The effect of technological parameters on functional, technological and physicochemical indicators of horse meat minces with added chicken combs

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ABSTRACT
This study aimed to determine the effect of technological parameters of the production of horse meat minces with the addition of protein-oil emulsion from chicken combs on the functional, technological and physicochemical indicators. Chicken combs were pre-treated with bacterial concentrate to improve their properties. Experimental approach: The ultimate shear stress and technological indicators – water holding capacity and oil holding capacity – were determined to set the optimal time for cutting raw materials. Physicochemical analyses of the meat minces were conducted. Results and conclusions: The research results have shown that the cutting time significantly affects the meat minces' rheological, functional and technological indicators. The optimum mixing time for meat minces is 6 min. Adding a protein-oil emulsion from biotechnologically processed chicken combs, cottonseed oil, and water into the minced horse meat does not significantly affect the nutritional value. Adding 15 – 20% protein-oil emulsion (POE) is recommended to get minced meat with optimal rheological parameters. Novelty and scientific contribution: The research results allow the rational use of poultry by-products.

Keywords: meat mince, chicken comb, horse meat, protein-oil emulsion, bacterial concentrate

INTRODUCTION
Industrial poultry breeding all over the world is gaining momentum. Complexes of various capacities appear in many countries, using modern technologies and highly productive crosses [1], [2], [3]. Today, most poultry processing enterprises face the problem of maximum and rational use of poultry by-products rich in proteins [4], [5], [6]. The stomach, legs, and combs are by-products obtained during the primary processing of poultry and are used to a limited extent in modern production. Various chicken by-products have been reported to contain significant amounts of proteins, enzymes, and lipids [7]. Their constituent connective tissue is a source of fodder protein and food protein [5]. Therefore, it is important to expand the use of collagen-containing poultry by-products. One of the fundamental ways to improve the properties of food-grade raw materials is their preliminary biotechnological processing [8], [9]. In the process of directed biotechnology, not only optimal functional and technological indicators of raw materials are formed, but also the biological value increases [10]. In technological practice, it is necessary to adjust the functional properties of meat systems when using different types of meat — the structure of the minced meat changes when the meat ingredients are combined. The interaction between individual structural elements is determined by the chemical composition, biochemical parameters, temperature, dispersion, and technological factors [11]. The muscle and connective tissue proteins are minced meat's main structural units. The quantitative content of protein in the system, its qualitative composition, and processing conditions predetermine the degree of stability of the resulting meat systems and affect the structural and mechanical properties [12], [13]. It is known that horsemeat has increased rigidity and is also a source of acid radicals (pH 5.65 – 5.95), which disrupts the acid-base balance in the human body, shifting it to the acidic side.
To improve the structural and mechanical characteristics of minced meat products based on horse meat, it is proposed to use a protein-oil emulsion from chicken combs pre-treated with a bacterial concentrate. Chicken combs contain more salt-soluble proteins, which can affect the hydrophilicity of the protein fraction, which causes a higher pH than in horse meat. Thus, combining various raw materials in the product allows for optimal-quality meat systems [16], [17], [18], [19].

The objective of this study was to determine the effect of technological parameters of the production of horse meat minces with the addition of the protein-oil emulsion from chicken combs on the functional, technological and physicochemical indicators.

**Scientific Hypothesis**

The scientific hypothesis of this work is the experimental determination of the effect of technological production parameters of the horse meat minces with the addition of the protein-oil emulsion from chicken combs on the formation of the functional, technological and physicochemical indicators.

**MATERIAL AND METHODOLOGY**

**Samples**

Horse meat, produced at the Arkalyk (Petropavlovsk, Republic of Kazakhstan) farm, was used chilled to make minced meat. The proximate composition of the horsemeat was as follows (%): protein – 19.5, fat – 9.9, moisture – 69.6, and ash – 1.0. To prepare the protein-oil emulsion (POE), chicken combs and cottonseed oil were used. The combs were separated from the heads of 42-day old broiler chicken immediately after the poultry slaughter at Ardager in Semey (Republic of Kazakhstan). The resulting combs were cooled at 2 ± 2 °C and transported chilled to the laboratory to obtain the POE. The cottonseed oil (Altyn may, Bagdaulet-1, Republic of Kazakhstan) was purchased locally and stored at room temperature until the POE was obtained.

**Chemicals**

All chemicals were purchased from the company Alfa-lab (Semey, Republic of Kazakhstan) and had the quality of analytical purity.

