1. Introduction

The global market of special, dietary foods has been developing rapidly recently. According to statistical forecasts, their share in the coming decades in developed countries will be up to 30 % of the total food market [1, 2]. Among dietary foods, a special place belongs to protein-free and gluten-free products, including bakery and confectionery products, the need for which is constantly growing.

The addition of the studied MPS leads to a slowdown in the staling processes of protein-free bread based on corn starch, as well as muffins based on wheat germ meal, during storage. It was found that in 24 hours of storage, the protein-free bread demonstrates a decrease in the moisture loss and crumbling index, as well as an increase in compressibility indicator, compared with control samples. It was also determined that gluten-free muffins with the addition of MPS lose moisture more slowly over 7 days of storage; they have lower crumbling and compressibility indices compared to the control. This is due to the high hydrophilic properties of the studied microbial polysaccharides, which can bind a significant amount of water and retain it during the storage of products. In addition, microbial hydrocolloids can envelop the gelatinized starch grains with a thin film, thereby helping inhibit the process of starch retrogradation.

The samples of bread and muffins containing MPS almost did not change their appearance, color, taste, and smell during the studied shelf-life, while the crumb of the examined samples demonstrated better elasticity and less crumbling.

All studied MPS exhibit the same nature of the effect on the quality indicators of products during storage with xampan exerting the greatest effect and gelan – the least.

Keywords: protein-free bread, gluten-free muffins, microbial polysaccharides, processes of staling, starch retrogradation, quality indicators

EFFECT OF MICROBIAL POLYSACCHARIDES ON THE QUALITY INDICATORS OF PROTEIN-FREE AND GLUTEN-FREE PRODUCTS DURING STORAGE

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rye, barley, and oatmeal, which contain gluten, are completely excluded for some people.

As gluten-free raw materials, the extraction cakes or meals, non-fat residues of oil raw materials, which are by-products in the technological process of obtaining oil by pressing or extraction, respectively, can be used.

The absence in the formulations of bakery and confectionery products of wheat flour, which is a source of gluten proteins, which play a major role in shaping the structure of the dough, leads to the need to involve other structure-forming agents. Among them, thickeners and gelling agents of polysaccharide nature are most often used. An effective structure-forming agent in gluten-free products is the microbial polysaccharide xanthan. However, new microbial polysaccharides (MPS) such as enposan and gelan have recently appeared in the market. These hydrocolloids not only participate in the formation of the structure of the dough and finished products but also affect the staling process during the storage of gluten-free products.

Therefore, it is a relevant task to study the processes that occur during the storage of gluten-free products.

2. Literature review and problem statement

When storing protein-free and gluten-free products, there is a decrease in their quality associated with the processes of drying and staling, accompanied by loss of moisture, increased stiffness, and reduced elasticity of the crumb. The taste and aromatic characteristics of the products also deteriorate [7]. Although staling processes have been studied for a long time, their mechanisms in dough systems that do not contain traditional types of flour for baking have not been fully elucidated.

According to the accepted theory of staling, the loss of moisture by products begins immediately after leaving the oven, which is due to the heat and mass transfer processes inside and on their surface. The main role in staling is assigned to the retrogradation of starch. During baking, the starch grains swell and partially gelatinize while the starch changes from a crystalline to an amorphous state. During storage, it is reversed to a crystalline state, called retrogradation. In the proteins of bread, denatured during baking, the hydration capacity is reduced during storage, which leads to the compaction of its structure [8, 9].

Since during the staling of bread, including protein-free, the changes in its properties are largely due to the partial loss of moisture, its binding and retention can employ various hydrocolloids such as hydroxypropylmethylcellulose (HPMC), carboxymethylcellulose (CMC), guar gum, xanthan, etc. [10]. They can also include hydrocolloids contained in concentrated plant raw materials [11, 12].

In the technology of protein-free bakery products, hydrocolloids together with starches play a major role in shaping the structure of products [13–15]. To produce gluten-free bread, cellulose derivatives are used, as well as plant-derived gums, agar, pectin [16, 17], or their combinations [18], to improve the structural-mechanical, physical-chemical, and organoleptic characteristics of its quality. However, such products stale quickly and their shelf life is shorter compared to traditional products, which leads to the search for hydrocolloids that help extend shelf life.

