UDC 664.644:664.665
DOI: 10.15587/1729-4061.2019.184464

INFLUENCE OF MICROBIAL POLYSACCHARIDES ON THE FORMATION OF STRUCTURE OF PROTEIN-FREE AND GLUTEN-FREE FLOUR-BASED PRODUCTS

Формування структури окремих дієтичних хлібобулочних і борошняних кондитерських виробів, які повинні виготовлятися без пшеничного борошна, ускладнено через відсутність клейковини. Постійно триває пошук ефективних структуроутворювачів взамін клейковинних білків. Досліджено вплив мікробних полісахаридів (МПС) ксантану, енпосану і гелану на формування структури безклейковинного безбілкового хліба і безклейковинних маффінів.

На фаринографі Брабендера досліджено здатність модельної безбілкової системи на основі кукурудзяного крохмалю утворювати тісто за умов додавання ксантану, енпосану і гелану в кількості 0,1...0,5 % до його маси. Встановлено, що за кількості 0,3...0,5 % усіх досліджених мікробних полісахаридів утворюється тісто з показниками, які забезпечують формування необхідної структури тіста за відсутності клейковини.

Досліджено вплив МПС на пружно-еластичні, пластично-в'язкі властивості безклейковинного тіста. Встановлено, що за рахунок додавання ксантану у безбілковому тісті зникають реопексні властивості. В'язкість безбілкового тіста з кількістю ксантану 0,3...0,5 % до маси крохмалю досягає значень, характерних для пшеничного хлібного тіста. У безклейковинному кондитерському тісті для маффінів достатньою є кількість МПС 0,1 % до маси готових виробів. При цьому ефективна в'язкість збільшується у 2...3 разу для усіх досліджених МПС, що забезпечує потрібну консистенцію тіста для формування способом відсаджування.

Досліджено показники якості випечених виробів з додаванням досліджуваних МПС. Показано, що їх використання у визначених кількостях приводить до збільшення питомого об'єму виробів і забезпечення пористої структури випечених виробів. Під час зберігання зменшується кришкуватість виробів, що свідчить про сповільнення процесів черствіння у безклейковинних системах з використанням ксантану, енпосану і гелану.

Усі досліджувані МПС виявляють однаковий характер впливу на ті чи інші показники, але найбільшу дію виявляє ксантан, найменшу – гелан

Ключові слова: безбілковий хліб, безклейковинні маффіни, мікробні полісахариди, структурно-механічні властивості, показники якості

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Received date 25.09.2019
Accepted date 14.11.2019
Published date 17.12.2019

1. Introduction

Gluten-free products form a separate group in bakery and flour confectionery products. They are necessary for diet and health-improving nutrition. They include protein-free and gluten-free products. The group can also include health-improving products based on highly nutritious oil meal and oilcake without wheat flour.

Protein-free products are necessary for patients with disorders of protein metabolism, in particular phenylketonuria. There are about 1.5 thousand people suffering from phenylketonuria in Ukraine today. The incidence of the disease is 1:10,000 of newborns on average in the world. There is a limitation for protein-free products for the total protein content, which is usually from 0.6 to 2.2 % in terms of solids [1]. Gluten-free products are necessary to feed patients with celiac disease, people with allergies to wheat flour and its intolerance. The exact number of celiac patients in Ukraine is not known, because it has large number of forms, different for children and adults, and it is difficult to diagnose. According to various data, the incidence of celiac disease ranges from 1:51 to 1:250 in different countries. There is also an increased public awareness of gluten intolerance and people choose gluten-free foods as an attribute of healthy eating. Consumption of gluten-free products is becoming the norm of life in western countries. Therefore, there is a growth of their production. Gluten-free products are quite popular in the USA, Europe, and Russia [2, 3].

The main difference between protein-free and gluten-free products is a type of flour and its quantity in a formulation. The main raw materials for protein-free products are various types of native starch with 5.0 to 10.0 % of flour added to enhance taste and aroma. The amount of protein introduced with flour is negligible. If flour is gluten-free, then such products will be suitable not only for a protein-free diet, but also for a gluten-free one. It is possible to add gluten-free products to a protein-free diet if they are low in protein. Conversely, protein-free products may also be suitable for a gluten-free diet, but only if they do not contain flours with high gluten content (wheat, rye, barley, and oat). However, protein-free products are poor in protein and they are not adequate products for daily consumption during a gluten-free diet.

Promising raw materials for production of gluten-free products may be oilcake and solvent cake (low fat residues of oil raw material, which remains after removal of the oil by pressing or extraction, respectively). It is possible to use the mentioned raw materials to enrich production with dietary fiber, vitamins, and minerals, or as a major ingredient. If oilcake and solvent cake are made of gluten-free raw materials, then their products are suitable for a gluten-free diet.

The main structure-forming agents are thickeners and gelling agents of polysaccharide nature in absence of gluten. The search for effective structure-forming agents, which would provide formation of dough structure and finished product, is constantly underway. Today, new types of thickeners and gelling agents are emerging on the market and we need research on their use in gluten-free products.

2. Literature review and problem statement

Authors of papers [3, 4] note that replacement of gluten is a serious technological task, since it plays a major role in formation of a dough structure and baked products. Formation of a structure of fibrin and protein free products and gluten-free products coincides largely. But it is more difficult to obtain a structure of protein-free bread in comparison with other products from a technological point of view, since requirements of the diet limit the protein content strictly and exclude all ingredients with its high content [5].

