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The journal “Theory and practice of meat processing” is an international peer-reviewed scientific journal covering a wide range of meat science issues.

We publish original results of the fundamental and applied research in the field of:

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- targeted modification (selection, hybridization, operative manipulation);
- processing of meat raw materials;
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- study of effects of meat and meat product consumption on human health;
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THE EFFECT OF PHYTOGENIC ADDITIVES ON BIOCHEMICAL PARAMETERS OF BROILER CHICKEN TISSUES

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Keywords: *broiler chickens, quercetin, cinnamaldehyde, 7-hydroxycoumarin, chemical composition, mineral composition*

Abstract

The aim of this study was to evaluate the effect of combinations of bioactive compounds of plant origin (cinnamaldehyde, quercetin and 7-hydroxycoumarin) on productivity, meat quality and mineral metabolism in broiler chickens. During a 35-day experiment, 180 broiler chickens of the Arbor Acres cross were divided into 4 groups ($n=45$): control (basal diet — BD) and three experimental treatments (BD with additives): I (cinnamaldehyde 30 mg/kg feed + quercetin 2.5 mg/kg feed), II (cinnamaldehyde 30 mg/kg feed + 7-hydroxycoumarin 0.3 mg/kg feed), III (combination of all three substances). Zootechnical parameters, chemical, amino acid and elemental composition of meat and liver from the experimental animals were assessed. Statistical significance was determined using the Mann-Whitney U-test ($p \le 0.05$). The greatest synergistic effect was demonstrated by the combination in the experimental group III. Compared to the control, in this group, the absolute live weight gain significantly increased by 880.7 g (by 51.4 %; $p \le 0.05$), and the average daily gain increased by 18.23 g (by 34.5 %; $p \le 0.05$). Feed conversion improved by 9.4 % (from 2.02 to 1.83 kg feed/kg weight gain), and the productivity efficiency index (EPEF) more than doubled by 257.04 points (from 209.37 to 466.41; $p \le 0.05$). The muscle tissue weight of carcasses in experimental group III was 246.5 g higher than the control value (31.9 %; $p \le 0.05$). A significant increase in the fat mass fraction was observed in the breast muscles of all experimental groups, e. g. in group I by 0.9 % ($p \le 0.001$). And in group II, an increase in the protein fraction by 2.2 % ($p \le 0.05$) was observed. The additives had a modulating effect on the mineral composition of tissues, causing, in particular, a decrease in the concentration of iron (Fe) in the breast muscles of groups I and III by 7.21 mg/kg (22.3 %; $p \le 0.001$) and 5.70 mg/kg (17.6 %; $p \le 0.001$), as well as an increase in the content of zinc (Zn) in the thigh muscles of groups I and II by 7.61 mg/kg (16.3 %; $p \le 0.05$) and 9.01 mg/kg (19.3 %; $p \le 0.01$), respectively. Thus, the combined use of cinnamaldehyde, quercetin and 7-hydroxycoumarin demonstrated a statistically significant positive effect on growth, feed efficiency and meat productivity of broilers, and also changed the biochemical profile of muscle tissue, which confirms the potential of this composition as an alternative to antibiotic growth promoters.

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Introduction

The intensification of poultry production to meet the growing demand for meat products faces significant challenges, including an increased risk of disease transmission and the need to maintain high rates of livestock growth. The established practice of addressing these challenges has been the use of antibiotics, not only for treatment but also as growth promoters [1,2,3]. However, the global problem of antibiotic resistance and consumer concerns about product safety have led to a ban on the use of antibiotic growth promoters and stimulated the search for effective and safe alternatives [4,5].

Plant-based alternatives to antibiotics represent a large and diverse group of phytobiotics, i. e. complexes of biologically active substances of plant origin. These include essential oils, tannins, saponins, flavonoids, alkaloids, and resin acids, which exhibit a broad spectrum of activity, including antimicrobial, antiviral, antioxidant, and anti-inflammatory effects [6]. However, phytobiotics are not the only possible alternative. Current research also focuses on probiotics, prebiotics, organic acids, enzymes, and bacteriophages. Each of these approaches has its own mechanism of action, and their effectiveness often depends on the synergistic effect of combined use. Therefore, the search for an optimal alternative lies not in opposing phytobiotics against antibiotics, but in a comprehensive assessment of the effectiveness of specific compounds or their combinations for modulating intestinal health, immune status, and poultry productivity.

This study aimed to investigate the effect of a specific composition of plant-based bioactive compounds on the performance and meat quality of broiler chickens. The selection of specific components — cinnamaldehyde, quercetin, and 7-hydroxycoumarin — was based on their unique and complementary properties, addressing key performance and health issues in poultry production [7].

Cinnamaldehyde, the main component of cinnamon essential oil, was included due to its potent antimicrobial properties against a wide range of pathogenic and opportunistic microorganisms [8]. Hypothetically, it should improve the microbiological status of the gastrointestinal tract, reducing microbial load and competitive nutrient uptake, which, in turn, could increase feed digestibility and the efficiency of energy use for growth.

Quercetin, one of the most common flavonoids, was selected for its pronounced antioxidant and anti-inflammatory activity [9–11]. According to the literature, quercetin's ability to inhibit proinflammatory signaling pathways (e. g., mTOR) and modulate the immune response should reduce the level of systemic inflammation often associated with intensive farming practices [12,13]. This, in turn, may redirect energy resources from immune defense to muscle growth and synthesis processes, as well as improve meat quality by reducing oxidative stress.

7-hydroxycoumarin was selected as a promising modulator of pathogenic microflora virulence. Its ability to inhibit quorum sensing and biofilm formation [14–19] potentially reduces bacterial pathogenicity without direct bactericidal pressure on them, minimizing the risk of resistance development [20–23]. This is expected to contribute to maintaining intestinal health and resistance to infections.

Although the effects of some individual phytogenic additives have been studied, the potential synergistic effect of this particular combination on product quality parameters (chemical, amino acid, and mineral composition of meat) remains unclear. We hypothesized that this combination would have a comprehensive positive effect on meat productivity and meat quality in broilers through simultaneous effects on the microbiota, antioxidant status, and inflammatory processes.

Thus, the scientific novelty of this study lies in its comprehensive assessment of the effects of a synergistic composition of bioactive compounds (cinnamaldehyde, quercetin, and 7-hydroxycoumarin) on productivity, meat quality parameters, and mineral metabolism in Arbor Acres broiler chickens.

Objects and methods

The study was conducted in the vivarium of the Federal Research Centre for Biological Systems and Agrotechnologies of the Russian Academy of Sciences (Orenburg) from February to August 2022. The objects of the study were Arbor Acres broiler chickens and bioactive compounds of plant origin (cinnamaldehyde, quercetin, 7-hydroxycoumarin).

