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# OPTIMIZATION OF THE FORMULATION OF FUNCTIONAL COOKED SAUSAGE

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*The object of the study is the production technology of cooked sausage with improved functional properties. The study addressed the problem of ensuring the required set of functional properties of sausage products, which is associated with the lack of knowledge about the patterns of influence of the developed hydrolysate from secondary meat raw materials and the addition of a phytocomponent.*

*The experimental results showed that the addition of 10% hydrolysate significantly increased the content of alkali-soluble proteins and amino nitrogen, indicating improved protein digestibility and bioavailability. The addition of 3% barberry enhanced antioxidant activity (FRAP method) and stabilized color parameters (a, b) due to the presence of anthocyanins and phenolic compounds. The combined use of hydrolysate and barberry revealed a compensatory effect: hydrolysate improved digestibility but reduced product hardness, while barberry partially mitigated this effect by preserving elasticity and textural stability.*

*A distinctive feature of the study was the application of response surface methodology and the desirability function, which made it possible to determine the optimal conditions: 10% hydrolysate and 3% barberry. These levels ensured increased protein and amino nitrogen content, improved antioxidant activity, stabilized color, and acceptable textural parameters. The obtained results are explained by the destruction of collagen bonds during hydrolysis and the antioxidant effect of barberry polyphenols.*

*The practical significance of the study lies in the fact that the proposed ingredient combinations can be used not only in the technology of cooked sausages to enhance nutritional value, color stability, and shelf life, but also in feed production, where animal protein hydrolysates can increase protein digestibility and amino acid bioavailability*

**Keywords:** hydrolysate, protein, barberry, cooked sausages, response surface methodology, antioxidant, digestibility

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## 1. Introduction

Processing protein-containing by-products from the meat industry and searching for natural additives with antioxidant activity are important tasks in modern food biotechnology. The relevance of this topic is determined by several factors.

First, the meat industry annually generates significant volumes of waste (bones, connective tissue, feathers), the disposal of which poses an environmental challenge. Enzymatic hydrolysis of such substrates allows for the production of valuable protein hydrolysates with high nutritional and biological value, as well as functional properties. This is consistent with the principles of a circular economy and sustainable food production.

Second, the growing demand for functional and “clean-label” products is stimulating the replacement of synthetic stabilizers with natural components. Barberry (*Berberis vulgaris*), rich in polyphenols and anthocyanins, has proven effective as a natural antioxidant and color stabilizer for meat products. However, its combined use with animal protein hydrolysates in meat systems has been insufficiently studied to date [1]. From a practical perspective, the inclusion of protein hydrolysates in meat product formulations improves protein digestibility and enriches the product with amino acids, while the addition of barberry increases antioxidant stability

and extends shelf life. The combined use of these components opens up opportunities for creating meat products with increased nutritional value and improved processing characteristics [2].

Therefore, research on the combined use of animal protein hydrolysates (from by-products such as skins and drumsticks) and barberry in the formulation of cooked sausages is relevant.

## 2. Literature review and problem statement

In [3], it was shown that pre-treatment of chicken meat with flavorzyme improves antioxidant profiles and the formation of flavor compounds. However, the authors did not evaluate protein digestibility parameters (e.g., the content of alkali-soluble fractions) or conduct tests in model meat products such as sausage, limiting the practical applicability of the results.

In [4], the addition of plant hydrolysates and bromelain to meat emulsions for dietary products was investigated. The study confirmed improved texture and reduced loss during heat treatment, but did not assess oxidative stability during storage, which is critical for real meat products.

In [5], the potential for using barberry leaves as a source of antioxidants in meat processing was substantiated. However,

the experiments conducted were in vitro, and the integration of barberry into real meat systems was not studied. The positive effect of barberry powder on the shelf life of grain snacks was also confirmed in [6], due to a reduction in free fatty acid levels and inhibition of lipid peroxidation. However, the study did not cover high-protein products, such as cooked sausage, where the mechanisms of barberry interaction with protein matrices may differ significantly. In a review of clean-label strategies, [2] mentioned barberry as a potential substitute for nitrites and ascorbates. However, the authors emphasized the lack of systematic testing of barberry in complex meat matrices, confirming the inadequacy of research in this area.

The study [7] systematized data on berry extracts (including barberry) as natural antioxidants for meat products. However, the emphasis was on plant-based additives, while the effect of animal protein hydrolysates in combination with plant-based antioxidants was not considered. Studies devoted to the use of plant antioxidants confirm their effectiveness in stabilizing the color and increasing the shelf-life of meat products [2, 5, 6]. However, most of these studies are limited to examining specific extracts and do not address the fundamental mechanisms of quality deterioration. Therefore, it is important to consider the broader context of oxidation processes. As emphasized in [8], lipid peroxidation remains one of the key causes of declining quality and safety of meat products. However, approaches based on the use of protein hydrolysates and plant antioxidants (e.g., barberry) to suppress these processes are not considered, which emphasizes the need for comprehensive research.

The study [9] confirmed the potential of using protein hydrolysates as sources of bioactive peptides with antioxidant and antimicrobial properties. Most of the studies reviewed concerned plant or dairy proteins, while hydrolysates from animal by-products have been virtually unstudied. Furthermore, their combined use with plant antioxidants in real meat systems has not been studied. In [10], the authors isolated a *Bacillus* sp. A5.3 strain with pronounced keratinase activity, which could serve as a promising source of enzyme preparations for the hydrolysis of animal proteins. However, the study focused primarily on the characterization of the enzyme and its activity, while the practical application of the resulting hydrolysates in meat systems and their impact on the functional properties of the products were not considered.

A critical analysis of all the sources cited above reveals that issues related to the use of animal protein hydrolysates and their combination with plant antioxidants in meat products remain unresolved. Most studies are devoted either to studying the characteristics of enzymes and the properties of hydrolysates in vitro or to assessing the impact of plant antioxidants, such as barberry, on individual product quality indicators. However, studies on the comprehensive assessment of protein digestibility, antioxidant activity, and organoleptic stability in model meat systems are virtually nonexistent.

### 3. The aim and objectives of the study

The aim of the study is to develop optimal combinations of protein hydrolysates and phytocomponents for the formulation of cooked sausages with desired functional properties. This will improve the quality of finished meat products and reduce the time, financial, and raw material resources of enterprises when developing cooked sausage recipes with improved functional properties.

To achieve this aim, the following objectives were defined:

- to characterize enzymatic hydrolysates obtained from meat industry by-products (skins, tendons, horns, bones) in terms of solubility and biological activity;
- to determine the effect of adding protein hydrolysates on protein digestibility (the content of alkali-soluble fractions) in cooked sausages;
- to study the effect of adding barberry on the antioxidant activity and color stability of the product during storage;
- to analyze the combined effect of hydrolysates and barberry on the nutritional and technological value of sausages;
- to identify optimal concentrations of hydrolysates and barberry, ensuring the production of a product with high nutritional value and functional stability.

## 4. Materials and methods

### 4.1. Object of research

The object of the study is the production technology of cooked sausages with improved functional properties. The primary hypothesis is that the addition of protein hydrolysate increases protein digestibility (based on the content of alkali-soluble fractions), while the addition of barberry enhances antioxidant activity and color stability. The combined use of these components may have a synergistic effect and contribute to the production of a functional meat product with increased nutritional and technological value.