All formulation components take part in formation of a structure in one way or another. Studies on the influence of different types of starch, such as rice, manioc, corn [6], tapioca [7], potato [8], and others [9] on formation of a dough structure in absence of wheat gluten are especially worth of attention. One should note that properties of grain and tuberous starches have certain differences and creation of a dough structure with the necessary properties has certain features. Papers [10, 11] show that combination of different types of starches or modification of their properties contributes to stabilization of a structure [12]. The studies show that the role of starch is important in such systems, but it is not possible to achieve the required quality of dough and finished products without use of structure-forming agents. Papers show the mechanism of gluten-free dough formation: swollen starch grains are embedded in a spatial grid created by swollen non-starch polysaccharides and gluten-free proteins in different ratios [13].

One can influence a structure of protein-free and gluten-free products using different types of flour [14, 15]. Starch is the main raw material in protein-free products usually, and it is possible to add flour in the amount of 5...10 % by weight of starch only. Flour has no significant effect on a structure of products and it is more a flavoring ingredient in this case. A share of gluten-free flour in a formulation can be 5.0...90.0 % by weight of starch or even 100 % in exchange of starch in gluten-free products. Studies [15–17] prove that each type of gluten-free flour causes certain structural-and-mechanical properties of dough and products. Creation of a structure of gluten-free products, unlike protein-free ones, becomes much easier due to the use of structure-forming protein-containing raw materials, such as egg, dairy, and fish products.

There is also instability in a structure of protein-free dough and finished products due to the use of flour raw materials and starches with different properties.

Studies [18, 19] show that improvement of structural-and-mechanical properties of dough and baked goods occurs due to selection of thickeners and gelling agents that play a role of the basic structure-forming agents in gluten-free systems. Such thickeners such as pectin, carboxymethylcellulose, hydroxypropylmethylcellulose, -glucan [20, 21], guar gum, and xanthan [22, 23] affect rheological properties of dough based on rice flour, corn, rice and other starches significantly.

An option to overcome difficulties in structure formation of gluten-free dough systems is optimization of the quantitative content of structure-forming agents [20]. Authors of work [24] show that presence of various hydrocolloids, such as hydroxypropyl methylcellulose (HPMC), carboxymethylcellulose (CMC), plantain gum, carob, guar gum and xanthan contribute to resistance to deformation and elasticity of gluten-free dough. Studies prove that xanthan has the greatest influence on viscous-elastic properties of dough. It causes its hardening. Studies established a line of positive influence of thickeners on a structure of dough: xanthan>CMC>pectin>agar>β-glucan.

Taking into account changing properties of structure-forming raw materials, it is necessary to study each composition to substantiate quantitative and qualitative ratios of hydrocolloids in formulations of gluten-free and protein-free products.

One should note that formulations of flour products include fats, baking powder, and flavor components, which also influence formation of their structure. Therefore, it is important to study indices of structure formation taking into account an ingredient composition of products.

In addition, formulations also include dietary fiber, vitamins, mineral premixes, amino acid mixtures, [25, 26], fruit and berry raw materials obtained using fundamentally new equipment [27–29] to improve the nutritional value of products. Authors of studies propose to use certain technological methods and raw materials, which slows down hardening processes to increase shelf life and reduce crumbling [14, 17, 30, 31].

Table 1 illustrates an analysis of the role of formulation ingredients that influence the formation of a structure of protein-free and gluten-free bakery products

Table 1

Ingredients of formulations that affect the formation of a structure of protein-free and gluten-free bakery products

No.	Name of raw material	Technological function	Reference
1	Starches – corn, potato, wheat, rice and other types of native and modified starches;	The main formulation component involved in formation of the spatial structure	[6–11]
2	Cereal flour for products: – protein-free – of all types of cereals, except legumes, in small quantities (5.0...10.0 % by weight of a formulation amount of starch); – gluten-free – of all types of cereals except wheat, rye, barley, and oats (5.0...90.0 % by weight of starch up to 100.0 % replacement of a formulation amount of starch)	Taste components, structure-forming agents, sources of enzymes and nutrition of yeast	[15–17]
3	Hydrocolloids (thickeners): non-starch polysaccharides – derivatives of cellulose, pectin, agar, gums vegetable and microbial, etc.)	Basic structure-forming agents	[18–24]
4	Biological and chemical baking powder (pressed yeast, dry, sodium bicarbonate, ferments)	Baking powder	[1, 17, 21, 23]
5	Sucrose, glucose, maltose	Sources of yeast nutrition, flavoring components	[20, 21]
6	Fats (oil, margarine, butter, confectionery fats, shortenings)	Plasticizers of dough	[23, 30]
7	Salt, seasonings, spices, spicy aromatic herbs, nuts, dried fruits	Flavorings and fillers	[1, 26]
8	Dietary fiber, vitamin and mineral premixes, phenylalanine-free amino acids, vegetable powders	Enriching additives	[25–29]

The most important factor in formation of a structure of protein-free and gluten-free products, in our opinion, is the use of hydrocolloids of different nature with substantiation of their quantitative ratio.