Work [18] showed that, when compared to other hydrocolloids, xanthan exerts the best effect on the viscoelastic properties of the dough, its resistance to deformation, and bread quality. The microbial polysaccharide xanthan (E 415) (other names: xampan, xanthan gum) belongs to the class of food additives, thickeners, and gelling agents, and is widely used in the food industry [16, 19]. Xanthan, produced by the bacteria Xanthomonas campestris, is made up of D-glucose, D-mannose, and D-gluconic acid. Its molecular weight is 1,000...2,000 kDa.

The authors of [20] proved that when 0.1% of xanthan is added to the dough after 24 h of storage, bread loses 1.0% less free moisture than with κ-carrageenan, carboxymethylcellulose (CMC), and alginate. It is shown that the addition of xanthan reduces the degree of hardness of the crumb and the rate of staling of wheat bread during storage. In work [21], this is explained by the fact that gums change the structure of the amorphous phase of the crumb due to the formation of macromolecular structures with starch. This is likely due to the fact that the anionic polysaccharide xanthan forms unstable compounds with protein molecules [18], as well as interacts with various starches [22–24]. In paper [25], it was also found that xanthan stabilizes starch gels due to intermolecular interactions, slowing down the retrogradation of lotus seed starch. However, data on changes in the quality of protein-free and gluten-free products with the addition of xanthan during storage are not generalized.

With the help of microorganisms, other polysaccharides have recently been obtained, which are similar in structure and properties to xanthan. Among such, of interest are the xanthan-type thickeners enposan and gelan, which are currently less studied.

The enposan heteropolysaccharide (also known as polyxian) is produced by Bacillus polymyxa bacilli. Its molecules consist of D-glucose, D-mannose, D-galactose, and D-galacturonic acid, and have a molecular weight of 1,000...1,500 kDa. In terms of its physicochemical properties, enposan is very close to xanthan [26, 27].

Gelan is a heteropolysaccharide produced by Sphingomonas Elodea (formerly Pseudomonas Elodea) that has a linear structure, its molecular weight is approximately 500 kDa. Macromolecules consist of repeating tetrasaccharide units of D-glucose, D-gluconic acid, and L-rhamnose. Gelan can form gels with almost all ions, including hydrogen (acidic media) but the affinity for divalent ions is much stronger than for monovalent [28, 29].

The effect of enposan and gelan, in comparison with xanthan, on the processes of structure formation in protein-free bread on corn starch and in gluten-free muffins based on wheat germ meal was studied in [30, 31]. It is shown that the use of all MPS in the protein-free dough system in the amount of 0.3% by weight of starch allows obtaining dough and bread with the necessary structural and mechanical parameters. To form the structure and high performance of gluten-free muffins, their rational amount is 0.1% by weight of finished products. However, the issues of the effect of xampan, enposan, gelan, which have hydrophilic properties, on the staling processes that occur during the storage of products, have not been studied in detail, which predetermines the expediency of research in this area.

At present, scientific approaches to inhibiting the staling processes of protein-free and gluten-free products are not fully defined. Since the deterioration of the quality of non-gluten-free products during storage is mainly due to staling processes, it is important to find effective ways to slow down these processes. Given this, microbial polysaccharides are
not only effective structure-forming agents but the substances that affect the processes of staling. Therefore, the research is important that could identify the most effective techniques, from this point of view, to expand the range of their application.

3. The aim and objectives of the study

The research aims to determine the effect of the microbial polysaccharides (MPS) xampan, enposan, and gelan on a change in the quality indicators of protein-free and gluten-free products during storage. This would allow a reasonable approach to the choice of hydrocolloids in the formulations of such products, in order to improve the efficiency of their manufacturing process and ensure the high quality of finished products, including during storage.

To accomplish the aim, the following tasks have been set:

- to determine a change in the physical-chemical, structural-mechanical properties of protein-free bread and gluten-free muffins, which contain the experimental microbial polysaccharides, during their storage;
- to determine a change in the organoleptic properties of protein-free bread and gluten-free muffins containing microbial polysaccharides during storage.