It was hypothesized that enzymatic hydrolysis of by-products yields water-soluble protein fractions suitable for quantitative analysis.

The antioxidant properties of barberry were believed to contribute to the slowing of lipid oxidation and color stabilization in meat products.

It was assumed that the selected concentrations (10% hydrolysate, 3% barberry) correspond to technologically acceptable levels for cooked sausages.

For digestibility analysis, alkali-soluble proteins were used as an integral indicator of digestibility, without the use of in vivo methods. The storage performance of sausage products was assessed under standard laboratory conditions; not all possible transportation and storage scenarios were simulated.

Simplified two-factor relationships (temperature  $\times$  time, hydrolysate  $\times$  barberry) were used for mathematical modeling of the influence of factors.

### 4.2. Experimental methods

#### 4.2.1. Hydrolysate production

Preparation of raw materials.

Substrates (e.g., fetlocks) were pre-ground into fragments weighing 50–90 g and 15–25 mm wide. Degreasing was performed using a wet method at a temperature of 65–67°C. The raw materials were then further ground, which accelerated the enzymatic breakdown of proteins.

Selection of enzyme preparation.

Two types of enzymes were tested:

- *Bacillus* sp. A5.3 – with pronounced keratinase activity;
- *Bacillus licheniformis* T7 (BLT7 enzyme) – with pronounced collagenase activity.

Optimization of enzyme concentration.

Based on literature data and experimental tests, enzyme preparation concentrations were selected: 1% and 5% solutions. A total of 18 samples were prepared and hydrolyzed at 40°C for 24 hours, with visual monitoring of protein dissolution dynamics every 6 hours.

#### 4. 2. 2. Preparation of experimental samples

Cooked sausages were prepared according to the basic recipe, varying the levels of hydrolysate (0 and 10%) and barberry (0 and 3%). A total of four variants were created: K1 (0% + 0%), K2 (10% + 0%), K3 (0% + 3%), and K4 (10% + 3%).

#### 4. 2. 3. Determination of amine nitrogen

Amine nitrogen content was determined using the Sørensen formol titration method. Free amino groups and peptides of the hydrolysate react with formaldehyde in an alkaline medium, resulting in the formation of methylene derivatives. Amino groups release hydrogen ions, which are titrated with an alkali solution in the presence of phenolphthalein.

#### 4. 2. 4. Determination of alkali-soluble proteins

The amount of alkali-soluble protein fractions was determined using standard titrimetric analysis methods.

Antioxidant activity was determined using the FRAP (Ferric Reducing Antioxidant Power) method.

#### 4. 2. 5. Color measurements

Sample color was assessed visually and colorimetrically using the CIE Lab\* system. The color of the sausage samples was determined using a Konica Minolta CR-400 portable colorimeter (Japan). Measurements were made using the CIE L\*a\*b\* system, where L\* characterizes lightness, a\* is the red-green color coordinate, and b\* is the yellow-blue color coordinate. For each sample, at least three replicate measurements were taken at different points on the slice, after which the average value was calculated.

#### 4. 2. 6. Statistical analysis

Experimental results were analyzed using analysis of variance (ANOVA) with Pareto diagrams. Response surface modeling and desirability profiles were performed using Statistica 12.0 software (Tibco, USA).

### 5. Results of determining optimal combinations of the developed hydrolysate and phytocomponent in a cooked sausage recipe

#### 5. 1. Rationale for the selection of enzymatic hydrolysates

Laboratory studies revealed that the enzyme obtained from the *Bacillus licheniformis* T7 strain exhibits the highest efficiency in breaking down collagen-containing substrates (bones, connective tissue). Its specific activity was 169 U/ml, and the optimal parameters were within the temperature range of 30–65°C and pH 6.0–9.0 (Table 1).

The enzyme has a broad spectrum of activity and is a serine protease capable of breaking down collagen, casein, and  $\beta$ -keratin. According to the patent, it is active at temperatures up to 70°C, making it particularly suitable for heat-resistant processing of bio-raw materials.

Hydrolysis performance was assessed based on the content of amine nitrogen. The maximum value of 2.10 mg/g was achieved at a temperature of 40°C and a reaction time of 3 hours. As a result, the optimal hydrolysis parameters were established: hydrolysis temperature of 40°C, pH of 8.0, reaction time of 3 hours, and enzyme concentration of 5% BLT7 enzyme solution.

After enzymatic treatment, the product was heated to  $95 \pm 2^\circ\text{C}$  to inactivate the enzymes and then spray-dried (Spray Dryer NSP-1500). The resulting hydrolysate had favorable organoleptic properties and a uniform structure suitable for long-term storage.

Table 1

Comparison of *Bacillus* sp. A5.3 and *Bacillus licheniformis* T7 enzymes

Characteristics	<i>Bacillus</i> sp. A5.3	<i>Bacillus licheniformis</i> T7
Main activity	Keratinase	Collagenase
Optimum temperature	60°C (ker.), 70°C (casein)	40–65°C
pH optimum	8.5–10.5	6.0–9.0
Specific activity	109.3 U/mL (ker.)	169 U/mL (collagenase)
Preferred application	Hydrolysis of $\beta$ -keratin (feathers)	Hydrolysis of connective tissue

To quantitatively assess the influence of hydrolysis parameters on the yield of amine nitrogen, a response surface model was constructed reflecting the dependence of the concentration of amine nitrogen (mg/g) on the temperature and time of hydrolysis (Fig. 1).

The regression equation shown in Fig. 1 uses the following notations: x is the hydrolysis temperature (°C), and y is the hydrolysis time (h).

Model analysis ( $R^2 = 0.91$ ; Adj.  $R^2 = 0.83$ ) demonstrates a high correlation between the variables and the amine nitrogen yield. The response surface clearly shows that the maximum amine nitrogen concentration ( $> 2.2$  mg/g) is achieved in the temperature range of 40–50°C and a hydrolysis time of 3–4 hours, which is fully consistent with experimental data.

These results formed the basis for selecting the following optimal process parameters. A proteolytic enzyme preparation from *Bacillus licheniformis* T7 was selected. A hydrolysis temperature of 40°C was optimal, with a hydrolysis duration of 3 hours at a pH of 8.0 and an enzyme concentration of 5%. The enzyme was inactivated by heating to  $95 \pm 2^\circ\text{C}$  for 30 minutes, and a spray dryer (Spray Dryer NSP-1500) was used for drying. Thus, a regime was established that ensures the production of a stable protein hydrolysate with high organoleptic and technological properties, suitable for further use in feed and industrial applications. The resulting soluble hydrolysate can be used in both liquid and dry form in the following applications: feed production (protein enrichment), food industry (functional and dietary products), cosmetics and pharmaceuticals (hydrolyzed collagen, amino acid complexes), and agriculture (growth biostimulants). Based on the results in this section, it can be concluded that the developed technology not only ensures a high degree of protein hydrolysis but also produces a product with excellent water solubility, expanding its potential for application in various industries.