Researchers consider xanthan (other names: xanthan, xanthan gum) an effective thickener of microbiological origin, and papers [32, 33] emphasize that it is a key ingredient for formation of a structure of gluten-free products. Xanthan exists as an extracellular heteropolysaccharide in nature. It is made of D-glucose, D-mannose and D-glucuronic acid. It appears during the life-cycle of *Xanthomonascampestris* bacteria. Its molecular weight is 1,000... 2,000 kDa.

However, there are other microbial polysaccharides and new ones emerged recently in the market. It is possible to use them in protein-free and gluten-free technologies due to their structure and properties. They include enposan and gellan. They are relatively new microbial thickeners and are less studied.

Enposan (another name is polymixan) is a heteropolysaccharide produced by *Bacilluspolymyxa*. Its molecules consist of D-glucose, D-mannose, D-galactose, and D-galacturonic acid. They have a molecular weight of 1,000...1,500 kDa. Enposan is very close to xanthan by its physicochemical properties [34, 35].

Gellan is a heteropolysaccharide produced by *Sphingomonas Elodea* (formerly *Pseudomonas Elodea*). It has a linear structure. Its molecular weight is approximately 500 kDa. The molecules consist of repeating tetra-saccharide units linked by pyranose rings of D-glucose, D-glucuronic acid, and L-rhamnose. It is capable of forming a jelly with almost all ions, including hydrogen ones (acidic media), but its affinity for divalent ions is much stronger than for monovalent ions [36, 37].

An analysis of information sources shows that there are not enough studies into a possibility of using enposan and gellan in technologies of dietary protein-free and gluten-free products. Enposan and gellan have structures and properties close to xanthan. However, there is no full assessment of their technological potential to stabilize a structure of dough systems, especially in gluten-free dough systems. The issues of studies on product quality indicators in presence of the mentioned microbial polysaccharides remain unresolved. There is no systematic understanding of an influence of enposan and gellan, comparing with xanthan, on processes of dough structure formation and formation of such structural-and-mechanical parameters as ability to form dough, resilience, and elasticity. This is a prerequisite for further research in these directions.

3. The aim and objectives of the study

The objective of the study is to substantiate a use of microbial polysaccharides (MPS) of xanthan, enposan, and gellan as structure-forming agents in protein-free bread and gluten-free muffins.

We set the following tasks to achieve the objective:

– investigation of the ability of a model protein-free system with addition of MPS of xanthan, enposan, and gellan to form dough on Brabender farinograph;

– studying of an influence of MPS of xanthan, enposan and gellan on structural-and-mechanical properties of protein-free bread dough and gluten-free muffin dough;

– investigation of a quality of baked protein-free bread and gluten-free muffins with addition of the studied MPS.

4. Methods to study the properties of dough and quality indicators of finished products

4. 1. Subjects of the study

The subjects of the study were:

– a model system of protein-free dough, which consisted of components in the following weight ratios: corn starch – 100.0, rye flour of peeled grinding – 5.0 and MPS – 0.1...0.5;

we took the system of protein-free dough without addition of MPS as the control one;

– protein-free bread dough, which consisted of components in the following weight ratios: corn starch – 100.0, rye flour of peeled grinding – 5.0, table salt – 2.5, sugar – 4.0, sunflower oil – 5.0 and MPS – 0.1...0.5; we took the protein-free dough made according to the same formulation without addition of MPS as control;

– gluten-free dough for muffins, it consisted of components in the following weight ratios: wheat germ meal – 37.0, white sugar – 18.0, margarine – 17.0, chicken eggs – 8.0, kefir – 18.0, vanilla sugar – 1.0, baking powder – 0.2, salt – 0.5, and MPS – 0.1...0.3; we took gluten-free muffin dough made according to the same formulation without addition of MPS as control;

– samples of baked protein-free bread and gluten-free muffins with addition of the investigated MPS; we took samples of baked products without addition of MPS as control.

We used the following microbial polysaccharides in the study: xanthan – TU U 88-105-001-2000, enposan – TU U 64-20100488.001 – produced by "Enzifarm", Ukraine, and gellan, produced by "CP Kelco ApS", Denmark.

4. 2. Methods for determining the structural-and-mechanical properties of dough for protein-free bread and gluten-free muffins

We determined capability of the model protein-free system to form protein-free dough using Brabender farinograph. The capability was defined by such parameters as duration of formation, resistance to mixing, resistive capacity, stability, degree of dilution and elasticity [38].

We measured structural-and mechanical properties of protein-free bread dough and gluten-free muffin dough at the Tolstoy flat-parallel elastoplastometer [39] defining the modulus of momentary resiliency, modulus of elasticity, and plastic viscosity. The fixed load value for all protein-free dough systems was 50 g, and it was 20 g for muffin dough.

We determined the shear stress (τ, Pa) from formula

$$\tau = \frac{m \cdot g}{F}, \quad (1)$$

where m is the weight of load, kg; g is the acceleration of free fall, m/s^2; F is the area of a plate, m^2.

The tangent shear stress was 327.0 Pa for all samples of protein-free dough, and 50.0 Pa for muffin dough samples. We maintained the same temperature of samples (20 °C) during the experiment, the height of samples of protein-free dough was 8 mm, the muffin dough – 6 mm.

