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## MORPHOLOGY OF THE SURFACE OF COOKED SAUSAGES MADE WITH THE COLLAGEN-CONTAINING PROTEIN ADDITIVE “BILKOZYNE”

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**Abstract.** The purpose of this study was to investigate the effect of the collagen-based additive *Bilkozyne* on the texture of cooked sausages. This additive is a food ingredient obtained by partial hydrolysis of a beef skin split. We suggested that *Bilkozyne* usage in the formulae of meat products will allow reducing production costs and improving the texture of the final product. We used scanning electron microscopy (SEM) regarded as a unique tool in the comprehensive research of the texture of food products. Prior to that, we had developed a protein stabiliser (PS), based on *Bilkozyne* and other hydrocolloids, in order to distribute fats uniformly in the protein matrix of mincemeat. The influence of PS on the surface morphology of dried samples of cooked chicken sausages made from the mincemeat has been investigated. The main component (60%) of the PS was collagen containing the protein *Bilkozyne*. The PS levels in the cooked sausages were 10% and 15%. Qualitative changes of the texture of cooked sausages have been examined by means of high resolution SEM. The following morphological parameters of the samples have been examined: the number of holes per 1000  $\mu\text{m}^2$  of the area of the sample; the average area of the holes,  $\mu\text{m}^2$ ; the holes ellipticity; the average area of the surface without the holes per 1000  $\mu\text{m}^2$  of the area of the sample. It has been shown that adding 10% of the PS in the hydrated form results in essential improvement of the morphological features of the sausages as compared to those made with non-hydrated PS. It has been found that an increase of the PS in the formulae from 10% to 15% has a negative impact on the texture of the final products, causing a significant decrease in the hydration degree of the myofibrillar proteins of the meat's muscle tissues. *Bilkozyne* has a positive effect on the distribution of fats and proteins in the mincemeat. This makes it possible not to change the source of fats in cooked sausages. Thus, the inclusion of *Bilkozyne* into the formula of cooked sausages results not only in the improvement of their texture, but also in the increase of essential amino acids content.

**Keywords:** cooked sausages, morphology, scanning electron microscopy, texture, collagen.

## ДОСЛІДЖЕННЯ МОРФОЛОГІЇ ПОВЕРХНІ ВАРЕНИХ КОВБАС, ВИГОТОВЛЕНИХ ІЗ КОЛАГЕНОВИМ БІЛКОВИМ ПРЕПАРАТОМ “БІЛКОЗИН”

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**Анотація.** Метою цієї роботи було дослідження впливу композицій на основі яловичого колагенового білка «Білкозин» на текстуру варених ковбас. Ця добавка представляє собою продукт, отриманий шляхом кислотного гідролізу спилку яловичої шкірки. Для аналізу поверхні варених ковбас використано скануючу електронну мікроскопію (СЕМ), що вважається унікальним інструментом у дослідженнях текстури харчових продуктів. Із метою рівномірного розподілу жирів у білковій матриці фаршу, попередньо розроблено білковий стабілізатор (БС) основою якого є «Білкозин» та інші гідроколоїди. Методом СЕМ досліджено вплив білкового стабілізатора з колагеновим білком «Білкозин» на морфологію поверхні висушених варених ковбас, у залежності від концентрації (10–15%) БС, а також отримано кількісні показники, що характеризують морфологію поверхні варених ковбас. Визначені такі морфологічні показники:

кількість порожнин на 1000  $\mu\text{m}^2$  площі зразку, середня площа порожнин  $\mu\text{m}^2$ , еліптичність порожнин, площа поверхні без порожнин на 1000  $\mu\text{m}^2$  площі зразку. Знайдено, що внесення білкового стабілізатора в кількості 10% в гідратованій формі приводить до істотного покращення морфологічних характеристик варених ковбас у порівнянні зі зразками, виготовленими з внесенням білкового стабілізатора в сухому вигляді. Знайдено, що збільшення концентрації БС в рецептурі з 10% до 15% негативно впливає на текстуру готових виробів, що проявляється в помітному зменшенні гідратації міофібрилярних білків. Встановлено, що використання «Білкозину» позитивно впливає на розподіл частинок жиру і білків в масі фаршу. Це дозволяє не змінювати джерело жирів у рецептурі варених ковбасних виробів. Таким чином, використання «Білкозину» в рецептурах варених ковбас дозволило не лише поліпшити текстуру готових виробів, а й підвищити в них вміст незамінних амінокислот.