4. Methods to study the quality indicators of protein-free bread and gluten-free muffins during storage

4.1. The study objects

Protein-free bread and gluten-free muffins with the addition of microbial polysaccharides, which were made according to the formulation and technology that we developed, were selected as the objects to study. Protein-free bread and gluten-free muffins without the addition of MPS served as control:

1. Protein-free bread, baked according to the following formulation (g): corn starch (100.0), rye flour (5.0), dry yeast (0.8), table salt (2.5), white sugar (4.0), sunflower oil (5.0), and MPS (0.3). The dough moisture content is 50.2 %. The yield is 167 %.

The protein-free bread was prepared as follows. Corn starch, peeled rye flour, white crystal sugar, table salt, and the MPS xampan, or enposan, or gelan were mixed. Pre-hydrated dry yeast was added to the resulting mixture and the dough was kneaded. The fermented dough was divided into pieces, placed in molds greased with oil. The dough was kneaded. The fermented dough was divided into pieces, placed in molds greased with oil. The dough was mixed, adding sunflower oil at the end of kneading. The kneaded dough was subjected to fermentation for 60 min at a temperature of 30…32 °C. During fermentation, the dough was kneaded. The fermented dough was divided into pieces, placed in molds greased with oil. The dough blanks were subjected to aging for 30 min at a temperature of 35…37 °C at W=75...85 %. The bread was baked at t=210…220 °C for 40…60 minutes.

2. Gluten-free muffins were baked according to the following formulation (g):

- wheat germ (38.0), white crystal sugar (17.5), chicken eggs (6.6), margarine (16.5), kefir (22.0), vanilla sugar (0.85), baking powder (0.25), MPS (0.1). The yield is 100 g.

Dry (meal, sugar, vanilla sugar, and baking powder, salt, the MPS xampan, or enposan, or gelan) and liquid ingredients (plasticized margarine, chicken eggs, kefir) were mixed separately. Next, the dough was kneaded and dough pieces were formed, which were baked at t=175…180 °C for 30…40 min, and cooled.

Wheat germ meal (hereinafter, meal) is a by-product in the technology of germ oil, which contains a significant amount of protein (37…43 %) and non-starch polysaccharides (22…26 %), mainly represented by cellulose and hemicelluloses. Besides, the meal contains mono- and disaccharides, vitamins PP, E, group B vitamins, carotenoids, and minerals [32].

Experimental dosages of MPS were selected on the basis of results from previous studies into the structural-mechanical [30], organoleptic, and physical and chemical properties of protein-free bread and muffin dough based on wheat germ meal [31].

In our studies, the following substances were used: corn starch, according to DSTU 3976-2000; wheat germ meal, according to TU U 20608169.002-99; xampan, according to TU U 88-105-001-2000; enposan, according to TU U 64-20100488.001, manufactured by Enzym Pharm (Ukraine); gelan, according to the quality certificate (manufactured by CP Kelco ApS, Denmark).

4.2. Methods for determining the MPS effect on the physical-chemical and organoleptic indicators of products during storage

Product indicators during storage were determined using the following methods:

- determining a change in the moisture content of products during storage was performed by drying to constant weight [33];
- measuring the degree of deformation of the product crumb (compressibility) – at the automated penetrometer made by Labor [33];
- determining the products' crumbliness based on the content of crumbs formed as a result of shaking in a vibratory mixer [34].

Changes in the organoleptic parameters of their quality were also determined [33].

Freshly baked products were analyzed not earlier than 3 hours after baking.

Statistical processing of experimental data was carried out by the method of Fischer-Student: the level of confidence was 0.95. The error of the experiment was 3…5 %.

5. Results of studying the effect of microbial polysaccharides on the products' properties during storage

5.1. Determining the effect of microbial polysaccharides on the properties of protein-free bread

It is known that during cooling and subsequent storage, bread loses moisture, which is a sign of its staling. Moreover, the more intense the loss of moisture, the faster the bread loses its freshness.

The protein-free bread was stored without packaging for 24 hours at a temperature of 18…20 °C at W=5…80 %.

Results of the experiments are shown in Fig. 1.

Fig. 1 shows that the addition of experimental MPS, especially xampan, helps slow down the loss of moisture by bread during storage. Thus, during 24 h of storage, the control sample lost 4.2 % of moisture; while when introduced with xampan, enposan, and enposan – 2.6, 2.9, and 3.3 %. These data are consistent with the results reported in [10, 15, 20, 28] on the effect of hydrocolloids on a change in the moisture content of bread during storage.
When bread is stored, there are changes in the structural and mechanical properties of the crumb, which also indicates its staling.

