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# DEVISING A TECHNOLOGY AND OPTIMIZING PROCESSING PARAMETERS FOR MAKING FUNCTIONAL BOILED SAUSAGE FORTIFIED WITH PROTEIN HYDROLYSATES

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***The object of this study is the production of cooked sausages fortified with protein hydrolysates obtained from collagen-containing raw materials (in particular, beef legs with fetlock).***

***The study is aimed at eliminating the lack of data on the effect of hydrolysate concentration and process parameters on the functional, physicochemical, and antioxidant characteristics of meat products. A multifactorial experiment was conducted according to the Box-Benkin design in order to optimize the conditions of enzymatic hydrolysis. A statistically significant second-order regression model was built ($R_2 = 0.83$; $F = 13.18$; $p < 0.05$). Response surface analysis made it possible to determine the optimal conditions: temperature treatment at 70.4°C, fermentation at 50°C, and a duration of 2 hours, at which the content of amino nitrogen reaches 2.00 mg/g.***

***In a single-factorial experiment, the effect of hydrolysate doses (0%, 10%, 15%) was studied. A strong linear relationship was found between the dose and water retention capacity ($R_2 = 0.98$), with an increase of 9.3%. Antioxidant activity also increased (DPPH up to 29.88%, FRAP up to 33.5 mg GAE/g). The amino acid profile was improved by increasing the levels of leucine, glycine, and arginine. However, at the 15% dose, a deterioration in organoleptic properties (taste and aroma) was observed, which reduced consumer acceptability.***

***The results confirm the effectiveness of combining mathematical modeling and biochemical analysis in the development of functional meat products based on collagen-containing by-products. Limitations of the hydrolysate application have been identified and directions for future research have been proposed: sensory validation, peptide fractionation, and testing of intermediate concentrations (12–14%) to achieve an optimal balance between functionality, quality, and taste***

***Keywords: protein hydrolysate, collagen, Box-Benkin design, optimization, amino nitrogen, water-holding capacity***

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## 1. Introduction

In the context of rapid population growth and global challenges related to food security, the development of technologies aimed at increasing the nutritional and biological value of products is of particular importance. The modern food industry is faced with the need to search for new functional ingredients that can not only improve technological properties but also have a positive effect on the health of consumers.

One of the promising areas is the use of protein hydrolysates obtained from collagen-containing raw materials. These compounds contain bioactive peptides that can exhibit antioxidant, antihypertensive, antimicrobial, and immunomodulatory activity. Research in recent years demonstrates that such peptides can participate in the prevention and adjuvant therapy of a number of socially significant diseases, including type 2 diabetes, hypertension, inflammatory processes, thrombosis and oncopathologies. According to [1], the annual production of collagen-containing by-products exceeds 2 million tons. At the same time, the demand for functional meat products has grown by 35% over the past 5 years.

The scientific literature offers a significant amount of data obtained in vitro, on animal models, and in limited clinical studies confirming the biological activity of protein hydrolysates. However, a number of key aspects concerning their use in real food matrices, as well as the influence of technological factors (heat treatment, fermentation, pH level, storage conditions) on the preservation and bioavailability of bioactive components, remain insufficiently studied. Particularly poorly represented are works evaluating the functional efficiency of hydrolysates in models close to real digestion, including in vitro simulation of the gastrointestinal tract.

In addition, a promising direction is the use of protein hydrolysates in the food industry. Their properties can be improved by optimizing the process parameters. For this purpose, modern methods of mathematical design of experiments are used. They make it possible to predict the behavior of bioactive components under various processing conditions.

Thus, the relevance of the scientific topic related to the study of protein hydrolysates as functional ingredients is due to the need to:

– increase the biological and technological value of food products;

– introduce ingredients with proven positive physiological effects;

– devise methodological approaches to assess the stability and effectiveness of bioactive components in products.

Consequently, research aimed at developing and substantiating the parameters for the use of protein hydrolysates in food systems is relevant.

## 2. Literature review and problem statement

Modern trends in sustainable production and functional nutrition contribute to the increasing interest in the use of protein hydrolysates in the meat industry. Paper [1] reports the results of studies on the functional and technological properties of protein hydrolysates when they are included in the recipes of meat products, in particular, cooked sausages. It is shown that hydrolysates have significant potential as sources of biologically active peptides with antioxidant, antimicrobial, and hypotensive activity, which opens up prospects for their use in the development of functional food products. Protein hydrolysates are a set of low-molecular peptides that are released during the enzymatic hydrolysis of food proteins [2]. However, the mechanisms that affect the distribution of biologically active peptides in complex food matrices remain incompletely understood. However, the mechanisms that affect the distribution of biologically active peptides in complex food matrices remain incompletely understood.

Milk, fish, meat, slaughter by-products, and plant proteins (legumes, cereals) can serve as sources of protein for obtaining hydrolysates. By-products of the food industry are increasingly being used as sustainable and cost-effective raw materials [3]. At the same time, the question of the influence of a specific type of protein raw material on the composition and functionality of the resulting peptides in the finished product remains open.

Current research focuses on the growing interest in peptides derived from food proteins and their potential for developing functional ingredients. Review [4] highlights that bioactive peptides from various sources have a wide range of physiological effects, including blood pressure regulation, antioxidant activity, and effects on intestinal microflora. However, despite the rapid development of this field, there remains insufficient data on the interaction of peptides with other components of food systems, especially in multicomponent matrices such as meat products. This limits the predictability of their activity in the finished product and requires clarification of the conditions of use.