**Ключові слова:** варені ковбаси, морфологія, скануюча електронна мікроскопія, текстура, колаген.

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## Introduction. Formulation of the problem

Meat proteins play a prominent role in human nutrition [1]. The most important ingredients in the formulae of cooked sausages are: mincemeat, fats, water, food additives, and fillers of plant origin. Uniform distribution of fat particles in the protein matrix has a strong effect on the texture and organoleptic properties of the final products [2]. Proteins of the muscle tissue are activated through sarcolemma destruction with myosin and actin released, followed by their solubilisation [3]. Myofibrillar proteins directly influence the fats emulsification and determine the moisture-retaining power of the food matrix.

It is known that meat proteins can stabilise a mincemeat emulsion [4]. It is necessary to distribute proteins uniformly in particles of fats prior to the thermal treatment stage. Myosin is the main structural protein of meat. It plays a significant part in the formation of emulsions, and determines the moisture-retaining power of semi-finished meat products. Myosin is regarded as a binder on the phase boundary: residues of non-polar amino acids interact with the particles of fats, whereas residues of polar amino acids bind themselves to the water phase. Not only proteins, but fats, too, have some important functions in meat products, directly influencing their softness and succulence.

Rheological parameters of sausages made on the basis of protein stabilisers and protein-in-oil emulsions mainly depend on the emulsifying ability and moisture-retaining power of meat proteins.

Several factors affect the quality of cooked sausages, in particular the type of meat (beef, pork, or chicken), its functional and technological characteristics, the origin of the fats, the degree of emulsion dispersion when composing mincemeat emulsions, the pH value, the duration and intensity of mincing the raw meat, its temperature during the composition and dispersion of the mincemeat emulsions [3,4].

The meat of broilers is widely used as the main ingredient of meat emulsions in manufacturing cooked sausages [5,6]. However, there are structural differences between the meat of broilers and traditional types of meat. That is why, it is necessary to study the

impact of its myofibrillar proteins on the hydration degree of partially hydrolysed beef collagen, which can be used as the main component of the protein stabiliser. In our previous studies, we found that the collagen-containing beef protein *Bilkozyne* was structurally similar to myofibrillar proteins of broiler meat, and its inclusion into the formula of cooked sausages had a positive effect on the functional and technological properties of meat emulsions [6]. In this work, we did a comparative study of the effect of the *Bilkozyne*-based protein stabiliser on the surface morphology of cooked sausages made of broiler meat.

## Analysis of recent research and publications

Changing the fats in the product's composition (for example, using sunflower and olive oil instead of animal fats [7]) is considered to be the main method of affecting the texture of cooked sausages. Unfortunately, this method has some disadvantages, such as the need of including antioxidants into the recipe in order to avoid oxidative spoilage, or the need for the appropriate amount of water used for proteins hydration, or the need for additional equipment [6].

We have suggested an alternative approach. It consists in using the collagen-containing beef protein *Bilkozyne* as the main ingredient of the PS to structure the mincemeat emulsion on the boundary of the hydrophobic and hydrophilic phases [6]. The surface of dry samples of the cooked sausages was studied by the method of scanning electron microscopy in order to examine *Bilkozyne*'s ability to be an effective stabiliser of meat emulsions.

The method of scanning electron microscopy has been widely used in meat technology to study the structural characteristics of the surface of raw meat and meat products. This method has been used in studying the surface morphology of fermented sausages with different levels of starch, fats, and egg whites in the formula [8], and in the analysis of the texture of frankfurter sausages with a modified fats profile [9]. Also, this method was useful in the analysis of the fats distribution in the polymer chains of meat proteins and other major components [10]. It is known that fat particles become covered with myofibrillar proteins on the stage

of cutting mincemeat. Particles of fats migrate into the disperse phase while proteins, water, and polysaccharides remain in the solid phase, which results in the inclusion of fats into the food matrix. That is why, residues of polysaccharides and connecting tissues can be seen in microphotographs of meat and mince samples [11]. There are two methods of obtaining microphotographs of meat and sausages samples, namely, freezing and drying [8-11]. With the latter method, there are holes present in the microphotographs. They are due to the evaporation of fat globules on the surface of fats. The number and quantity of the holes is a measure of food quality.

