar dosage, improver dosage, type of bread making methods and the amylolytic activity of five different types of wheat flours on the sugar content and the bread quality. The sugar content in the bread crumb was determined using the Bertrand's method and was counted for sucrose. When the dough was prepared using accelerated technology, the improver affected the sugar content in the bread due to the starch enzymatic hydrolysis. The effect of improver dosages and sugar dosages on the sugar content in the bread was established. When using the improver, the sugar content exceeded the permitted amount in 1.25 times. No correlation was found between sugar dosage in recipe and bread quality when accelerated bread making way was used because of short fermentation time. The influence of wheat flour amylolytic activity (falling number) on the sugar content in bread was established, including when sugar was absent in the formulation. When sugar presented at bread formulation, the flour amylolytic activity did not significantly affect the bread quality, except the acidity. The bread making way had a greater influence on bread quality than falling number of flour. When sugar absent at bread recipe, the higher was the flour amylolytic activity, the higher was the sugar content in bread made by traditional way due to the starch deterioration. Obtained data have shown that when a baking method is selected, the flour amylolytic activity must be taken into account.

Key words: bread, sugar content, sucrose, bread making technology.

#### **INTRODUCTION**

Sugars currently used by the bread making industry are sucrose, glucose, glucose syrup, fructose and lactose. Sucrose is most commonly used in many types of bread as an ingredient in the formulation. In bread, sugars may act as sweeteners, texture modifiers, bulking agents, flavor enhancers, flavour precursors, colour precursors and appearance modifiers (Puchkova et al., 2005; Cauvain & Young, 2007; Trinh et al., 2016).

In the Russian Federation, Ukraine and Belarus, the sugar content in the bakery products is regulated by normative documentation. Practice has shown that even if the sugar dosage was in accordance with the recipe, bread sugar dosage could not conform to the standard. It is one of serious problems to the bread making industry. Regardless of the type of sugar used in bread making process, the sugar content is determined in terms of sucrose. Methods for determining the mass fraction of sugar is based on the Bertrand reaction end the results obtained during the reaction are recounted for sucrose. That is why not only added sugar, but also flour's own sugars can contribute to the result. The flour contains reducing sugar (glucose, fructose and maltose disaccharide) and sugars that become reducing after hydrolysis of starch, sucrose, arabinose (Puchkova et al., 2005; Struyf et al., 2017).

Flour amylases make a big contribution to sugar content in the bread. The majority of fermentable and reducing sugars in dough are generated by  $\alpha$ - and  $\beta$ -amylases degradation of damaged starch (Van der Maarel et al., 2002; Cauvain & Young, 2007; Codina & Leahu, 2009; Struyf, N. et al., 2016).  $\alpha$ -amylases and  $\beta$ -amylases, both endogenously present in wheat, have been described extensively in literature (Cauvain & Young, 2007; Struyf, N. et al., 2016).  $\alpha$ -amylases are endo-amylases that hydrolyze the  $\alpha$ -(1, 4)-linkages inside the starch chain more or less randomly, thereby generating oligosaccharides and a-limit dextrins. The  $\beta$ -amylases are exoenzymes that cleave the penultimate  $\alpha$ -(1, 4)-inkage from the nonreducing end of the polymeric chains and release the disaccharide maltose.  $\beta$ -amylase, acting alone, can degrade amylose completely to maltose.  $\alpha$ -amylase degrades starch to smaller dextrins, thereby generating more non-reducing ends susceptible to  $\beta$ -amylase attack (Van der Maarel et al., 2002; Struyf, N. et al., 2016). Maltose has a reducing form, as one of the two units may have an open-chain form with an aldehyde group. Maltose is involved in the Bertrand's reaction, so it is included in the total amount of sugar in bread.

The  $\alpha$ -amylase activity in flour is often estimated by the Falling Number (FN) test. The FN represents an important quality characteristic of grain products. The FN of flour is related to the amount and activity of cereal enzyme  $\alpha$ -amylase, which is present in the wheat after harvesting. Flours with a low FN result insufficient bread quality (low specific volume, sticky crumb). Every & Ross (1996) have shown that FN less than 250 s leads to sticky crumbs. Puckova et al. (2005) have meant about FN less than 200 s. In accordance with the Russian standard for flour with an ash content 0.55–0.75%, the FN should be no less than 200 s (State Standard of the Russian Federation, 2017).

Flours with a high FN (more than 350 s) have a reduced capacity to form fermentable sugars (Codina & Leahu, 2009; Struyf et al., 2016). To avoid quality deterioration, wheat with a high FN is sourced by the miller and  $\alpha$ -amylases are added (Goesaert et al., 2009; Popper et al., 2006).

 $\alpha$ -amylase is consistently used by the baking industry to improve dough properties and bread quality. It can be used as pure enzyme and as part of an improving additive. The beneficial effect of improvers with  $\alpha$ -amylase on bread texture and elasticity is well known (Patel et al., 2012; Barrera et al., 2016). A fungal  $\alpha$ -amylase from or malt are frequently used for this purpose (Goesaert et al., 2005; Cauvain & Young, 2007; Codina & Leahu, 2009). When the amylase is used, it leads to the maltose formation and to sugar content increasing in bread. That is why the aim of present work was to study the effect of the sugar dosage, improver dosage, type of bread making methods and the amylolytic activity of five different types of wheat flours on the sugar content and the bread quality.

## MATERIALS AND METHODS

#### **Characteristic of ingredients**

Five wheat flour samples obtained from different milling companies located in Russia were used for this study. Flour had different amylolytic activity (FN). The higher was the FN value, the lower was the amylolytic activity of flour.

The ash content and the FN were determined according to ICC approved method no: 104/1 and 107/1 respectively (ICC-standard methods: 104/1 (1990), 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 Industry.