The remaining scientific gaps may be due to both the fundamental complexity of modeling the behavior of peptides in multicomponent food systems and the lack of standardized conditions for obtaining hydrolysates for different types of raw materials. This limits the predictability of their activity in the finished product and requires clarification of the conditions of use.

The process of obtaining hydrolysates is carried out using enzymes – proteases. The choice of protease (e.g., trypsin, papain, subtilisin) affects the depth and profile of hydrolysis, and hence the biological activity of the resulting peptides [5]. Despite the wide choice of enzymes, it is not always clear which combination of conditions make it possible to achieve maximum peptide activity without loss of product quality.

These properties are critical when developing product recipes with specified characteristics. The most commonly used are subtilisin, trypsin, papain, flavorcym (a mixture of endo- and exoproteases), as well as enzymes of microbial and plant origin, such as neutrases and alcalases. According to [6], the addition of hydrolysates in an amount of 5–15% increased the antioxidant activity of meat products by 20–45%. However, the mechanism of degradation of these peptides during heat treatment remains insufficiently studied. Each enzyme has a unique specificity and depth of hydrolysis, which determines the composition and properties of the resulting hydrolysate.

The advantage of enzymatic hydrolysis compared to acid or thermal hydrolysis is the preservation of the biological activity of amino acids and selective cleavage of protein without destroying sensitive components. In addition, in silico design methods of peptides and proteolytic pathways are widely used to predict biological activity and accelerate the mass production of bioactive fractions. The functional properties of protein hydrolysates depend on the amino acid composition, the length of the peptides, and the degree of hydrolysis. For example, short peptides containing hydrophobic amino acids often exhibit antioxidant and hypotensive activity [7]. However, the question of how stable these properties are when peptides are included in real formulations and after heat treatment remains unresolved.

One of the ways to overcome these limitations is to use mild temperature conditions, combined proteases, and encapsulation of peptides to increase their stability and mask the bitter taste.

However, the use of protein hydrolysates in food systems is associated with a number of technological difficulties. These include reduced stability during heat treatment, peptide bitterness, and a possible decrease in activity during storage. Therefore, scientists are actively developing encapsulation and mild processing methods that help preserve bioactivity until consumption [8]. However, the effectiveness of such methods in specific food systems (including meat products) requires additional testing.

Taking into account the above, the use of protein hydrolysates in cooked sausages is a promising direction. They make it possible not only to improve the nutritional value of the product but also to use by-products of processing, such as collagen-containing raw materials. Thus, this study, aimed at optimizing the recipe for sausages with protein hydrolysates, fully fits into the modern concept of functional nutrition and sustainable production.

## 3. The study materials and methods

The objective of our study is to devise a technology for producing cooked sausages with the addition of protein hydrolysate. This will make it possible to scale up the study for production without losing the quality of sausages, while the addition of hydrolysates will make it possible to increase the shelf life and improve the value of sausages.

To achieve the goal, the following tasks were set:

– to obtain protein hydrolysate from collagen-containing raw materials (beef legs with fetlock) in accordance with the established technological conditions and conduct its physicochemical characteristics;

– to study the effect of raw material heat treatment conditions and fermentation mode (temperature, duration) on the degree of hydrolysis according to the "amine nitrogen" indicator;

– to build a regression model for the dependence of amine nitrogen content on technological factors based on the Box-Benkin plan and determine the optimal parameters;

– to evaluate the effect of protein hydrolysate dose on the functional properties of cooked sausages: moisture-holding capacity, amino acid composition, and antioxidant activity.

## 4. The study materials and methods

### 4. 1. The object and hypothesis of the study

The object of our study is the technology for production of cooked sausages with the addition of protein hydrolysate obtained from collagen-containing raw material – beef legs with fetlock.

The introduction of protein hydrolysate obtained from by-product collagen-containing raw materials into the recipe of cooked sausages will improve their functional characteristics (water-holding capacity, antioxidant activity, amino acid composition) while optimization of the conditions of enzymatic hydrolysis will ensure the maximum content of amino nitrogen without deterioration of organoleptic properties.

The following assumptions were accepted. The pH level during enzymatic hydrolysis was maintained constant (about 7.0). The effect of other components of minced meat on the degree of hydrolysis was not considered. The main factors affecting the efficiency of hydrolysis are temperature and duration of fermentation.

The following simplifications were adopted:

– the use of one enzyme preparation (subtilisin), without comparison with others;

– the degree of hydrolysis was assessed only by the content of amino nitrogen;

– the sensory assessment was preliminary and did not include an expert panel.

### 4. 2. Research materials

The following materials were used as objects of research:

– collagen-containing raw materials - beef legs with fetlock joint;

– protein hydrolysate in powder form, obtained by enzymatic hydrolysis;

– minced meat for the production of cooked sausage.

### 4. 3. Research methods

Obtaining hydrolysate: enzymatic hydrolysis of raw materials at 50°C, pH ~7.0, for 3–4 hours, followed by drying to a powder state.

Determination of amine nitrogen: Sorensen formol titration method.

Determination of water-holding capacity: centrifugation of samples according to a standard methodology.