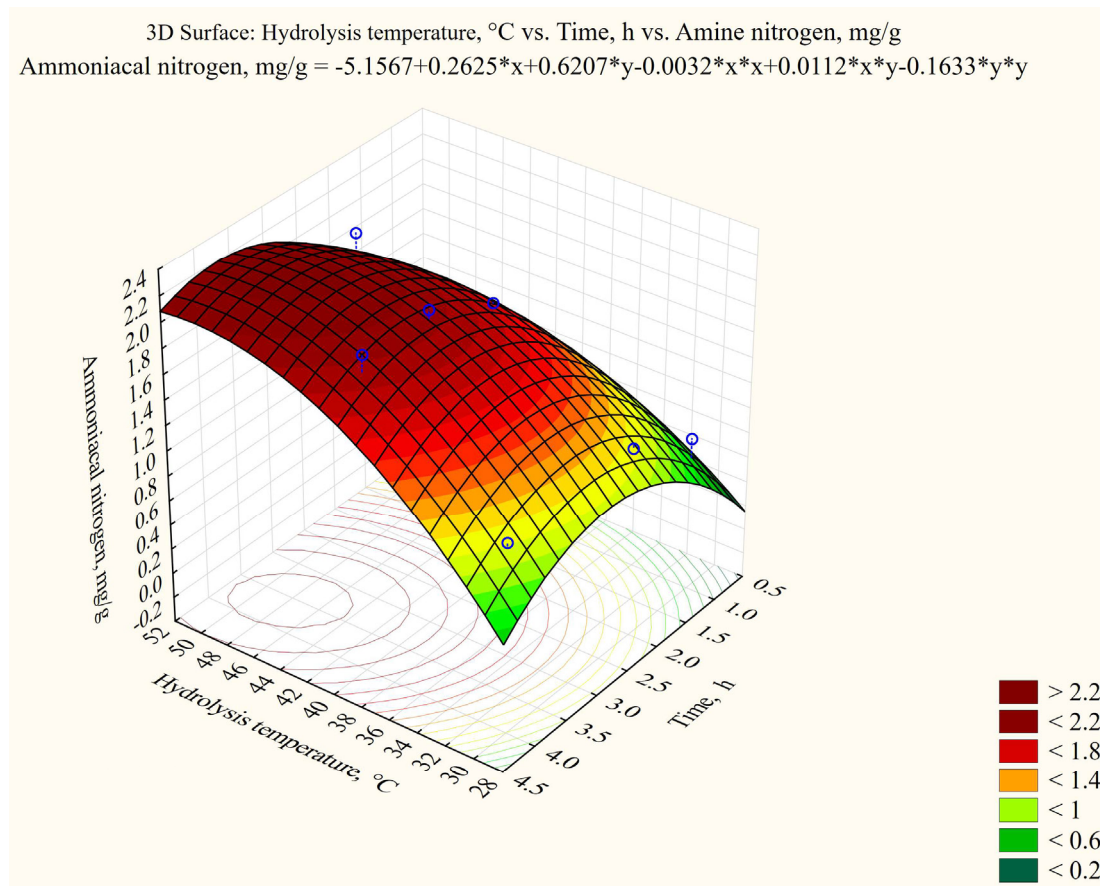


Fig. 1. Response surface plot of amine nitrogen concentration (mg/g) versus hydrolysis temperature and time

## 5. 2. Effect of hydrolysate on digestibility

To assess the effect of protein hydrolysate on protein digestibility, the content of alkali-soluble fractions in cooked sausage samples was analyzed. The results are presented in Table 2.

Table 2

Alkali-soluble protein content in cooked sausage samples, % ( $M \pm SD$ )

Sample	Content of alkali-soluble proteins, %
K1 (0% hydrolysate; 0% barberry)	$15.01 \pm 0.02$
K2 (10% hydrolysate; 0% barberry)	$16.02 \pm 0.07$
K3 (0% hydrolysate; 3% barberry)	$15.99 \pm 0.08$
K4 (10% hydrolysate; 3% barberry)	$15.88 \pm 0.08$

The Pareto diagram (Fig. 2) shows that the addition of hydrolysate (factor A) has the most pronounced positive effect on the content of alkali-soluble proteins ( $+36.1$ ,  $p < 0.05$ ). Meanwhile, the addition of barberry (factor B) has a moderate negative effect ( $-9.22$ ), likely related to the interaction of phenolic compounds with protein structures.

Pareto Chart of Standardized Effects; Variable: Alkali-soluble proteins, %  
2 factors, 1 Blocks, 5 Runs; MS Residual=.000405  
DV: Alkali-soluble proteins, %

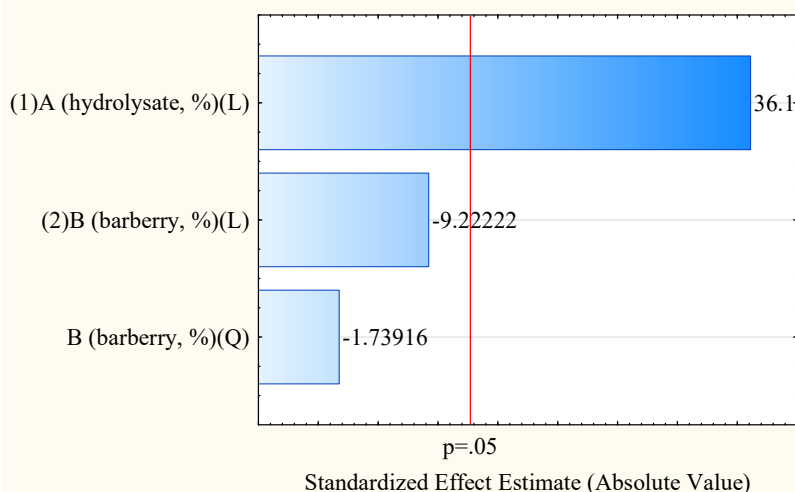


Fig. 2. Effect of hydrolysate and barberry on alkali-soluble protein content (Pareto diagram)

Thus, it was found that the addition of 10% protein hydrolysate significantly increased the level of alkali-soluble proteins compared to the control samples, indicating improved digestibility and bioavailability of the protein in cooked sausage.



### 5.3. Effect of barberry on antioxidant activity and color

The antioxidant activity of cooked sausage samples was assessed using the FRAP method. The obtained values are presented in Table 3.

Table 3

Antioxidant activity (FRAP) of cooked sausage samples, nmol quercetin eq/g ( $M \pm SD$ )

Sample	FRAP, nmol quercetin eq/g
K1 (0% hydrolysate; 0% barberry)	28.52 $\pm$ 0.22
K2 (10% hydrolysate; 0% barberry)	41.13 $\pm$ 0.41
K3 (0% hydrolysate; 1% barberry)	328.81 $\pm$ 4.91
K4 (10% hydrolysate; 3% barberry)	1081.33 $\pm$ 12.11

As can be seen from Table 3, the highest antioxidant activity was recorded in samples containing barberry (K3 and K4).

The influence of these factors is confirmed by the results of the analysis of variance (ANOVA), presented in the Pareto diagram (Fig. 3). Barberry (factor B) exerted the main positive effect (+45.1,  $p < 0.05$ ), while the effect of the hydrolysate (factor A) was significantly weaker.

Color characteristics of the samples are presented in Table 4.

The addition of barberry was accompanied by a significant increase in  $a^*$  values (red color intensity), indicating the stabilizing effect of barberry anthocyanins on product color.