The results of determining the crumb compressibility of the control and experimental samples of protein-free bread at the penetrometer are shown in Fig. 2.

Data in Fig. 2 show a decrease in this indicator of all bread samples during storage, which indicates the loss of freshness. The control sample has the lowest initial compressibility, which decreases slightly over 24 hours of storage. It should be noted that the addition of microbial polysaccharides, especially xampan, leads to a significant increase in this indicator, which, although declining rapidly during storage, remains higher than in the control sample. Thus, after 24 hours of storage, the compressibility of protein-free bread crumbs was higher than that in the control sample by 3.4; 2.8, and 1.4 times when using xanthan, enposan, and gelan, respectively.

The freshness of bread is also determined by the ability of the crumb to crumble.

The experimental data characterizing the change in the crumbliness of protein-free bread during 24 h of storage are shown in Fig. 3.

Fig. 3 shows that the crumbliness indicator of the control bread sample increases by 4.0 times during 24 hours of storage. Whereas the bread crumbs with the addition of xampan, enposan, and gelan increase to a lesser extent, by 2.5...3.6 times. Samples of bread with the addition of xampan have the lowest crumbliness.

The results of experimental studies into the effect of xampan, enposan, gelan on the process of staling are confirmed by organoleptic characteristics. There was no significant difference between their effects on the organoleptic characteristics.

The organoleptic characteristics of baked protein-free bread after 24 hours of storage are given in Table 1.

| Characteristic | Protein-free bread without MPS (control) | Protein-free bread with the addition of 0.3 % of MPS to the starch mass |
|---------------|------------------------------------------|-------------------------------------------------------------|
| Appearance    | Proper, hilly, torn with large cracks and explosions | Proper, smooth, without cracks and explosions |
| Color of the crumb crust | Light-yellow, white, evenly colored | Light-yellow, evenly colored |
| State of crumb | Inelastic, small uneven, poorly developed, thick-walled, rigid | Elastic, medium porosity, uniform, well developed, thin-walled, soft |
| Taste and smell | with starch flavor and smell | inherent in bread, without extraneous flavors and smells |

It should be noted that the freshly baked sample of protein-free bread without the addition of MPS demonstrated significantly worse organoleptic characteristics compared to the samples with the addition of xampan, enposan, gelan. Thus, the shape of the...
bread was correct, but hilly with torn and large cracks and explosions, and its appearance remained the same after 24 hours of storage. At the same time, freshly baked samples with the addition of MPS were characterized by the correct shape, without cracks, explosions, which do not change during storage. The color of both freshly baked samples and those stored for 24 hours did not change.

Freshly baked bread without the addition of MPS had an inelastic, uneven, poorly developed, thick-walled hard crumb that grew harder during storage. The addition of MPS ensures the formation of the required porosity and crumb structure of freshly baked bread and helps preserve the soft structure over 24 hours of storage.

Freshly baked bread sample without the addition of MPS is characterized by a starchy flavor and smell that are not felt when adding MPS. This trend is observed during the studied shelf life.

5.2. Determining the effect of microbial polysaccharides on the properties of gluten-free muffins

The examined samples of muffins were stored in plastic bags for 7 days at a temperature of 18...20 °C and W of the air not higher than 75%. Changes in moisture, compressibility, crumbliness, and organoleptic characteristics of muffins were also investigated during storage.

The results of studying the changes in the mass fraction of moisture during storage are shown in Fig. 4.

The data in Fig. 5 demonstrate that during storage the control samples lose moisture less intensely than the experimental muffins.

Indeed, after storage for 1...7 days, the moisture content in products without MPS is reduced by 2.4...7.5%, while in muffins with the addition of xampan – by 0.7...4.1%, with the addition of enposan – by 1.4...4.8%, gelan – by 2.0...5.8%, respectively. The products with the addition of xampan lose the least moisture, those with gelan – the most.

Changes in the compressibility of the crumb of the control and experimental muffins over 7 days of storage are shown in Fig. 5.

The results of studying the changes in the mass fraction of moisture during storage are shown in Fig. 4.

