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JUSTIFICATION OF THE USE OF A TECHNOLOGICAL FOOD ADDITIVE WITH TRANSGLUTAMINASE FOR STABILIZATION OF MINCE SYSTEMS OF COOKED SAUSAGE PRODUCTS

The object of the research is the technology for producing minced meat products using a functional additive. The need to form stable technological and structural indicators of emulsion sausages requires a comprehensive solution consisting in combining structure-forming raw materials of different origins.

Cooked sausage "Olivier" minced systems were developed using a functional system consisting of transferase, hydrocolloids and a protein-containing additive. As a research result of minced meat systems, it was determined that the viscosity of the resulting minced meat increases with an increase in the content of the functional additive. This indicates an increase in intermolecular interaction and compaction of the protein matrix. A comprehensive assessment of active acidity before and after heat treatment indicates the possibility of stabilizing this indicator with enzymatic action on the raw materials. The textural characteristics of the finished product show a similar pattern, so the hardness of the sample without the use of the additive was 1575 g/cm², and when using 1% of the additive it increased by 32.9% and amounted to 2093 g/cm². Also heat treatment losses decreased from 22.00% to 15.11%.

The use of hydrocolloids and transglutaminase makes it possible to increase the protein and fat content in the products due to the formation of a strong matrix, which significantly reduces losses during heat treatment and prevents phase separation.

The obtained results made it possible to establish the relationship between the contraction of the technological additive with transglutaminase on the structural-mechanical indicators and chemical composition of minced systems. The practical value of the work lies in the possibility of using an ingredient complex to increase stability of minced systems, optimize the technological process and reduce losses.

Keywords: meat products, minced meat systems, transglutaminase, structure stabilization, hydrocolloids, emulsion sausages.

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1. Introduction

Viscoplastic characteristics directly affect consumer evaluation of the product, since this characteristic acts as a connecting link between structural-mechanical and organoleptic indicators [1]. Predictability of changes in viscoplastic characteristics during technological processing and storage is critically important for meat products [2]. The formation of such properties is determined by the composition of muscle tissue, structural organization of proteins and technological processing. Assessment of textural properties of the product is one of the main sensory characteristics for consumers, which is determined by a combination of rheological and structural characteristics [3]. The use of functional food ingredients is designed to regulate and optimize these characteristics for minced meat products [4]. In particular, the use of hydromodules allows the formation of a stable protein-fat matrix in the product [5]. The use of plant raw materials is widely represented in the meat processing industry in order to influence the technological characteristics of the product and increase stability. Plant fibers are often used to bind moisture in the product and increase yield. Researchers have proven [6] that apple and citrus fibers improve the juiciness and texture of low-fat meat products and provide antioxidant effects. It is noted that the addition of carboxymethyl cellulose in an amount of 0.5–1.5 g/100 g

of product improves the gel strength of surimi-type minced meat by 25–30% compared to the control [7].

Polysaccharides represent a separate group of functional components used to form the texture and stabilize meat systems. Starches, in particular, contribute to the improvement of the gel matrix and increase the density of meat products. Pueraria starch in duck-based sausages has been determined to increase the overall structural and technological performance of the product [8]. Studies of gels with corn starch show high stability under the influence of external factors, which allows the formation of viscoplastic characteristics of products using it [9].

A separate group is represented by polysaccharides that exhibit the ability to form a hydrocolloid structure. One of these is carrageenan, which has high hydrophilicity, which contributes to gel formation and moisture retention. The formation of a hydroprotein matrix in sausages and salted meat products with the participation of carrageenan is accompanied by a decrease in losses during heat treatment and stabilization of the phase composition [10]. Emulsion gels structured with plant hydrocolloids, in particular agar, konjac glucomannan and gum arabic, are considered as an effective alternative to animal fat in meat products [11]. Such systems may contain vegetable oils (e. g. grape seed oil) and natural emulsifiers, in particular aquafaba, which allows to reproduce the solid structure, texture and sensory properties characteristic

of animal fat, while reducing the overall calorie content and saturated fat content [12]. According to research, partial or complete replacement of pork fat with such gels can lead to an increase in the hardness of the finished product [13].