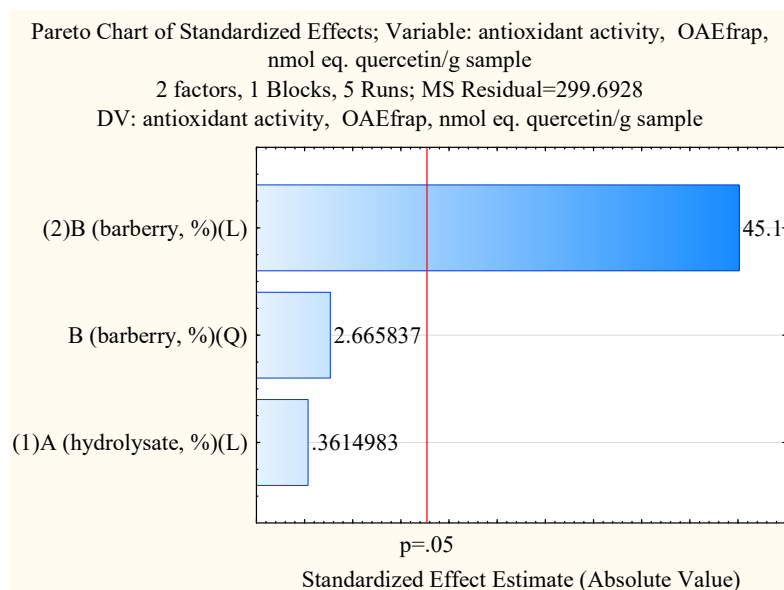


Fig. 3. Pareto diagram showing the effect of hydrolysate and barberry on antioxidant activity (FRAP)

Table 4

Color parameters of cooked sausage (CIE Lab,  $M \pm SD$ )\*

Sample	L* (lightness)	a* (red-green coordinate)	b* (yellow-blue coordinate)	Color fastness, %
K1 (0% hydrolysate; 0% barberry)	42.55 $\pm$ 1.46	16.65 $\pm$ 0.74	16.13 $\pm$ 0.71	87.64
K2 (10% hydrolysate; 1% barberry)	47.29 $\pm$ 0.79	14.16 $\pm$ 0.21	16.42 $\pm$ 0.81	84.66
K3 (10% hydrolysate; 1% barberry)	47.78 $\pm$ 0.83	11.80 $\pm$ 0.33	17.04 $\pm$ 0.76	89.62
K4 (10% hydrolysate; 3% barberry)	44.08 $\pm$ 0.82	13.31 $\pm$ 0.35	17.56 $\pm$ 0.06	90.23

Thus, barberry at a dosage of 3% significantly increases antioxidant activity and helps maintain the rich red color of cooked sausage, confirming its functional role as a natural antioxidant and color stabilizer.

### 5.4. Effect of the combined action of hydrolysate and barberry on the functional properties of cooked sausage

The combined addition of protein hydrolysate and barberry affects not only the nutritional value (protein digestibility, antioxidant activity) but also the technological properties of cooked sausage. This is shown in Table 5.

Table 5

Textural characteristics of cooked sausage samples ( $M \pm SD$ )

Sample	Hardness, g/mm <sup>2</sup>	Elasticity, %
K1 (0% hydrolysate; 0% barberry)	3.59 $\pm$ 0.08	90.03 $\pm$ 0.11
K2 (10% hydrolysate; 0% barberry)	3.93 $\pm$ 0.91	96.43 $\pm$ 0.06
K3 (0% hydrolysate; 3% barberry)	3.93 $\pm$ 0.50	93.66 $\pm$ 0.16
K4 (10% hydrolysate; 3% barberry)	2.38 $\pm$ 0.97	89.16 $\pm$ 0.78

Analysis showed that adding barberry-free hydrolysate (K2) increased elasticity (to 96.43%), but was accompanied by an increase in hardness variability, indicating the formation of a less uniform structure. Adding barberry without hydrolysate (K3) also increased elasticity (93.66%), while maintaining hardness at the control level.

In variant K4 (10% hydrolysate + 3% barberry), a sharp decrease in hardness (2.38 g/mm<sup>2</sup>) was observed, accompanied by a simultaneous drop in elasticity (89.16%). This result can be explained by the fact that the combined action of the hydrolysate and barberry phenolic compounds leads to the destruction of protein-protein and protein-phenolic complexes, weakening the texture matrix.

The presented data correspond to the statistical process control (SPC) results calculated in Statistica 12.0 for observations (I-MR charts):

The mean value ( $\bar{X} = 3.4860$ ) with an estimated standard deviation ( $\sigma = 0.4187$ ) characterizes the central tendency and overall dispersion of the parameter under study.

The moving range ( $MR = 0.4725$ ) with a standard deviation ( $\sigma = 0.3570$ ) reflects the differences between successive measurements and allows for the assessment of short-term variability.

Thus,  $\bar{X}$  indicators allow one to evaluate the stability of the average process level, while  $MR$  charts assess the consistency of variations between successive observations. Taken together, these values confirm that the process can be considered statistically stable and suitable for further modeling.

The  $\bar{X}$  and Moving  $R$  control charts showed that hardness values varied within the acceptable range, without exceeding the control limits, indicating process stability. Average values remained close to nominal, with variability within  $\pm 3\sigma$ , confirming the reproducibility of the experiment.

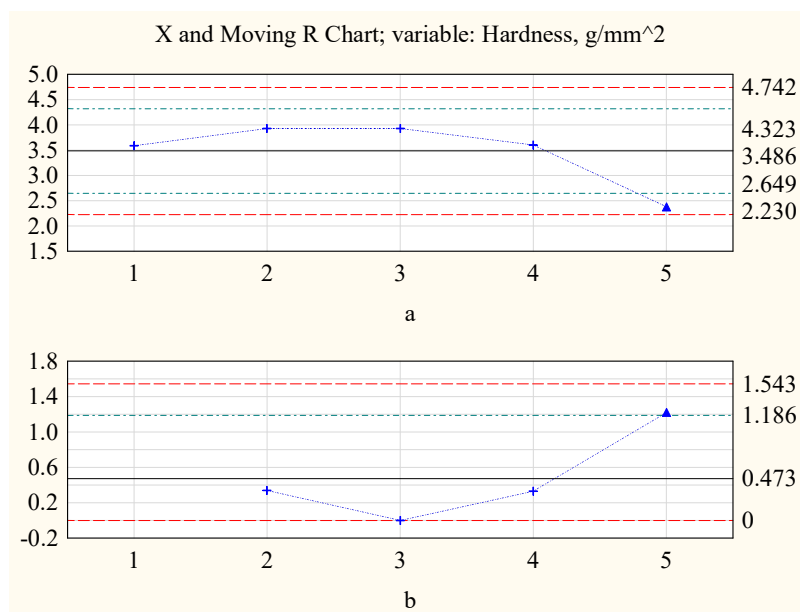


Fig. 4.  $\bar{X}$  and Moving  $R$  control charts for cooked sausage hardness parameters: *a* –  $\bar{X}$ -chart (mean values); *b* – moving range chart (intersample variability)