The amount of gluten was determined in the following way. Crude gluten was washed from dough mixed with flour and water, which has been aged in water for hydration and the formation of intra- and intermolecular bonds in substances that form gluten (mainly proteins – gliadin and glutenin), followed by washing by a working body of a mechanized device (MOK–1M, Russia) using water to remove watersoluble 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 compression strain index of raw gluten under the influence of a load (120 g) for a 30 s. It reflects the degree of gluten deformation. A special device IDK (Russian) was used for this. The results of the gluten elastic properties measuring were expressed in arbitrary units of the device IDK. According State Standard (State Standard of the Russian Federation GOST 26574, 2017) 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 characterized the wheat flour are presented in Table 1.

Indicators	Wheat flour							
Indicators	F1	F2	F3	F4	F5			
Falling number, s	$352\pm13^{\mathrm{a}}$	$318\pm8^{b}$	$304 \pm 7^{\circ}$	$282 \pm 10^{d}$				
Ash content, %	$0.53\pm0.03^{\rm a}$	$0.71\pm0.03^{\text{b}}$	$0.55\pm0.03^{\text{a}}$	$0.69 \pm 0.03$	$^{b}0.74 \pm 0.04^{b}$			
Wet gluten, %	$29.2\pm1.9^{\rm \ a}$	$29.9\pm2.0^{a}$	$27.8 \pm 1.6^{b}$	$30.3\pm1.6^{\rm c}$	$25.0\pm2.5^{\text{d}}$			
Gluten deforma tion index,	$65\pm3^{a}$	$65\pm3^{a}$	$65\pm3^{a}$	$65 \pm 3^{a}$	$60 \pm 3^{b}$			
units of device IDK								

Table 1. Quality parameters of wheat flour

 $a-c = Means \pm SD$  within the same row with different lowercase superscript letters are significantly different ( $p \le 0.05$ ).

Potable grade water was used in the study, as well as edible sodium chloride (Russia), dried or pressed baker's yeast (Lesaffre, Russia), complex baking improver Magimix 'Soft sandwich bread' (Lesaffre, France), enzyme 'Fungamil Super AX' with fungal  $\alpha$ -amylase activity of 60 FAU/h (Russia), sunflower oil and sugar (sucrose). Mass fraction of sucrose in sugar 99.80%.

#### **Bread preparation**

The investigation was carried out in two steps. At the first step the impact of sugar dosage, improver dosage, type of bread making methods on the sugar content and bread quality was investigated. At the second step the impact of the amylolytic activity of five different types of wheat flours on the sugar content and bread quality was studied.

I. Bread making process when investigated impact of the impact of sugar dosage, improver dosage, type of bread making methods on the sugar content and bread quality

When investigated the impact of the technology, sugar dosage and improver dosage on sugar content and bread quality, the dough was prepared by the next ways.

Only one sample of flour was used in this study (F1 with falling number 352 s).

The dough was prepared using three techniques widely used at Russian bread industry:

(I) one-step method using dried baker's yeast. All ingredients were mixed and the dough was then fermented for 3 h;

(II) two-step method using a prefermented dough. The prefermeted dough was prepared on the first step using a bakery yeast, 50% of wheat flour and yeast. The dough was prepared on second step using prefermented dough and other ingredients (Table 2);

(III) accelerated way of bread making with liquid sourdough (10% of dough mass) and improver Magimix 'Soft sandwiche bread'. Dough was prepared using a two-step method with sourdough and bakery yeast. The first step was the preparation of starter. The sourdough was prepared at first step using commercial starter (1% of total flour mass), 5% of the total amount of flour (6 g) and 17% of the total amount of water (9 g). These ingredients were carefully mixed, then fermented for 24 h at 37 °C in a thermostat (TC/80, Russia). After that, part of the sourdough was used to knead the dough.

Commercial starter for sourdough (State research institute of baking industry, Russia) contained a mixture of *Lactobacillus casei*, *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus brevis*.

It should be noted that the rest of the sourdough is mixed with flour and water and fermented. Part of fermented sourdough is usually used for bread making and small part is used for new sourdough. Sourdough allows getting the dough and bread in a quick way. Although the sourdough ferments for a long time, the method of dough preparing is very fast. And since sourdough is always continuously conducted at bakeries enterprises it can be used for bread making at any time. So the dough is prepared very quickly. Therefore, this method is called accelerated.

Dough was prepared by adding flour, water, improver Magimix 'Soft sandwiche bread', yeast and other ingredients (Table 2).

Improver Magimix 'Soft sandwiche bread' was used only in bread making process III, because of the deterioration in the dough quality in accelerated method. Magimix 'Soft sandwiche bread' is a bread improver used for preparing quality, standardized bread. The composition includes a complex of enzymes, emulsifier, ascorbic acid, L-cysteine.

T L' ( 1 )	Ι	II		III	
Ingredients and parameters		1 <sup>st</sup> step	2nd step	1 <sup>st</sup> step	2 <sup>nd</sup> step
Ingredient amounts					
Wheat flour, g	100	50	50	6	94
Dried yeast, g	0.7	0.3	0.2	-	1.0
Salt, g	1.5	-	1.5	-	1.5
Sugar, g	4.0	-	4.0	-	4.0*)
Sunflower oil, g	3.0	-	3.0	-	3.0
Starter sourdough, g				0.06	
Sourdough, g	-	-	-	-	15
Prefermented dough, g	-	-	78.7	-	-
Magimix 'Soft sandwiche bread', g	-	-	-	-	$0.7^{**)}$
Water, g	54.4	28.9	25.3	9	45.4
Process parameters					
Moisture content, %	41.5	45	41.5	-	41.5
Fermentation time, h	3	3	1	24	0.17
Proofing time	71	-	82	-	64
(fermentation before baking), min					

Table 2.	Formulations	of the	dough
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\*) In this study, when the impact of sugar dosage was investigated, the sugar content in the composition was decreased by 0, 10, 20, 50%. I.e. the sugar content in the formulation was 4 g, 3.6 g, 3.2 g, 2 g, while the dosage of the other ingredients was not changed.

\*\*) the dosage of improver Magimix 'Soft sandwiche bread' was decreased on 15, 30, 50 and 100% in this study. I.e. the improver content in the formulation was 0.60 g, 0.21 g, 0.35 g, 0 g while the dosage of the other ingredients was not changed.