Determination of amino acid composition: liquid chromatography after acid hydrolysis (6N HCl, 110°C, 24 h).

Evaluation of antioxidant activity: DPPH and FRAP methods.

Statistical processing: Statistica 12.0 and Microsoft Excel software; building models using the least squares method, ANOVA analysis.

### 4. 4. Experimental design

The multifactorial experiment was performed according to the Box-Benkin design with three variable technological factors:

$X_1$ – heat treatment temperature, °C (range: 65–95);

$X_2$ – fermentation temperature, °C (30–50);

$X_3$ – fermentation time, *h* (1–4);

*Y* – response: amino nitrogen content, mg/g.

In addition, a single-factorial experiment was conducted with varying the concentration of protein hydrolysate (0%, 10%, 15%) in the sausage recipe and assessing the functional characteristics of the products.

## 5. Results of the study of sausage technology with the addition of hydrolysates

### 5. 1. Obtaining protein hydrolysate and its effect on the characteristics of sausages

To obtain a functional ingredient, enzymatic hydrolysis of beef legs with fetlock was carried out at a temperature of 50°C and pH 7.0 for 3 hours. The result was a powdery fraction of protein hydrolysate used in the production of sausages.

Adding hydrolysate in an amount of 10% and 15% affected the sausage indicators, the results are given in Table 1.

Table 1

Effect of protein hydrolysate dose on the physicochemical and antioxidant parameters of cooked sausages ($M \pm SD$, $n = 3$)

| Indicator | Control | 10% H | 15% H |
|---|---|---|---|
| Alkali-soluble proteins, % | 1.49 ± 0.05 | 10.07 ± 0.21 | 11.25 ± 0.18 |
| Water-holding capacity (WHC), % | 55.9 ± 1.1 | 58.81 ± 0.9 | 61.13 ± 1.0 |
| Shear stress, kPa | 60.4 ± 1.5 | 88.2 ± 1.8 | 91.6 ± 1.6 |
| Antioxidant activity (DPPH), % | 10.23 ± 0.25 | 25.77 ± 0.31 | 29.88 ± 0.28 |
| Reducing capacity (FRAP), mg GAE/g | – | 33.5 ± 1.2 | 30.5 ± 1.1 |

*Note: values are given as mean±standard deviation ($M \pm SD$) at $n = 3$.*

As can be seen from the results given in Table 1, the introduction of protein hydrolysate into the sausage recipe increases the moisture-holding capacity, structural strength, and antioxidant activity, confirming its effectiveness as a functional ingredient.

### 5. 2. Effect of process parameters on the degree of protein hydrolysis

The effect of processing temperature, fermentation temperature and fermentation duration on the degree of protein hydrolysis expressed through the content of amino nitrogen was studied.

A Pareto diagram was constructed to determine the significance of the influence of the studied factors and their interactions on the content of amino nitrogen in the hydrolysate (Fig. 1). The Pareto diagram clearly demonstrates the contribution of each factor and combination of factors to the change in response (*Y*, mg/g) based on standardized regression coefficients.

According to the results of ANOVA and Pareto diagram, the greatest influence on the degree of hydrolysis was exerted by the duration of fermentation ($p < 0.05$), followed by the fermentation temperature and the processing temperature. The observed trend showed an increase in the content of amino nitrogen with an increase in the duration of fermentation to a certain optimum.

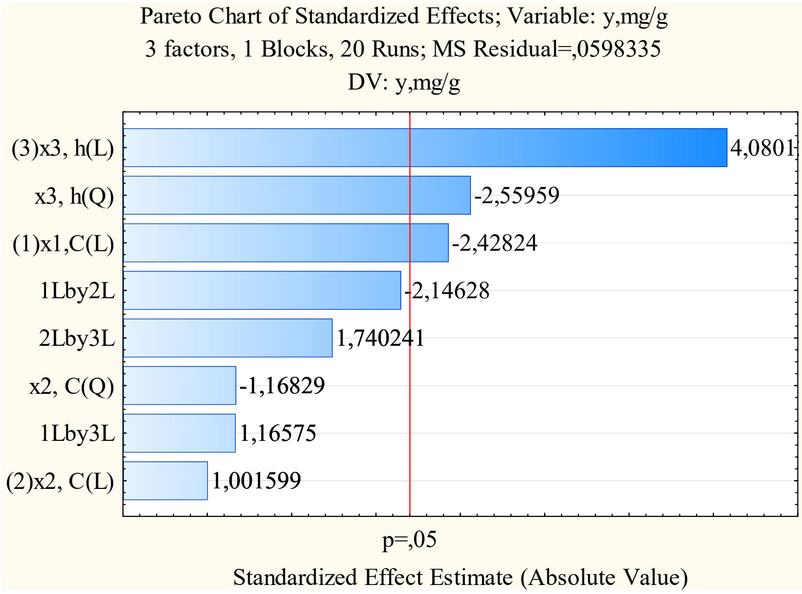


Fig. 1. Pareto diagram of standardized effects of factors influencing the content of amino nitrogen (*Y*, mg/g)

**5. 3. Modeling the degree of hydrolysis and optimizing process parameters for the content of amino nitrogen**

The quadratic effect of fermentation time $\left(X_3^2\right)$, exceeding the level of statistical significance at $p = 0.05$, has the greatest impact on the response. Also significant are the linear and quadratic effects of fermentation temperature ($X_3$), as well as the linear effect of raw material heat treatment temperature ($X_1$). The influence of the interactions $X_1 \times X_2$ and $X_2 \times X_3$ was also found to be moderately significant. The effects located to the right of the vertical line are statistically significant, while those located to the left do not have a significant impact on the result at the selected confidence level.

