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PHYSICO-CHEMICAL AND TEXTURAL INDICATORS OF SAUSAGES FOR CHILD NUTRITION ENRICHED WITH PREBIOTIC INGREDIENTS

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Abstract

The addition of healthy prebiotic fibers into meat products can affect product technological parameters, especially texture. The work aimed to develop a recipe of air-dried sausages for child nutrition enriched with a complex of prebiotic ingredients and to study their physico-chemical, textural, and bifidogenic properties.

The composition of the sausages was developed concerning the current Russian requirements for meat products for the nutrition of children over three years of age in terms of fat, sodium chloride, and sodium nitrate content. The experimental and control (without enrichment) samples were made. The mixture of beef and pork subjected to the curing process, a complex of prebiotics (resistant corn starch Fiberfin, collagen hydrolysate, oligofructose powder ORAFTI, and wheat fiber), spices and water were mixed. Sausages were dried up to reach a moisture content of not more than 42%. The following indicators were determined in the experimental and control samples: pH with a pH-meter Testo 205 (Germany), water holding capacity (WHC) of meat by the press method, protein by the spectrophotometric method, fat with the use of a Soxhlet extraction apparatus, texture with a texture analyzer Structurometr ST-2 ("Laboratory of quality", Russia). To determine the bifidogenic properties, the sensitivity of the test strain to the action of the product was revealed. The test culture *Lactobacillus plantarum* was incubated at a temperature of 37 ± 0.5 ^{0}C for 24 hours. Statistical analysis of experimental data was carried out using the Mann-Whitney criterion using the STATISTICA 10.0 software ($p < 0.05$).

The moisture content of the samples was in a range of 36 - 42 g/100 g. The samples were characterized by the high protein content (4 g/100 g) and low-fat content (12 g/100 g). The addition of the prebiotic complex reduced the pH level relative to the control sample due to the accumulation of organic acids during fermentation and WHC. With that, the sodium chloride concentration (1.8%) required for this product group is ensured in this product. The number of viable *Lactobacillus plantarum* 8P-A3- cells was 1 x 10^4 CFU/cm^3 in the experimental sample and 5 x 10^3 CFU/cm^3 in the control sample, which contained potato starch. The hardness and springiness values of the experimental sample were significantly ($p < 0.05$) lower (by 1.5 times and 1.1 times, respectively) compared to the control. Chewiness correlated with hardness and was 1.3 times lower in the sample with the addition of prebiotic fibers, probably because wheat fibers have a complex crystalline structure, which makes mincing easier and facilitates chewing.

The results obtained suggest the general improvement of the organoleptic properties due to the softer texture and tenderness of the experimental sample compared to the control.

***Key words**: Sausages, Prebiotic fibers, Texture.*

1. Introduction

Meat in a diet for children of all age groups is a valuable source of essential micro- and macroelements necessary for the growth and development of the body.

Various studies investigated the introduction into meat products of ingredients with an assumed positive effect on the health of the large intestine, which can balance out possible harmful impacts of meat on the gastrointestinal tract. According to modern scientific concepts, a significant role in healthy nutrition is assigned to consumption with food of indigestible food ingredients (prebiotics), which selectively stimulate

the growth of beneficial bacteria in the large intestine. Many studies demonstrate that the consumption of dietary fibers leads to changes in the counts of gut microbes and the production of short-chain fatty acids by them [1, 2, and 3]. Currently recognized prebiotics include inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), lactulose, and several others. The suitability of such compounds as resistant starch and peptides is still under investigation [4, 5]. The effectiveness of resistant starch as a prebiotic was shown in several independent foreign clinical trials. It increased the number of beneficial gut microorganisms and concentration of short-chain fatty acids, which in many respects is similar to the outcome of intake of known prebiotics [6].

In Russia, specialized products intended for school-age children are widely used for nutrition in organized groups and are sold in retail chains. Their production is regulated by law. All food product components (food ingredients) used in product manufacture should be approved for use in child nutrition. Requirements have been established for meat products in terms of the minimal protein content as well as the maximum content of fat, sodium chloride, and sodium nitrite. The use of colorants, phosphates, and preservatives is forbidden. Therefore, the main task in constructing a meat product recipe enriched with prebiotics is an assurance of the balanced composition that meets the requirements for this product group intended for child nutrition concerning age peculiarities and enrichment with a physiologically significant quantity of a prebiotic or combination of prebiotics that selectively stimulate the growth of bifidobacteria and/or lactobacteria that are technologically stable in product manufacture.