Fermentation time was different for each technique. Dough with sourdough was fermented only for 10 min because sourdough usage allowed obtaining high dough acidity. If the fermentation time was increased, the bread could have too high acidity, exceeding the requirements of Russian standards.

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 cooked in an oven SvebaDahlen (Sweden) at 200 °C for 23 min.

# **II.** Bread making process when investigated impact of the flour amylolytic activity and type of bread making methods on the sugar content and bread quality

When investigated the influence of the amylolytic activity of five different types of wheat flours and tipy of technology on sugar content and bread quality, the dough was prepare by three ways:

(IV) one-phase method using pressed baker's yeast. All ingredients were mixed and the dough was fermented for 1 h;

(V) two-phase method using a prefermented dough. The prefermeted dough was prepared using bakery yeast, 50% of wheat flour and yeast. The dough was prepared on second step using prefermented dough and other ingredients (Table 3);

(VI) one-phase method using pressed baker's yeast and improver – fungal  $\alpha$ -amylase 'Fungamil Super AX'. Fungal  $\alpha$ -amylase was used only in this method to avoid quality deterioration due to accelerated technology.

	IV			VI
Ingredients and parameters		1 <sup>st</sup> step	2 <sup>nd</sup> step	
Ingredient amounts				
Wheat flour, g	100	50	50	100
Pressed yeast, g	1.5	0.5	1.0	1.5
Salt, g	1.5	-	1.5	1.5
Sugar, g	4.0	-	4.0	4.0
Sunflower oil, g	3.0	-	3.0	3.0
Prefermented dough, g	-	-	79	-
Enzyme 'Fungamil', g	-	-	-	0.002
Water, g	53.0	28.5	25.0	25.0
Process parameters				
Moisture content, %	41.5	45	41.5	41.5
Fermentation time, h	3	3	1	1
Proofing time (fermentation before baking), min	71	-	82	82

Table 3.	Formulations	of the	dough

#### Assessment of baked bread

The quality of bread was evaluated by following parameters.

Mass proportion of moisture of the dough was determined by drying at a temperature of 130 °C during 40 minutes in drier (SHS–1M, Russia). The mass fraction of moisture was calculated as the ratio between the mass of evaporated water and the initial mass and was multiplied by 100%.

Acidity was determined by titration, using 0.1 N solution of NaOH (State Standard of the Russian Federation, 1996). For determination of titratable acidity, a 10 g sample was mixed with 100 mL of distilled water and the suspension was titrated to a final pH of 8.5 with 0.1N NaOH (PH-meter-millivoltmeter, Russia). One degree of acidity equal to the 1 ml of 0.1 N NaOH consumed. Acidity analysis were done in duplicate.

Porosity – was determined as the ratio between pore volume and the total volume of products, pore volume – as the difference between the volume of product and the volume of non-porous mass, specific volume – as the ratio between product volume and mass of whole bread (cm  $g^{-1}$ ) (Puchkova, 2004).

Compressibility was determined on the automatic penetrometer Labor (Hungary) (Puchkova, 2004). To determine compressibility, 40 mm thick slice was cut from the middle of the bread loaf (cut planes must be strictly parallel). The plastic penetrating body of the device (diameter 25 mm, weighing 300 g) was raised up to the upper position. Units of device were measured. The bread slice was put on the surface of the lifting table. The lifting table of the penetrometer was raised until the penetrating body was in contact with the bread. For a 5 s, the body penetrated bread. Units of device were measured again. The compressibility of bread was calculated as a difference between the initial and final unints of the device.

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

#### The sugar content deterioration

The sugar content in the bread crumb was determined in accordance with the Russian standard using the Bertrand's method (State Standard of the Russian Federation, 1968).

Bertrand's method of analysis remains a widely used industrial method to estimate the total sugar and reducing sugar content of different food products and bread. In this work, the assay itself involves collecting the precipitate of cuprous oxide formed by reduction of the copper-alkaline liquor in the presence of reducing sugars and assayed by the manganimetric method.

To determine the sugar 300 g of bread without crust was used.

The bread was thoroughly crushed. 25 g of crushed bread was introduced into a volumetric flask with a capacity of 200 cm<sup>3</sup>. Water was added into the flask over 2/3 of the volume and flask was left to stand for 5 minutes with frequent shaking. After that, 10 cm<sup>3</sup> of a 15% solution of ZnSO<sub>4</sub> and 10 cm<sup>3</sup> of a 4% solution of NaOH were added into the flask, mixed well. Then water was addes until the mark of 200 cm<sup>3</sup>, flask was shaked again and left for 15 minutes. Then liquid was filtered through filter paper into a dry flask. For hydrolysis of sucrose, 50 cm<sup>3</sup> of the filtrate was taken into a volumetric flask with a capacity of 100 cm<sup>3</sup> and 5 cm<sup>3</sup> of 20% hydrochloric acid was added. The flask was kept at 70 °C for 8 minutes. Then it was quickly cooled to room temperature  $(20 \pm 1)$  °C. Solution was neutralized with a 10% sodium hydroxide solution. 20 cm<sup>3</sup> of the solution, 20 cm<sup>3</sup> of a 4% solution of CuSO<sub>4</sub> and 20 cm<sup>3</sup> of an alkaline solution of potassium-sodium tartrate were introduced into a conical flask. The flask was heated to a boil. They boiled for exactly 3 minutes from the moment of bubble formation. The liquid was filtered through an asbestos filter. The precipitate in the flask and on the filter was washed several times with hot water.

The Cu<sub>2</sub>O precipitate was dissolved in 20 cm<sup>3</sup> of an ammonium-iron sulfate (III) solution. The solution was filtered by suction and the precipitate was washed. The resulting green colored solution in a suction flask was titrated with Potassium permanganate until a faint pink color. Bertrand tables in Russian Standard (State Standard of the Russian Federation, 1968) give a direct correspondence between the volume of potassium permanganate (0.1 N) used and the sucrose content of the sample.