A separate direction is the use of protein components. The use of compositions based on proteins of different origins in combination with structure-forming agents improves the stability of water-fat complexes in the composition of emulsion sausages [14]. Proteins of vegetable origin in the meat processing industry are widely represented by soy proteins and isolates [15]. The use of vegetable proteins can increase the hardness of minced meat, increasing moisture binding in the product [16]. Preparations based on proteins of animal origin, hydrocolloids, are more often used as emulsifiers [17]. However, proteins are not the same in molecular structure, which causes a heterogeneous technological effect in the composition of the product. Thus, collagen increases the elasticity of the product, and sodium stearate, on the contrary, densifies the structure [18]. According to studies [19], whey proteins have a high ability to gel when used as a partial replacement for meat raw materials. It has also been determined that native whey protein isolate is more stable under thermal influence than soy protein isolate.

The formation of structural and technological characteristics occurs under the significant influence of enzymatic processes. In the technology of meat products, microbial enzymatic preparations are widely used, which exert a controlled effect on the structure of muscle fibers.

Proteolytic enzymes (proteases) have become widely used in the meat processing industry due to the ability to carry out controlled hydrolysis of myofibrillar proteins [20]. However, it is this transformation that causes a weakening of the muscle structure and a decrease in stiffness [21]. At the same time, the formation of a stable spatial structure of minced systems of sausage products is a priority technological task. Excessive proteolytic influence can cause a weakening of the protein matrices of minced meat and complicate the formation of a dense structure.

Accordingly, enzymes of the transferase class, in particular transglutaminase, are considered effective for forming the structure of emulsified sausage products. This enzyme is widely used in the meat industry due to its ability to catalyze protein crosslinking, which promotes the formation of a gel structure, increases structural stability and modifies the texture of the product [22]. Its mechanism of action is to catalyze the formation of intermolecular covalent bonds between glutamine and lysine residues, as well as between myofibrillar protein chains [23].

Transglutaminase improves the properties of gels, strengthens the interconnections between molecules and forms a dense three-dimensional structure in soy isolate-based gels [24]. The addition of transglutaminase promotes myosin cross-linking and stabilizes protein structures in meat products [25]. It has been found that induced protein aggregation gradually increases with increasing enzyme concentration [26].

The use of transglutaminase enhances the interaction between animal and plant proteins, contributing to the formation of a stable protein network and improving the structural and textural properties of sausage products [27].

The combination of microbial transglutaminase with pig blood plasma increases moisture binding, which leads to an increase in the yield of smoked sausages by 5% [27]. It was determined that such a combination contributed to the formation of a solid colloidal structure with increased resistance to thermal stress and binding of moisture and fat [28].

In minced meat, the addition of recombinant microbial transglutaminase promotes the formation of high-molecular protein complexes of actin and myosin, which improves the rheological properties of the product. The use of enzymatic preparations also shows an improvement in the stability of the shape of meat products and reduces the rate of lipid oxidation [29].

Different functional ingredients show an effect on different product characteristics. Fermented additives can improve organoleptic characteristics, but have no effect on microbial spoilage of the product [30].

In turn, dietary fibers effectively improve and bind moisture in the product [31]. Various characteristics and properties of functional food ingredients encourage a comprehensive approach to the formation of quality characteristics of meat products. It is the combination of enzymatic preparations, protein components and polysaccharides that can effectively contribute to the formation of stable structural and mechanical characteristics of emulsion products.

The object of research is the technology of manufacturing minced meat systems using a functional additive.

The aim of research is to substantiate the use of a technological food additive with transglutaminase to stabilize minced meat systems of cooked sausage products.

Research objectives:

- to determine the parameters of model minced meat of cooked sausages before heat treatment;
- to investigate changes in pH indicators, textural characteristics and weight loss after heat treatment;
- to determine the chemical composition of the obtained cooked sausages and evaluate the use of the food mixture for the effectiveness of stabilizing their quality indicators.

2. Materials and Methods

For research on the recipe, boiled sausage "Olivier" was chosen as an analogue according to TU-TI TU U 15.1-31256979-007-2003 for the production of "Boil sausages, sausages, sausages and meat loaves". The recipe contains chicken breast meat in the amount of 18%, deveined chicken meat – 50%, mechanically deboned meat – 25%, refined sunflower oil – 2%, potato starch – 3% and chicken eggs – 2%.

Based on the analysis of literature sources, in order to improve the structural characteristics of boiled sausages, a complex functional additive for emulsion sausages was developed. The recipe composition of the additive is presented in Table 1.