Experimental design

180 7-day-old broiler chickens were divided into 4 groups using the analog method ($n = 45$): Control (C): Basal diet (BD); Experimental group I (EXP-I): BD + cinnamaldehyde (30 mg/kg feed) + quercetin (2.5 mg/kg feed); Experimental group II (EXP-II): BD + cinnamaldehyde (30 mg/kg feed) + 7-hydroxycoumarin (0.3 mg/kg feed); Experimental group III (EXP-III): BD + cinnamaldehyde (30 mg/kg feed) + quercetin (2.5 mg/kg feed) + 7-hydroxycoumarin (0.3 mg/kg feed) (Table 1).

Table 1. Experimental design

Objects of the study	Group	Experimental period	
		Preparation	Experiment
		Days	
		7	35
Arbor Acres broiler chickens	Control ($n = 45$)	BD	BD
	Experimental I ($n = 45$)		EXP-I
	Experimental II ($n = 45$)		EXP-II
	Experimental III ($n = 45$)		EXP-III

Husbandry and feeding

Husbandry conditions were identical for all groups. Diets were formulated according to the recommendations by the All-Russian Poultry Research and Technological Institute [24]. Feeding was twice daily, and intake was monitored daily.

Sampling

On the 42nd day, the test animals were slaughtered by the internal method. Samples of the breast and thigh muscles, as well as the liver, were collected immediately after slaughter and frozen (–18 °C).

Biosubstrate analysis

The samples were analyzed for their chemical composition: content of moisture (dried at 150 ± 2 °C), crude fat (Soxhlet extraction), crude protein (Kjeldahl method), and ash. Amino acid composition was determined by capillary electrophoresis on Kapel-105M system after acid hydrolysis of the samples and the production of FTC derivatives. Elemental composition: ICP-MS on Agilent 7900 ICP-MS mass spectrometer.

Chemical analysis of biosubstrates was performed using standardized methods at the testing center of the Federal Research Centre for Biological Systems and Agrotechnologies of the Russian Academy of Sciences.

Carcass morphometry

Eviscerated carcasses (standard definition: internal organs removed, head, skinless neck, feet up to the tarsal joint ± 20 mm) were weighed (Mercury 327 ACP LCD electronic scales, Russia, with an accuracy of ± 2 g). Cutting was performed according to the recommendations by the All-Russian Poultry Research and Technological Institute [24], with the main parts (breast, drumstick and thigh, wings, forequarters, and dorsal-scapular portion) isolated. The parts were anatomically deboned, and the isolated tissues (muscle, skin, veins, fat, and bones) were weighed (MW-II laboratory scales, China, with an accuracy of ± 0.01 g) to calculate the yield.

Determining the moisture content

To determine the moisture content of the test sample, 0.2 kg samples were taken. The biosubstrate samples were freed from their coverings (including the skin), then homogenized (repeatedly minced using a meat grinder), and then thoroughly mixed (the temperature of the resulting test sample was approximately 25 °C). Next, 0.01 kg ± 0.001 kg of purified sand and a glass rod for mixing the sand with the sample were transferred to a weighing cup, after which they were dried in a drying oven (SHC-80–01, Russia) at a temperature of 150 °C for 0.5 h. Then, the dried cups were closed with lids and placed in a desiccator, where they cooled to 20–22 °C, after which they were weighed to the third decimal place on electronic scales (VM-153, Russia). Prepared samples of the tested biosubstrates (3000 mg) were placed in the cups, after which the total mass of the cup with the contents was weighed. The samples with sand were thoroughly mixed using a glass rod and transferred to a drying oven at a temperature of 150 °C for 60 min for drying. After drying, the cups were covered with lids and transferred to a desiccator to cool to 20–22 °C. The cups were then weighed to the third decimal place. To calculate the moisture content in%, the following formula was used:

$$A = ((a - b)/(a - c)) \times 100\,\%, \tag{1}$$

where:

A is the moisture mass fraction;

a is the weight of the cup containing biosubstrate sample, glass rod, and sand, g;

b is the weight of the cup containing biosubstrate sample, glass rod, and sand after drying, g;

c is the weight of the cup containing the glass rod and sand, g.

Determining the ash content

To determine the ash content, a weighed portion of the air-dried sample (1–2 g ± 0.0001 g) and 3 ml of ashing agent (magnesium acetate in ethyl alcohol with added iodine) were placed into calibrated crucibles. The ashing agent was ignited, and after it burned out, the crucibles were placed in a furnace (Natbertherm GmbH, Germany) at 500–550 °C until a grayish-white ash was obtained. The crucibles were then annealed to a constant weight. To calculate the ash content in%, the following formula was used:

$$A = (a - b) \times 100\,\%/c, \tag{2}$$

where:

A is the ash mass fraction;

a is the weight of the ash in the biosubstrate sample, g;

b is the weight of the ash from the ashing agent, g;

c is the weight of the initial portion of the biosubstrate, g.

Determining the fat content

To determine the fat content in the experimental biosubstrates, we used a method of repeated extraction of fat from a dried sample using a solvent in a Soxhlet extraction apparatus. The solvent was then removed from the samples, and the resulting fat was dried to a constant weight. A 5000 mg portion of the prepared sample was placed on a watch glass and dried in a drying oven for 60 minutes at 105 °C (SHC-80-01, Russia). The dried test sample was then placed in a specially prepared filter paper sleeve with a cotton wool base. Next, the watch glass was soaked in diethyl ether and transferred to a prepared sleeve. The sleeve was then sealed and transferred to a special desiccator in a Soxhlet apparatus. The extraction process was carried out for approximately 6 hours (the extract was poured off at an average rate of 7 times per hour) using an extraction flask and a water bath. The process was considered completed when no greasy stain from the extract remained on the filter paper. Upon completion of the extraction, the solvent was distilled from the extraction flask. The extraction flask, containing the fat after extraction, was then dried in a drying oven at 105 °C to a constant weight. To calculate the fat content in%, the following formula was used:

$$A = ((a - b) \times 100\,\%)/c, \tag{3}$$

where:

A is the fat mass fraction;

a is the weight of the extraction flask containing the fat, g;

b is the weight of the empty extraction flask, g;

c is the weight of the biosubstrate sample, g.

Determining the protein content

This method allows for the determination of the total nitrogen content in a sample, including nitrogen in various forms, such as ammonia. For this purpose, a weighed portion of the sample (2 g ± 0.001 g) was transferred to a Kjeldahl flask, anhydrous potassium sulfate (15 g) and concentrated sulfuric acid (25 ml) were added, everything was mixed, and the flask was placed on a heating device to dissolve the sample. After the sample was completely dissolved, the mineralization process was continued at boiling until the contents of the flask became transparent and then turned a pale green-blue color. After the flask contents became completely colorless, it was boiled for another 1.5 hours, with the total mineralization time being approximately 2 hours. The Kjeldahl flask containing the sample was then cooled (40 °C) and distilled water (50 ml) was added. The flask was then stirred and cooled to a temperature of 20–22 °C. To calculate the protein content in%, the following formula was used:

$$A = (0.0014 \times (a - b) \times c \times 100)/d \times 6.25, \tag{4}$$

where:

A is the protein mass fraction;

a is the amount of hydrochloric acid solution (0.1 mol/dm^3) used to titrate the test sample, cm^3;

b is the amount of hydrochloric acid solution (0.1 mol/dm^3) used to titrate the control sample, cm^3;

c is the correction factor to the nominal concentration of the hydrochloric acid solution;

0.0014 is the amount of nitrogen equivalent to 1 cm^3 of hydrochloric acid solution (0.1 mol/dm^3), g;

100 is the conversion factor for%;

d is the weight of the test sample, g;

6.25 is the conversion factor for protein.