We determined momentary resiliency modulus from formula:

$$G_r = \frac{\tau}{\gamma_0}, \quad (2)$$

where τ is the tangent shear stress, Pa; γ_0 is the relative conditional moment deformation.

We determined the modulus of elasticity from formula:

$$G_{el} = \frac{\tau}{\gamma_{he}}, \quad (3)$$

where τ is the tangent shear stress, Pa; γ_{he} is the relative high-elastic deformation.

We determined the plastic viscosity from formula:

$$\eta_0^* = \frac{\tau}{\mathrm{tg}\alpha}, \quad (4)$$

where η_0^* is the plastic viscosity, Pa·s; tgα is the tangent of an angle of inclination of a finite linear section of a curve to the axis of abscissa.

We determined viscosity of dough for bread and muffins at the "Reotest-2" rotary viscometer (Germany) with an extended rotor speed range of 0.001...100 s^{-1} [39]. The liquid limit or a transition point was determined from the plastic state to the fluid state of substance at low revolutions of the measuring cylinder.

4. 3. Methods for determining the quality indicators of protein-free bread and gluten-free muffins

We determined the quality of products by indicators of specific volume and crumbling after 24 hours of storage according to the methods described in a work [40]. The organoleptic characteristics of products were also defined.

We calculated the specific volume of baked product samples from formula

$$V_{sp} = V/m, \text{ cm}^3/\text{g}, \quad (5)$$

where V is the volume of a sample, cm^3; m is the weight of a sample, g.

We calculated a crumbling indicator of baked products from formula

$$C = (b/a)\ 100\ \%, \quad (6)$$

where C is crumbling, %; a is the weight of a portion of cut cubes, g; b is the weight of crumbs formed due to friction of cubes, g.

The error value for all studies was σ=3...5 %, the number of experiment repetitions was n=5, the probability was P≥0.95. We processed the experimental data statistically by the Fischer-Student method at a reliability level of 0.95. We calculated the results of studies as an average of at least five repetitions. The MS Office software package was used, including MS Excel, and the standard Mathcad software package to process the experimental data.

5. Results of studies on properties of dough and quality indicators of finished products

5. 1. Results of studies on the influence of microbial polysaccharides on structural-and-mechanical parameters of protein-free dough

We established the ability of a model protein-free system to form dough at Brabender farinograph at the beginning of the research. The model system, which consisted of starch, rye flour and microbial polysaccharides in the mentioned ratios, played a role of "protein-free flour". We investigated its ability to form dough under an influence of different amounts of MPS.

The results of studies showed that the obtained farinograms did not have a typical appearance compared to farinograms of wheat flour. They were more similar to farinograms of rye flour, especially in the part of a curve, which characterized stability of dough. Table 2 gives properties of the model systems of protein-free dough according to indicators of farinograms.

Table 2 shows that addition of MPS leads to a decrease in duration of formation of dough. Thus, xanthan has the greatest effect. The addition of 0.3 % by weight of starch reduces duration of dough formation by 44.0 %, and in the amount of 0.5 % by weight of starch – by 56.0 % comparing to the control sample without MPS. Gellan demonstrates the least effect comparing to xanthan and enposan – duration of formation of dough at its amount of 0.5 % by weight of starch reduces by 17.0 %.

Table 2

Indicators of model systems of protein-free dough according to farinograms n=5, $P \geq 0.95$, σ=3...5 %

Dough properties	Amount of microbial polysaccharide, % by weight of starch						
	control sample (without additives)	xanthan		enposan		gellan	
		0.3	0.5	0.3	0.5	0.3	0.5
Duration of dough formation, min	1.80	1.00	0.80	1.10	0.8	1.50	1.00
Resistance of a system to mixing, min	Not detected	0.75	1.00	0.70	0.90	0.70	0.90
Resistance of dough, min	0.80	0.70	0.50	0.70	0.55	0.60	0.55
Degree of dissolution, FU	370	285	220	275	240	270	250
Elasticity, mm	3.0	6.0	10.0	6.0	9.0	5.0	8.0
Stability of dough, min	0.25	0.70	0.90	0.65	0.85	0.60	0.70

It should be noted that we did not observe stability of protein-free dough samples to mixing in dough samples with the MPS content less than 0.3 % by weight of starch, such dough did not keep consistency and, after reaching a peak, began to subside. Instead, addition of xanthan in the amount of 0.3 % led to the emergence of system resistance to mixing for 0.75 min, and in the amount of 0.5 % – for 1 min. Addition of enposan and gellan had the same effect on the system's resistance to mixing, which was slightly less comparing to xanthan.

The degree of dilution of dough, which corresponded to the value of the fall of the curve after 12 min. after start of dilution, decreased at addition of MPS. We established that introduction of additives in the amount of 0.1 % by weight of starch did not reduce the degree of dilution of dough significantly comparing with the control dough without MPS. However, one can see from Table 2 that larger quantities reduced the degree of dilution significantly. For example, introduction of xanthan in the amount of 0.3 % by weight of starch led to a decrease in the degree of dilution of dough by 23.0% and in the amount of 0.5% by weight of starch – by 40.0 %. Xanthan in the amount of 0.5 % by weight of starch had the best effect on reduction of the degree of dilution of dough.