The **purpose** of this study is to investigate the effect of the collagen-containing beef protein *Bilkozyne* on the texture of cooked sausages, with the aid of scanning electron microscopy.

#### Objectives:

- to prove the effectiveness of the SEM method in studying the texture of cooked sausages;
- to obtain quantitative parameters of the surface morphology of cooked sausages made with the use of the proteins stabiliser (PS) high in *Bilkozyne* in

dry and hydrated forms;

- to describe the surface morphology of the cooked sausages produced with different concentrations (10% and 15%) of the protein stabiliser;
- to determine the most effective form and quantity of the PS in the cooked sausages formula to produce final products of the best quality as for its surface morphology parameters.

### Research Materials and Methods

The cooked sausages *Kuryachi* were manufactured according to the demands of the standard technological instruction TU U 15.1-19492247-013-2003 developed in the National University of Food Technologies.

The content of the components in the mixture of hydrocolloids of the PS was as follows: dried collagen-based protein *Bilkozyne* – 60% (*Bilkozyne* Co., Pryluky, Ukraine), dried whey – 20%, carboxymethyl-cellulose – 5%, guar gum – 10%, xanthan gum – 5% (Roeper, Germany) [12]. The formulae of the cooked sausages are given in Table 1.

**Table 1. Formulae of the cooked sausages *Kuryachi* made with the use of the hydrated protein stabiliser (PS)**

Ingredients	Amount, %			
	Sample 1	Sample 2	Sample 3	Sample 4
Meat of broilers, %	70	70	80	80
Pre-hydrated proteins stabiliser (PS) based on the collagen-containing additive <i>Bilkozyne</i>	15	15	10	10
Soybean isolate preliminary mixed with water (1:5)	15	15	10	10
Silica A300, %	0.1	0.1	0.1	0.1
Salt, % per 100 g	2.1	2.1	2.1	2.1
Sodium nitrite, g per 100 g	0.005	0.005	0.005	0.005
Water, %	30	30	30	30
Spice mixture <i>Frankfurter</i> sausages	1.0	1.0	1.0	1.0

The cooked sausages were prepared in two ways. According to the first method, the PS was pre-hydrated, whereas in the second method, the PS, composed on the basis of the collagen-containing additive *Bilkozyne*, was introduced into the meat emulsion in the dry form, and the water used for the PS production was taken from the total water from the formulae given in Table 1.

We compared the cooked sausages *Kuryachi* and *Molochni*. The latter sausages were manufactured according to the state standard DSTU 4436:2005, which was obtained from the local supermarket chain *Auchan*. The samples of the cooked sausages had been dried in the drying chamber at a temperature of  $50 \pm 1^\circ\text{C}$  for 8 days prior to the experiments.

The morphology of the surface of the samples was studied with the use of the scanning electron microscope JSM-6700F (JEOL, Japan) in M. P. Semenenko Institute of Geochemistry, Mineralogy, and Ore Formation. The samples had been coated with a platinum layer, 10 nm in depth, prior to the experiments. The photos of the samples were taken, the accelerating voltage being 15 kV, and the measure current 0.65 nA.

Dried samples of the cooked sausages ( $0.5 \times 0.5$  cm), 1 mm in depth, were fixed with a steel holder and vacuumised before taking photos at the temperature  $20 \pm 0.1^\circ\text{C}$

The following morphological parameters have been determined: the number of the holes (NH) in  $1000 \mu\text{m}^2$  of the area of the sample; the average area of the holes (AH,  $\mu\text{m}^2$ ); the holes ellipticity expressed as the ratio  $D_{\text{max}}/D_{\text{min}}$ ; the surface without holes (SWH) per  $1000 \mu\text{m}^2$  of the area of the sample. The ImageJ software was used for the morphological analysis of the surface of the samples. With the P values being  $< 0.05$ , all the data obtained were considered statistically significant.

