Optimization of the proteolysis process of connective tissue proteins in ostrich meat

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Abstract. A promising area of research for the development of functional food products of mass consumption is the use of non-traditional sources of raw meat of domestic production, including ostrich meat, which has a high nutritional and biological value. However, ostrich meat is characterized by an increased content of connective tissue, which causes its rigidity. The use of proteolytic enzymes will increase the functional and technological properties of meat and the assimilation of hard-to-digest proteins, in particular collagen. The aim of the study is to optimize the technological parameters of biomodification of the properties of ostrich meat raw materials using collagenase. The object of the study is the meat of the femoral part of an ostrich grown on the territory of the Leningrad region. To optimize the technological parameters of minced meat fermentation, the fractional replica method was used to study the effect of three factors on the response functions: the mass fraction of collagenase (C, encoded variable $X_1$), the duration ($\tau$, encoded variable $X_2$), and the temperature of minced meat aging (t, encoded variable $X_3$). The response functions are the values of the moisture-holding capacity ($Y_1$) and the content of amine nitrogen ($Y_2$). The regression equations describing the dependence of the response functions on the factors under study have been compiled. The technological parameters of the fermentation of minced meat based on ostrich meat with the use of collagenase have been optimized: the mass fraction of the enzyme is 0.05%, the holding time of the minced meat is 4.5 hours at $t=13^\circ C$, allowing one to obtain minced meat with high organoleptic characteristics and functional and technological properties.

1. Introduction

The nutrition structure of the majority of the population of the Russian Federation does not correspond to the concept of a balanced diet, as evidenced by systematic studies conducted by the Institute of Nutrition of the Russian Academy of Medical Sciences. In this regard, it is advisable to develop functional food products for mass consumption based on non-traditional sources of raw meat of domestic production, including ostrich meat, which has a high nutritional and biological value [1-4].

Russian ostrich farming is a relatively young and dynamically developing branch of agriculture. Great interest in the cultivation of ostriches is due to their high productivity in comparison with other agricultural animals. It should be noted that ostrich meat is dietary, has no religious and national restrictions.
In terms of organoleptic quality indicators and chemical composition, it is not inferior, and in some respects exceeds traditional types of meat raw materials. Ostrich meat is characterized by a low cholesterol content, a high protein content-up to 21%, a fatty acid composition favorable for the human body's absorption and a low-calorie content. In addition, it is a source of vitamins PP, E, group B, mineral elements iron, selenium, calcium, phosphorus, potassium. However, ostrich meat is characterized by a high content of connective tissue and defective collagen protein, which gives it rigidity and reduces the digestibility of proteins.

Domestic and international experience shows the feasibility of using enzyme preparations for the biomodification of the properties of low-grade raw materials in the meat industry, and its use in the technology of functional products for the intended purpose.

The aim of the study is to optimize the technological parameters of biomodification of the properties of ostrich meat raw materials using collagenase.

2. Materials and methods

The object of the study is the meat of the femoral part of an ostrich grown on the territory of the Leningrad region (Beloostrov village).

The slaughter and exsanguination of the bird was carried out without prior electro-silencing. Then the carcass of the bird was cooked, the plumage was removed by hand and gutted. To avoid microbiological damage, the surface of the carcass after evisceration was treated with a 1% solution of acetic acid. After deboning, the meat of the ostrich thigh was cooled to a temperature in the center (2±2) °C. For the study, meat raw materials with pH and moisture retention values of 6.2 and 92.4%, respectively, were used [5-9].

Tables 1 and 2 show the total chemical composition, vitamin, mineral composition, amino acid composition of proteins, of chilled ostrich meat, respectively, used for fermentation.