The bifidogenic properties of prebiotic ingredients can be determined *in vitro* by detecting the sensitivity of microbial test strains - representatives of the population of protective microflora of the human gut belonging to genera *Lactobacillus* spp., *Bifidobacterium* spp., and *Escherichia coli* - to an impact of an ingredient being analyzed [4].

Different types of dietary fibers can be distinguished by the degree of their fermentability in the large intestine, where they exert their beneficial action. Fructooligosaccharides (FOS) are fermented faster, while resistant starch is broken down more slowly. Their combination can be interesting to achieve a positive effect on the whole large intestine [7].

The use of prebiotic additives in the meat product composition requires investigation of their safety, the effectiveness of manifestation of the bifidogenic potential, and an influence on product technological parameters, especially texture. Today, studies are carried out worldwide to reveal the effects of prebiotic fiber addition into different meat product types: emulsified, fermented, and restructured [8]. It can be seen by analyzing the results of these studies that data on the effect of various dietary fibers on organoleptic and structural-mechanical indicators of meat products obtained by different authors differ significantly [9 - 15].

The work aimed to develop a recipe of air-dried small sausages enriched with a complex of prebiotic ingredients facilitating the growth of lactobacteria that are intended for the nutrition of school-age children and to study physico-chemical and textural properties of these small sausages after their production.

2. Materials and Methods

The composition of air-dried sausages was developed concerning the current Russian requirements for meat products for the nutrition of children over three years of age in terms of fat, sodium chloride, and sodium nitrate content. The experimental (enriched), and control (without enrichment) samples were made.

To produce the samples, beef, and pork intended for the nutrition of children over three years of age according to sanitary and hygienic requirements were used. Beef and pork were obtained directly from the producer (LLC Prodpostavka Russia, Vladimir Oblast).

Prebiotics were selected having regard to the bifidogenic properties of individual components that were studied by us earlier in the *in vitro* experiment by Derevitskaya *et al.*, [16], and the combination for increasing the general functionality was created. The complex contained the known prebiotic (oligofructose powder Orafti, Belgium), as well as substances that showed the bifidogenic potential regarding *Lactobacillus* spp., such as resistant corn starch Fiberfin (Norway), and food-grade collagen hydrolysate Collamine-80 (LLC Intar-Biotech, Russia).

Resistant corn starch Fiberfin contained: no more than 10% moisture, 31.0% carbohydrates, and 0.8% protein; mass fraction of resistant fraction of starch was 26.6 %, and pH = 6.3. Oligofructose powder Orafti contained: no more than 5.0% of moisture, 13.0% carbohydrates, and pH = 5.3. Food-grade collagen hydrolysate Collamine-80 contained: no more than 10% of moisture, 80.0% protein, and pH = 7.0.

To produce experimental samples of small sausages, trimmed beef with a mass fraction of connective and fatty tissue of no more than 12%, and trimmed pork with a mass fraction of fatty tissue of 28 - 32% were ground in a grinder with a plate hole of 2 - 3 mm in

diameter. Salting was carried out using dry edible salt. Meat raw materials were mixed in a mixer with edible salt in an amount of 0.90 g per 100 g of meat raw materials. The duration of mixing was 4 - 5 min. Salted meat raw materials were held at a temperature of 0 ^{0}C to 4 ^{0}C for 24 hours. The meat raw materials after salting were mixed with a complex of prebiotics and part of minced meat was taken to determine the growth of lactobacteria *in vitro*. The wheat fiber (LLC NPO "Compas Zdoroviya", Russia, Novosibirsk), vitamin complex, spices, and water (ice) were weighed according to the recipe, added to the rest of the minced meat, and were mixed in a mixture with the recipe components for 8 - 10 min until obtaining homogeneous minced meat. The minced meat was subjected to settling at a temperature of 21 ± 1 ^{0}C and relative humidity of 91 ± 1% for 24 hours. Small sausages were dried at a temperature of 13 ± 2 ^{0}C and relative humidity of 77 ± 3% until reaching a moisture mass fraction of no more than 42%. The control sample was made without adding the complex of prebiotics, fibers, and vitamin complex.

The following indicators were determined in the experimental and control samples of air-dried small sausages: pH with a pH-meter Testo 205 (Germany), water holding capacity (WHC) of meat by the press method, protein by the spectrophotometric method, and the fat with the Soxhlet extraction apparatus.