To assess the quality of model fit, a dependence plot of the observed values on those predicted by the model was constructed (Fig. 2). Ideal fit is shown by a diagonal line; deviations from it reflect the model error.

Most of the experimental points are located near the ideal fit line, which confirms the sufficient accuracy of the model and its adequacy for predicting the amino nitrogen content. The spread in the area of average values may be due to the nonlinearity of the response or incomplete consideration of the interactions of factors.

To visualize the second-order model and identify optimal conditions, a response surface was constructed reflecting the dependence of the amino nitrogen content (Fig. 3).

To quantitatively describe the relationship between technological factors (processing temperature, fermentation temperature and duration) and the response (amino nitrogen content, mg/g), a second-order regression model was constructed based on the Box-Benkin plan. The resulting equation takes the following form

$$z = 5.6187 + 0.0457x - 1.0547y - 0.14y^2 - 0.0175x^2 + 0.0041xy + 0.0120y + 7.4347, \quad (1)$$

where $z$ is the content of amino nitrogen, mg/g (response); $x$, $y$ are the coded values of the factors.

The model includes linear, quadratic and interacting terms, reflecting the complex nature of the hydrolysis process. Significant coefficients were determined by the least squares method at a significance level of $p < 0.05$ (Table 2). The adequacy of the model is confirmed by the determination coefficient $R^2 = 0.83$, which indicates a high degree of correspondence between the experimental and theoretical values.

Thus, this model can be used to predict and optimize the conditions of enzymatic hydrolysis in order to obtain a protein hydrolysate with specified properties.

The plot clearly shows the response function maximum at $X_1 \approx$ 70–75°C and $X_2 = 50$ °C. A nonlinear dependence and the effect of interaction of factors are observed. This analysis makes it possible to determine the optimal parameters for achieving the maximum level of amino nitrogen in the product – 2.00 mg/g.

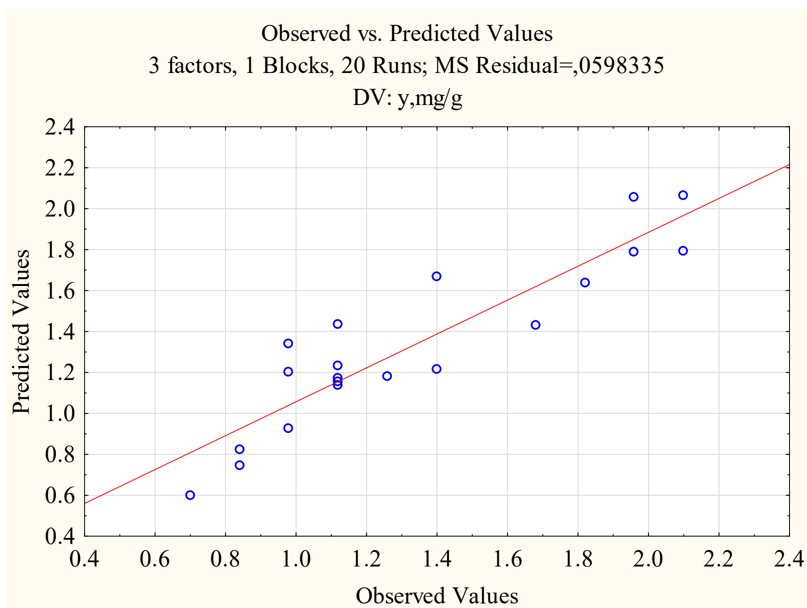


Fig. 2. Comparison of observed and predicted values of amino nitrogen (*Y*, mg/g)

To determine the optimal process parameters and visualize their impact on the amino nitrogen content, a desirability function was used, combining all factors into a single criterion (Fig. 4).

The maximum of the desirability function (0.99) is achieved at a processing temperature of 70.4°C, a fermentation temperature of 50°C and a fermentation time of 3.25 h. This plot confirms the selected optimal conditions and strengthens the reliability of the optimization performed.

The significance of the model and individual factors was tested using analysis of variance (ANOVA). The results are given in Table 2.

The following have statistically significant effects on the response: linear ($p = 0.034$) and quadratic ($p = 0.026$) effects of fermentation time ($X_3$), as well as raw material processing temperature ($X_1$, $p = 0.034$). Other interactions and quadratic terms did not reach the significance level ($p > 0.05$), but the interaction $X_1 \times X_2$ is close to significant ($p = 0.055$). The model is characterized by a good degree of explanation ($R^2 > 0.8$) and can be used for predictions and optimization.