**Table 1. General chemical composition of ostrich meat [11]**

| Moisture content, % | Content protein, % | Fat, % | Energy value, kcal/kJ |
|---------------------|---------------------|--------|----------------------|
| 75.1                | 22.0                | 1.0    | 97/406               |

Vitamin composition of ostrich meat, mg/100 g of meat

| B₁, mcg | B₂, mcg | PP, mg | B₅, mg | B₆, mg | B₉, mcg | B₁₂, mcg |
|---------|---------|--------|--------|--------|---------|----------|
| 0.55    | 0.48    | 2.97   | 1.1    | 0.53   | 5.5     | 0.65     |

Mineral composition of ostrich meat, mg/100 g of meat

| Na | K | Ca | Mg | P | Fe | Zn | Se |
|----|---|----|----|---|----|----|----|
| 55 | 320 | 10 | 17 | 249 | 4.4 | 2.4 | 0.024 |
Table 2. Amino acid composition of ostrich meat proteins [11]

| Essential amino acids | Content g/100 g of meat; mg / g of protein | Amino Acid score, % |
|-----------------------|------------------------------------------|---------------------|
| Valine                | 1.20 53.0                                | 106                 |
| Leucine               | 1.96 87.0                                | 124                 |
| Isoleucine            | 1.00 44.0                                | 110                 |
| Lysine                | 2.00 90.0                                | 164                 |
| Methionine+cystine    | 0.95 42.0                                | 120                 |
| Threonine             | 1.15 51.0                                | 128                 |
| Tryptophan            | 0.23 10.2                                | 102                 |
| Phenylalanine+tyrosine| 1.82 81.0                                | 135                 |

| Interchangeable amino acids | Content g/100 g of meat | mg / g of protein |
|-----------------------------|-------------------------|-------------------|
| Alanine                     | 1.35                    | 60.0              |
| Arginine                    | 1.40                    | 62.0              |
| Histidine                   | 0.50                    | 22.0              |
| Serin                       | 0.95                    | 42.0              |
| Aspartic acid               | 2.20                    | 98.0              |
| Glutamic Acid               | 3.35                    | 149.0             |
| Glycine                     | 1.37                    | 61.0              |
| Proline                     | 1.10                    | 49.0              |

As an enzyme preparation, collagenase (TU 9154-032-11734126-10), obtained from the hepatopancreas of the king crab, with a proteolytic activity of 130 PE / mg of the drug, PH = 6.5, was used as an enzyme preparation.

Collagenase is a proteolytic enzyme that effectively destroys peptide bonds in natural collagen, inhibits the synthesis of metalloproteases and prevents the destruction of proteoglycans, as well as cartilage tissue collagen.

The content of amine nitrogen was determined by the formol titration method, the moisture-holding capacity was determined by pressing, the pH value was determined by the potentiometric method, the protein content was determined by the Kjeldahl method, and the fat content was determined by the Soxlet method according to the standard methods described in the work [11].

To optimize the technological parameters of fermentation of minced ostrich meat using collagenase in the study of the effect on the response functions of three factors (n), we used the method of fractional replicas. That allows us to study the simultaneous effect of several factors on the process during a relatively small number of experiments N (N=2^n-1); to detect the effect of interaction of factors under their joint influence; to construct a mathematical description of the process under study (mathematical model). This allows us to optimize the output parameter without conducting additional experiments [12].

The moisture-holding capacity and the content of amine nitrogen were determined depending on the mass fraction of collagenase introduced, the duration and temperature of the minced meat exposure in order
to optimize the technological parameters of the use of collagenase in minced meat, as well as organoleptic parameters (color, consistency, appearance, smell).

3. Results and discussion
In the process of optimizing the technological parameters of biomodification of the properties of ostrich meat, the influence of the mass fraction of collagenase (C, encoded variable $X_1$), the duration of exposure ($\tau$, encoded variable $X_2$) and the temperature of exposure ($t$, encoded variable $X_3$) on the change in the moisture retention capacity and the content of amine nitrogen, selected as the response functions ($Y_1$) and ($Y_2$), respectively, was studied.

The parameters of the fermentation of minced meat at the main level and the variation interval are as follows: $C_0=0.04\%$, $\Delta C=0.02\%$; $\tau_0=4$ h, $\Delta \tau=2$ h; $t_0=12^\circ C$, $\Delta t=7^\circ C$.

The matrix of experiment planning by the method of fractional replicas is made, presented in table 3.