### Results of the research and their discussion

The *Bilkozyne*-based PS composition was introduced into the mincemeat in the dry and hydrated forms. As it was established earlier, an increased hydration level results in the full destruction of helical polymers in *Bilkozyne* and makes it possible to distribute it more uniformly in the mincemeat emul-

sion [6,13]. Thus we obtain a more even texture of the mincemeat samples, with fewer holes (NH value), which are smaller in area (AH) [13]. The texture parameters of the cooked sausages manufactured with the PS added in the dry form (sample 1) and hydrated form (sample 2) have been assessed by SEM. Besides, we have analysed the surface morphology of the dried

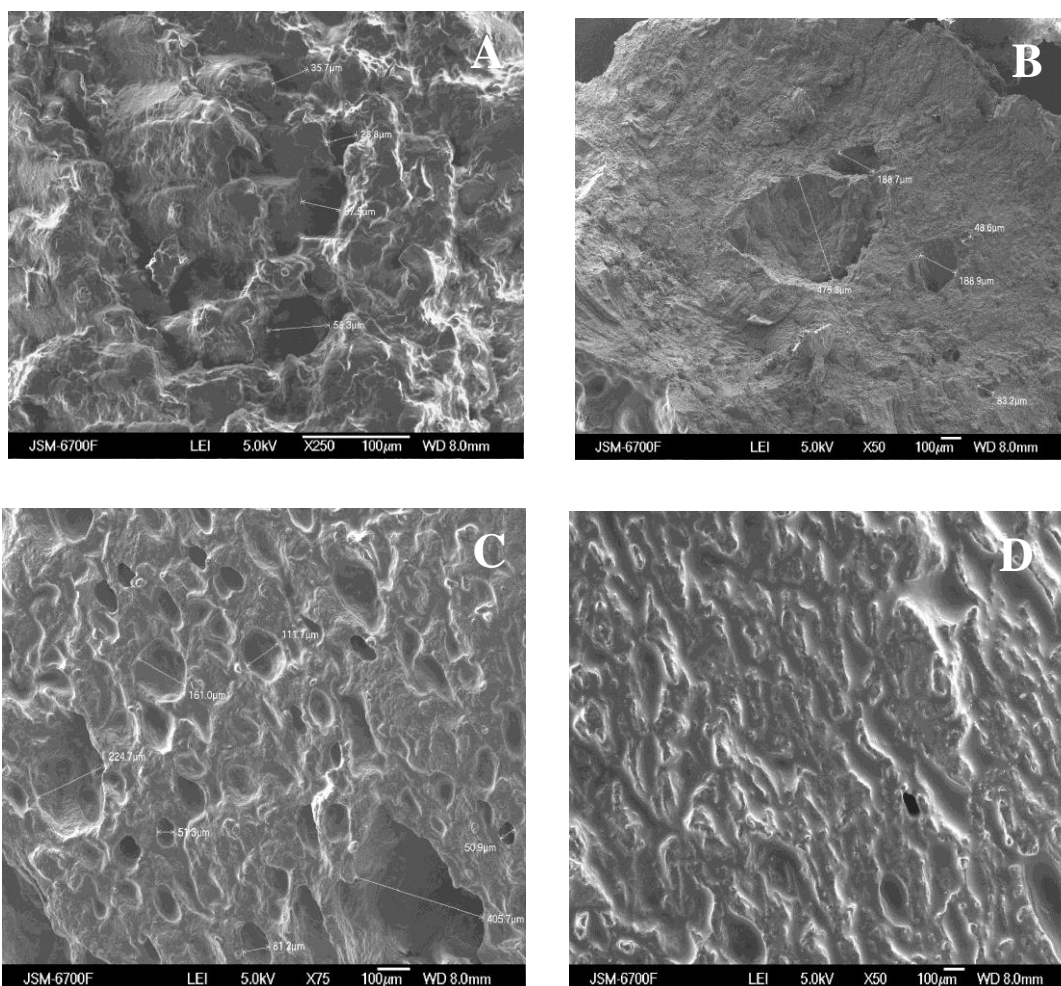
samples of cooked sausages manufactured with a 5% lower level of dry PS (sample 3), and hydrated PS (sample 4), as well as of the sample manufactured according to the state standards without adding *Bilkozyne* (*Molochni* sausages, sample 5). The parameters of the surface morphology of all the samples are given in Table 2.