Table 1

Recipe composition of the functional food additive

Raw materials	Quantitative content, %
Extra potato starch (vimal)	28.00
Demineralized dry whey	20.00
E415 (xanthan)	18.00
Transferase enzyme (activity 100–120)	15.00
E466 (carboxymethylcellulose 6000)	13.00
E551 (Silicon dioxide)	5.00
E425 (konjac gum)	1.00

The developed functional system was introduced in dry form at the first stage of cutting in the amount of 0.5; 0.75; and 1% per 100 g of unsalted raw materials. The resulting recipes for boiled sausages are presented in Table 2.

The technology for making sausages involves grinding broiler chicken meat in a HKN-225S meat grinder (China) with a grate diameter of 4–8 mm and salting agents. When preparing minced meat on a Talsa K15neo-m cutter (Spain), first, low-fat raw materials are ground, adding a phosphate-containing flavoring composition and water in the form of ice. At the first stage of dry cutting, the developed functional additive is introduced in dry form. At the second stage, fatty raw materials, chicken eggs, starch and remaining ice are introduced. Cutting is carried out under temperature control, which should not exceed 12°C.

Heat treatment was carried out using a steam-convection oven Unox XEVC0511E1R (Italy). The technology includes a precipitation stage to stabilize the minced meat system. The next stage is drying at a temperature of 50–60°C at humidity of 10–30% without steam supply.

Bringing to a state of culinary readiness takes place in the humid convection mode at 75–85°C and a humidity of 80–10%. The end of the cooking process is considered to be the achievement of a temperature of $71 \pm 2^\circ\text{C}$ in the center of the loaf. Cooling was carried out by dousing with cold water.

Table 2

Recipe composition of boiled sausages "Olivier" using the functional system

Components	Recipe options			
	Sample 1	Sample 2	Sample 3	Sample 4
Unsalted raw material, kg per 100 kg				
Broiler chicken breast meat	18	18	18	18
Broiler chicken meat with veins	50	50	50	50
Mechanically deboned meat	25	25	25	25
Refined oil	2	2	2	2
Potato starch	3	3	3	3
Chicken egg	2	2	2	2
Spices and materials in g, per 100 kg of unsalted raw material				
Functional system	–	700	1050	1400
Maxspice Furshetna* (0.6% to the total mass of minced meat)	823	831	833	834
Table salt	2350	2350	2350	2350
Sodium nitrite in 2.5% solution, ml	450	450	450	450
Amount of water (ice) % to the mass of unsalted raw material	35	35	35	35

Note: * – Maxspice Buffet combined mixture from Macros LLC (Vasylkiv, Ukraine) with the taste of classic spices and nutmeg for the production of boiled sausages, sausages and sausages, as well as for semi-smoked sausages. Ingredients: acidity regulators (E450, E451), salt, flavor and aroma enhancer (E621, E627, E631), antioxidant (E300), yeast extract, spice extracts (nutmeg, pepper, etc.), anti-caking agent (E551)

The samples were tested for nutritional and energy value. The protein content was determined by the Kjeldahl method (DSTU ISO 937:2005). The total fat content was determined by the Soxhlet method (DSTU ISO 1443:2005).

The amount of ash was determined after mineralization of the samples using a muffle furnace (SNOL-1.6, Lithuania) at 520°C.

Technological characteristics of minced meat systems and finished products were determined using laboratory equipment.

Determination of moisture content in the test product was carried out according to DSTU ISO 1442:2005 by drying in a drying oven at 105°C to constant mass with subsequent calculation of the change in initial and final mass. The mass was determined on analytical balances from RADWAG (Poland) with an accuracy of 0.0001 g.

The results were calculated according to

$$W = \frac{m_1 - m_2}{m_1 - m} \quad (1)$$

where W – moisture content, %; m – mass of dry box, g; m_1 – mass of box with sample, g; m_2 – mass of box with sample after drying, g.

pH was determined by determining active acidity using a pH meter Hanna HI98107 (Italy) with an accuracy of 0.1 pH. The study was carried out with an exposure of 30 min (DSTU ISO 2917-2001).

Viscosity and hardness of test samples were measured using a Shimadzu EZ-LX Texturometer (Japan) 0.001 gf, g/cm². The equipment is equipped with an appropriate load sensor and software for structural-mechanical and rheological studies.

Losses during heat treatment are obtained by calculating the ratio of the change in the mass of the product after heat treatment to the mass

of the product before it. According to this, it is possible to obtain the calculation formula

$$X = \frac{M_c - M_f}{M_c} \quad (2)$$

where X – losses after heat treatment; M_c – mass of the product before heat treatment; M_f – mass of the finished product after heat treatment.