Fig. 4. Change in the muffin moisture content during storage: 1 – muffins without MPS (control); muffins with the addition of: 2 – xampan, 3 – enposan, 4 – gelan

The data demonstrate that with the addition of all MPS the reduction in this indicator for muffins is less intense than that in control products.

Thus, after 1...7 days of storage, compressibility decreases in muffins without additives by 9.8...41.1%, while in samples with the addition of xampan – by 5.1...20.7%, enposan – by 5.2...25.8%, gelan – by 12.0...32.8%, respectively. Xampan exerts the best effect on maintaining the soft structure during storage: gelan – the worst. At the end of the experiment, the compressibility of muffins with the addition of the xampan, enposan, and gelan polysaccharides was 1.3, 1.4, and 1.3 times higher than in the control sample, respectively.

The results from determining the crumbliness of muffins during storage are shown in Fig. 6.

Fig. 6 shows that during storage the crumbliness of the samples increases. Thus, after 7 days of storage, the crumbliness of the control sample increases by 1.8 times compared to the first day of storage, in products with the addition of xampan – by 1.4 times, enposan – by 1.6 times, gelan – by 1.7 times, compared to the original. Products with the addition of MPS have lower crumbliness compared to the control.

The results of studying the MPS effect on the organoleptic characteristics of the baked gluten-free muffins after 7 days of storage are given in Table 2.

It should be noted that the organoleptic characteristics of freshly-baked muffins, both for the control sample and those with the addition of MPS, had the greatest differences in the appearance and condition of the crumb. The addition of MPS contributes to the absence of explosions of the crust during baking, as well as significantly improves the structure of the products. The crumb becomes elastic, well fluffy, soft, not brittle. Table 2 illustrates that the addition of MPS does not change the color, taste, and smell of gluten-free muffins during storage. However, adding them helps preserve the soft and non-brittle structure of gluten-free muffins.

Based on the results from determining the effect of xampan, enposan, gelan on the organoleptic characteristics of gluten-free muffins, no significant difference was found.
light brown, golden brown
gelan
Enposan

slow down their compaction due to the steric hindrance of hydroxyl groups of the hydrocolloids while enveloping par
gen bonds, thereby inhibiting retrogradation processes. It is inferred that the longer hydrocolloids – amylpectin and starch

are related to their interaction with amylopectin and starch amylose. Microbial polysaccharides can bind to the branched
chains of amylopectin molecules to form associates, as well as to the linear chains of amyllose molecules to form hydrogen bonds, thereby inhibiting retrogradation processes. It is also possible that the hydrocolloids while enveloping partially gelatinized starch grains, slow down their compaction during the crystallization of amyllose and amylopectin [23].

The results from a set of our studies suggest the possibility of using experimental microbial polysaccharides to slow the staling of not only gluten-free products but also traditional products based on wheat flour.

In the future, studies are planned to tackle the effect of experimental microbial polysaccharides on changes in water state in protein-free and gluten-free bakery and confectionery products, which would expand the understanding of the mechanism of their action in these systems.

7. Conclusions

1. The addition of the microbial polysaccharides xampan, enposan, and gelan slows down the staling processes of protein-free bread based on corn starch and muffins based on wheat germ meal during storage. This has been proven by the fact that at the end of shelf life the moisture losses by bread are reduced by 2.6, 2.9, and 3.3 %, and muffins – by 7.5, 4.1, and 5.8 %, compared to the control sample, respectively. In this case, the compressibility of bread crumbs containing xampan, enposan, and gelan, respectively, is 3.4, 2.8, and 1.4 times higher, for muffins – 1.5, 1.4, and 1.3 times higher than that in control products. The crumbliness of the control bread sample increases by 4.0 times, while this indicator for products with the addition of experimental MPS increases to a lesser
extent, by 2.5...3.6 times. The crumbliness of muffins with the addition of microbial polysaccharides increases by 1.4...1.7 times, while in the control sample – by 1.8 times. The samples of bread and muffins with MPS almost do not change during the studied shelf life in appearance, color, taste, and smell, while the crumb of the tested products has better elasticity and less crumbliness. Our results indicate the significant potential of the studied microbial polysaccharides as substances that contribute to the prolongation of product freshness.

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