**Table 2. Influence of the form of including *Bilkozyne* on the surface morphology of the cooked sausages**

Sample	NH	AH $\times 10^3$ , $\mu\text{m}^2$	E	SWH, $\mu\text{m}^2$
1	211 $\pm$ 2.2	1.4 $\pm$ 0.9	1.2 $\pm$ 0.05	675 $\pm$ 23
2	83	35.1	1.48 $\pm$ 0.04	541 $\pm$ 23
3	104 $\pm$ 1.4	12.3 $\pm$ 1.2	1.6 $\pm$ 0.06	840 $\pm$ 35
4	0.2	0.03	1.96 $\pm$ 0.06	5 $\pm$ 2
5	0.4	8.5 $\pm$ 0.1	1.87 $\pm$ 0.05	275 $\pm$ 23

From Table 2 it is obvious that the inclusion of 15% of *Bilkozyne* into the formulae of cooked sausages, whether in the dry or in the hydrated form, results in a non-uniform texture of the final product. It is especially true for the samples manufactured with hydrated

PS, where the holes, though fewer in number, can be as big as 475  $\mu\text{m}$  in diameter (Fig. 1B), as compared with the samples made with the addition of non-hydrated PS (Fig. 1A).



**Figure 1. SEM microphotographs of cooked sausages containing 15% of PS in the dry (A) and hydrated (B) forms, and the samples made with the addition of 10% of PS in the dry (C) and hydrated (D) forms**

In our opinion, fibres of collagen were destroyed because of the preliminary hydration, but the hydration

degree was not enough to provide the cooked sausages with a uniform texture.

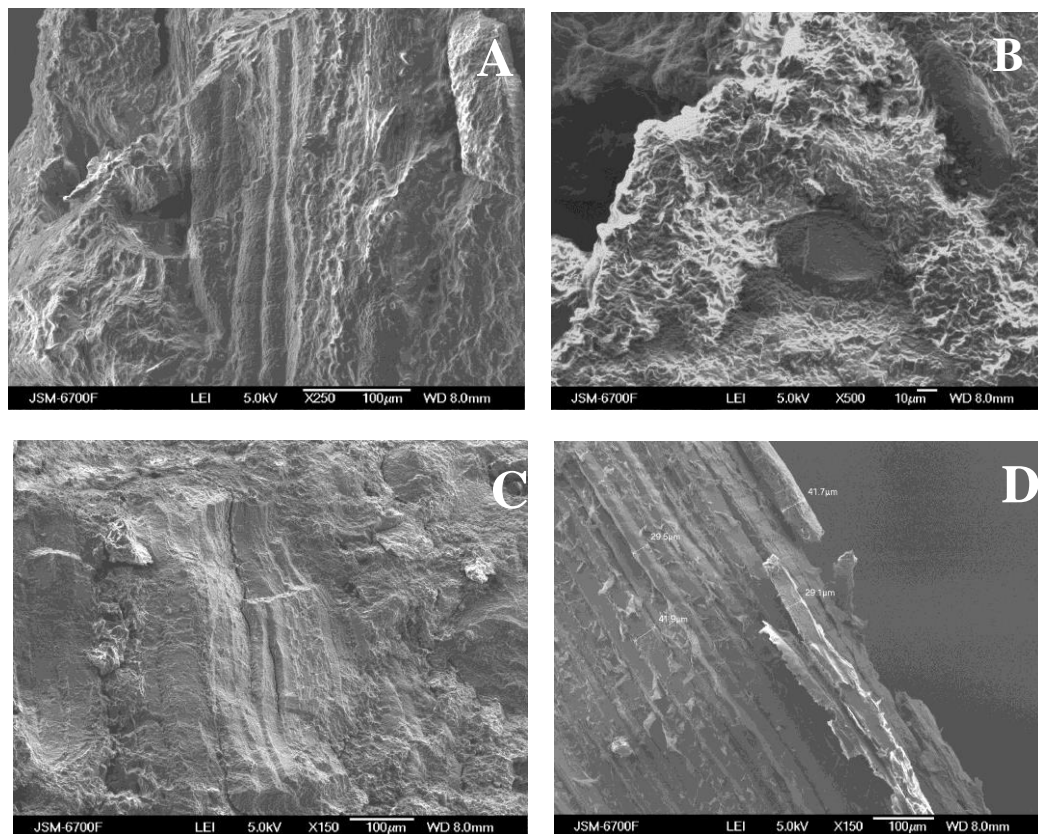
We lessened the amount of PS in the formula to avoid the above-mentioned faults in the texture. An analysis of Table 2 shows that adding 10% of PS leads to markedly fewer holes. This indicates that fats have high migrating ability inside the food matrix.