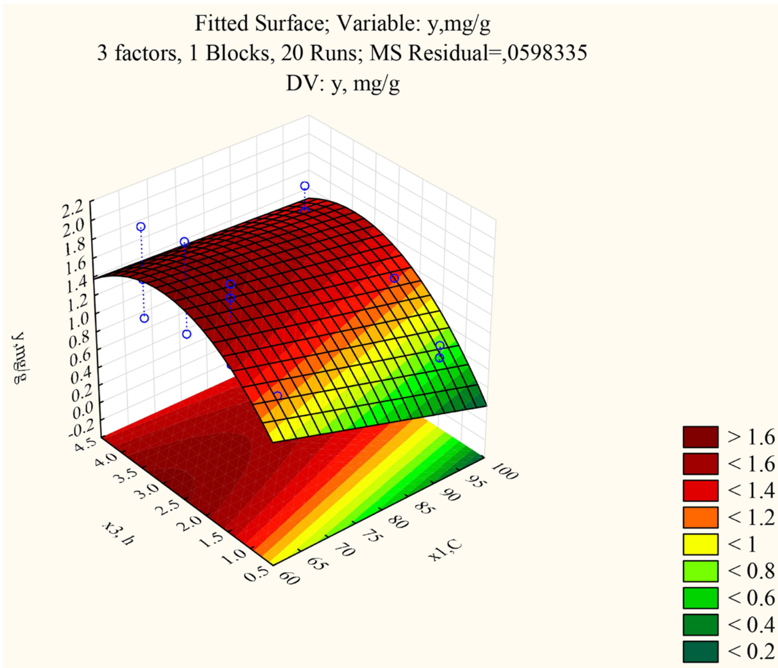


Fig. 3. Response surface characterizing the effect of processing temperature ($X_1$) and fermentation ($X_2$) on the content of amino nitrogen ($Y$, mg/g)

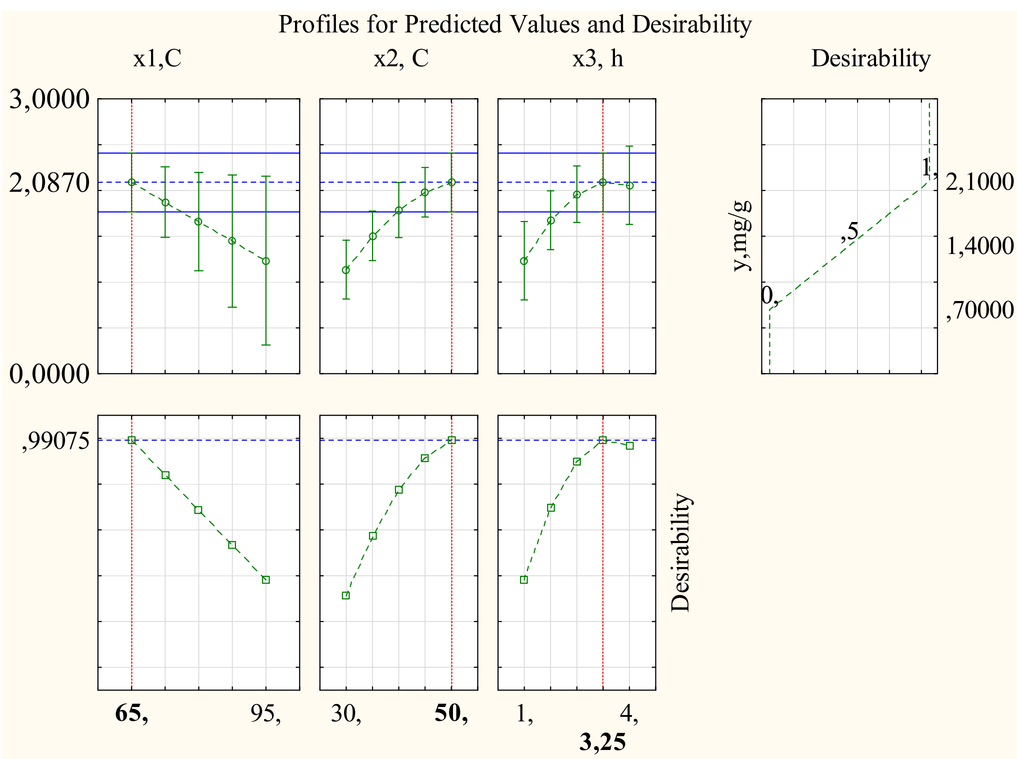


Fig. 4. Predicted value profiles and desirability functions for parameters $X_1$, $X_2$, $X_3$

Table 2

Results of the analysis of variance

| Factor | ANOVA; Var.: *y*, mg/g; R-sqr = 0.82779; Adj: 0.70255 (Spreadsheet5) 3 factors, 1 Blocks, 20 Runs; MS Residual = 0.0598335 DV: *y*, mg/g | | | | |
|---|---|---|---|---|---|
| | SS | df | MS | F | p |
| (1)x1,C(L) | 0.352800 | 1 | 0.352800 | 5.89637 | 0.033514 |
| (2)x2, C(L) | 0.060025 | 1 | 0.060025 | 1.00320 | 0.338061 |
| x2, C(Q) | 0.081667 | 1 | 0.081667 | 1.36490 | 0.267387 |
| (3)x3, h(L) | 0.996072 | 1 | 0.996072 | 16.64741 | 0.001820 |
| x3, h(Q) | 0.392000 | 1 | 0.392000 | 6.55152 | 0.026538 |
| 1L by 2L | 0.275625 | 1 | 0.275625 | 4.60654 | 0.055001 |
| 1L by 3L | 0.081312 | 1 | 0.081312 | 1.35897 | 0.268371 |
| 2L by 3L | 0.181202 | 1 | 0.181202 | 3.02844 | 0.109679 |
| Error | 0.658168 | 11 | 0.059833 | nd | nd |
| Total SS | 3.822000 | 19 | nd | nd | nd |

**5. 4. Effect of protein hydrolysate dose on the functional properties of cooked sausages**

Evaluation of the finished product with the addition of hydrolysate revealed an increase in the amino acid content and an improvement in the structural-mechanical and antioxidant properties (Table 3).