**Animals and Biological Material**

For biotechnological treatment, we used the bacterial concentrate Bifilakt-AD (Experimental Biofactory, Uglich, Russian Federation), which includes the following types of bacteria: *Lactococcus lactis subsp. Diacetilactis, Streptococcus thermophiles, Lactobacillus acidophilus, Bifidobacterium bifidum, B. longum*, and *B. teenis*.

**Instruments**

The grinder (Fimar 32/RU Unger, Italy), cutter (CFS/GEA Cutmaster), devices DK6, UDK129 (VELP SCIENTIFICA, Italy), centrifuge (Orbita, Russian Federation), consistometer (KF40, Brookfield), structometer ST-2 (Quality Laboratory, Russian Federation) were used in this research.

**Laboratory Methods**

Physicochemical analyses of the meat mince samples were conducted to standard methods:

- Total nitrogen content was assayed by the Kjeldahl method using devices DK6, UDK129 (VELP SCIENTIFICA, Italy), an automated incinerator, and a distillation apparatus [20].
- Moisture was determined by drying the sample in a metal bottle at a temperature of 105 °C to constant weight [21].
- Total fat was determined via the Soxhlet method [22].
- The ash content was determined via the dry ashing method.

The water-holding capacity (WHC) was determined by the gravimetric method proposed by Zhang et al. [23]. The oil-holding capacity (OHC) was determined by the method described by Salavatulina [24]. The meat minces' ultimate shear stress (USS) was determined on the consistometer (KF40, Brookfield).

The adhesion was determined on the structometer ST-2 (Quality Laboratory, Russian Federation) by measuring the force of separation of a specially selected plate from the test specimen. The measure of adhesion is the amount of peel force per unit contact surface [25].

**Description of the Experiment**

**Sample preparation**: To increase the biological value of the combs, they were pre-treated with a bacterial concentrate Bifilakt-AD (Experimental Biofactory, Uglich, Russian Federation). Before processing, the bacterial concentrate was activated in pre-sterilized skim milk at 37 ± 1 °C for 5 – 6 h until the acidity reached 80 °T. Chicken combs were passed through a grinder (Fimar 32/RU Unger, Italy) fitted with a plate with 3-mm diameter holes, and activated sourdough was added to them in 20% of the raw material weight. After mixing, the combs were kept in a thermostat at 37 ± 1 °C for 3 – 4 h until the raw material was tenderized.
After biotechnological treatment, chicken combs were finely ground in a cutter (CFS/GEA Cutmaster) with water and cottonseed oil in a ratio of 75:10:15, respectively, to obtain the POE. During the preparation of test samples of the minces, the POE was added to the chopped horse meat in an amount of 10 to 25% (Figure 1).

**Figure 1** Samples of minced horse meat.

**Number of samples analyzed:** 5 batches of samples were made for research: a control sample of minced horse meat, 4 test samples with the addition of 10, 15, 20 and 25% POE to minced horse meat.

**Number of repeated analyses:** each of analyse was measured three times.

**Number of experiment replication:** analyses were carried out in five replicates.

**Design of the experiment:** The change in the chopped horse meat's functional, technological, and physicochemical indicators was studied.

The WHC was determined by the gravimetric method. In centrifuge tubes (Orbita, Russian Federation), samples were centrifuged at 70 × g for 15 min at 4 °C. Then the samples were heated at 75 °C and weighed after separating the supernatant. After ageing at 4 °C for 24 hours, the samples were centrifuged again, the resulting supernatant was separated, and samples were weighed again. Water-holding capacity (%) was calculated by Equation 1:

\[
\text{WHC} (\%) = \left( \frac{m_2 - m}{m_1 - m} \right) \times 100\%
\]

Where:
m – the mass of the sample in g; m1 is the mass of the sample after heating and decanting the supernatant in g; m2 – the mass of the sample after centrifugation and removal of the resulting supernatant in g.

The OHC was determined as a difference between the oil mass fraction in the mince and the quantity of oil, separated by heat treatment on the water bath.

The meat minces’ ultimate shear stress (USS) was determined on the consistometer (KF40, Brookfield). The container for the product was filled with the test sample, the surface was levelled with a spatula, and its level was set relative to the zero division of the instrument scale. The depth of immersion of the cone in the product (mm) was determined by a scale, setting, and selecting a certain weight. The USS (Pa) was calculated by Equation 2:

\[
\text{USS} = K \times \frac{M}{h^2}
\]

Where:
K is the cone constant, for α = 60°, K = 2.1 m/kg; M is the cone's mass with a rod and additional weight in kg; h is immersion depth of the cone, m.