#### 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.

The accuracy of the experimental data was evaluated by using mathematical statistical methods in Microsoft Excel (2010 version) at a theoretical frequency of 0.95. Results were given as mean  $\pm$  standard deviation.

## **RESULTS AND DISCUSSION**

For research, bread 'Nareznoi' traditional and commonly produced in Russia, Ukraine and Belarus was used. The mass fraction of sugar in the dry matter of the wheat long-loaf bread 'Nareznoi' from flour of the first or higher grade should not exceed  $4.2 \pm 1\%$  according Russian Standard (State Standard of the Russian Federation GOST 27844–88, 1988). The effects of the technology, sugar dosage and improver dosage on sugar content and bread quality were investigated. The sugar content depended on the method of the dough preparation (technology).

The sugar quantity didn't exceed the high normative level in breads prepared without improver by one-stage technology and two-stage technology with prefermented dough (Formulation at Table 2). When using sourdough and improver (III way according Table 2), the sugar content was the highest and exceeded the standard highest level by 1.17–1.25 times (Table 4). This may be due to the fact that the dough with the sourdough was fermented for only 10 minutes. Sugar was not fermented by yeast during such a short time (Verheyen, 2016). And the improver affected the sugar content in the bread due to the starch enzymatic hydrolysis (Van der Maarel et al., 2002). In terms of porosity, specific volume, shape stability and compressibility of the crumb, this sample was slightly inferior to bread prepared using a baking improver.

The bread making procedure using sourdough and short fermentation is commonly use in Russian industry, therefore the bread with excess sugar content may be found on the shelves. That is why the effects of dosage of sugar and improver on sugar content and bread quality when using this dough preparing method were investigated.

When the sugar dosage was reduced on 10 and 20%, the sugar content in bread steel exceeded the standard norm in 1.25 times (Table 4). Only when the sugar dosage was reduced by 50%, the amount of sugar in the bread met the standard requirements and was 4.1% of dry weight.

It should be noted that the sugar dosage reduction did not significantly affect the porosity, specific volume and compressibility of the crumb during 5 days of storage. This may also be due to the short dough fermentation time: sugar did not participate in fermentation, therefore, did not contribute to the formation of carbon dioxide, which determines the porosity and specific volume (Verheyen et al., 2015; Trinh et al., 2016; Verheyen, 2016). So, when sugar was reduced or eliminated it did not affect the fermentation and carbon dioxide formation.

When reducing the improver dosage by 15, 30 and 50%, the sugar content practically unchanged and exceeded standard norm in 1.25 times. The sugar content met the requirements only when improver was completely excluded. Porosity, specific volume, shape stability and compressibility of bread crumb were worse when not using the improver. This is understandable with such a short dough fermentation time (Verheyen, 2016; Van der Maelen et al., 2017).

Indicators	Ι	II	III							
			dosage of sugar, % of the amount according to the recipe			dosage of improver, % of the amount according to the recipe				
			100	90	80	50	85	70	50	0
Mass proportion of moisture, %	$39.7 \pm 0.3^{a}$	$39.4\pm0.3^{\text{a}}$	$39.0\pm0.2^{\text{a}}$	$39.4\pm0.3^{a}$	$39.5\pm0.3^{\text{a}}$	$39.4 \pm 0.3^{a}$	$38.2 \pm 0.3^{b}$	$38.2 \pm 0.3^{b}$	$38.4 \pm 0.3^{b}$	$39.6 \pm 0.3^{\circ}$
Mass proportion of sugar, %	$3.3\pm0.1^{a}$	$4.2\pm0.2^{b}$	$6.5\pm0.3^{\circ}$	$6.5 \pm 0.2^{\circ}$	$6.3 \pm 0.2^{\circ}$	$4.1 \pm 0.2^{b}$	$6.2 \pm 0.2^{\circ}$	$6.2 \pm 0.3^{\circ}$	$6.1 \pm 0.3^{\circ}$	$4.9\pm0.2^{d}$
Acidity, degrees N	$1.0 \pm 0.1^{a}$	$1.0\pm0.1^{\text{a}}$	$1.4\pm0.1^{b}$	$1.6 \pm 0.2^{\circ}$	$1.4 \pm 0.1^{b}$	$1.5 \pm 0.1^{\circ}$	$1.4 \pm 0.1^{b}$	$1.4 \pm 0.1^{b}$	$1.4 \pm 0.1^{b}$	$1.6 \pm 0.1^{\circ}$
Porosity, % Compressibility, units of the device	$83\pm2^{a}$	$82\pm2^{a}$	$85 \pm 2^{b}$	$83 \pm 1^{a}$	$85 \pm 2^{b}$	$84 \pm 2^{b}$	$85\pm3^{b}$	$85 \pm 2^{b}$	$83\pm2^{a}$	$80\pm2^{c}$
20h after baking	$64\pm3^{a}$	$52 \pm 2^{b}$	$69 \pm 4^{\circ}$	$68 \pm 3^{\circ}$	$71\pm4^{d}$	$80 \pm 4^{e}$	$98\pm5^{\rm f}$	$84\pm3^{\rm g}$	$66\pm3^{\rm h}$	$29\pm2^{i}$
48 h after baking	$48\pm3^{a}$	$37\pm2^{\rm b}$	$71 \pm 3^{\circ}$	$59\pm4^{\text{d}}$	$49\pm2^{a}$	$58\pm3^{\text{d}}$	$74\pm5^{e}$	$78\pm4^{\rm f}$	$52\pm3^{\rm g}$	$20\pm2^{\rm h}$
120 h after baking	$31\pm2^{a}$	$27\pm2^{b}$	$41 \pm 3^{\circ}$	$42 \pm 3^{\circ}$	$45\pm3^{d}$	$48\pm3^{e}$	$34\pm3^{\rm f}$	$32\pm4^{a}$	$27\pm2^{b}$	$15\pm2^{\text{g}}$
Specific volume, cm <sup>3</sup> g <sup>-1</sup>	$3.8\pm0.2^{\rm a}$	$3.7\pm0.2^{\mathrm{a}}$	$4.1 \pm 0.3^{b}$	$4.0\pm0.3^{\text{b}}$	$4.0\pm0.4^{b}$	$4.1\pm0.3^{\text{b}}$	$4.3\pm0.3^{\rm c}$	$4.5\pm0.2^{d}$	$4.0\pm0.3^{\text{b}}$	$3.1\pm0.3^{\text{e}}$
Shape stability indicator, H : D	$0.46 \pm 0.05$	$a^{a}0.44 \pm 0.04^{a}$	$0.45\pm0.05^{a}$	$0.45 \pm 0.05$	$^{a}0.45 \pm 0.06^{a}$	$0.48 \pm 0.04$	$^{b}0.50 \pm 0.05$	$^{b}0.49 \pm 0.05$	$^{b}0.43 \pm 0.02$	$a^{a}0.40\pm0.0$