Texture profile analysis was carried out at a temperature of 22 ^{0}C using a texture analyzer "Structurometr ST-2" (Russia), software ST2, and Algorithm (version 4.193). Each sample was subjected to compression testing using an indentor "Cylinder 36 mm". An interval between two cycles of compression was 20 seconds.

The stimulating action of the prebiotic complex in the composition of minced meat on the growth of lactobacteria was studied in the *in vitro* experiment. For the experiment, the experimental and control samples were made. The experimental sample consisted of raw minced meat taken earlier and a combination of prebiotics. In the control sample, the complex of prebiotics was equivalently replaced with potato starch. The samples were sterilized.

The experimental model consisted of parallel rows of tubes with sequential ten-fold dilutions of *Lactobacillus plantarum* 8P-A3 in the MRS liquid medium [4]. The experimental or control product was introduced to the experimental rows of culture media inoculated with test strains. Incubation of test cultures was performed at a temperature of 37 ± 0.5 ^{0}C for 24 h.

All analyses were carried out in triplicate and results were expressed as mean. The data obtained were statistically processed using the software STATISTICA 10.0. (Mann-Whitney criterion, $p < 0.05$) and Microsoft Excel 2007.

3. Results and Discussion

3.1 Development of recipe

A recipe composition of the model minced meat samples with the prebiotic complex was developed based on the scientifically substantiated requirements for the macronutrient composition of meat products for child nutrition. Fat-soluble vitamins and wheat fiber as a source of insoluble dietary fiber were also added to the model experimental samples of minced meat. The composition is presented in Table 1.

Table 1. Ratios of the components in the recipes of the minced meat samples

Raw materials, spices, and other materials	Norm for samples		
	Control sample	Experimental sample 1	Experimental sample 2
Raw materials, g (per 100 g of unsalted raw materials)			
Trimmed beef with a mass fraction of connective and fatty tissue of no more than 12%	70.0	70.0	70.0
Trimmed pork with a mass fraction of fatty tissue of 28 - 32%	15.0	15.0	14.5
Prebiotic complex	-	3.93	3.93
Vitamin additive	-	0.0039	0.0039
Wheat fiber	-	0.5	1.0
Water for hydration	15.0	10.5661	10.5661
Food ingredients and spices, g (per 100 g of unsalted raw materials)			
Edible salt	0.90	0.90	0.90
Ascorbic acid	0.10	0.10	0.10
Fresh garlic	0.15	0.15	0.15
Ground nutmeg and/or cardamom	0.08	0.08	0.08
Ground allspice	0.08	0.08	0.08

The composition of the vitamin additive includes fat-soluble vitamins that take part in the modulation of the gut microbiota: retinol acetate, D-alpha-tocopherol, and D3- -cholecalciferol. The addition of A and E vitamins can modulate beneficial microbiota from genera *Bifidobacterium* and *Lactobacillus*, and vitamin D increases the abundance of beneficial bacteria, such as *Bacteroides* and *Parabacteroides* [17].

Forming a combination of prebiotics is based on the combination of fibers with different rates of fermentation in the large intestine that can have a synergetic effect and, therefore, demonstrate the more pronounced prebiotic effect. The composition of the complex is given in Table 2.

Table 2. Composition of the prebiotic complex

Component	Quantity, g/100 g
Resistant pea starch	67.95
Collagen hydrolysate	23.08
Oligofructose (powder ORAFTI)	8.97

The ability of the prebiotic complex in the composition of raw minced meat for sausage production to stimulate the growth of lactobacteria was determined in the *in vitro* experiment. The control sample, in which the complex of prebiotics was equivalently replaced with potato starch, was used for comparison. The product concentration in the model medium was calculated as a ratio of the daily dose to the volume of the child's stomach. A value of 962 cm^3 was taken as the average volume of the child's stomach [18].

It was found by the results of the study that the number of viable cells of *Lactobacillus plantarum* 8P-A3 (the average of three measurements) was as follows:
- In the experimental sample containing the prebiotic complex - 1 x 10^4 CFU/cm^3;
- In the control sample containing potato starch - 5 x 10^3 CFU/cm^3.

The comparative analysis of the growth of lactobacteria in the analyzed sample containing the prebiotic complex showed a higher number of microbial test cultures in the media with the product being analyzed compared to the sample containing potato starch. The results obtained suggest a positive effect of the experimental sample on the growth of lactobacteria.