Increasing the hydrolysate dose has a positive effect on the amino acid profile, which confirms its role as a source of biologically active compounds. To visualize the multifactorial relationship between the concentration of protein hydrolysate and the quality indicators of sausages, a principal component analysis (PCA) bioplot was constructed. The plot (Fig. 5) reflects the distribution of samples in the coordinates of the principal components and the direction of the influence of responses on overall quality.

The greatest contribution to the *t*1 component was made by the total protein and total amino acid content, while the *t*2 component mainly reflected changes in the water-holding capacity and arginine content. The negative direction of the protein and leucine vectors may indicate a redistribution of the amino acid composition with an increase in the degree of hydrolysis.

Table 3

Amino acid composition of sausages with the addition of hydrolysate

| Amino acid | Control | 10% H | 15% H |
|---|---|---|---|
| Glycine, g/100 g | 0.78 | 0.81 | 0.87 |
| Arginine, g/100 g | 1.31 | 1.32 | 1.37 |
| Leucine, g/100 g | 0.98 | 1.11 | 1.37 |
| Total AA, g/100 g | 14.25 | 13.21 | 15.45 |

In order to comprehensively assess the effect of protein hydrolysate concentration on the quality and functional properties of cooked sausages, a plot of the predicted value profiles and the desirability function was constructed (Fig. 6). It makes it possible to reflect the dependence of responses (water-holding capacity, protein content, amino acid composition, and antioxidant activity) on the studied factor, as well as to determine the optimal hydrolysate level taking into account the overall objective function.

Green lines show predicted response values (with confidence intervals) when changing the hydrolysate dose. The right column shows the corresponding desirability function. The highest desirability is achieved at a concentration of 15%. Analysis of the predicted value profiles revealed that increasing the protein hydrolysate dose has a positive effect on the amino acid profile (glycine, arginine, leucine), water-holding capacity, and total amino acid content. The desirability function reaches a maximum at 15% of the additive, indicating that this concentration is optimal for the combination of technological and nutritional properties.

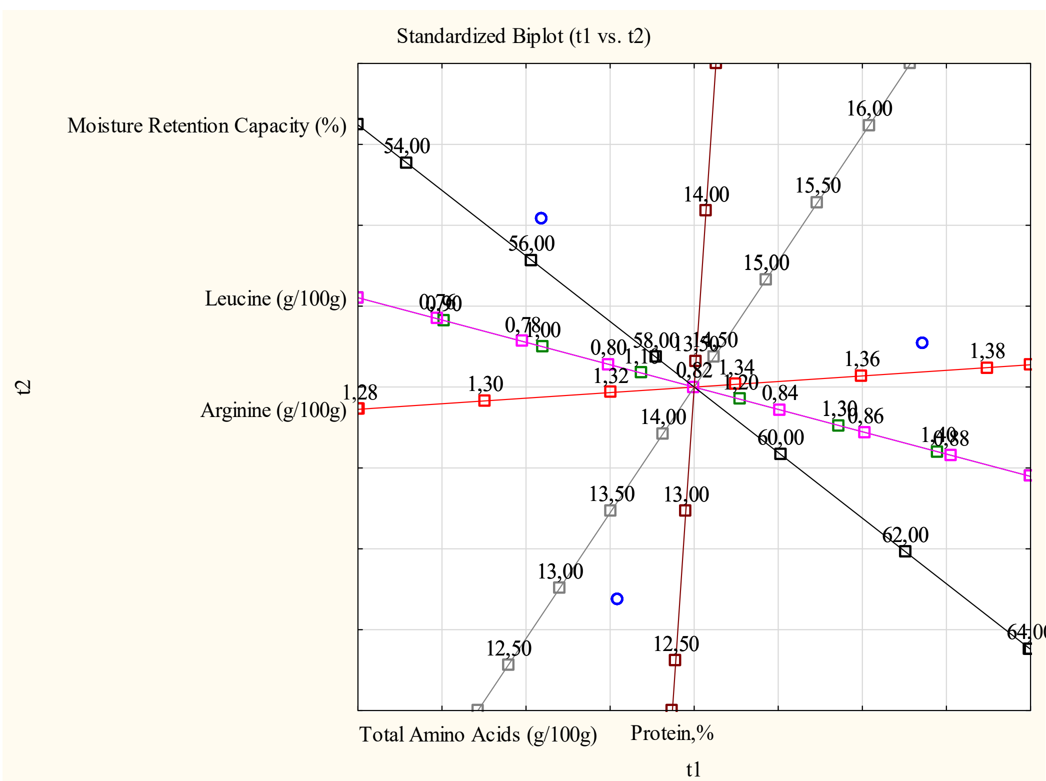


Fig. 5. Bioplot of major components: relationships between quality indicators and hydrolysate levels