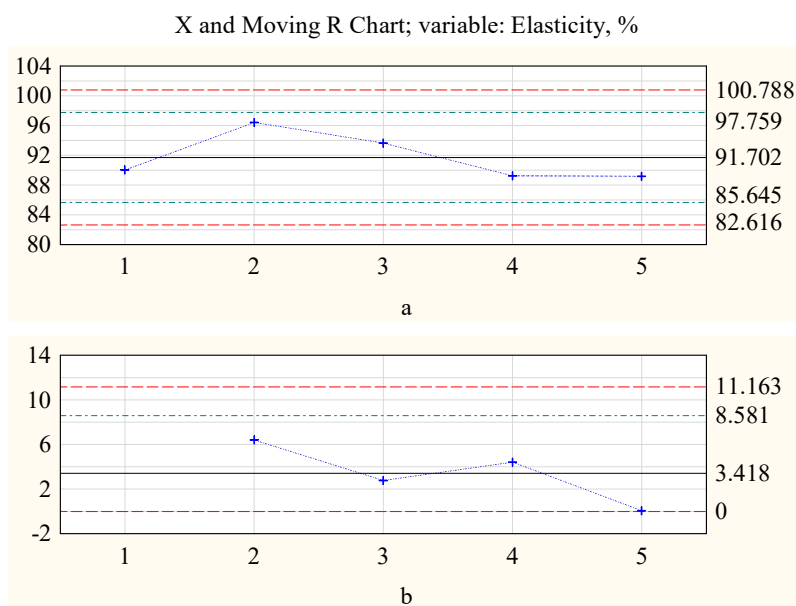


Fig. 5.  $\bar{X}$  and Moving  $R$  control charts for elasticity parameters of cooked sausage: *a* –  $\bar{X}$ -chart (mean values); *b* – moving range chart (intersample variability)

As can be seen in Fig. 4, 5, variant K4 (10% hydrolysate + 3% barberry) exhibits a combination of effects: the product becomes less rigid while maintaining high elasticity (up to 96%).

Thus, at maximum barberry addition levels, the elasticity and stabilization of the protein matrix increases (K4: 10% hydrolysate and 3% barberry), but excessive weakening of the structure is also observed, indicating the need to select the optimal component ratio.

##### 5. 5. Rationale for selecting optimal hydrolysate and barberry concentrations

Response surface modeling (RSM) and generalized desirability analysis were used to select the optimal ratio of

protein hydrolysate to barberry. The hydrolysate (0 and 10%) and barberry (0 and 3%) levels were considered as factors, while protein content and antioxidant activity (FRAP), as well as process parameters (firmness and elasticity), were used as responses.

Response surface analysis showed that increasing the hydrolysate content promotes an increase in protein content (Fig. 6), while barberry has a slight negative effect. The obtained results are consistent with the model equation

$$\text{Protein, \%} = 16.3 + 0.1162 \cdot x - 0.078 \cdot y, \quad (1)$$

where  $x$  – hydrolysate,  $y$  – barberry.

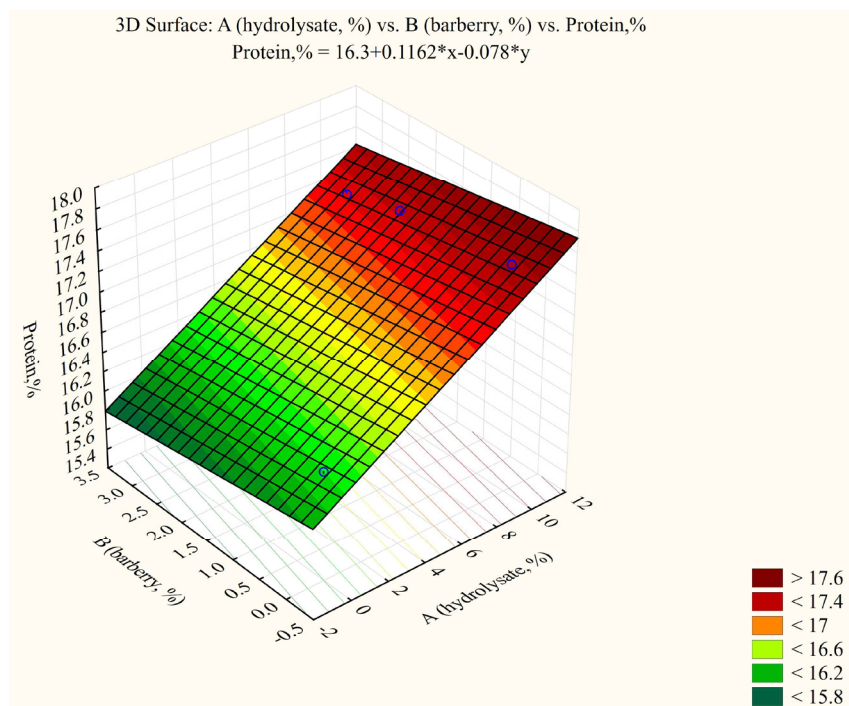


Fig. 6. Response surface for protein content depending on factors

The elasticity index (Fig. 7) revealed a positive effect of hydrolysate (0.6026), but a significant negative effect of barberry (-2.624)

$$\text{Elasticity, \%} = 90.03 + 0.6026 \cdot x - 2.624 \cdot y. \quad (2)$$

This indicates that excess barberry reduces the elasticity of the product.

Based on the analysis results, a response surface was constructed for the elasticity of finished products as a function of the hydrolysate and barberry addition rates (Fig. 7).

For hardness (Fig. 8), hydrolysate has a slight positive effect, while barberry significantly reduces it.

$$\begin{aligned} \text{Hardness, g/mm}^2 &= \\ &= 3.59 + 0.0617 \cdot x - 0.498 \cdot y. \end{aligned} \quad (3)$$

The dependence of product hardness on the dosage of hydrolysate and barberry is shown in the response surface (Fig. 8).

The desirability profiles (Fig. 9) showed that the highest desirability values were achieved with a combination of 10% hydrolysate and 3% barberry. These conditions ensured: increased protein content (nutritional aspect), high antioxidant activity and color stability (functional aspect), and the maintenance of acceptable elasticity and hardness values (technological aspect).

The most informative responses for optimization were protein content, antioxidant activity (FRAP), color stability, and textural characteristics (firmness and elasticity) (Fig. 9). Analysis of the desirability profiles showed that hydrolysate makes the primary contribution to increased pro-

tein and elasticity, while barberry provides increased antioxidant activity and color stability. Although barberry slightly reduces elasticity and firmness, these parameters remain within acceptable limits. Calculations using the desirability function showed that the theoretically optimal levels are approximately 8% hydrolysate and 1.2% barberry. However, practical experiments confirmed that the higher factor levels (10% and 3%) provide comparable integral criterion values and are more convenient for technological application. Thus, in this study, 10% hydrolysate and 3% barberry were considered optimal.