Further mixing of the protein-free dough system resulted in formation of new consistency with certain elasticity. Introduction of MPS increased elasticity of dough. For example, addition of xanthan led to an increase in the elasticity parameter from 3 mm (a control sample) to 10 mm (a sample with addition of xanthan in the amount of 0.5 % by weight of starch). One can see that addition of enposan provided values of elasticity close to xanthan. Addition of gellan had the least effect on elasticity comparing to xanthan and enposan.

An indicator of preservation of dough consistency level characterizes stability of dough. Table 2 shows that the stability of dough with addition of MPS increased. Thus, introduction of additives in the amount of 0.5 % by weight of starch led to an increase in the stability of dough comparing with the control sample. When we added xanthan, it increased in 3.6 times, enposan – in 3.4 times, gellan – in 2.8 times.

We should note that such indicators of farinograms were similar to indicators of farinograms of rye dough and provided for formation of a certain susceptible structure in absence of gluten.

Protein bread dough contains other formulation components usually. Therefore, we investigated an influence of MPS on the structure of protein-free bread dough, which contained salt, sugar, oil and MPS in the ratios specified in paragraph 4, in further research.

Resilient-elastic and plastic-viscous properties determine structural-and-mechanical indicators of dough traditionally.

Studies of resilient-elastic properties of protein-free dough at the Tolstoy elasto-plastometer showed that the momentary resilience modulus increased with addition of MPS at the constant tangential shear stress. Thus, addition of xanthan in the amount of 0.5 % by weight of starch led to an increase in the modulus of momentary resilience by 11.0 % comparing with the control sample (Table 3). Addition of enposan and gellan in the same amount increased the momentary resilience modulus by 9.3 % and 6.3 %, respectively.

Table 3

Structural-and-mechanical indicators of protein-free dough with different amounts of a structure-forming additive n=5, $P \geq 0.95$, σ=3...5 %

Indicator	Without additive (control sample)	Amount of MPS, % by weight of starch					
		xanthan		enposan		gellan	
		0.3	0.5	0.3	0.5	0.3	0.5
Modulus of momentary resilience, $\times 10^{-2}$ Pa	6.4	6.8	7.1	6.6	7.0	6.5	6.8
Elasticity modulus, $\times 10^{-2}$ Pa	9.1	15.3	29.2	14.8	29.0	12.0	14.0
Plastic viscosity, $\times 10^{-3}$ Pa·s	13.20	22.86	41.53	21.30	40.10	18.50	32.80

Addition of MPS led to an increase in the modulus of elasticity. Thus, the indicator for protein-free dough with addition of the investigational preparations in the amount of 0.5 % by weight of starch was 3.2 times higher than the sample without additive for xanthan and enposan, and 1.5 times higher for gellan.

Moreover, the value of the modulus of elasticity was an order of magnitude higher than the value of the modulus of resilience, which indicated the dominance of resilient properties over elastic ones in the dough.

One can also see that as the amount of MPS increased, the plastic viscosity of the protein-free dough increased. Thus, comparing to the control sample, the dough with addition of structure-forming agents in the amount of 0.5 % by weight of starch led to a three-fold increase in the plastic viscosity in case with xanthan and enposan, and in 2.5 times in case of using of gellan.

An indicator of effective viscosity characterizes plastic-viscous properties of dough usually. Effective viscosity is the main characteristic of structural-and-mechanical properties of dispersed systems. This indicator describes the equilibrium state between processes of restoration and destruction of a structure in a fixed flow [33]. Studies on an influence of all investigated MPS on the effective viscosity showed that the dependences are the same. We present the data on xanthan as an example (Fig. 1).

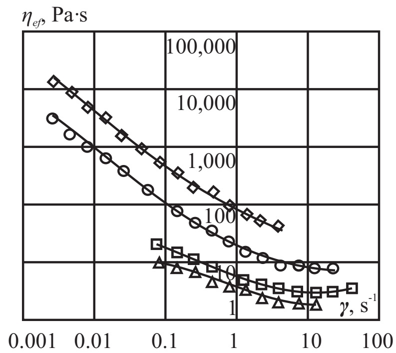

Fig. 1. Dependence of effective viscosity of protein-free dough on the shear rate by the amount of xanthan, % by weight of starch: ▲ – 0 (control); □ – 0.1; ○ – 0.3; ◊ – 0.5

Based on data shown in Fig. 1, one can say that we observed reopex properties of dough in the study of the control sample of the protein-free dough without xanthan. The viscosity decreased at low shear rates (from 1 to 10 s^{-1}), and the viscosity increased as the rotor speed increased. The test sample with the amount of xanthan of 0.1 % by weight of starch behaved similarly. The properties of this dough are quite close to the control dough, but the reopex properties began to show up at higher shear rates than in the control sample (greater than 10 s^{-1}). The protein-free dough with 0.3 % and 0.5 % by weight of starch behaved as a non-Newtonian fluid. We did not observe rheopexy in these dough samples.

One can see in Fig. 1 that the highest effective viscosity in the protein-free dough sample with addition of xanthan in the amount of 0.5 % by weight of starch ($1.5\cdot10^4$ Pa·s) was at the shear rate of 0.0028 s^{-1}. The dough sample with 0.3 % of xanthan by weight of starch had lower viscosity and reached a value of $3.0\cdot10^3$ Pa·s at the same shear rate. We know that the structure of wheat bread dough of high-grade flour has the viscosity of 10^3...10^4 Pa·s at a small gradient of shear rate (0.003 s^{-1}), and the structure of rye dough – the viscosity of 105 Pa·s. Based on the above data, one can say that the viscosity of protein-free dough approaches the viscosity of wheat dough at low shear rate.