The coded variables varied at two levels - upper (+1) and lower (-1) and were determined by the formulas:

$$X_1 = \frac{C-C_0}{\Delta C}; \quad X_2 = \frac{\tau-\tau_0}{\Delta \tau}; \quad X_3 = \frac{t-t_0}{\Delta t}.$$  

Table 3. Planning matrix and experiment results

| No. of experiment | $X_1$ | C, % | $X_2$ | $\tau$, h | $X_3$ | t, $^\circ C$ | $X_1X_2$ | $y_1$ | $\bar{y}_1$ | $y_2$ | $\bar{y}_2$ | $S_1^2$ | $S_2^2$ |
|------------------|------|-----|------|---------|------|--------------|----------|-----|----------|-----|----------|--------|--------|
| 1                | -1   | 0.02| -1   | 2       | +1   | 19           | +1       | 91.9| 92.1     | 92.0| 0.46     | 0.50   | 0.48   | 0.02 | 0.0010 |
| 2                | +1   | 0.06| -1   | 2       | -1   | 5            | -1       | 89.8| 90.4     | 90.1| 0.51     | 0.53   | 0.52   | 0.18 | 0.0002 |
| 3                | -1   | 0.02| +1   | 6       | -1   | 5            | -1       | 87.1| 87.5     | 87.3| 0.77     | 0.81   | 0.79   | 0.08 | 0.0010 |
| 4                | +1   | 0.06| +1   | 6       | +1   | 19           | +1       | 82.7| 83.3     | 81.0| 0.95     | 0.99   | 0.97   | 0.18 | 0.0010 |
| 5                | 0    | 0.04| 4    | 0       | 12   | 0            | 0        | 87.3| 87.7     | 87.5| 0.60     | 0.64   | 0.62   | 0.08 | 0.0020 |

To check the reproducibility of the experiments, the following parameters are calculated:
- the arithmetic mean of the response function of the moisture-holding capacity and the content of amine nitrogen;
- estimation of variance for each series of parallel experiments;
- Cochran criterion ($G_p$) - the ratio of the largest of the variance estimates to the sum of all variance estimates:

$$G_p = \frac{\max S_j^2}{\sum j S_j^2}.$$  

The values of the Cochran criterion ($G_p$) and $G_{p2}$ are determined for the total number of variance estimates $\sum S_j^2 = 0.46$ and $\sum S_2^2 = 0.0032$, the number of degrees of freedom $f=1$.

Since $G_{p1}=0.39$ and $G_{p2}=0.31$ are less than $G_{tab}=0.907$ with a confidence probability of 0.95 and $f=1$, the experiments are reproducible, and the estimates of the variances are homogeneous.

To optimize the technological parameters of fermentation of meat raw materials, a regression equation of the following form was used:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3;$$

where $Y$ is the response function;
The regression coefficients for the moisture holding capacity are determined by the formulas:

\[ b_0 = \frac{1}{N} \sum \bar{y}_i = 88.1; \]
\[ b_1 = \frac{1}{N} \sum x_1 \bar{y}_i = -1.55; \]
\[ b_2 = \frac{1}{N} \sum x_2 \bar{y}_i = -2.95; \]
\[ b_3 = \frac{1}{N} \sum x_3 \bar{y}_i = -0.6. \]

Regression coefficients for the amine nitrogen content: \( b_0 = 0.69, b_1 = 0.06, b_2 = 0.19, b_3 = 0.04. \)

To determine the significance of the coefficients of the regression equation, an estimate of the variance is calculated (\( s_b^2 \)):

\[ s_b^2 = \frac{s^2}{N}. \]

The coefficient of the regression equation is significant if \( |b| \geq |s_b t_{a/2} \) where \( t_{a/2} = 2.776 \) is the Student's test value with a confidence probability of 0.95 and \( N = 4 \).

For the product \( X_1 X_2 \) and the factor \( X_3 \), the columns are the same, so the coefficients \( b_{12} \) and \( b_3 \) cannot be defined separately. The coefficients \( b_{13}, b_{123}, \) and \( b_{23} \) are not significant.

To check the adequacy of the regression equation to the process under study, an estimate of the adequacy variance (\( s_{ad}^2 \)) is calculated using the formula:

\[ s_{ad}^2 = \frac{1}{N-B} \sum_{j=1}^{N} (y_j^p - y_j^p)^2 \]

where \( B \) is the number of regression coefficients of the desired equation, including the free term; \( y_j^p - y_j^p \) - experimental and calculated value of the response function in the \( j \)-th experiment;

\( N \) is the number of experiments of the full factorial experiment.