The adhesion was determined on the structurometer ST-2 (Quality Laboratory, Russian Federation) by measuring the force of separation of a specially selected plate from the test specimen. The measure of adhesion is the amount of peel force per unit contact surface [18].

**Statistical Analysis**

The data were analyzed using Statistica 12.0 (STATISTICA, 2014; StatSoft Inc., Tulsa, OK, USA). The values are presented as the mean ± SEM. The differences were considered to be statistically significant at \( p \leq 0.05 \). The data were analyzed by One-way ANOVA using free web-based software offered by Assaad et al. [26].
RESULTS AND DISCUSSION

The meat minces have a plastic-viscous structure characterized by a set of properties: the ultimate shear stress [13], [27]. The consistency of the meat minces directly depends on the moisture and fat content and the degree of grinding [28], [29]. The ultimate shear stress is most sensitive to changes in technological and mechanical factors. Therefore, this indicator is used to evaluate the minced meat during manufacture. Pelenko et al. [30] determined the effect of processing time of the grinding, forming, or mixing on the properties of minced meat. Kabulov et al. [31] detected the effect of combined processes on the quality of minced meat. The amount and quality of protein in the food system and processing conditions affect the degree of stability of the resulting meat systems and structural and mechanical properties [32]. The resulting product's nutritional value, processing, sensory and rheological characteristics vary depending on the added ingredients, water, and fat content [12], [33]. Kumar et al. [34] noted that plant oils in the composition of meat products significantly affect the technological properties of meat emulsion. It is important to set rheological indicators. Rivas and Sherman [35] noted that water-soluble meat proteins exhibited important viscoelastic properties. Water-soluble proteins demonstrate strong adhesion adsorbed on the surface of contacting oil droplets when oils are added to the meat system. The results of determining the USS of the test samples of the minces with the addition of the POE are shown in Figure 2. Based on experimental data. The mixing process is divided into 4 periods. In the 1st period, particles are crumpled, moisture transitions from a free to a more bound state, and the structure of the minced meat is strengthened. With further mixing (2nd period), the temperature of the minced meat increases. The meat system is aerated, and the constituent particles of the minced meat are emulsified [36]. In the third mixing period, further strengthening of the structure occurs, and the USS decreases (Figure 2). At the same time, the fat droplets merge with the protein fraction, strengthening the structure; more persistently retaining moisture. The fourth period is characterized by a decrease in the values of the USS, which is associated with the loosening of the minced meat.

![Figure 2](image-url)

**Figure 2** The dependence of the USS of the meat minces on the duration of cutting at different amounts of the POE.

The addition of the POE to the meat minces in an amount of 10 to 25% affects the decrease of USS (Figure 2). The addition of the POE to the meat minces in an amount of 10 to 25% affects the decrease of USS (Figure 2). The USS is significantly (p ≤0.05) reduced up to 6 minutes of mixing, a monolithic structure is formed, and further mixing leads to a slight decrease of the USS. The control sample of the meat minces, consisting of horse meat, showed higher USS values compared to all test samples of the meat minces. Adhesion is of great importance in forming a monolithic structure of minced meat. This indicator is characterized by the force of interaction between the surfaces of the interacting structural material and the product during
separation. The stickiness of raw meat is caused by the accumulation of salt-soluble proteins on the surface of the meat [25].

Figure 3 The adhesion of the meat minces is dependent on the duration of cutting at different amounts of the POE.

Figure 3 shows the results relative to the adhesion of the meat minces. Adhesion increases with an increase in the amount of the POE in the formulation. It was also noted that after 6 – 8 minutes of minced meat cutting, adhesion increases insignificantly in all samples. The lowest adhesion values were noted for the control sample, regardless of the cutting time.

Results of rheological studies have shown that an increase in the amount of the POE in the composition of the meat minces leads to a significant decrease in its strength characteristics, an improvement in plasticity, and an increase in stickiness and adhesion.

One of the most important technological properties of meat and minced meat is its WHC [37]. Many authors noted that the higher this indicator, the less moisture loss during heat treatment, and the higher the yield of finished products and their sensory characteristics: tenderness, juiciness, and taste [38], [39], [40], [41], [42].