5. 2. Results of studies on the influence of microbial polysaccharides on structural-and-mechanical properties of gluten-free muffin dough

We substantiated the use of microbial polysaccharides as structure-forming agents in gluten-free flour confectionery products on the example of muffins. Cupcakes, butter biscuits, biscuit cookies, and others have a structure similar to muffins. Wheat germ meal (WGM) was used as gluten-free flour in muffin formulations.

Muffins with full replacement of wheat flour with wheat germ meal have good organoleptic quality indicators. However, the products have a small volume, inelastic and too fragile crumb due to absence of gluten proteins and starch of wheat flour, which are responsible for a structure of products. Therefore, we proposed using microbial polysaccharides such as xanthan, enposan and gellan as structure-forming agents to give dough necessary properties. Previous laboratory baking showed that muffins with full replacement of wheat flour with wheat germ meal with addition of experimental additives in the amount of 0.1 % by weight of a finished product had the best organoleptic and physical-and-chemical quality indicators.

Table 4 gives the results of measurement of structural-and-mechanical properties of gluten-free dough for muffins with addition of 0.1 % of MPS by weight of a finished product. We used two control samples to compare structural-and-mechanical properties of gluten-free dough and gluten-containing dough in the experiment. Control No. 1 was the dough mixed with wheat flour without additives, and control No. 2 was the dough with wheat germ meal without investigated additives.

Table 4

Structural-and-mechanical properties of gluten-free dough for muffins n=5, $P\geq0.95$, σ=3...5 %

Dough sample based on	Values of indicators		
	Modulus of momentary resilience, $\times10^{-2}$ Pa	Modulus of elasticity, $\times10^{-2}$ Pa	Plastic viscosity, $\times10^{-3}$ Pa·s
wheat flour (control No. 1)	6.1	3.9	3.9
wheat germ meal without additives (control No. 2)	33.5	13.2	31.4
wheat germ meal with addition of:			
xanthan	20.3	10.2	10.6
enposan	19.5	9.4	10.2
gellan	21.1	9.7	15.7

Table 4 data shows that the wheat germ meal dough (control No. 2) had a momentary elasticity modulus by 5.4 times, an elastic modulus by 4 times, and a plastic viscosity indicator by 8 times, higher than parameters of the wheat dough (control No. 1), which contributed to formation of excessively elastic-viscous dough and complicates development and formation of dough pieces by distribution into muffin molds.

Addition of all investigated MPS to the dough with meal contributed to decrease in the value of the modulus of momentary resilience by 1.6...1.7 times, the modulus of elasticity by 1.3...1.4 times and the plastic viscosity by 2.0...3.0 times comparing to control No. 2. We could distribute the dough for muffins with all MPS in the amount of 0.1 % by weight of a product into molds easily. We can state that the use of structure-forming agents can improve structural-and-mechanical properties of gluten-free dough, bringing them closer to the properties of wheat dough typical of muffins.

Fig. 2 shows results of research into the viscous-plastic properties of gluten-free confectionery dough with full replacement of flour with wheat germ meal based on effective viscosity.

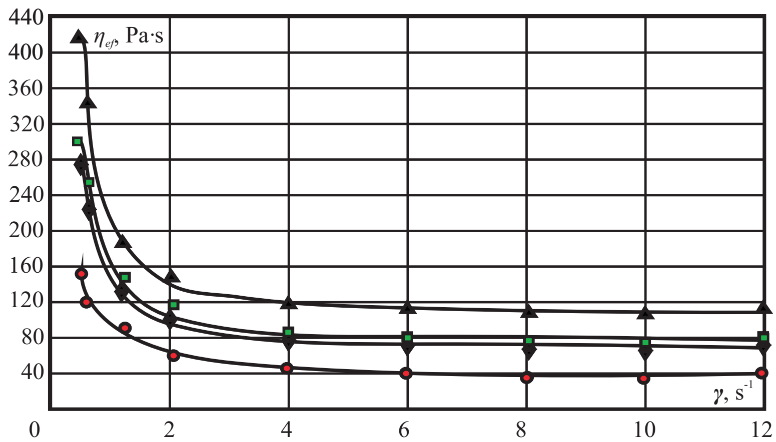

Fig. 2. Dependence of effective viscosity (Pa·s) on the shear rate of gluten-free dough for muffins with addition of 0.1 % of MPS by weight of product: ● – control sample (without additive); ▲ – xanthan; ■ – enposan; ♦ – gellan

Fig. 2 shows that the curves of the effective viscosity of the investigated samples of muffin dough as well as the curve of the control sample are typical of non-Newtonian fluids. A sharp drop in the effective viscosity of dough with a slight increase in a shear rate, and a further increase in a shear rate (greater than 2.0 s^{-1}) resulted in a slight decrease in its values. The use of xanthan increased the viscosity of the dough samples by 3.0 times, and addition of enposan and gellan increased the indicator almost by 2 times comparing to the control sample. An increase in the effective viscosity by 2...3 times in the presence of all investigated MPS contributed to formation of the necessary structural-and-mechanical properties for formation of dough by distribution.