Determining the amino acid composition

The analysis was performed using capillary electrophoresis on Kapel-105M device manufactured by Lumex-Marketing LLC (Russia) according to the standard procedure (M 04-38-2009). The amino acid composition was determined using the following mass fractions: arginine, lysine, tyrosine, phenylalanine, histidine, leucine and isoleucine (total), methionine, valine, proline, threonine, serine, alanine, and glycine in the form of phenylisothiocarbamyl derivatives. Samples (0.1 g) were placed in vials with heat-resistant screw caps and fluoroplastic liners for hydrolysis. 10 ml of HCL (1:1) was added and mixed. Acid hydrolysis was performed in a drying oven (SHC-80-01, Russia) for 15 ± 2 h, at a temperature of 110 °C. After that, the vials were removed from the cabinet and cooled to a temperature of 20–22 °C, then the contents were filtered (using a blue-ribbon filter). The resulting hydrolysate (50 mm^3) was transferred to glass weighing bottles and evaporated to dryness in a stream of warm air. Then the FTC derivative was obtained by adding 150 mm^3 of sodium carbonate solution and 300 mm^3 of phenyl isothiocyanate solution in isopropyl alcohol to the dry residue, stirring until the precipitate dissolved, closing the lid and leaving for 35 min at room temperature. Then the solution was evaporated to dryness in a stream of warm air. The dry residue was dissolved in 500 mm^3 of distilled water. The resulting solution for analysis was transferred to an Eppendorf tube and centrifuged (5 min, 5000 rpm) in a centrifuge (Eppendorf AG MiniSpin, Germany). The following formula was used to calculate the mass fraction of each amino acid in the sample (%):

$$A = 10 \times C/m, \tag{5}$$

where:

A is the mass fraction of amino acids, %;
10 is the volume conversion factor;
C is the measured mass concentration of the amino acid in the solution;
m is the weight of the test sample.

Determining the elemental composition

Pre-homogenized sample in its dried or native form (weighing from 0.1 to 0.3 g) was placed in TFM container and weighed to an accuracy of 0.0001 g (Pioneer PX 224 laboratory scales, USA). Nitric acid, hydrogen peroxide, and, if necessary, hydrofluoric or hydrochloric acid were added. Degradation of the organic matrix was performed in TOPEX+ microwave sample preparation system (PREEKEM, China) at the appropriate temperature and pressure (the degradation program is selected depending on the sample type). Elemental analysis was performed on Agilent 7900 ICP-MS single-quadrupole inductively coupled plasma mass spectrometer (Agilent, USA). Fe and Zn were analyzed in helium mode using a collision cell. Standard solutions were prepared from a multicomponent mixture of 23 elements from Merck (Germany) with the addition of additional reference samples. Statistical data were accumulated through a series of measurements across the entire mass range in duplicate. The analysis result is the concentration of the target elements, expressed as mg of element per kg of sample, taking into account the sum of instrumental and methodological errors.

Ethics statement

The experiments were carried out in accordance with the requirements of the Federal Law of the Russian Federation[1], the Declaration of Helsinki[2], the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes[3].

Statistical analysis

The significance of differences was assessed using the Mann-Whitney U-test (Statistica 10.0 package). The significance level was $p \leq 0.05$.

Results and discussion

The obtained research results revealed that the experimental groups of broiler chickens showed a trend toward an increase in average daily weight gain by 4.5–34.5 % compared to the control group. The greatest effect was observed in experimental group III ($p \leq 0.05$). This indicates a synergistic effect from the combined use of all three studied additives. In terms of absolute weight gain, the pattern completely replicates the dynamics of average daily weight gain, as these indicators are directly related. Experimental groups I and III demonstrated significantly higher absolute weight gain (by 34.4 % and 51.4 %, respectively) compared to the control group (Table 2).

The feed intake is crucial for interpreting the results. For example, in experimental group I, feed intake was 3,171.3 g (a decrease of 8.5 % relative to the control), in experimental group II it was 3,468.4 g (similar to the control), and in experimental group III it was 3,959.0 g (an increase of 14.2 % relative to the control). Thus, the improved gains in the experimental groups are not due to a simple increase in feed intake. Moreover, experimental group I demonstrated better gains while consuming less feed. This clearly indicates that the primary mechanism of action of phytogenic additives is improved nutrient digestibility, not appetite stimulation. Feed consumption per 1 kg of gain (feed conversion ratio, kg) is an integral indicator of feed efficiency. In the control group, this parameter was 2.02 kg of feed/kg of gain, which was higher than in the experimental groups by 6.9 % in group I, 6.4 % in group II, and 9.4 % in group III, respectively. All experimental diets reduced feed consumption per unit of gain. The best feed conversion was

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[1] Federal Law of the Russian Federation dated December 27, 2018 No. 498-FZ "On the responsible treatment of animals and on amendments to certain legislative acts of the Russian Federation." Retrieved from https://docs.cntd.ru/document/552045936. Accessed May 17, 2025 (In Russian)

[2] WMA Declaration of Helsinki — ethical principles for medical research involving human subjects Retrieved from https://www.wma.net/policiespost/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/ Accessed May 17, 2025

[3] ETS No. 123, Strasbourg, 1986) (European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. Retrieved from https://rm.coe.int/168007a67b. Accessed May 17, 2025

Table 2. Growing efficiency of test animals

Parameter	Group			
	Control	I	II	III
Average daily gain over 5 weeks, g	52.91 ± 10.6	60.90 ± 0.6	55.27 ± 3.7	71.14 ± 4.9^{a}
Absolute gain, g	1714.8 ± 311.6	2304.8 ± 65.9^{a}	1934.5 ± 127.8	2595.5 ± 166.1^{a}
Feed intake, g	3467.0 ± 630.0	3171.3 ± 90.7	3468.4 ± 229.2	3959.0 ± 253.3
Feed consumption per 1 kg of live weight gain, g	2.02	1.88	1.89	1.83
Livability, %	96	98	98	98
EPEF	209.37 ± 66.32	442.57 ± 4.45^{a}	308.28 ± 20.37^{a}	466.41 ± 32.14^{a}

Note: ^{a} — $P \leq 0.05$ when compared to the control group.

achieved in group III, confirming the synergistic effect of the combination and its high cost-effectiveness.