The Fischer criterion \( (F_p) \) is calculated using the formula:

\[ F_p = \frac{\max s_{ad}^2}{\min s_{ad}^2} \]

The obtained values of the Fisher criterion \( (F_{p1} \) and \( F_{p2} \)) were compared with its table value \( (F_{table} = 7.71) \). Since \( F_{p1} \) and \( F_{p2} \) are less than \( F_{table} \), the equations adequately describe the process under study.

Thus, the regression equations have the following form:

\[ Y_1 = 88.1 - 1.55X_1 - 2.95X_2 - 0.6X_3 \]
\[ Y_2 = 0.69 + 0.06X_1 + 0.19X_2 + 0.04X_3. \]

The fundamental work of R. Fischer, D. Box, Y. Adler and other researchers on the mathematical planning of experiments allowed the use of highly efficient planning schemes, such as the method of steep ascent/steepest descent. This method has found application in solving problems of optimization of technological processes in food technologies.

An important advantage of mathematical planning of experiments in comparison with classical research methods is the possibility of simultaneous influence on the technological process of a large number of factors. In addition, this method allows, along with the quantitative consideration of each individual factor, to establish the presence of interfactory interactions in the system and assess the influence of the latter, as well as to determine the value of the parameters for optimal process efficiency [13].

Optimization of the parameters of fermentation of ostrich meat by the method of steep ascent is presented in table 4.
Table 4. Characteristics and results of the experiment

| Characteristics and No. of experience | C, % | τ, h | t, °C | X1 | X2 | X3 | y₁ | y₂ |
|--------------------------------------|------|------|-------|----|----|----|----|----|
| Main level                           | 0.04 | 4    | 12    | -  | -  | -  | -  | -  |
| Variation interval                   | 0.02 | 2    | 5     | -  | -  | -  | -  | -  |
| Movement step                        | 0.01 | 0.5  | 1     | -  | -  | -  | -  | -  |
| Steep climb                          |      |      |       |    |    |    |    |    |
| 1                                    | 0.05 | 4.5  | 13    | 0.5| 0.25| 0.2| 86.5| 0.78|
| 2                                    | 0.06 | 5.0  | 14    | 1.0| 0.50| 0.4| 84.8| 0.86|
| 3                                    | 0.07 | 5.5  | 15    | 1.5| 0.75| 0.6| 83.2| 0.95|
| 4                                    | 0.08 | 6.0  | 16    | 2.0| 1.00| 0.8| 81.6| 1.03|

It is assumed that the optimization ends at the following values: moisture retention capacity of $y_1 = 86.5\%$, amine nitrogen content of $y_2 = 0.80$ mg/100 g.

The organoleptic evaluation of the quality of fermented minced meat was carried out on a five-point scale, which accepted the following descriptors: smell, appearance, consistency, color. The profilogram of organoleptic quality assessment of minced meat without fermentation (control) and fermented minced meat is shown in figure 1.

![Figure 1. Profilogram of organoleptic evaluation of the quality of minced meat without fermentation and fermented minced meat](image)

The following parameters of fermentation of minced meat based on ostrich thigh meat were selected: mass fraction of collagenase $C = 0.05\%$, minced meat holding time $\tau = 4.5$ h at a temperature $t = 13\degree C$. A further increase in the collagenase concentration, the holding time and the temperature of the minced meat leads to a decrease in the moisture retention capacity, a dilution of the consistency of the minced meat and a decrease in the total protein content from 22.0% to 14.0% as a result of protein proteolysis. These changes are probably associated with a decrease in the content of high-molecular proteins, an increase in the number
of peptides of different molecular weights and free amino acids, which is accompanied by a decrease in the pH value from 6.2 to 5.7.

4. Conclusion
The optimal technological parameters of biomodification of the properties of ostrich meat with the use of collagenase are proposed: the mass fraction of collagenase is 0.05%, the duration of minced meat aging is 4.5 hours at t=13 ° C. That allows one to obtain minced meat with high organoleptic parameters and functional and technological properties compared to the control sample. The resulting fermented minced meat under the selected modes is recommended for use in the technology of chopped semi-finished products, cooked sausages, minced meat and vegetable canned food.

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