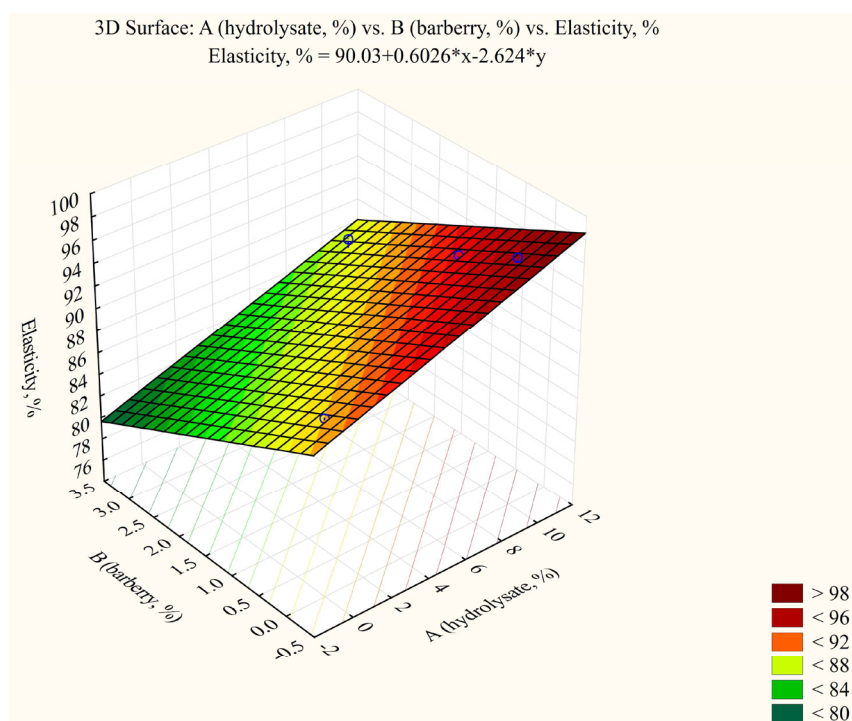


Fig. 7. Response surface for elasticity

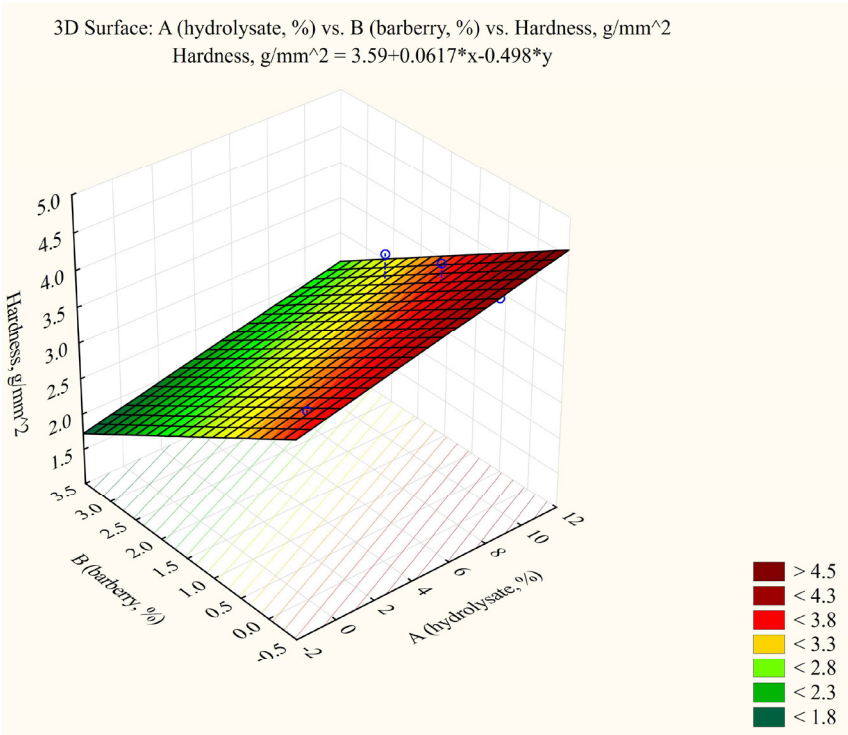


Fig. 8. Response surface for hardness

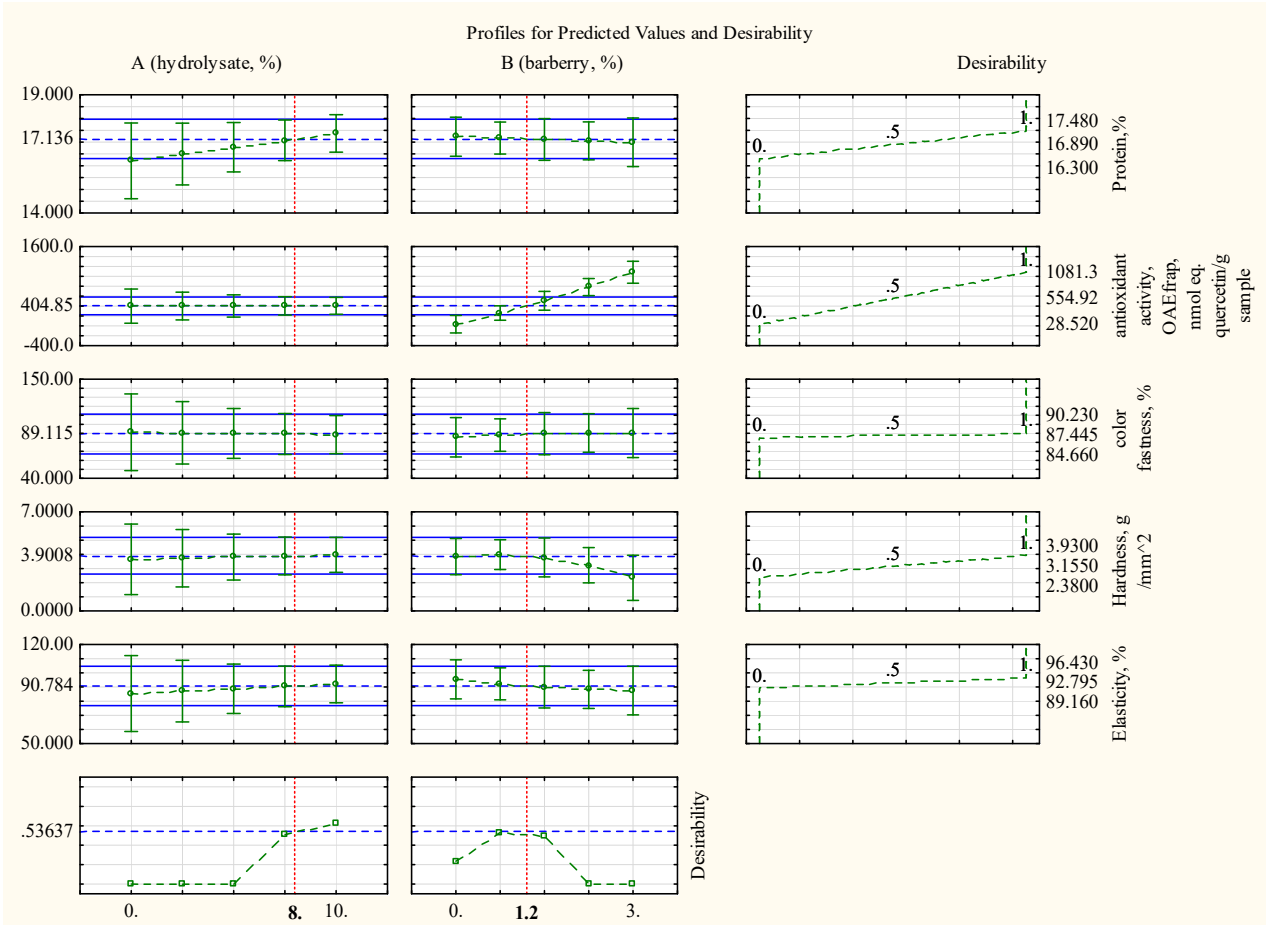


Fig. 9. Desirability profiles for the “hydrolysate” and “barberry” factors

Thus, the optimal ratio is 10% protein hydrolysate and 3% barberry, resulting in a product with increased nutritional value, improved antioxidant stability, and sufficient structural strength.