Livability was very high in all groups (96–98 %), indicating good housing conditions and the absence of a negative impact of the additives on the animals' health. The Production Efficiency Factor (EPEF) is a complex parameter combining growth, livability, and feed conversion. In the control group, it was 209.37, while a significant increase was observed in all experimental groups ($p \leq 0.05$).

Thus, the use of experimental phytogenic additives, particularly the combination of cinnamaldehyde, quercetin, and 7-hydroxycoumarin (group III), significantly improved all key livestock performance indicators: increased the rate and absolute live weight gain, significantly improved feed conversion and overall production efficiency (EPEF). Importantly, this positive effect was achieved primarily by increasing feed digestibility, not by increasing feed intake.

A study of meat productivity indicators in test animals revealed a significant advantage of using phytogenic additives in their diets only for group III (Table 3).

The control slaughter revealed the following. High pre-slaughter live weights were observed in experimental groups I and III, exceeding those of the control group by 13.1 % and 30.8 % ($p \leq 0.01$). Experimental group III significantly exceeded the control group ($p \leq 0.05$) in terms of eviscerated carcass weight by 31.5 %, muscle tissue by 31.9 %, and edible portion by 33.4 %, respectively. Feeding phytogenic additives to chickens in experimental groups II and III also demonstrated a beneficial effect on slaughter yield, which increased by 0.8–0.9 % relative to the control value.

Similar results were obtained in other studies involving the introduction of phytobiotics in broiler chicken diets. Thus, it was established that with the addition of a plant-based additive from the extract of edible chestnut wood, a high pre-slaughter live weight was observed relative to the control group, and the weight of eviscerated chickens from the experimental groups with the highest level of chestnut extract exceeded similar groups with the lowest level of this substance in the diet by 6.07 % ($p \leq 0.05$) and 9.48 % ($p \leq 0.05$), respectively [25]. Similar results were obtained in broiler chickens for the weight of semi-eviscerated carcasses in the work by Gheisar et al. [26]. This was probably due to the greater digestibility of nutrients in the experimental groups following the inclusion of phytobiotics, which contributed to a more intensive increase in live weight. Younis and Abdel-Latif previously reported that the slaughter yield of broiler chickens was significantly ($p \leq 0.05$) higher when using water supplemented with 1 % red hot pepper extract [27]. High slaughter yields were also observed in test animals supplemented with turmeric [28].

Analysis of the chemical composition of breast tissue in animals from the experimental groups revealed an increase in fat concentration by 0.2–0.9 % ($p \leq 0.001$) relative to the control group. In experimental group II, protein and dry matter concentrations also increased relative to the control group by 2.2 % ($p \leq 0.05$) and 2.4 % ($p \leq 0.01$) (Table 4).

We hypothesize that the increased fat content in the breast muscles of the experimental groups observed in the study is a direct consequence of the combined effects of the phytogenic additives on the metabolism of the test animals. This is not a random result, but a predictable phenomenon, explainable in terms of their biological activity, as their primary mechanisms involve improving nutrient absorption and energy metabolism. The greatest increase in breast muscle fat was observed in group I (cinnamaldehyde + quercetin). This is an interesting observation, suggesting that this combination most effectively triggers

Table 3. Slaughter parameters of test animals at the end of the experiment

Parameter	Group			
	Control	I	II	III
Pre-slaughter live weight, g	2135.5 ± 168.23	2415.3 ± 28.20	2236.0 ± 118.53	2794.0 ± 176.59^{b}
Eviscerated carcass, g	1568.8 ± 185.77	1733.3 ± 21.73	1647.5 ± 79.53	2062.7 ± 149.03^{a}
Muscle tissue, g	772.4 ± 143.32	823.2 ± 13.32	810.9 ± 43.24	1018.9 ± 15.19^{a}
Bone tissue, g	451.0 ± 84.16	511.5 ± 1.39	470.8 ± 22.29	591.4 ± 23.31
Edible portion, g	1370.2 ± 140.72	1546.3 ± 20.77	1439.7 ± 70.60	1827.9 ± 150.00^{a}
Inedible portion, g	634.4 ± 113.28	716.2 ± 13.84	655.0 ± 42.26	837.3 ± 24.29
Edible portion / Inedible portion	2.16 ± 0.045	2.16 ± 0.016	2.20 ± 0.035	2.18 ± 0.115
Slaughter yield, %	72.9 ± 1.10	71.8 ± 0.50	73.8 ± 0.86	73.7 ± 0.71

Note: ^{a} — $P \leq 0.05$; ^{c} — $P \leq 0.01$ when compared to the control group.

Table 4. Analysis of the chemical composition of breast muscles in test animals

Mass fraction	Group			
	Control	I	II	III
Moisture, %	76.6±2.40	75.1±2.55	74.2±2.47	74.8±2.26
Dry matter, %	23.4±0.69	24.9±0.65	25.8±0.55^{b}	25.2±0.88
Fat, %	0.7±0.03	1.6±0.06^{c}	1.0±0.04^{c}	0.9±0.05^{c}
Ash, %	0.99±0.05	0.98±0.04	0.99±0.03	0.99±0.05
Protein, %	21.7±0.51	22.3±0.65	23.9±0.88^{a}	23.3±0.80

Note: ^{a} — $P \leq 0.05$; ^{b} — $P \leq 0.01$; ^{c} — $P \leq 0.001$ when compared to the control group.

lipogenesis mechanisms in this particular muscle. Cinnamaldehyde likely plays a key role in stimulating digestion and enzyme secretion, while quercetin ensures metabolic stability through its antioxidant action. The best results in protein concentration were shown by groups II and III contained 7-hydroxycoumarin, which may indicate its special role in optimizing protein metabolism.

Thus, an increase in the fat content of breast muscles is a marker of improved overall metabolic status in animals. The phytogenic additives studied optimize digestion and energy absorption. Excess absorbed energy is naturally deposited as fat in tissues, including muscles.

The results showed that introducing the experimental additives into the diet did not significantly affect the amino acid composition of breast muscles in broiler chickens (Table 5).

At the same time, in the breast muscles of animals from the experimental groups, a tendency towards increasing concentrations was observed: arginine by 0.1% and 0.2% (groups II and III), lysine by 0.4% and 0.2% (groups II and III), tyrosine by 0.2% (II group), phenylalanine by 0.2% (II group), histidine by 0.1% (II group), leucine + isoleucine by 0.3% (II group), methionine by 0.1% (groups II and III), threonine by 0.1% (groups II and III), serine by 0.1% (groups II and III), alanine by 0.1% (II group) and glycine by 0.1% (groups II and III) in comparison with the control.

The obtained results are consistent with literature data demonstrating that flavonoids in animal diets improve meat quality by optimizing the amino acid profile [29]. A likely reason is the pronounced antioxidant activity of flavonoid-containing plants, which reduces the level of free radicals and thereby promotes the preservation of amino acids in muscle tissue [30]. Our data also correlate with the findings by Haščík et al. [31], who demonstrated an increase in the amino acid concentration in chicken meat with the addition of a probiotic with anthocyanins and propolis extract, and Omar et al. [32], who noted improved growth in broilers under the influence of phenol-containing onion extracts due to increased amino acid digestibility and improved intestinal health.