The dependence of the WHC of the test meat minces on the duration of cutting for different amounts of the POE is shown in Figure 4. The results show that the optimal value of the WHC for the test samples of meat minces varied from 90.5 to 93.9 (p ≤0.05) with cutting for 6 min.

The results of Danilov et al. [9] indicated improvement of functional and technological properties of heat-treated horse mince with an increasing amount of the paste made from the fermented rumen. The authors noted that defibrillation of modified collagen fibres improves the functional and technological properties [9], [43], [44], [45].
Figure 4 The dependence of the WHC of the meat minces on the duration of cutting at different amounts of the POE.

With an increase in the POE content in the meat minces, there is an increase in the WHC. This can be explained by a decrease in the amount of a well-connected protein-oil-water system in the meat minces, which is stable during heat treatment. The results of determining OHC, presented in Figure 5, showed that adding more than 20% of the POE into the minced meat contributes to a slight decrease in OHC. The same dependence was noted for the cutting time – the optimal interval is from 4 to 6 min. Further cutting leads to local overheating of the minced meat and a decrease in the ability of horse meat proteins to bind the added oil. Gombozhapova et al. [46] explain the decrease in moisture-binding and water-retaining capacity with a long time of massaging by partial denaturation of proteins. Kotliar et al. [47] determined an increase in water retention capacity of the meat paste sample with added protein-fat emulsion on a vegetable oils basis.

The results of the physicochemical analyses are presented in Table 1. The results show that with an increase in the POE content, the protein content in the meat minces decreases, but POE remains at a high level – 16.3% \((p \leq 0.05)\) even in the sample with the addition of 25%. The fat content in the meat minces increased from 10.1 to 15.1% \((p \leq 0.05)\). With increasing POE content, the moisture content changed insignificantly in samples with the added 15, 20, and 25% POE. The approximate composition of the POE can explain this: protein – 9.65%, fat – 15.1%, moisture – 74.26%, and ash – 0.99. The given POE indices differ from the horse meat indices, and therefore the replacement of a part of the horse meat in the minced meat with POE leads to a decrease in protein. The sample of pate with the addition of gizzard and 15.36% olive oil was found to be similar in protein content [48].

The research results showed that the rheological and functional-technological indicators of the meat mince change during the cutting process. The amount of POE injected was also affected.
Figure 5 The dependence of the OHC of the meat minces on the duration of cutting at different amounts of the POE.

Table 1 Physicochemical indicators of the meat mince with the addition of the POE.

| Indicators | 10% POE | 15% POE | 20% POE | 25% POE | Control |
|------------|---------|---------|---------|---------|---------|
| Moisture   | 68 ±0.0872<sup>b</sup> | 67 ±0.0812<sup>c</sup> | 66.8 ±0.112<sup>c</sup> | 66.5 ±0.086<sup>c</sup> | 69.1 ±0.333<sup>a</sup> |
| Protein    | 17.9 ±0.132<sup>b</sup> | 17.4 ±0.129<sup>b</sup> | 16.8 ±0.0843<sup>c</sup> | 16.3 ±0.086<sup>c</sup> | 18.4 ±0.14<sup>a</sup> |
| Fat        | 11.6 ±0.0493<sup>c</sup> | 14 ±0.0927<sup>b</sup> | 14.8 ±0.0922<sup>a</sup> | 15.1 ±0.0374<sup>a</sup> | 10.1 ±0.203<sup>d</sup> |
| Ash        | 1.81 ±0.0102<sup>b</sup> | 1.78 ±0.0202<sup>b</sup> | 1.55 ±0.0224<sup>d</sup> | 1.43 ±0.0152<sup>d</sup> | 2.43 ±0.0129<sup>a</sup> |

Note: Values are means ±SEM, n = 5 per treatment group. Means in a row without a common superscript letter differ (p < 0.05) as analysed by one-way ANOVA and the TUKEY.

CONCLUSION
The research results have shown that cutting time significantly affects meat minces' rheological, functional, and technological indicators. The optimum mixing time for meat minces is 6 min. Adding a protein-oil emulsion obtained from biotechnologically processed chicken combs, cottonseed oil, and water into the minced horse meat does not significantly affect the product's nutritional value but, at the same time, allows the rational use of poultry by-products. To obtain minced meat with optimal rheological parameters, adding 15 – 20% POE is recommended.

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