Table 4. Impact of type of bread making methods on physico-chemical indicators of the bread

 $a-k = Means \pm SD$  within the same row with different lowercase superscript letters are significantly different ( $P \le 0.05$ ).

Compressibility decrease rand crumbs become more firm, hard and crumbly during the bread storage, due to the changes in starch fractions (Gray & Bemiller, 2003). These changes are mainly ascribed to gradual amylopectin retrogradation, which occurs during the five storage days. Generally, the best crumbs compressibility (which characterizes the freshness of the product) had bread made with sourdough and improver (III). The crumb compressibility of bread made using the one-stage technology (I) was better than that of bread with prefermented dough. This can be explained by a more uniform thinwalled structure of porosity resulting from longer dough fermentation. Bread prepared in one stage had a large specific volume and better porosity. This means that a more uniform thin-walled porosity of the crumb contributes to a better preservation of the freshness of the bread.

It should be noted that the bread prepared with long fermentation had a distinct pleasant aroma in comparison with the loaves prepared with sourdough and improver, despite the fact that the sourdough contributes to the formation of taste and smell (Jensen et al., 2011; Onishi et al., 2011; Plessas et al., 2011; Demin et al., 2013, Savkina et al., 2019). The fermentation was too short, and the sourdough dosage was too small for enough aromatic substances accumulating in the dough (Birch et al., 2013; Verheyen, 2016).

Wheat	Way of	Acidity,	Porosity,	Specific	Compres-sibility	, Shape stability
flour	bread	degrees N	%	volume,	units	indicator,
(FN, s)	making*	-		$cm^3 g^{-1}$	of the device	H : D
F1 (352 s)	IV	$1.0 \pm 0.2^{a}$	$83\pm2^{a}$	$3.8\pm0.3^{\mathrm{a}}$	$47\pm3^{a}$	$0.46\pm0.05^{\text{a}}$
	V	$1.0\pm0.2^{a}$	$81 \pm 2^{b}$	$3.7\pm0.2^{\rm a}$	$45\pm2^{b}$	$0.42\pm0.04^{b}$
	VI	$0.8\pm0.1^{b}$	$83\pm2^{\rm a}$	$3.5\pm0.2^{b}$	$34\pm3^{\circ}$	$0.41\pm0.03^{b}$
		х	х	Х	Х	Х
F3 (318 s)	IV	$1.6 \pm 0.2^{c}$	$85\pm2^{\circ}$	$4.0\pm0.3^{\text{c}}$	$52 \pm 2^{d}$	$0.43\pm0.03^{b}$
	V	$1.4 \pm 0.2^{d}$	$80\pm3^{b}$	$3.7\pm0.2^{a}$	$42 \pm 1^{e}$	$0.39\pm0.05^{\text{c}}$
	VI	$1.3 \pm 0.1^{d}$	$81 \pm 2^{b}$	$3.4\pm0.2^{d}$	$40 \pm 2^{e}$	$0.38\pm0.04^{\text{c}}$
		у	х	Х	Х	Х
F2 (304 s)	IV	$1.2 \pm 0.1^{e}$	$85 \pm 2^{\circ}$	$3.9\pm0.2^{\rm c}$	$52 \pm 3^{d}$	$0.40\pm0.02^{\rm d}$
	V	$1.4 \pm 0.2^{d}$	$80 \pm 2^{b}$	$3.7\pm0.1^{a}$	$42 \pm 2^{e}$	$0.34\pm0.04^{e}$
	VI	$1.0 \pm 0.1^{a}$	$81 \pm 2^{b}$	$3.8\pm0.2^{a}$	$42 \pm 3^{e}$	$0.38\pm0.05^{\rm c}$
		Z	х	Х	Х	Х
F4 (282 s)	IV	$1.7 \pm 0.2^{\mathrm{f}}$	$83\pm3^{\rm a}$	$3.8\pm0.1^{a}$	$48 \pm 2^{\mathrm{f}}$	$0.42\pm0.05^{\text{b}}$
	V	$1.6 \pm 0.2^{\text{g}}$	$81 \pm 2^{b}$	$3.5\pm0.2^{b}$	$35 \pm 2^{\circ}$	$0.41 \pm 0.04^{b}$
	VI	$1.5 \pm 0.2^{d}$	$81 \pm 2^{b}$	$3.2\pm0.2^{d}$	$30\pm2^{\mathrm{g}}$	$0.43\pm0.05^{b}$
		xy	х	Х	Х	Х
F5 (216 s)	IV	$1.8\pm0.1^{\rm f}$	$82 \pm 2^{b}$	$3.8\pm0.1^{a}$	$52 \pm 3^{d}$	$0.42\pm0.04^{b}$
	V	$1.7 \pm 0.2^{\mathrm{f}}$	$81 \pm 2^{b}$	$3.7\pm0.2^{a}$	$42 \pm 2^{e}$	$0.42\pm0.03^{b}$
	VI	$1.6\pm0.2^{\rm g}$	$82\pm3^{\text{d}}$	$3.8\pm0.2^{a}$	$40 \pm 3^{e}$	$0.48\pm0.04^{\text{a}}$
		ху	Х	Х	Х	Х

**Table 5.** Impact of the flour amylolytic activity (FN) and type of bread making methods on physico-chemical indicators of the bread

\*according Table 3:  $a-g = Means \pm SD$  within the same column with different lowercase superscript letters denote significantly different among dough types ( $P \le 0.05$ ) while letters 'x-z' denote significantly different values among type of flours (Tukey's test, p < 0.05).