It has also been previously established that many compounds of natural origin, especially those obtained from natural essential oils, have a positive effect on both carcass and meat quality parameters. An example of this is 1,8-cineole (eucalyptol), the main component of rosemary and eucalyptus essential oils, which, when added to broiler feed, increases body weight and weight gain, improves feed conversion ratio, and has a beneficial effect on the quality characteristics of the resulting meat [33]. Phytoncides have also been shown to significantly increase the amino acid concentration in broiler meat, indicating their role in improving meat quality [34,35]. Furthermore, it has been demonstrated that dietary supplementation with natural antioxidants may improve meat quality by preventing lipid peroxidation and protein denaturation, ultimately improving the fatty acid and amino acid profile of meat [36].

Analysis of the mineral composition of the breast muscles of the animals studied revealed intergroup differences in the concentrations of certain chemical elements (Table 6).

Thus, a reliable decrease in Ca was established in all experimental groups by 20.0% ($p \leq 0.01$), 13.3% ($p \leq 0.05$),

Table 5. Amino acid content of breast muscles in test animals

Amino acid	Group			
	Control	I	II	III
Arginine, %	5.3±0.11	5.1±0.16	5.4±0.18	5.5±0.14
Lysine, %	6.9±0.15	6.8±0.11	7.3±0.20	7.1±0.13
Tyrosine, %	4.1±0.18	4.1±0.16	4.3±0.19	4.1±0.22
Phenylalanine, %	3.0±0.09	3.1±0.11	3.2±0.08	3.0±0.12
Histidine, %	2.9±0.07	2.7±0.06^{a}	3.0±0.07	2.9±0.04
Leucine+Isoleucine, %	10.2±0.25	10.2±0.28	10.5±0.31	10.1±0.33
Methionine, %	2.1±0.07	2.1±0.10	2.2±0.11	2.2±0.11
Valine, %	4.2±0.15	4.0±0.14	4.2±0.15	4.1±0.09
Proline, %	2.8±0.12	2.8±0.17	2.8±0.11	2.8±0.15
Threonine, %	3.1±0.18	3.1±0.14	3.2±0.15	3.2±0.07
Serine, %	2.8±0.14	2.7±0.12	2.9±0.15	2.9±0.12
Alanine, %	7.1±0.25	6.8±0.27	7.2±0.19	7.1±0.21
Glycine, %	3.4±0.16	3.3±0.16	3.5±0.17	3.5±0.11

Note: ^{a} — $P \leq 0.05$ when compared to the control group.

Table 6. Elemental composition of breast muscles in test animals

Element	Group			
	Control	I	II	III
Ca, g/kg	0.30 ± 0.02	0.24 ± 0.01^{b}	0.26 ± 0.01^{a}	0.21 ± 0.01^{c}
P, g/kg	9.00 ± 0.37	8.43 ± 0.38	8.51 ± 0.29	9.17 ± 0.38
Na, g/kg	1.98 ± 0.07	1.78 ± 0.07^{a}	1.52 ± 0.06^{c}	1.93 ± 0.08
K, g/kg	14.98 ± 0.78	14.26 ± 0.60	14.63 ± 0.61	15.34 ± 0.63
Mg, g/kg	1.12 ± 0.05	1.07 ± 0.04	1.11 ± 0.04	1.15 ± 0.04
B, mg/kg	1.85 ± 0.10	1.39 ± 0.06^{c}	1.80 ± 0.14	1.49 ± 0.06^{b}
Mn, mg/kg	0.67 ± 0.02	0.55 ± 0.02^{c}	0.63 ± 0.03	0.62 ± 0.02
Co, mg/kg	0.02 ± 0.003	0.01 ± 0.001^{b}	0.02 ± 0.001	0.02 ± 0.001
Fe, mg/kg	32.39 ± 1.10	25.18 ± 1.38^{c}	32.08 ± 3.40	26.69 ± 1.20^{c}
Zn, mg/kg	25.65 ± 0.85	26.65 ± 0.93	24.26 ± 1.04	25.22 ± 0.86
Se, mg/kg	0.69 ± 0.062	0.77 ± 0.091	0.73 ± 0.265	0.90 ± 0.356
Cu, mg/kg	1.12 ± 0.05	1.25 ± 0.04^{a}	1.08 ± 0.03	1.07 ± 0.04

Note: ^{a} — $P \leq 0.05$; ^{b} — $P \leq 0.01$; ^{c} — $P \leq 0.001$ when compared to the control group.

30.0 % ($p \leq 0.001$) and Na by 10.1 % ($p \leq 0.05$) in group I and 23.2 % ($p \leq 0.001$) in the group II, relative to the control. Similarly, B decreased by 24.9 % ($p \leq 0.001$) and 19.5 % ($p \leq 0.01$) in experimental groups I and III, Mn and Co by 17.9 % ($p \leq 0.001$) and 50.0 % ($p \leq 0.01$) in group I, and Fe by 22.3 % ($p \leq 0.001$) and 17.6 % ($p \leq 0.001$) in groups I and III, relative to the control. It should be noted that in the breast muscles of the studied chickens, a reliable decrease in Ca concentration due to the introduction of the tested phytogenic additives may be associated with the formation of tannin-calcium complexes in the gastrointestinal tract of broilers [37].

The thigh muscles of experimental broiler chickens also showed a tendency toward changes in chemical composition (a significant increase in fat in group I, a decrease in protein in all groups). The dynamics in the thigh muscles differ from those in the breast muscles, which is physiologically justified, since these muscles have different fiber types (white/glycolytic in the breast muscles, red/oxidative in the thigh muscles) and different metabolism. A significant increase in fat by 0.4 % ($p \leq 0.001$) in group I is consistent with the mechanisms described above for the breast muscles. Enhanced lipogenesis and lipid transport affect all muscles. The lack of significant changes in groups II and III may indicate that the additive combinations affect metabolism differently in different muscle types. Thigh muscles, which are more metabolically active due to their oxidative nature, may utilize incoming lipids differently. The decrease in the mass fraction of protein in all experimental groups (by 0.6 %-1.6 %) was not significant (Table 7).

Table 7. Analysis of the chemical composition of the thigh muscles in test animals

Mass fraction	Group			
	Control	I	II	III
Moisture, %	76.1 ± 2.41	76.8 ± 2.58	77.5 ± 2.38	76.6 ± 2.37
Dry matter, %	23.9 ± 0.57	23.2 ± 0.65	22.5 ± 0.48	23.4 ± 0.62
Fat, %	1.5 ± 0.06	1.9 ± 0.07^{a}	1.6 ± 0.05	1.6 ± 0.06
Ash, %	0.99 ± 0.02	0.98 ± 0.01	0.98 ± 0.03	0.98 ± 0.01
Protein, %	21.5 ± 0.47	20.3 ± 0.65	19.9 ± 0.96	20.9 ± 0.51

Note: ^{a} — $P \leq 0.001$ when compared to the control group.