3. Results and Discussion

3.1. Research on the indicators of the obtained minced meat for boiled sausages

According to the experimental research plan, 4 experimental samples of minced meat for emulsion sausages were produced and their technological indicators were determined. The results of the research are presented in Table 3.

Table 3

Technological characteristics of minced meat systems using a complex of transglutaminase and hydrocolloids

Indicators	Sample 1	Sample 2	Sample 3	Sample 4
Ground meat pH	6.2 ± 0.01	6.18 ± 0.02	6.15 ± 0.01	6.06 ± 0.02
Mush meat viscosity, mPas	4.86 ± 0.02	5.10 ± 0.03	5.26 ± 0.01	6.50 ± 0.03

From the obtained research results presented in Table 3, the use of a functional mixture with transglutaminase in the minced meat for boiled sausages shows a pronounced effect on the studied indicators. The active acidity in the samples of meat systems has a pronounced decrease, which correlates with an increase in the proportion of the studied complex. When the maximum concentration is reached at the level of 1%, the studied indicator is within 6.06 ± 0.02 , which is 2.26% lower than sample No. 1 where the developed system was not used. Such a decrease is explained by the acid-buffer characteristics of the components. In particular, with a change in protein structures under the action of transferases. The viscosity of minced meat systems changes significantly under the influence of changes caused by the addition of a mixture of enzymes and hydrocolloids. An increase in the proportion of the studied additive in the product composition causes a progressive increase in the viscosity index. Thus, for Sample No. 1 it was 4.86 ± 0.02 mPas, and when using a functional additive in an amount of 0.5% it increases by 4.9%. However, the most pronounced effect on the studied indicator is found precisely in the addition of 1% of the developed stabilization system, where the value reached 6.50 ± 0.03 mPas, which is an increase of 33.7% compared to sample No. 1.

Such results are due to the synergistic interaction of the components of the food additive. Whey proteins and transglutaminase modify the functional properties of proteins and their buffering capacity. Hydrocolloids intensively bind moisture, increasing the hydration of protein matrices and changing the ratio of free and bound moisture. At the same time, silicon dioxide ensures the stability of the mixture and its uniform distribution.

3.2. Research on the quality indicators of finished cooked sausages

The next stage of the research was to bring the sausage products to culinary readiness and conduct product research. The resulting sausage products can be evaluated, in particular, by their appearance on the cut. Cooked sausages should be homogeneous, without visible inclusions and not divided into ingredients. The resulting sausages are presented in Fig. 1.

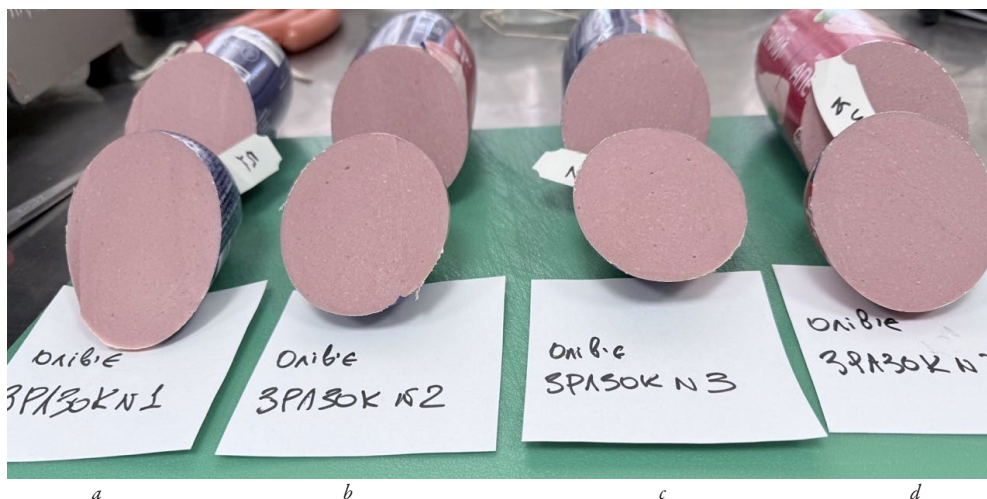


Fig. 1. View of the cross-section of sausage products: *a* – sample No. 1; *b* – sample No. 2; *c* – sample No. 3; *d* – sample No. 4

As can be seen in Fig. 1, all the obtained samples of cooked sausages have a homogeneous structure with a uniform distribution of technological components. The use of a food additive with transglutaminase and hydrocolloids did not cause the formation of local seals or aggregates, which indicates the stability of the structure formation and the absence of deterioration of the organoleptic characteristics of the finished product.