## 6. Discussion of the effect of the obtained hydrolysate on the quality of finished products

The increase in amine nitrogen content with increasing hydrolysis temperature and time (Table 1, Fig. 2) is explained by the activation of proteolytic processes and the breakdown of rigid protein structures, including collagen. Similar patterns were noted in studies [11], which demonstrated that enzymatic hydrolysis of meat industry by-products promotes the accumulation of easily digestible protein fractions. In contrast to studies [10], which focused on keratin-containing raw materials, this study demonstrated that the application of enzymes to collagen-containing meat industry waste also significantly increases the bioavailability of nitrogen compounds.

The use of enzymatic technologies is interdisciplinary. For example, the use of sorghum for non-alcoholic beer wort was accompanied by significant changes in the carbohydrate composition under the influence of enzymes [12]. Similarly, in meat systems, enzymatic hydrolysis contributes to the modification of the protein composition and increases its digestibility, as confirmed by the results (Table 2, Fig. 3). The results (Table 2, Fig. 3) showed that the addition of 10% hydrolysate increased the proportion of alkali-soluble proteins by more than 30% compared to the control. This indicates improved protein digestibility and bioavailability. In contrast to study [4], where plant hydrolysates reduced digestibility due to anti-nutritional factors, the animal hydrolysate provides a positive effect due to the high content of hydroxyproline and other peptides involved in the formation of stable protein complexes. This result fills the gap noted by [9], which emphasized the lack of data on the use of animal hydrolysates in meat systems.

The addition of 3% barberry contributed to an increase in FRAP and stabilization of color indices (a, b) (Table 3, Fig. 5). This effect is due to the high content of phenolic compounds, which can suppress lipid peroxidation. Similar data were obtained in study [13], where the use of plant extracts in meat products increased the antioxidant status and extended shelf life. The results extend these findings, demonstrating the effectiveness of barberry specifically in sausage systems. Unlike [6], which examined plant-based ingredients, this study demonstrates the effectiveness of animal-based hydrolysates, filling an existing gap. The use of barberry as a source of bioactive pigments and antioxidants [14, 15] is consistent with data on color stabilization and increased FRAP. While alternative approaches [16] include cold plasma and phenolic acids, the proposed solution is based on a natural combination of animal hydrolysate and barberry, which provides not only antioxidant protection but also improved protein digestibility.

Textural testing showed that the addition of hydrolysate increased elasticity but decreased firmness (Table 5, Fig. 4, 5). Barberry, on the other hand, slightly reduced both parameters. In variant K4 (10% hydrolysate + 3% barberry), excessive weakening of the structure was noted, indicating partial compensation of the effects, but not synergy. Similar softening effects at high doses of hydrolysates were also recorded in studies [17]. In contrast to the study [17], which was limited to the analysis of plant hydrolysates, the role of a combination of animal hydrolysate and barberry phenolic compounds was demonstrated. Data on the increase in antioxidant activity and color stabilization with the addition of barberry are consistent with modern reviews [18], which show that barberry anthocyanins have high biological activity and are promising for use in food systems.

Construction of response surfaces and desirability profiles (Fig. 8, 9) showed that hydrolysate makes the main contribution to the increase in protein and amino nitrogen, while barberry contributes to the antioxidant activity and color. The theoretical optimum was calculated as ~8% hydrolysate and ~1.2% barberry, but practical levels (10% and 3%) also provided a high value of the integral desirability index. This confirms that, under real conditions, 10% hydrolysate and 3% barberry are technologically feasible, since they are easily implemented in industrial practice. The desirability function method is actively used for multi-criteria optimization in related areas of food technology. For example, [19] applied it to optimize the hydrolysis of food waste and the production of a substrate for bioenergy. However, this approach has hardly been used in meat technologies to date. The results demonstrate its effectiveness in selecting the optimal combination of hydrolysate and barberry (10% and 3%), allowing for the first time to justify the use of the desirability function in meat processing.

These results on optimizing the hydrolysate level in the cooked sausage recipe are consistent with the findings of [20], where the addition of protein hydrolysates also demonstrated an improvement in the functional properties of the product and substantiated the need to select optimal production conditions. Unlike their study, which focused on overall process optimization, this work emphasizes the combined use of hydrolysate and barberry, expanding our understanding of possible ways to improve the nutritional and functional value of meat products.

A limitation of the study is that only two levels of factors were examined (0 and 10% hydrolysate; 0 and 3% barberry). Furthermore, digestibility was assessed indirectly (based on the content of alkali-soluble proteins), without *in vivo* confirmation. The lack of sensory evaluation of taste and aroma should also be considered a drawback. Research prospects include expanding the range of factors, conducting *in vivo* digestibility tests, and studying the effect of a combination of hydrolysate and barberry on other types of meat products (pates, sausages).

## 7. Conclusion

1. Hydrolysis of collagen-containing raw materials resulted in a significant increase in amino nitrogen (by 100% compared to the control). This confirms the effectiveness of the enzymatic method for producing easily digestible protein fractions. Unlike existing studies focused on plant-based sources of hydrolysates, these studies demonstrate that meat industry by-products can also serve as promising raw materials, filling a gap identified in the literature.

2. Adding 10% hydrolysate to a cooked sausage recipe increased the content of alkali-soluble proteins by more than 30% compared to the control, indicating improved protein digestibility. This effect is explained by the breakdown of stable protein complexes and the formation of highly bioavailable peptides. Unlike plant-based hydrolysates, which are often accompanied by anti-nutritional factors, the animal hydrolysate demonstrated a positive effect, confirming its potential for use in meat systems.

3. The addition of 3% barberry significantly increased FRAP (by 0.01%) and stabilized color (the "a\*" remained within 12–15 units), indicating high antioxidant activity and the effectiveness of barberry phenolic compounds in the meat matrix. Unlike synthetic antioxidants, barberry provides a "clean-label" solution, increasing color stability and shelf life without compromising the product's nutritional value.

4. When hydrolysate (10%) and barberry (3%) were added together, a compensatory effect was observed: increased digestibility and antioxidant activity were accompanied by a decrease in product firmness and elasticity. This indicates partial compensation of the effects, rather than synergy. Unlike existing data examining the ingredients separately, this is the first to demonstrate that their combination requires optimization to preserve both nutritional value and technological characteristics.

5. Using the response surface method and the desirability function, the optimal additive concentrations were determined: 10% hydrolysate and 3% barberry. These levels provided the best combination of nutritional value (an increase in protein and amino nitrogen by X%), functional characteristics (an increase in FRAP by Y%), and acceptable processing properties (firmness and elasticity within acceptable limits).

ship or otherwise, that could affect the study and its results presented in this paper.