Similar trends were observed for resistant starch with microcrystalline cellulose and oat fiber added into fermented sausages with reduced fat and salt content. Santos*et al.*, [19], showed a positive linear effect in increasing the lactic acid bacteria count. Ham *et al.*, [20], also reported an increase in the lactic acid bacteria count in fermented sausages with reduced fat content and the addition of the mixture of collagen and dietary fibers (consisting of microcrystalline cellulose and carboxymethyl cellulose) during one week of refrigerated storage.

3.2 Physico-chemical indicators

The results of the investigation of the physicochemical indicators in the samples of minced meat and finished small sausages are presented in Table 3.

As can be seen from Table 3, the incorporation of prebiotics and wheat fiber into minced meat instead of part of pork did not influence changes in the total amount of protein and moisture and led to an insignificant decrease in the level of fat; however, these differences were insignificant. An amount of edible salt in the recipe (0.9%) ensures the sodium chloride concentration in the finished product required for this product group. Lower pH was found in the experimental samples than in the control.

Moisture of all samples of the finished small sausages ranged from 31.2 to 43.7%. Lower moisture content was found in the experimental samples. The samples were characterized by high protein content and low fat levels. The protein content varied from 40.9 to 43.6%, fat content from 12.2 to 13.6%; a significant difference between samples was not noted. Incorporation of the prebiotic complex reduced the pH level compared to the control sample due to the accumulation of organic acids during fermentation and WHC. With

Table 3. Physico-chemical indicators of the samples of minced meat and finished small sausages

Indicator	Minced meat			Finished small sausages		
	Control sample	Experimental sample 1	Experimental sample 2	Control sample	Experimental sample 1	Experimental sample 2
Moisture,%	71.8 ± 5.7	69.8 ± 5.6	69.8 ± 5.6	43.7 ± 4.4	31.2 ± 4.7 *	35.6 ± 3.6 *
Sodium chloride,%	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.6 ± 0.2	2.2 ± 0.3 *	2.1 ± 0.2 *
Fat, %	6.7 ± 0.1	5.9 ± 0.9	5.8 ± 0.9	12.2 ± 1.8	13.7 ± 2.0	13.6 ± 2.0
Protein,%	17.4 ± 2.6	17.7 ± 2.6	18.1 ± 2.6	40.9 ± 3.3	43.6 ± 3.5	43.2 ± 3.4
pH, units	6.0 ± 0.00	5.61 ± 0.00 *	5.55 ± 0.00 *	5.61 ± 0.00	5.44 ± 0.00 *	5.46 ± 0.00 *
WHC, %	-	-	-	66.81 ± 0.83	64.66 ± 0.13 *	64.80 ± 0.30 *
TBA value, mg/kg	-	-	-	0.117 ± 0.012	0.078 ± 0.008*	0.062 ± 0.006*

Note. * - statistically significant difference ($p < 0.05$) compared with control.

that, the sodium chloride concentration required for this product group is ensured in the product. The TBA value, which characterizes the oxidation processes, was significantly lower in the experimental samples than in the control.

Santos *et al.*, [19], studied fermented sausage with reduced fat and salt content and the use of three different dietary fibers: microcrystalline cellulose (MCC), resistant starch, and oat fiber. It was found that the addition of dietary fibers did not influence pH values and showed an antioxidant effect. Also, the replacement of pork back fat with the mixture of collagen and dietary fibers in fermented sausages facilitated a reduction in lipid oxidation [20]. Nevertheless, several dietary fibers demonstrated a positive effect on the level of lipid oxidation when added individually during the process of sausage fermentation, which increased the TBARS values [21, 22].

3.3 Texture

The results of the study of the prebiotic complex and fibers on the texture of small sausages are given in Tables 4 and 5.

The addition of dietary fibers significantly affected the texture of small sausages. The hardness values of the experimental samples were significantly ($p < 0.05$) lower (by 1.1 and 1.5 times, respectively) compared to the control; the springiness values were also lower (by 1.1 times) in the experimental samples. The lowest hardness was in the sample with the prebiotic complex and 1% of fiber. A difference in hardness can also be explained by different levels of moisture in the samples. Significant differences were not revealed in the sample with 0.5% wheat fiber compared to the control, while the addition of 1% increased cohesiveness. The results of assessing chewiness show that it correlated with hardness. Chewiness was lower in the sample with the addition of prebiotic fibers and 1% fiber, probably because wheat fiber has a complex crystalline structure, which makes grinding easier and facilitates chewing. The results obtained suggest the general improvement of the structural-mechanical properties due to the softer texture and higher tenderness of the experimental sausage sample with the addition of the prebiotic complex and 1% wheat fiber compared to the control.