The influence of the flour amylolytic activity on sugar content and physicochemical quality indicators in wheat bread was studied. In this study, the dough was prepared in a according to the formulation in Table 3. Flour samples with different amylolytic activity (FN) were used.

Flour amylolytic activity did not significantly affect the bread quality, except the acidity. The bread making way had a greater influence (Table 5). The data obtained confirm that there is no significant correlation between falling number and any of the quality traits, except acidity (Newberry et al., 2018).

The higher was the flour amylolytic activity, the higher was the bread acidity. This may be due to the fact that as a result of amylolysis, own sugar (maltose) was formed from flour starch. Maltose participated in lactic acid and alcohol fermentation in the dough and acidity was accumulated (Table 5). Porosity was higher when one-stage bread making way (IV) was used. This may be due to the dough fermentation propagation. In a two-stage process, the prefermented dough fermented without sugar. When the dough is kneaded using prefermented dough and sugar, it was fermented for only 1 hour. And with method IV, the dough with was fermented sugar for 3 hours. Sugar was fermented by yeast and was involved in the formation of gas to loosen the dough.

It was found that the higher was the flour amylolytic activity, the higher was the sugar content in bread (Fig. 1). Sugar exceeded the norm in any bread making way when flour had high amylolytic activity (FN 216 s).

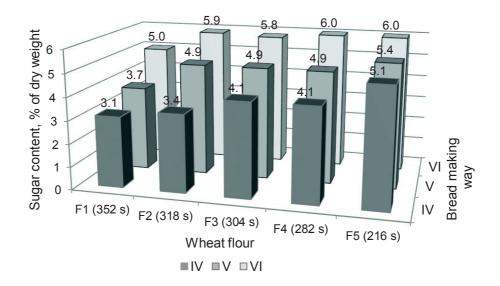


Figure 1. Sugar content in bread from different types of flour.

When bread was made using fungal amylase and flour possessing FN equal or lower then 318 s, the sugar content didn't depend on the flour amylolytic activity. Significant influence on the sugar content had only flour possessed FN more than 318 s.

When the amylolytic enzyme (VI) was used, the sugar content in all samples, regardless of the flour amylolytic activity, corresponded to the upper limit of the norm or exceeded it.

Sugar content was at the lower level of standard norm when bread was made by traditional way (IV) without fungal amylase and using flour possessing low amylolytic activity (falling number 352 s). It can be assumed that if the flour FN will be greater (amylolytic activity will be worse), then the sugar content will be below normal. A decrease in sugar content will lead to a decrease in the nutritional value of bread.

Flour possessing a FN greater than 216 s may be recommended for bread made with sugar in the recipe. If the flour has a higher amylolytic activity (the FN is equal to or less than 216 s), then it is not recommended to use an improver with amylolytic activity.

The influence of amylolytic activity of wheat flour on the sugar content and bread quality was established in bread prepared without sugar in the formulation (Table 6, Fig. 2). When sugar (sucrose) was excluded from the (Fig. 2), sugar was still found in bread. It was because the flour enzymes (for way IV, V) and fungal amylase (for VI way) affected the sugar generation in the dough from the starch (Van der Maarel et al., 2002; Cauvain & Young, 2007; Codina & Leahu, 2009; Struyf, N. et al., 2016).

Wheat flour	Way of	Acidity,	Porosity,	Specific	Compressi-	Shape stability
(Falling	bread	degrees N	%	volume,	bility, units	indicator,
number, s)	making*	-		$cm^3 g^{-1}$	of the device	H : D
F1 (352 s)	IV	$1.0\pm0.2^{a}$	$77 \pm 3^{a}$	$3.2 \pm 0.3^{a}$	$36 \pm 2^{a}$	$0.32\pm0.05^{a}$
	V	$0.8\pm0.1^{b}$	$82 \pm 3^{b}$	$3.4\pm0.3^{b}$	$32 \pm 2^{b}$	$0.38\pm0.05^{b}$
	VI	$0.8\pm0.1^{\text{b}}$	$79 \pm 2^{\circ}$	$3.5\pm0.2^{\rm b}$	$34\pm3^{\mathrm{a}}$	$0.40\pm0.04^{\text{c}}$
		Х	х	Х	Х	Х
F3 (318 s)	IV	$1.4 \pm 0.3^{\circ}$	$84 \pm 2^{d}$	$4.0\pm0.3^{\circ}$	$49 \pm 4^{c}$	$0.42\pm0.03^{\text{d}}$
	V	$1.4 \pm 0.2^{\circ}$	$80\pm2^{a}$	$3.4\pm0.2^{b}$	$39\pm4^{d}$	$0.40\pm0.04^{\text{c}}$
	VI	$1.4\pm0.2^{\rm c}$	$80\pm2^{a}$	$3.0\pm0.3^{\rm d}$	$31 \pm 3^{b}$	$0.44\pm0.03^{e}$
		У	У	У	У	у
F2 (304 s)	IV	$1.2\pm0.1^{\rm d}$	$83\pm3^{d}$	$3.1\pm0.2^{\mathrm{a}}$	$42 \pm 4^d$	$0.35\pm0.04^{\rm f}$
	V	$1.0\pm0.2^{a}$	$81 \pm 1^{a}$	$3.1 \pm 0.1^{d}$	$36 \pm 2^{a}$	$0.47\pm0.05^{\text{g}}$
	VI	$1.0\pm0.1^{a}$	$82 \pm 2^{b}$	$3.5\pm0.2^{\rm d}$	$33\pm3^{a}$	$0.35\pm0.03^{\rm f}$
		Z	у	Х	Z	Z
F4 (282 s)	IV	$1.5 \pm 0.2^{\circ}$	$82 \pm 3^{b}$	$4.0\pm0.3^{\circ}$	$48 \pm 3^{\circ}$	$0.42\pm0.04^{\text{d}}$
	V	$1.5 \pm 0.2^{\circ}$	$80\pm2^{a}$	$3.2\pm0.3^{\text{d}}$	$37\pm4^{d}$	$0.43\pm0.05^{c}$
	VI	$1.6\pm0.3^{e}$	$79 \pm 2^{c}$	$2.9\pm0.2^{\text{d}}$	$30\pm3^{b}$	$0.42\pm0.03^{\text{d}}$
		ху	у	У	у	ху
F5 (216 s)	IV	$1.8\pm0.2^{\rm f}$	$83 \pm 2^{d}$	$4.1 \pm 0.3^{\circ}$	$48 \pm 4^{\circ}$	$0.43 \pm 0.04^{e}$
	V	$1.7\pm0.3^{\rm f}$	$81\pm2^{\mathrm{a}}$	$3.3\pm0.3^{\rm b}$	$39\pm3^{d}$	$0.42\pm0.03^{\text{d}}$
	VI	$2.0\pm0.2^{\text{g}}$	$80\pm2^{a}$	$3.3\pm0.2^{\text{b}}$	$31 \pm 2^{b}$	$0.48\pm0.02^{\rm g}$
		ух	у	Z	у	ух