5. 3. Results of studies of the influence of microbial polysaccharides on the quality indicators of baked protein-free bread and gluten-free muffins

Table 5 gives the results from studies on the influence of microbial polysaccharides on quality indicators of baked protein-free bread.

Table 5

Quality indicators of protein-free bread with addition of xanthan and enposan, n=5, P≥0.95, σ=3...5 %

Indicator	Without an additive (control sample)	Value of an indicator with addition of 0.3 % of MPS by weight of starch		
		xanthan	enposan	gellan
Specific volume, cm^3/g	1.8	2.45	2.43	2.4
Crumbling, %	5.7	2.5	2.6	2.8

As one can see in Table 5, addition of MPS in the amount of 0.3 % by weight of starch increased the specific volume of products comparing to the control sample. Xanthan had the greatest influence – the indicator increased its value by 36 %. Gellan had the smallest influence – the indicator increased its value by 33 %. The products with addition of all microbial polysaccharides had well-developed porosity and larger volume comparing with the control sample. They had no cracks on a surface. The bread crumb was similar to the wheat bread crumb in organoleptic terms. The product without addition of MPS had poorly developed porosity and large cracks on its surface.

Addition of MPS affects also storage of products. We can characterize storage by crumbling of crumb after 24 hours of storage. We can see that the crumbling decreased with addition of MPS comparing to the control sample. Thus, the crumbling decreased by 2.3 times with addition of xanthan, by 2.2 times –with addition of enposan and by 2.0 times – with addition of gellan. The data indicated a slowdown in hardening due to the addition of microbial polysaccharides.

Table 6 gives the results from studies of the influence of microbial polysaccharides on the quality indicators of baked gluten-free muffins with wheat germ meal. One can see in Table 6 that the addition of MPS in the amount of 0.1 % by weight of a finished product increased the specific volume of a product and reduced the crumbling value. Thus, addition of xanthan increased the specific volume of products by 14.3 %, enposan – by 10.7 %, gellan – by 3.5 %, and the crumbling decreased by 50.9 %, 50.4 % and 49.1 %, respectively. Muffins with MPS had an excellent appearance, which is characteristic for these products, cracks on their surface characteristic for this type of products and soft, elastic crumb, which was not brittle and did not crumble. Xanthan in gluten-free muffin dough had also the greatest influence, and gellan – the least one.

Table 6

Quality indicators of muffins with wheat germ meal with addition of xanthan, enposan and gellan n=5, P≥0.95, σ=3...5 %

Indicator	Without an additive (control sample)	Value of an indicator with addition of 0.1 % of MPS, by weight of a finished product		
		xanthan	enposan	gellan
Specific volume, cm^3/g	2.8	3.2	3.1	2.9
Crumbling, %	22.0	10.8	10.9	11.2

6. Discussion of results of studies on properties of dough and quality indicators of finished products

Studies on the model protein-free dough system (Table 2) at a farinograph showed that addition of MPS leads to a decrease in duration of dough formation. MPS has good moisture-binding and moisture-retaining properties of MPS, so dough interacts immediately with water in the first minutes of dough mixing and creates a stable colloidal system into which starch grains are embedded during further mixing.

Addition of MPS in quantities greater than 0.3 % by weight of starch leads to an increase in resistance of protein-free dough samples to mixing. At the same time, there is no resistance to mixing at all at addition of MPS in the amount of 0.1 %. Therefore, small quantities of all tested MPS do not form the desired structure of protein-free dough and the amount of an additive should be at least 0.3 % by weight of starch.

The degree of dilution of dough, which corresponds to the magnitude of the fall of the curve after 12 min after the beginning of dilution, decreases significantly with an increase in the amount of MPS, apparently, due to the branched structure of biopolymer molecules, especially xanthan. Addition of them causes structuring of a protein-free dough system and dilution decreases.

Further mixing of a protein-free dough system leads to formation of new consistency with a certain elasticity due to swelling and insignificant action of hydrolytic enzymes. The elasticity increases with an increase in the amount of MPS (Table 3). An increase in the elasticity indicator with an increase in the amount of additives is obviously related to formation of an elastic gluten-like structure in dough and appearance of elasticity and resilience in it. Therefore, we can increase stability of dough by introduction of structure-forming agents in the process of mixing of a protein-free starch-based dough system, which differs from the traditional one by absence of gluten.

It is clear that the dough forming process occurs due to binding of water by dry components of a protein-free dough system. Addition of MPS accelerates formation of protein-free dough due to their good hydration ability. Elastic protein-free dough will promote formation and preservation of its porous structure during its fermentation, separation, and baking.