The study of structural, mechanical and technological indicators is a key task in assessing the influence of ingredients on the quality of the finished product. During the research, the change in active acidity in the finished product, technological losses during cooking and the hardness of the resulting product were determined. The results obtained are presented in Table 4.

Table 4
Technological characteristics of finished sausages using a technological additive

Indicators	Sample 1	Sample 2	Sample 3	Sample 4
pH	6.12 ± 0.01	6.10 ± 0.01	6.08 ± 0.02	6.07 ± 0.02
Loss after heat treatment %	22.00 ± 0.02	18.02 ± 0.03	16.12 ± 0.02	15.11 ± 0.01
Hardness (gf, g/cm ²)	1575 ± 0.01	1645 ± 0.01	1723 ± 0.01	2093 ± 0.01

The studies presented in Table 4 indicate that in sausages brought to a state of culinary readiness, the pH does not significantly decrease with an increase in the proportion of the mixture used. At the same time, in samples No. 3 and No. 4, in which the proportion of the stabilizing mixture with transglutaminase was at the level of 1.0–1.4%, the quantitative pH value is within the statistical error, which indicates the stabilization of the buffer capacity of minced meat when adding a functional mixture at the level of 1% to stabilize the active acidity of sausage products. These data are consistent with the researchers [27], where the use of transglutaminase in products allowed to obtain stable active acidity during the fermentation process.

The reduction in losses after heat treatment indicates a pronounced effect of the use of a mixture of transferase and hydrocolloids on the stability of moisture binding. Regarding Sample No. 1, where the losses were 22.00 ± 0.02%, and the addition of the technological mixture reduces these losses by 18.1–31.3%. Such results are explained by the increased moisture-holding capacity achieved by the use of hydrocolloids. An additional effect is exhibited by transferase, which promotes the formation of additional bonds between protein molecules, which leads to the compaction of the matrix and a decrease in moisture release

under the influence of heating. An additional agent is dry demineralized whey, which promotes gelation in the product. The combined synergistic effect leads to the possibility of reducing technological losses during cooking. A similar effect of individual components was noted in their works [13, 28], which indicates the relevance of intensifying the technological effect when combining raw materials.

Similar to the previous results, the hardness of the studied sausages increased with an increase in the amount of the enzyme complex and hydrocolloids in the composition. Thus, Sample No. 1 had indicators at the level of 1575 ± 0.01 g/cm², and the introduction of 0.5% hydrocolloid increased this indicator by 4.4%, 0.75% by 9.4%. The greatest increase in hardness to 2093 ± 0.01 g/cm² was shown by sample No. 4, which is 32.9% higher than the sample without the use of a technological additive. The increase in product hardness is due to the complex action of the ingredients of the mixture. Transglutaminase catalyzes the formation of ε-(γ-glutamyl)-lysine bonds between muscle proteins, which leads to increased elasticity and the formation of a denser structure. The hydrocolloids included in the composition swell under the influence of temperatures and form an additional gel framework that integrates with the protein matrix. Such a common action of all components allows to obtain a product with a stable structure and improved textural characteristics.

3.3. Assessment of the chemical composition of the obtained cooked sausages

An important characteristic in the development of food products using new ingredients is the determination of their chemical composition and energy value, which will allow assessing the complex changes in the product. The results of the conducted studies are presented in Table 5.

Table 5
Chemical composition of the developed sausages

Sample	Mass fraction, %			
	Moisture	Protein	Fat	Ash
Sample 1	75.40 ± 0.20	17.50 ± 0.15	4.30 ± 0.15	2.88 ± 0.10
Sample 2	74.51 ± 0.20	17.61 ± 0.20	4.55 ± 0.10	2.94 ± 0.10
Sample 3	74.82 ± 0.25	17.82 ± 0.20	4.75 ± 0.15	3.05 ± 0.10
Sample 4	74.65 ± 0.30	18.37 ± 0.15	6.13 ± 0.15	3.07 ± 0.11

In the studied samples of boiled sausages, the total moisture content, protein content, fat content and ash content were determined. According to the obtained research results, the use of 0.5–1% of the

stabilization system does not significantly affect the total moisture content in the product. A slight decrease in this indicator is caused by an increase in the proportion of dry matter.