**Table 6.** Impact of the flour amylolytic activity (FN) and type of bread making methods on physico-chemical indicators of the bread made without sucrose

\*according Table 3:  $a-g = Means \pm SD$  within the same column with different lowercase superscript letters denote significantly different among dough types ( $P \le 0.05$ ) while letters 'x-z' denote significantly different values among type of flours (Tukey's test, p < 0.05).

Deterioration in the specific volume, shape stability and compressibility of the crumb for bread made using flour F1 and F3 was noted. These flour samples had a high falling number and low ash content. Therefore, we can conclude that when sugar was

excluded from the recipe, there was not enough flour own sugar (maltose) for fermentation. And since yeast needs minerals for life and fermentation (Kurtzman, 2011), the low ash content, together with the low maltose content in the flour, led to a deterioration in fermentation and a decrease in specific volume and compressibility.

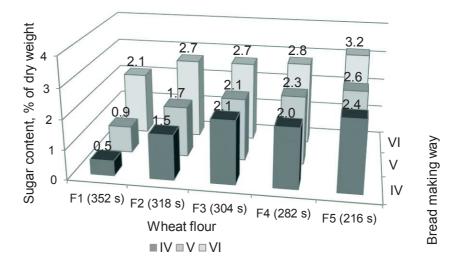


Figure 2. Sugar content in bread prepared without sugar in the formulation from different types of flour.

But it should be noted, that al parameters met the requirements of the standard for this bread (State Standard of the Russian Federation GOST 27844–88, 1988).

When flour with a falling number of 216 s (F5) and fungal amylase was used, the sugar content was at the lower limit of the standard norm for this type of bread (Fig. 2). Any wheat flour possessing low FN or high  $\alpha$ -amylase levels is automatically considered a poor bread wheat (Newberry et al., 2018). But obtained data allows to assume that when the flour possessing FN below 216 s will be used with fungal amylase in the dough, the bread 'Nareznoi' can be prepared without sugar in the recipe. And in this case, the bread quality will not deteriorate, and the sugar content will meet the requirements of the standard.

#### CONCLUSIONS

The sugar content in the bread met the requirements of regulatory documents when dough was prepared in the traditional way. The sugar content exceeded the permitted amount in 1.25 times when bread was made by accelerated way using improver. This may be due to the starch amylolytic hydrolysis.

It was shown that sucrose dosage in formulation had not a significant effect on the porosity, specific volume and compressibility of the crumb during 5 days of bread storage when bread was made by accelerated method. The sugar content in the bread met the requirements only when the improver was completely excluded, but at the same time, the bread physico-chemical indicators (porosity, specific volume and crumb compressibility) were worse.

The bread making way had a greater influence on bread physico-chemical indicators (except the acidity) than FN of flour.

When sugar was excluded from formulation, sugar was still found in bread, because the flour enzymes activity and fungal amylase affected the sugar generation in the dough from the starch.

The deterioration in the specific volume, shape stability and compressibility of the crumb was observed when flour possessed a high FN and low ash content. It may be due to the deterioration of fermentation because of lower feed content for yeast.

In order to meet normative documentation requirements in sugar content, it is necessary to take into account the flour amylolytic activity (falling number) and bread making method.