As in the breast muscles, ash content remained stable and unchanged across all groups. Unlike the breast muscles, the thigh muscles showed less sensitivity to the additives. The only significant effect was an increase in fat concentration in the group receiving the combination of cinnamaldehyde and quercetin.

Thus, the phytogenic additives studied exert a complex effect on metabolism, enhancing feed digestibility, energy metabolism, and lipogenesis, which is reflected in changes in the chemical composition of both the breast and thigh muscles.

Relative to the control, the amino acid composition of the thigh muscles (Table 8) was distinguished by an increase in concentrations in the experimental group I: arginine by 0.4 %, lysine, tyrosine, phenylalanine, proline, serine by 0.1 %, leucine + isoleucine and alanine by 0.2 %, in the experimental group III: methionine by 0.1 % and arginine by 0.2 %.

Table 8. Amino acid content of thigh muscles in test animals

Amino acid	Group			
	Control	I	II	III
Arginine, %	5.1 ± 0.10	5.5 ± 0.23	5.2 ± 0.06	5.3 ± 0.07
Lysine, %	7.1 ± 0.11	7.2 ± 0.17	6.8 ± 0.14	7.1 ± 0.21
Tyrosine, %	3.1 ± 0.11	3.2 ± 0.15	3.0 ± 0.17	3.0 ± 0.22
Phenylalanine, %	3.0 ± 0.06	3.1 ± 0.13	3.0 ± 0.10	3.0 ± 0.07
Histidine, %	2.4 ± 0.04	2.4 ± 0.09	2.3 ± 0.05	2.4 ± 0.06
Leucine + Isoleucine, %	10.0 ± 0.27	10.2 ± 0.24	9.7 ± 0.24	10.0 ± 0.25
Methionine, %	2.1 ± 0.09	2.1 ± 0.15	2.1 ± 0.08	2.2 ± 0.07
Valine, %	4.0 ± 0.11	4.0 ± 0.13	3.8 ± 0.12	3.9 ± 0.11
Proline, %	2.8 ± 0.08	2.9 ± 0.15	2.7 ± 0.17	2.8 ± 0.10
Threonine, %	3.4 ± 0.06	3.4 ± 0.09	3.2 ± 0.16	3.3 ± 0.12
Serine, %	3.0 ± 0.17	3.1 ± 0.08	2.9 ± 0.11	3.0 ± 0.12
Alanine, %	6.1 ± 0.16	6.3 ± 0.15	6.0 ± 0.19	6.1 ± 0.10
Glycine, %	3.6 ± 0.19	3.6 ± 0.23	3.5 ± 0.17	3.5 ± 0.11

In contrast, in the experimental group II, decreased levels of lysine, tyrosine, histidine, leucine + isoleucine, methionine, proline, threonine, serine, alanine, and glycine were observed. However, these changes were insignificant.

The overwhelming majority of previously cited studies have found positive dynamics in the accumulation of macro- and microelements in the muscle and liver tissues of farm animals as a result of the use of a mixture of phytogenic additives (a mixture of oregano, anise, and citrus essential oils) in their diets [38]. Similar results were obtained in our study, when analyzing the elemental composition of the thigh muscles of experimental broilers (Table 9).

As a result of a comparative analysis of the concentrations of chemical elements in the thigh muscles of experimental broilers, a reliable increase in Cu content was established in all experimental groups by 22.6 % ($p \leq 0.001$), 14.7 % ($p \leq 0.01$), 15.3 % ($p \leq 0.01$) relative to the control. In the experimental group I, an increase in the content of Mn, Fe and Zn was noted by 57.4 % ($p \leq 0.001$), 27.2 % ($p \leq 0.001$) and 16.3 % ($p \leq 0.05$) relative to the control. In experimental group II, the levels of Ca, Mn, Fe, and Zn increased by 13.8 % ($p \leq 0.01$), 22.1 % ($p \leq 0.001$), 18.1 % ($p \leq 0.01$), and 19.3 % ($p \leq 0.01$), respectively, relative to the control. In experimental group III, a 50.0 % decrease in Co content ($p \leq 0.001$) and a 17.2 % increase in Ca content ($p \leq 0.001$) relative to the control values were recorded.

Analysis of the liver chemical composition revealed a decrease in the moisture content in animals from all experimental groups compared to the control (Table 10).

All experimental groups showed increases in dry matter by 0.77 %, 2.91 % ($p \leq 0.05$), and 1.88 %. A statistically significant increase in fat content ($p \leq 0.05$) was also noted in all experimental groups by 0.39 %, 1.68 %, and 0.64 % relative to the control. The greatest effect was observed in group II, where fat content reached 4.31 % (versus 2.63 % in the control). Protein content also increased relative to the control value by 0.38 %–1.24 %. However, the data were not significant. Ash content remained unchanged.

All studied bioactive compound compositions had a significant effect on the liver, increasing fat and dry matter content. This may indicate activation of metabolic processes in the liver or changes in lipid metabolism. The most potent effect was demonstrated by the combination of cinnamaldehyde and 7-hydroxycoumarin (group II).

A comparative analysis of amino acid concentrations in the liver of test animals revealed no significant differences between groups (Table 11).

In all experimental groups, the dynamics of increasing content was recorded: lysine by 0.11 %–0.66 %, tyrosine by 0.05 %–0.18 %, phenylalanine by 0.02 %–0.27 %, histidine by 0.01 %–0.19 %, leucine + isoleucine by 0.06 %-0.66 %, valine by 0.03–0.32 %, proline by 0.03 %–0.19 %, threonine by 0.01 %-0.19 %, glycine by 0.08 %–0.3 %, respectively, relative to the control. In experimental group I, a slight increase in serine and alanine concentrations was observed by 0.01 % and 0.04 %, respectively. In experimental group III, arginine, serine, and alanine increased by 0.4 %, 0.17 %, and 0.4 %, respectively. Methionine levels remained virtually unchanged across all experimental groups.

It was found that the use of experimental additives in the diets also had a certain effect on the elemental composition in the liver of experimental broiler chickens (Table 12).