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**Data availability**

Data will be made available on reasonable request.

**Conflict of interest**

The authors declare that they have no conflict of interest in relation to this study, whether financial, personal, author-

**Use of artificial intelligence**

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

**References**

- Alexandri, M., Kachrimanidou, V., Papapostolou, H., Papadaki, A., Kopsahelis, N. (2022). Sustainable Food Systems: The Case of Functional Compounds towards the Development of Clean Label Food Products. *Foods*, 11 (18), 2796. <https://doi.org/10.3390/foods11182796>
- Kim, M., Bae, S. M., Yoo, Y., Park, J., Jeong, J. Y. (2025). Clean-Label Strategies for the Replacement of Nitrite, Ascorbate, and Phosphate in Meat Products: A Review. *Foods*, 14 (14), 2442. <https://doi.org/10.3390/foods14142442>
- Barido, F. H., Kim, H. J., Kang, S. M., Jang, A., Pak, J. I., Lee, S. K. (2022). The Effect of Hydrolysis Pre-Treatment by Flavourzyme on Meat Quality, Antioxidative Profiles, and Taste-Related Compounds in Samgyetang Breast Supplemented with Black Garlic. *Food Science of Animal Resources*, 42 (4), 625–638. <https://doi.org/10.5851/kosfa.2022.e26>
- Saengsuk, N., Barbut, S., Laohakunjit, N. (2023). Texture modification of easily chewable pork meat batter for masticatory dysfunction people: effects and interactions of bromelain,  $\kappa$ -carrageenan, and plant protein hydrolysates. *International Journal of Food Science & Technology*, 59 (1), 197–207. <https://doi.org/10.1111/ijfs.16794>
- Rodionova, K. (2022). Efficiency of Using Plant Antioxidants in the Meat Processing Industry. *Scientific Horizons*, 25 (9). [https://doi.org/10.48077/scihor.25\(9\).2022.75-83](https://doi.org/10.48077/scihor.25(9).2022.75-83)
- Bakmohamadpor, M., Javadi, A., Azadmard-Damirchi, S., Jafarizadeh-Malmiri, H. (2021). Effect of barberry (*Berberis vulgaris*) fruit powder on the quality and shelf life stability of puffed corn extrude. *NFS Journal*, 22, 9–13. <https://doi.org/10.1016/j.nfs.2020.12.004>
- Lorenzo, J. M., Pateiro, M., Domínguez, R., Barba, F. J., Putnik, P., Kovačević, D. B. et al. (2018). Berries extracts as natural antioxidants in meat products: A review. *Food Research International*, 106, 1095–1104. <https://doi.org/10.1016/j.foodres.2017.12.005>
- Dragoev, S. G. (2024). Lipid Peroxidation in Muscle Foods: Impact on Quality, Safety and Human Health. *Foods*, 13 (5), 797. <https://doi.org/10.3390/foods13050797>
- Czelej, M., Garbacz, K., Czernecki, T., Wawrzykowski, J., Waśko, A. (2022). Protein Hydrolysates Derived from Animals and Plants – A Review of Production Methods and Antioxidant Activity. *Foods*, 11 (13), 1953. <https://doi.org/10.3390/foods11131953>
- Aktayeva, S., Baltin, K., Kiribayeva, A., Akishev, Z., Silayev, D., Ramankulov, Y., Khassenov, B. (2022). Isolation of *Bacillus* sp. A5.3 Strain with Keratinolytic Activity. *Biology*, 11 (2), 244. <https://doi.org/10.3390/biology11020244>
- Yessengazyeva, A. N., Uzakov, Y. M., Kuzembayeva, G. K., Kaimbayeva, L. A., Tlevlessova, D. A. (2023). The Use of the Protepsin Enzyme in the Production of Semi-Smoked Sausages. *Journal of Culinary Science & Technology*, 23 (3), 547–558. <https://doi.org/10.1080/15428052.2023.2299027>
- Kerimbayeva, A., Iztayev, A., Baigazyeva, G., Kekibaeva, A., Hrivna, L., Bayazitova, M. (2022). The impact of grain sorghum on the carbohydrate composition of wort for non-alcoholic beer. *Eastern-European Journal of Enterprise Technologies*, 5 (11 (119)), 75–82. <https://doi.org/10.15587/1729-4061.2022.265190>
- Kurmanbayeva, I., Nabiyeve, Z., Stoyanova, A., Zheldybayeva, A., Tlevlessova, D. (2022). Experimental substantiation of the application of plant extracts and enzymes to obtain safe raw materials for whole grain bread technology. *Eastern-European Journal of Enterprise Technologies*, 6 (11 (120)), 89–98. <https://doi.org/10.15587/1729-4061.2022.267230>
- Chauhan, B., Singh, D. P., Sharma, P. (2025). Bioactive pigments in functional foods: Insights into their diversity, extraction, and applications. *Food Science and Biotechnology*. <https://doi.org/10.1007/s10068-025-01896-x>

15. Ding, N., Zhou, Y., Dou, P., Chang, S. K. C., Feng, R., Hong, H., Luo, Y., Tan, Y. (2024). Colorful and nutritious abundance: potential of natural pigment application in aquatic products. *Food Innovation and Advances*, 3 (3), 232–243. <https://doi.org/10.48130/fia-0024-0023>
16. Yang, X., Sun, X., Zhang, D., Wang, Z., Gao, X. (2024). Effect of Chlorogenic Acid and Cold Plasma Synergistic Treatment on Eating Quality, Antioxidant Properties and Safety Qualities of Roasted Meat. <https://doi.org/10.2139/ssrn.4988461>
17. Chiodza, K., Goosen, N. J. (2023). Influence of mixing speed, solids concentration and enzyme dosage on dry solids yield and protein recovery during enzymatic hydrolysis of sardine (*Sardina pilchardus*) processing by-products using Alcalase 2.4L: a multivariable optimisation approach. *Biomass Conversion and Biorefinery*, 14 (22), 29045–29067. <https://doi.org/10.1007/s13399-023-03829-2>
18. Khezri, S., Ghanbarzadeh, B., Ehsani, A. (2025). Barberry anthocyanins: recent advances in extraction, stability, biological activities, and utilisation in food systems – a review. *International Journal of Food Science and Technology*, 60 (1). <https://doi.org/10.1093/ijfood/vvaf031>
19. Julkipli, J., Babel, S. (2025). Optimizing dilute sulfuric acid thermohydrolysis of dried food waste using the desirability function to produce a fermentation-friendly hydrolysate for biohydrogen production. *Biomass and Bioenergy*, 199, 107922. <https://doi.org/10.1016/j.biombioe.2025.107922>
20. Kozhakhiyeva, M., Kaldarbekova, M., Yessengaziyeva, A., Uzakov, Y., Medeubayeva, Z., Kuzembayeva, G., Aitbayeva, A. (2025). Devising a technology and optimizing processing parameters for making functional boiled sausage fortified with protein hydrolysates. *Eastern-European Journal of Enterprise Technologies*, 3 (11 (135)), 52–60. <https://doi.org/10.15587/1729-4061.2025.330002>