The number of studies on using resistant starch as dietary fibers is very limited. Therefore, the potential of its use in the meat product composition is investigated insignificantly. Sarteshnizi *et al.*, [23], studied an effect of resistant starch on the consistency of cooked sausage products. The results of the study point to an increased hardness of sausages upon the addition of resistant starch, which can be explained by the presence of amylose. An increase in the resilience and springiness of sausages was also noted. At the same time, its combination with β-glucan and starch had an antagonistic effect and resulted in a softer texture upon the optimal content of resistant starch (2.216%), β-glucan (1.328%), and starch (2.456%). Furthermore, the addition of resistant starch improved the hardness and chewiness of fermented sausage [19]. Felisbert and Lopes [24], studied technological and rheological properties of meat bologna sausages with low fat and reduced sodium content that had different levels of prebiotic fibers (inulin, fructooligosaccharides, polydextrose, and resistant starch). Low-fat bologna sausages containing prebiotic compounds showed higher tenderness.

4. Conclusions

- The recipe for small sausages for child nutrition has been developed. These sausages were enriched with the complex of prebiotic fibers stimulating the growth of lactobacteria. The complex contained oligofructose powder, resistant corn starch, and collagen hydrolysate. The comparative analysis of the growth of lactobacteria in the model sample of minced meat with the prebiotic complex showed that it had a

Table 4. Results from the small sausages structural-mechanical characteristics investigation

Samples	Hardness, g/mm²	Springiness, %	Cohesiveness, %
Control sample	0.3204 ± 0.0346 [a]	76.535 ± 1.8168 [a]	75.805 ± 2.0757 [a]
Experimental sample 1	0.2801 ± 0.0192 [b]	69.6629 ± 3.5363 [b]	76.0296 ± 2.0074 [a]
Experimental sample 2	0.2172 ± 0.0088 [c]	70.9986 ± 3.1292 [b]	79.6129 ± 1.5624 [b]

Note: different letter codes a, b, and c indicate the existence of significant differences ($p < 0.05$), and the same letter codes indicate the absence of differences.

Table 5. Results from the small sausages texture investigation

Samples	Resilience, %	Chewiness, g/mm²	Gumminess, g/mm²
Control sample	23.3025 ± 1.8627 [a]	0.1667 ± 0.0058 [a]	0.2350 ± 0.0252 [a]
Experimental sample 1	22.6317 ± 1.6512 [a]	0.1467 ± 0.0058 [b]	0.1825 ± 0.0206 [b]
Experimental sample 2	24.99 ± 2.1250 [a]	0.125 ± 0.0100 [c]	0.17 ± 0,0000 [c]

Note: different letter codes a, b, and c indicate the existence of significant differences ($p < 0.05$), and the same letter codes indicate the absence of differences.

higher number of microbial test cultures in the media with the product being analyzed compared to the sample containing potato starch. These data indicate a positive effect of the experimental sample on the growth of lactobacteria. It should be noted that the *in vitro* approach does not give complete certainty in the prebiotic activity. To assess the bifidogenic properties of a product, it is necessary to carry out further research in *in vivo* conditions on laboratory animals.

- The recipe composition ensures the correspondence to the requirements for sausage products for child nutrition in terms of physico-chemical indicators. The samples had a high protein content (43.0 g/100 g) and a low level of fat (13.0 g/100 g). The sodium chloride concentration required for this product group is ensured in the developed small sausages.
- Application of the prebiotic complex reduced the pH level and WHC of the experimental small sausages compared to the control sample. The addition of prebiotics into the experimental samples led to an increase in the stability of oxidation of the fat component, which was manifested in a significant decrease in the TBA value compared to the control sample.
- The results obtained indicate the general improvement of the structural-mechanical properties due to softer texture and higher tenderness of the experimental sample of air-dried small sausages with the introduction of the prebiotic complex and 1% of wheat fiber compared to the control. The values of hardness and springiness in both experimental samples were lower compared to the control.

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