Table 9. Elemental composition of thigh muscles in test animals

Element	Group			
	Control	I	II	III
Ca, g/kg	0.29 ± 0.01	0.30 ± 0.01	0.33 ± 0.01^{b}	0.34 ± 0.01^{c}
P, g/kg	8.78 ± 0.30	8.67 ± 0.29	8.51 ± 0.28	8.26 ± 0.39
Na, g/kg	2.56 ± 0.14	2.68 ± 0.09	2.74 ± 0.10	2.70 ± 0.09
Mg, g/kg	1.09 ± 0.03	1.08 ± 0.05	1.05 ± 0.04	1.02 ± 0.04
K, g/kg	14.97 ± 0.61	14.65 ± 0.47	14.74 ± 0.52	13.71 ± 0.44
B, mg/kg	1.78 ± 0.10	1.69 ± 0.13	1.88 ± 0.11	1.76 ± 0.11
Mn, mg/kg	0.68 ± 0.02	1.07 ± 0.05^{c}	0.83 ± 0.03^{c}	0.65 ± 0.03
Co, mg/kg	0.02 ± 0.001	0.02 ± 0.001	0.02 ± 0.001	0.01 ± 0.001^{c}
Fe, mg/kg	35.79 ± 1.18	45.51 ± 1.96^{c}	42.27 ± 1.86^{b}	34.23 ± 2.67
Zn, mg/kg	46.76 ± 2.24	54.37 ± 2.17^{a}	55.77 ± 2.18^{b}	50.80 ± 1.68
Se, mg/kg	0.84 ± 0.367	0.76 ± 0.064	0.59 ± 0.065	0.77 ± 0.114
Cu, mg/kg	1.90 ± 0.06	2.33 ± 0.10^{c}	2.18 ± 0.08^{b}	2.19 ± 0.09^{b}

Note: ^{a} — $P \leq 0.05$; ^{b} — $P \leq 0.01$; ^{c} — $P \leq 0.001$ when compared to the control group.

Table 10. Analysis of the chemical composition of the liver in test animals

Mass fraction	Group			
	Control	I	II	III
Moisture, %	82.42 ± 2.32	81.65 ± 2.51	79.51 ± 2.49	80.54 ± 2.19
Dry matter, %	17.58 ± 0.71	18.35 ± 0.58	20.49 ± 0.86^{a}	19.46 ± 0.76
Fat, %	2.63 ± 0.09	3.02 ± 0.06^{a}	4.31 ± 0.36^{a}	3.27 ± 0.16^{a}
Ash, %	0.97 ± 0.006	0.97 ± 0.04	0.96 ± 0.06	0.97 ± 0.08
Protein, %	13.98 ± 0.46	14.36 ± 0.54	15.22 ± 0.68	15.22 ± 0.89

Note: ^{a} — $P \leq 0.05$ when compared to the control group.

Table 11. Amino acid content of the liver in test animals

Amino acid	Group			
	Control	I	II	III
Arginine, %	4.15 ± 0.18	4.03 ± 0.21	3.88 ± 0.19	4.55 ± 0.17
Lysine, %	4.12 ± 0.23	4.39 ± 0.31	4.23 ± 0.19	4.78 ± 0.28
Tyrosine, %	2.08 ± 0.13	2.19 ± 0.18	2.13 ± 0.14	2.26 ± 0.21
Phenylalanine, %	2.71 ± 0.09	2.76 ± 0.06	2.74 ± 0.09	2.98 ± 0.12
Histidine, %	1.59 ± 0.05	1.65 ± 0.07	1.6 ± 0.06	1.78 ± 0.07^{a}
Leucine + Isoleucine, %	7.9 ± 0.32	8.07 ± 0.29	7.96 ± 0.33	8.56 ± 0.37
Methionine, %	1.57 ± 0.07	1.59 ± 0.06	1.57 ± 0.04	1.57 ± 0.05
Valine, %	3.53 ± 0.16	3.56 ± 0.19	3.56 ± 0.14	3.85 ± 0.15
Proline, %	2.64 ± 0.10	2.68 ± 0.12	2.67 ± 0.09	2.83 ± 0.12
Threonine, %	2.69 ± 0.12	2.7 ± 0.11	2.7 ± 0.16	2.88 ± 0.18
Serine, %	2.71 ± 0.15	2.72 ± 0.12	2.68 ± 0.16	2.88 ± 0.14
Alanine, %	4.37 ± 0.19	4.41 ± 0.22	4.37 ± 0.16	4.77 ± 0.19
Glycine, %	2.95 ± 0.24	3.03 ± 0.19	3.03 ± 0.21	3.25 ± 0.17

Note: ^{a} — $P \leq 0.05$ when compared to the control group.

Table 12. Elemental composition of liver in test animals

Element	Group			
	Control	I	II	III
Ca, g/kg	0.36 ± 0.01	0.36 ± 0.02	0.39 ± 0.01^{a}	0.41 ± 0.02^{a}
P, g/kg	11.86 ± 0.55	12.18 ± 0.71	11.36 ± 0.37	11.71 ± 0.50
B, mg/kg	1.65 ± 0.07	1.58 ± 0.06	1.83 ± 0.12	1.61 ± 0.12
Na, g/kg	4.14 ± 0.18	4.77 ± 0.25^{a}	4.06 ± 0.14	4.15 ± 0.14
K, g/kg	11.60 ± 0.65	11.91 ± 0.73	10.76 ± 0.43	11.18 ± 0.40
Mg, g/kg	0.79 ± 0.03	0.86 ± 0.05	0.78 ± 0.02	0.79 ± 0.03
Mn, mg/kg	8.19 ± 0.48	7.51 ± 0.43	8.60 ± 0.28	7.46 ± 0.27
Fe, mg/kg	342.79 ± 14.40	224.10 ± 7.17^{c}	256.18 ± 9.22^{c}	307.84 ± 13.24
Zn, mg/kg	107.78 ± 3.99	111.12 ± 4.67	88.00 ± 3.08^{c}	126.02 ± 5.54^{b}
Co, mg/kg	0.07 ± 0.003	0.07 ± 0.004	0.06 ± 0.003^{a}	0.07 ± 0.004
Cu, mg/kg	14.94 ± 0.58	12.53 ± 0.43^{b}	12.08 ± 0.39^{c}	12.70 ± 0.46^{b}
Se, mg/kg	2.52 ± 0.26	2.60 ± 0.21	2.40 ± 0.37	2.64 ± 0.43

Note: ^{a} — $P \leq 0.05$; ^{b} — $P \leq 0.01$; ^{c} — $P \leq 0.001$ when compared to the control group.

Thus, a reliable decrease in Cu was noted in all experimental groups by 16.1 % ($p \leq 0.01$), 19.1 % ($p \leq 0.001$), 15.0 % ($p \leq 0.01$) relative to the control values. In the experimental group I, an increase in Na content by 15.2 % ($p \leq 0.05$) and a decrease in Fe by 34.6 % ($p \leq 0.001$) were observed. In the experimental group II, Fe, Zn and Co contents decreased by 25.3 % ($p \leq 0.001$), 18.4 % ($p \leq 0.001$) and 14.3 % ($p \leq 0.05$), and Ca content increased by 8.3 % ($p \leq 0.05$). In experimental group III, Ca and Zn contents increased by 13.9 % ($p \leq 0.05$) and 16.9 % ($p \leq 0.01$) compared to the control group. These results are consistent with previous studies showing that the inclusion of coumarin and *Bacillus cereus* probiotic in animal diets promotes an increase in Ca and Zn levels in liver tissue [39].