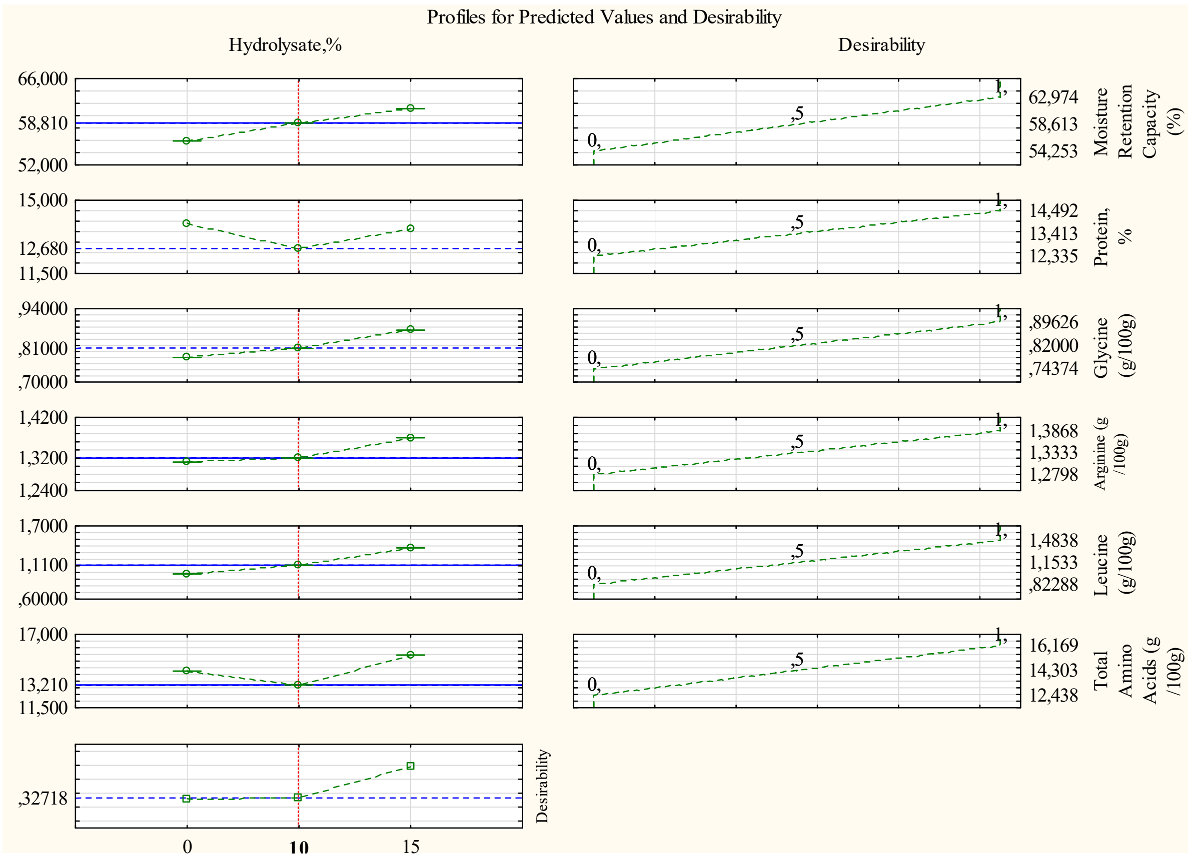


Fig. 6. Response profiles and desirability functions depending on protein hydrolysate concentration

## 6. Discussion of the technological and functional effects of protein hydrolysates in cooked sausages

According to the data in Table 1, the addition of protein hydrolysate leads to a reliable improvement in the textural and functional properties of the product. Thus, the water-holding capacity (WHC) increases from 55.9% (control) to 61.13% with the addition of 15% hydrolysate, indicating an improvement in the water-binding capacity of the protein matrix. Similarly, the antioxidant activity for DPPH increases almost threefold (to 29.88%), indicating the presence of bioactive peptides in the hydrolysate. The increase in the shear stress (from 60.4 to 91.6 kPa) reflects an increase in the structural density of the product. This makes the sausage more technologically advanced and stable during storage.

The data in Table 2 confirm that the addition of hydrolysate improves the amino acid profile of the product. In particular, there is an increase in the content of leucine (from 0.98 to 1.37 g/100 g), arginine (from 1.31 to 1.37 g/100 g) and glycine. This is especially important in terms of the formation of the functional properties of the product and maintaining collagen synthesis in consumers. The total content of amino acids increases to 15.45 g/100 g, which makes the product more biologically complete. Despite the active use of hydrolysates, their stability in complex food matrices and during heat treatment remains a subject of study [9–11].

As can be seen from the Pareto diagram (Fig. 1), the greatest influence on the amine nitrogen value is exerted by the fermentation time, followed by the quadratic effect of the fermentation temperature and the linear effect of the processing temperature. This suggests that the improvement of the hydrolysis degree is impossible without precise selection of the time parameters and temperature regime. Significant interactions confirm the need for multivariate modeling. Fig. 2 demonstrates good agreement between the observed and predicted values of the amine nitrogen content. Most of the points are located near the line of perfect match, indicating a high predictive ability of the model ($R^2 = 0.83$). This confirms that the model can be used to control the process. The response surface (Fig. 3) makes it possible to visually determine the zone of optimal process parameters. The maximum value of amine nitrogen is achieved at a processing temperature of about 70°C, a fermentation temperature of 50°C and a time of about 2–3 h. This confirms both the nonlinear nature of the process and the need to coordinate the modes. According to the desirability profile (Fig. 4), the best combination of all indicators is achieved at a hydrolysate concentration of 15%. This value provides optimal levels of amino acids, antioxidant activity, and water-holding capacity. The desirability function D reaches 0.99, which makes it possible to recommend this level as optimal for industrial use.