As one can see from Fig. 1C and 1D, using 10% of PS that had been hydrated prior to the experiment resulted in a far better texture of the product, which manifested itself in the morphological change of the dried samples of the cooked sausages from coarse to smooth. The most uniform surface was observed in the sample made with hydrated PS (Fig. 1D). In fact, the samples of the sausages, in which the PS had been hydrated prior to the experiment, had almost no “visible” holes on the surface. In other words, this type of emulsifying the mincemeat emulsions was the most effective, and the sausages manufactured using this type of meat emulsion were of the highest quality among all the samples. An essential increase in the NH and AH values in the sausage sample produced with the incorpora-

tion of 10% of PS in the dry form (Fig. 1C) can be explained by insufficient hydration of the PS components, especially *Bilkozyne*. The result is less uniform mincemeat emulsions, and a lower quality of the final product.

The data given in Table 2 revealed that the AH value of the cooked sausage sample made with 15% of preliminary hydrated PS ( $5\pm 2$ )  $\mu\text{m}^2$  is higher than that of the sample made with 10% of hydrated PS ( $541\pm 23$ )  $\mu\text{m}^2$ . Therefore, the hydration degree of the collagen-containing protein *Bilkozyne*, as well as *Bilkozyne*-based compositions, plays a key role in the formation of the surface structure of cooked sausages and can be regarded as the most important factor of the dispersion degree and stability of mincemeat emulsions.

The presence of non-hydrated myofibrillar proteins in the final product can affect the hydration of meat proteins. The samples of cooked sausages manufactured from broiler meat with 15% of PS are given in Fig. 2.

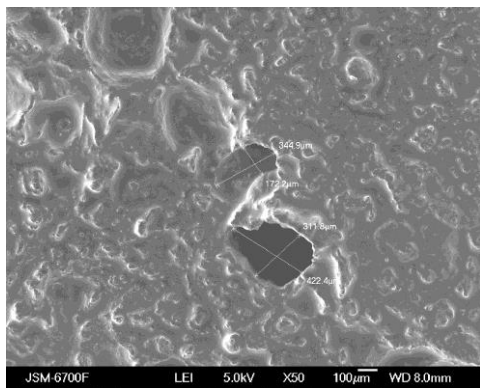


**Figure 2. SEM microphotographs of the samples of the cooked sausages that were manufactured with 15% of PS incorporated in the dry form (A); hydrated (B and C) forms; the broiler meat sample (D)**

As can be seen from Fig. 2A, residues of fibres whose structure and sizes are similar to those of broiler meat (Fig. 2D) are presented on the surface of the sample of the cooked sausages. Similar residues were observed in the samples of the cooked sausages produced with 15% of PS (Fig. 2B and Fig. 2C).

Microphotographs of the sample of cooked sausages *Molochni* (Fig. 3) manufactured according to the

state standard were taken in order to compare their morphological characteristics with those of *Bilkozyne*-based samples. Irregular distribution of holes and presence of huge holes on the surface of *Molochni* sausages (Fig. 3) indicate a violation of the manufacturing technology on the stage of mincemeat production.



**Figure 3. SEM microphotograph of the sample of cooked sausage *Molochni***

In our opinion, the hydration of muscle tissue proteins is a key factor of the texture formation in cooked sausages. This can be achieved both by manipulating the hydration degree and by changing the PS components. It should be noted that the samples of sausages produced with 10% of PS had no myofibrillar fibres of broiler meat, no matter by which method the PS had been incorporated in the meat emulsion.

A data analysis of Table 2 and scanning electron microphotographs revealed that using *Bilkozyne*-based

protein stabilisers is a cost-effective approach of texture improvement of cooked sausages.

### Conclusions

Thus, it has been shown that the SEM method can be regarded as an effective tool in the analysis of the surface of cooked sausages, manufactured with the use of the protein stabilisers based on the collagen-containing protein *Bilkozyne*, which indicates the technological necessity of preliminary hydration of PS prior to compilation of meat emulsions.

It has been found that the most effective level of *Bilkozyne*-based PS in manufacturing cooked sausages was 10%. The additive must be hydrated prior to the incorporation in the meat emulsion. This results in the cooked sausages with a uniform surface, and thus, in high sensory properties of the product.

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