The study on the effective viscosity of protein-free dough (Fig. 1) showed that it exhibits rheopexy properties without addition of xanthan. A sharp increase in the shear rate leads to a sharp increase in the dough viscosity, which can lead to overload and equipment failure. Addition of xanthan in the amount 0.3 % and 0.5 % by weight of starch will eliminate reopex properties. Dough will behave like a non-Newtonian liquid. We examined such samples of protein-free dough at low shear rates ($\gamma<1\ s^{-1}$). The samples exhibited viscous-plastic properties, which corresponds to the Ostwald dependence. An increase in the shear rate leads to a gradual destruction of the structure and achievement of a constant final viscosity of the Newtonian fluid. The viscosity of protein-free dough at low shear rate approaches the viscosity of wheat dough at addition of xanthan in such quantities. One can predict that protein-free dough will exhibit the same properties as wheat dough when processing it at the bakery. Almost parallel displacement of the curves of dependencies of the effective viscosity on the shear rate for protein-free dough samples with xanthan in the amount of 0.5 % and 0.3 % indicates that there is almost constant coefficient between these dependencies, which is approximately 4.7±0.2. This can indicate identical mechanisms of structure formation in the dough samples.

Studies (Fig. 1) showed that addition of xanthan in the amount of 0.3...0.5 % by weight of starch strengthens significantly a protein-free system under conditions of the same shear rate. And the effective viscosity of dough increases significantly. Protein-free dough with xanthan exhibits properties of non-Newtonian liquids, so it can refer to highly concentrated dispersed systems with a coagulation structure. Interaction between elements (starch grains, flour particles, etc.) occurs through a thin layer of dispersion medium (swollen xanthan) and it is conditioned by van der Waals forces in such structures.

According to ideas about structure formation in gluten-free systems, we can say that xanthan acts as a hydrocolloid in dough. It envelops starch grains and forms a stable structure similar to the gluten structure of dough. The reason for an increase in viscosity may be that xanthan can reduce the amount of free moisture in protein-free dough, since it has a strong moisture-binding and moisture-retention capacity. In general, we can say that MPS take part in formation and maintenance of the spatial structure of dough and thus provide formation of rheological properties of protein-free dough similar to traditional bread wheat dough.

Muffin dough is significantly different from protein-free bread dough in structure. It contains more moisture, includes protein-containing dairy and egg products that are involved in formation of the spatial structure of gluten-free confectionery dough. Studies showed that a sufficient number of MPS is 0.1 % by weight of a finished product. The effective viscosity (Fig. 2) increases by 2...3 times when using the investigated MPS at the same shear rate in this case. Thus, we obtain the desired consistency of dough for molding by distribution from a confectionary bag. The results of studies on elasticity and resilience of gluten-free dough for muffins with MPS confirmed the above (Table 4).

We know the ability of gluten and other proteins included in a dough system to preserve the swelled structure during baking determines the specific volume of flour products. As addition of MPS improves the specific volume of products (Tables 5, 6), we can assume that swollen microbial polysaccharides together with proteins of other components act as gluten and provide formation of a swollen structure for products in the gluten-free dough system.

Crumbling of flour products occurs usually due to formation of air layers, because of reduction of the volume of starch grains due to their crystallization. Air layers are more noticeable in stale bread. Most likely, the ability of microbial polysaccharides to reduce crumbling of protein-free bread (Table 5) and muffins (Table 6) may be related to enveloping of partially gelatinized starch grains and slowing down of their compaction due to crystallization of amylose and amylopectin during storage. We can explain the effect by slow formation of air layers between a hydrocolloid and partially gelatinized starch grains.

The obtained results show that enposan and gellan exhibit a similar effect on formation of a gluten-free dough structure as xanthan does. However, different types of dough require different amounts of MPS, depending on availability of protein-containing raw materials, which can influence formation of the structure significantly. However, the mechanism of linkage formation in the spatial structure of dough in presence of microbial polysaccharides and their influence on formation of physical-and-chemical parameters of baked products remain not fully studied. Therefore, further studies on the influence of MPS on organoleptic quality indicators, and structural-and-mechanical properties of baked goods, including storage period, are promising.

7. Conclusions

1. We established that the presence of xanthan, enposan and gellan in the amount of 0.3...0.5 % by weight of starch in dough leads to formation of a susceptible dough structure in

the absence of gluten. Duration of dough formation decreases by 17...56 %. System resistance to mixing and elasticity increase, and a degree of dilution of dough decreases by 23...43 %. The stability of dough increases also by 2.4...3.6 times comparing to dough without addition of MPS.

2. It was found that an increase in the amount of MPS from 0.1 to 0.5 % by weight of starch leads to improvement of resilient-elastic and plastic-viscous properties of protein-free bread dough. We established that addition of xanthan to a protein-free dough system influences a change in the effective viscosity indicator significantly. Reopex properties of dough disappear due to introduction of additives. It acquires properties of a non-Newtonian fluid, and the viscosity of protein-free dough with xanthan content of 0.3...0.5 % by weight of starch reaches the values typical of traditional wheat dough.

The amount of MPS of 0.1 % by weight of a finished product is sufficient for gluten-free confectionery dough for muffins. In this case, the effective viscosity increases by 2...3 times for all studied MPS, which provides the required consistency of dough for its formation.

3. The study showed that the use of xanthan, enposan, and gellan leads to an increase in the specific volume of products and to a porous structure during baking. We established that crumbling decreases during storage, which indicates slowing of hardening processes in gluten-free systems with addition of MPS. We obtain products with good organoleptic characteristics. They have well-developed porosity. They have a surface, taste and aroma, which are characteristic for the products.

All the investigated MPS have the same influence on certain indicators, but xanthan exhibits the greatest effect, and gellan – the least one.

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