The bioplot (Fig. 5) shows the directions and degree of correlation between the parameters. The WHC, total amino acid content, and antioxidant activity are directed in the same direction and are positively related. The opposite direction of the total protein vector may indicate a redistribution of protein fractions towards peptides upon the introduction of hydrolysate. Samples with 15% hydrolysate form a cluster characterized by improved functional properties. The obtained data on water-holding capacity are consistent with the results demonstrating the ability of collagen hydrolysates to reduce interfacial tension and improve the stability of emulsion systems [12].

Based on statistical analysis, multifactorial visualization, and an optimization model, it was found that the introduction of protein hydrolysate in an amount of 15% provides the maximum desirability function ($D = 0.99$). This level provides the best combination of technological (high water-holding capacity, strength), nutritional (increased amino acid content), and functional (antioxidant activity) properties of the product. Thus, a concentration of 15% is optimal in terms of comprehensive improvement of the quality of sausage products. Despite the positive effect of protein hydrolysate on the technological and functional properties of sausage products, the study revealed a number of limitations.

The data obtained during the study confirm and develop existing ideas about the biological value and technological efficiency of collagen hydrolysates. In particular, an increase in the content of alkali-soluble proteins to 11.25% and the total amino acid level to 15.45 g/100 g corresponds to previously published results, which report high bioavailability of amino acids from enzymatically cleaved collagen, including arginine, glycine, and proline [13].

Our regression model, demonstrating statistical significance ($R^2 = 0.83$; $F = 13.18$), is in good agreement with the findings reported in [14], which showed that rationalization of the combination of temperature and proteolysis time promotes stabilization of enzyme activity and an increase in the yield of peptides. The observed key role of fermentation time in the accumulation of amino nitrogen is similar to the results obtained from the models of kinetics of mild enzymatic hydrolysis.

In addition, the results of assessing the dose-dependent effect of hydrolysate on the properties of cooked sausages (an increase in moisture-holding capacity up to 61.13%, antioxidant activity up to 29.88%) confirm the versatility of the approach used in other studies. For example, work [15] demonstrated that the addition of protein hydrolysates improves the textural and functional characteristics of meat products, including sausages based on non-traditional raw materials, such as squid. Thus, the established dependences and observed effects fit into the overall scientific pattern, emphasizing the prospects for using hydrolysates in the technology of functional meat products.

The organoleptic factor turned out to be limiting with an increase in the hydrolysate dose. Despite the improvement of such indicators as water-holding capacity, antioxidant activity, and amino acid profile, already at a concentration of 15% there is a noticeable change in taste and smell, perceived by tasters as unpleasant or specific. At doses above 15%, the deterioration of organoleptic characteristics becomes even more pronounced, which limits the practical use of high doses even with favorable functional indicators. One of the promising areas is the encapsulation of peptides, which makes it possible to preserve their activity and level out undesirable taste properties [16].

A limited range of hydrolysate concentrations was used (0%, 10%, 15%), without intermediate values (e.g., 12–14%), which could provide a more balanced result between functionality and taste profile. Similar improvements in texture and antioxidant activity due to the addition of protein hydrolysates were also observed in squid sausage recipes, which confirms the versatility of the approach described in [17].

Taste and smell stability during storage were not assessed, which may have an additional impact on consumer perception of the product with hydrolysate.

In the future, it is necessary to study the effect of aromatic masking additives (natural extracts, spices, fats) to level out the specific smell at concentrations $> 10\%$. To test optimal additive levels in the range of 12–14%, which could potentially provide a compromise between improved functional properties and acceptable taste characteristics.

## 7. Conclusions

1. A highly soluble and functionally active powdered hydrolysate has been obtained. Chemical and biochemical analysis revealed an increase in the alkali-soluble protein content to 11.25% and the total amino acid content to 15.45 g/100 g.
2. Relationships were established between technological parameters (processing temperature, fermentation temperature and duration) and the degree of hydrolysis expressed through the content of amine nitrogen. Fermentation time had the greatest influence ($p < 0.05$), which was confirmed by Pareto analysis and ANOVA.
3. A second-order regression model was constructed according to the Box–Benkin plan, describing the dependence of amine nitrogen content on technological factors. The model turned out to be statistically significant ($R^2 = 0.83$, $F = 13.18$, $p < 0.05$). Optimum parameters: processing temperature 70.4°C; fermentation temperature 50°C; fermentation time 3.25 h; at which the content of amino nitrogen of 2.00 mg/g is achieved.
4. The effect of the protein hydrolysate dose (0%, 10%, 15%) on the functional characteristics of cooked sausages was assessed. At 15% of the additive, an increase in the moisture-holding capacity to 61.13%, antioxidant activity by DPPH to 29.88%, as well as the content of leucine, glycine, and arginine were recorded. The desirability function method showed that a concentration of 15% provides the best balance of functional indicators ($D = 0.99$).

## 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 reported in this paper.

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## Data availability

Data will be provided on reasonable request.

## Use of artificial intelligence

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

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