#### REFERENCES

- Barrera, G.N., Tadini, C.C., Leon, A.E. & Ribotta, P.D. 2016. Use of alpha-amylase and amyloglucosidase combinations to minimize the bread qualityproblems caused by high levels of damaged starch. J. Food Sci. Technol. 53. 3675–3684. doi: 10.1007/s13197-016-2337-2
- Birch, A.N.; Petersen, M.A.; Arneborg, N.; Hansen, Å.S. 2013. Influence of commercial baker's yeasts on bread aroma profiles. *Food Res. Int.* **5**.
- Cauvain, S.P. & Young, L.S., 2007. *Technology of Breadmaking*, second ed. Springer, BakeTran, UK., 397 pp.
- Codina, G.G. & Leahu, A. 2009. The improvement of the quality of wheat flour with a lower content of alpha-amylase trough the addition of different enzymatic products. *Lucrari Stiintifice Ser. Agron.* **52**, 629–635.
- Demin, M., Popov-Raljić, J., Lalicić-Petronijević, J., Rabrenović, B., Filipčev, B. & Šimurina, O. 2013. Thermo-mechanic and sensory properties of wheat and rye breads produced with varying concentration of the additive. *Hem. Ind.* 67(3), 455–463.
- Every, D. & Ross, M. 1996. The role of dextrins in the stickiness of bread crumb made from preharvest sprouted wheat or flour containing exogenous alpha amylase. J. Cereal Sci. 23, 247–256.
- Goesaert, H., Brijs, K., Veraverbeke, W.S., Courtin, C.M., Gebruers, K. & Delcour, J.A. 2005. Wheat flour constituents: how they impact bread quality, and how to impact their functionality. *Trends Food Sci. Technol.* 16, 12–30.
- Goesaert, H., Slade, L., Levine, H. & Delcour, J.A. 2009. Amylases and bread firming an integrated view. J. Cereal Sci. 50, 345–352.
- Gray, J.A. & Bemiller, J.N. 2003. Bread staling. Molecular basis and control. *Comprehensive* reviews in food science and food safety **2**, 1–21.
- ICC-standard methods: 104/1. 1990. Determination of ash in cereals and cereal products.
- ICC-standard methods: 107/1. 1995. Determination of the 'falling number' according to Hagberg as a measure of the degree of alpha-amylase activity in grain and flour.
- Jensen, S., Oestda, H., Skibsted, L., Larsen, E. & Thybo, A. 2011. Chemical changes in wheat pan bread during storage and how it affects the sensory perception of aroma, flavour, and taste. *Journal of Cereal Science* **53**(2), 259–268.
- Kurtzman, C., Fell, J.W. & Boekhout, T. 2011. *The Yeasts, a taxonomic study*, 5th Edition. Elsevier, Amsterdam, 2354 pp.
- Newberry, M., Zwart, A.B., Whan, A., Mieog, J.C., Sun, M., Leyne, E., Pritchar, J., Nicolas Daneri-Castro, S., Ibrahim, K., Diepeveen, D., Howitt, C.A. & Ral, J.–P.F. 2018. Does Late Maturity Alpha-Amylase Impact Wheat Baking Quality? *Frontiers in Plant Science* 9, 1356. doi:10.3389/fpls.2018.01356

- Onishi, M., Inoue, M., Araki, T., Iwabuchi, H. & Sagara, Y. 2011. Odorant transfer characteristics of white bread during baking. *Bioscience, Biotechnology, and Biochemistry* 75(2), 261–267.
- Patel, M.J., Ng, J.H.Y., Hawkins, W.E., Pitts, K.F. & Chakrabarti-Bell, S. 2012. Effects of fungal alpha-amylase on chemically leavenedwheat flour doughs. J. Cereal Sci. 56, 644–651. doi: 10.1016/j.jcs.2012.08.002
- Plessas, S., Alexopoulos, A., Bekatorou, A., Mantzourani, I., Koutinas, A. & Bezirtzoglou, E. 2011. Examination of freshness degradation of sourdough bread made with kefir through monitoring the aroma volatile composition during storage. *Food Chemistry* 124(2), 627–633.
- Popper, L., Schafer, W. & Freund, W. 2006. Future of Flour: a Compendium of Flour Improvement. Verlag Agrimedia, Kansas City, USA, 419 pp.
- Puchkova, L. 2004. Laboratory Workshop on Bakery Technology. Moscow, pp. 264. (in Russian).
- Puckova, L., Polandova, R. & Matveeva, I. 2005, *Technology of bread, pastry and pasta. Part 1. Bread technology*. Russian, Moscow, GIORD, pp. 559. (in Russian).
- Savkina, O., Kuznetsova, L., Parakhina, O., Lokachuk, M. & Pavlovskaya, E. 2019. Impact of using the developed starter culture on the quality of sourdough, dough and wheat bread. *Agronomy Research* 17(S2), 1435–1451. https://doi.org/10.15159/AR.19.138
- State Standard of the Russian Federation GOST 5672-68. 1968. Bread and bakery products. Methods for determining the mass fraction of sugar, pp. 10 (in Russian).
- State Standard of the Russian Federation GOST 27844–88, 1988. Rolls and buns. Specifications, pp.12 (in Russian).
- State Standard of the Russian Federation GOST 27842–88, 1996. Bread, rolls and buns. Methods for determination of acidity, pp. 5 (in Russian).
- State Standard of the Russian Federation GOST 27839–2013. 2013. Wheat flour. Methods for determining the quantity and quality of gluten, pp. 18 (in Russian).
- State Standard of the Russian Federation GOST 26574–2017. 2017. Baking wheat flour. Technical specifications, pp. 12 (in Russian).
- Struyf, N., Verspreet, J. & Courtin, C.M. 2016. The effect of amylolytic activity and substrate availability on sugar release in non-yeasted dough. *Journal of Cereal Science* 69, 111–118. doi: 10.1016/j.jcs.2016.02.016
- Struyf, N., Laurent, J., Lefevere, B., Verspreet, J., Verstrepen, K.J. & Courtin, C.M. 2017. Establishing the relative importance of damaged starch and fructan as sources of fermentable sugars in wheat flour and whole meal bread dough fermentations. *Food Chemistry* 218, 89–98.
- Trinh, L., Campbell, G.M. & Martin, P.J. 2016. Scaling down bread production for quality assessment using a breadmaker: Are results from a breadmaker representative of other breadmaking methods? *Food and Bioproducts Processing* 100, 54–60. doi: 10.1016/j.fbp.2016.06.004
- Van der Maarel, M.J.E.C., Van der Veen, B., Uitdehaag, J.C.M., Leemhuis, H. & Dijkhuizen, L. 2002. Properties and applications of starch-converting enzymes of the alpha-amylase family. J. Biotechnol. 94, 137–155.
- Van der Maelen, E., Hemdane, S., Verspreet J., Verstrepen, K.J.& Courtin, C.M. 2017. Bread Dough and Baker's Yeast: An Uplifting Synergy. *Comprehensive Reviews in Food Science* and Food Safety 16, 850–867.
- Verheyen, C. 2016. *Structural investigations of yeasted wheat dough the impact of CO*<sub>2</sub> *and glutathione*. Technische Universität, München, pp. 90.
- Verheyen, C., Albrecht, A., Elgeti, D. & Jekle, M. 2015. Impact of gas formation kinetics on dough development and bread quality. *Food Research International* 76(3), 860–866. doi:10.1016/j.foodres.2015.08.013