The mass fraction of protein shows a tendency to increase with an increase in the content of the mixture from $17.6 \pm 0.20\%$ to $18.3 \pm 0.15\%$. The increase in this indicator is associated with an increase in the proportion of dry demineralized whey and a decrease in the loss of nitrogenous substances during heat treatment due to the compaction of the product structure under the action of transferase.

The mass fraction of fat in the product increases similarly to the protein content. Such results indicate the stabilization of the protein-fat structure under the action of hydrocolloid and protein components. In turn, the compacted matrix of the product is capable of better retention of the fat phase during thermal exposure.

The increase in the mass fraction of ash is directly related to the increase in mineral substances when adding dry whey and silicon oxide to the product. Also, reducing losses during heat treatment allows to preserve a larger share of mineral components.

3.4. Limitations and directions of research development

From a practical point of view, it should be noted that the use of the developed mixture with transglutaminase in an amount of 0.5–1% allows, under production conditions, to ensure the stability of functional-technological and structural-mechanical indicators of minced cooked sausages and to reduce the percentage of losses of sausage products of the cooked group, increasing the profitability of the production of this group of sausage products.

These research data and results relate to the use of a composite mixture with transglutaminase for first-grade cooked sausage products based on meat raw materials from the processing of broiler chickens, taking into account the functional-technological characteristics of this type of minced meat systems.

Further research into the possibility of using a composite mixture with transglutaminase will be aimed at adapting and substantiating its use for stabilizing minced meat emulsions of cooked sausage products based on meat raw materials obtained during the processing of beef and pork.

4. Conclusions

1. The study of minced meat systems for cooked sausages before heat treatment showed that the use of a functional additive in an amount of 0.5 to 1% reduces the active acidity by 0.02–0.05 pH. The obtained dynamics of the decrease in active acidity with increasing additive concentration leads to the conclusion that increasing the proportion of the proposed additive can reduce pH to values not typical for meat products, therefore it is not rational. The effective viscosity of the developed minced meat systems significantly increases to 6.50 ± 0.03 mPas when using 1% of the developed mixture. Such data prove the effectiveness of combining hydrocolloids and enzymes in the composition of food additives in order to form a stable structure of sausage products.

2. The obtained results of studies of the obtained cooked sausages indicate that the use of a technological additive has a stabilizing effect on pH indicators. It was determined that heat treatment for samples using 0.75% and 1% of the functional additive causes stabilization of active acidity. Thus, samples 3 and 4 demonstrate values at the level of 6.08 and 6.07, respectively, which are in the optimal range for this type of product. The textural characteristics of the obtained products prove a synergistic effect on the formation of hardness and appearance on the cut. The combination of the properties of hydrocolloids to form gels and transglutaminase to form covalent intermolecular bonds allows to increase the hardness of sausages and reduce losses after heat treatment by 18.1–31.3% relative to the sample without the mixture.

The introduction of a complex mixture using transglutaminase and hydrocolloids increases the textural characteristics in proportion to the increase in its share in the product. As a result of the conducted studies, it was determined that the components of the proposed technological additive exhibit synergistic qualities in the formation of the product structure and technological parameters.

3. Introduction of the developed technological mixture in an amount of 0.5; 0.75 and 1% by weight of unsalted raw materials does not significantly affect the change in the chemical composition of sausages. The use of dry demineralized whey, hydrocolloids and transferase in the composition allows to increase the protein and fat content. Strengthening intermolecular bonds and compacting the structure significantly increases the retention of protein and fat phases during temperature exposure. The total content of minerals also increases with an increase in the proportion of silicon dioxide and demineralized whey and ensures high quality indicators of sausage products.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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Data availability

Manuscript has no associated data.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the presented work.

Authors' contributions

Vasyl Pasichnyi: Conceptualization, Methodology, Validation, Formal analysis, Data curation; **Andrii Marynin:** Validation, Formal analysis, Data curation, Writing – original draft, Writing – review and editing; **Sergii Iepishkin:** Investigation, Validation, Formal analysis, Data curation; **Yevheniia Shubina:** Investigation, Validation, Formal analysis, Data curation; **Olena Moroz:** Validation, Formal analysis, Data curation.

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