Since coumarin is a component of many plants, our results on the effect of 7-hydroxycoumarin on mineral metabolism may be compared with studies of plant extracts. For example, the addition of *Boswellia serrata* essential extract to broiler diets resulted in increased Ca levels in breast and thigh muscles and liver, and Mg levels in thigh muscles and liver, while simultaneously reducing Cu content in these tissues [40]. In our study, the addition of 7-hydroxycoumarin caused a similar effect on Ca (in thigh muscles and liver) and Cu (in the liver). Data on the direct effect of the studied bioactive compounds (cinnamaldehyde, quercetin, 7-hydroxycoumarin) on the mineral composition of broiler tissues are limited. However, it is known that the concentration of elements in the liver of farm animals depends on feeding and the environment [41]. Overall, our results confirm the ability of phytogenic additives to specifically influence the accumulation of microelements in animal tissues, where a moderate correlation with the total polyphenol content was observed [42]. This effect may be due to the antagonism between the components of the extracts and metal ions, as well as their ability to compete in the formation of metal complexes or chelate the transition metal ions [13, 43, 44], which ultimately modulates the accumulation of the latter in muscles and liver.

Conclusion

The study demonstrated the high efficacy of a combination of bioactive compounds (cinnamaldehyde, quercetin, and 7-hydroxycoumarin) in broiler chicken diets. The complete combination (group III) demonstrated the greatest synergistic effect, as evidenced by a significant increase in absolute weight gain by 51.4 % ($p \leq 0.05$) and a more than twofold increase in the EPEF.

The improved productivity of broiler chickens in the experimental groups was accompanied by positive changes in meat quality: a significant increase in breast muscle fat content by 0.2–0.9% ($p \leq 0.001$) and protein content by 2.2% ($p \leq 0.05$) in group II. The additives modulated mineral metabolism, causing statistically significant changes in Fe, Zn, and Cu concentrations in muscle tissue and liver ($p \leq 0.05$ to $p \leq 0.001$).

Thus, the obtained results demonstrate that the developed composition of bioactive substances, especially the complete composition (cinnamaldehyde, quercetin, and 7-hydroxycoumarin), is highly effective for stimulating growth, improving meat quality, and optimizing metabolic processes in broiler chickens. This combination is a promising alternative to antibiotic growth promoters in modern poultry farming.

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CAMEL HOMEOSTASIS AND MEAT QUALITY AFFECTED BY STRESS BEFORE SLAUGHTER- A LITERATURE REVIEW

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Keywords: *stress indicators, transport, handling, welfare, oxidant stress, Morocco*

Abstract
The welfare of dromedaries before and at slaughter influences their homeostasis and the organoleptic characteristics, quality, and commercial shelf life of their meat. Transportation, stocking density, travel time, waiting time at the slaughterhouse, water and food deprivation, and the slaughter procedure are the most important stressors for this species. In dromedaries, these events trigger behavioral, physiological, hematological, biochemical, hormonal, and muscular responses that could be assessed using reliable and specific biomarkers, namely, heart rate, respiratory rate, rectal temperature, hemolysis, neutrophil/lymphocyte ratio, enzymes, glycolytic potential, malondialdehyde, thiols, carbonyls, catecholamines, cortisol, and thyroid hormones. The dromedary, a large and difficult to handle animal, is faced with a lack of adopted transport vehicles, equipment, resources and training of breeders, drivers, and technicians on animal welfare, and with the slaughter procedure, which favors even more stressful situations. This literature review analyzes the impact of stress induced by the different stages of handling before slaughter on homeostasis and meat composition in dromedaries, using the results provided by recent work carried out on this theme. The dromedary is subjected to more stressful handling compared to ruminant species, which begins at the farm and then at the market, and continues with loading, transportation, distance traveled, stocking density during transportation, unloading, receiving, waiting time in slaughterhouses, deprivation of food and water, and the method of slaughter. These conditions do not meet international animal welfare standards resulting in significant alterations of homeostasis and meat quality of this species. Legislation on the welfare of dromedaries at all stages of the pre-slaughter process must be developed.

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Introduction

The one-humped dromedary (*Camelus dromedarius*) is considered a fundamental element of the economy and culture of arid and semi-arid environments, capable of providing various services, as it is used for transportation, recreation, racing, and production of meat, milk, leather, and fiber [1,2]. Historically, the dromedary was widely used as the main means of transporting humans and goods between countries, hence its nickname "ship of the desert" [3]. Thanks to its physiological adaptations to heat and dehydration, this animal thrives in dry, semi-arid, and tropical climates of Africa, Asia, and Oceania [4,5]. Furthermore, this species provides additional zootechnical value to people living in these environments [6].

The phases preceding slaughter, including capture, loading, transport [7], housing at the slaughterhouse, deprivation of water and feed [8,9,10], and stocking density [11,12] are considered major stress factors for dromedary camels and can have adverse effects on their health, welfare, and other aspects determining meat quality. It is therefore not surprising that camels must also experience positive emotions to achieve their well-being of which health is an important element [13,14,15]. However, while laws have been established in developed countries to protect the welfare of farm animals at various stages of production, transport, and slaughter [16,17], in Africa and Asia, there is no legislation or standards governing and protecting their welfare.

Camels share many similarities with other ruminants, but their specific needs are distinct due to their anatomical, physiological, and behavioral differences [1]. However, they are social, calm, docile, and peaceful herd animals that maintain strong bonds within the group and preserving their welfare requires experience with them, their handling and management, as well as knowledge of animal welfare, which recommends training in animal behavior and welfare for those handling and managing dromedaries [13,14]. Padalino et al. [18] recently surveyed 61 employees at the Birqash camel market in Cairo, Egypt, and found that the understanding of animal welfare was low and its meaning seemed to be ignored. In dromedaries, various markers have been identified as strong indicators of pre-slaughter stress, including physiological, biochemical, hematological and endocrine changes [7,8,9], leading to increased cortisol levels and lipid and protein oxidation [11,19], behavioral variations [20], changes in blood concentrations of several metabolites [21] and decreased immunity, which could have serious consequences for their welfare and immune function, as well as for the quality of their products [22,23]. Thus, early detection of stress is one of the most effective strategies to counter these disorders. In this species, the most common pre-slaughter stressors are loading, road transport, unloading, stocking density, stabling, water and food deprivation and ambient heat. However, the combination of behavioral, hematological, physiological, biochemical and hormonal measures will be of great use to reliably assess transport-induced stress in dromedaries. The present literature review discusses aspects and concerns about the impact of stress occurring before slaughter during transport, loading, unloading and lairage handling, and bleeding, on the homeostasis and meat composition of camels. This review exposes health and welfare, neurophysiology and assessment of stress, behavioral, hematological, hormonal biomarkers of stress, oxidant, thermal and pathological stress, and meat alteration by stress in camels, food safety of the consumer, legislation, conclusion and recommendations for protecting the welfare of camels during transport and at slaughterhouses.

Objects and methods

This manuscript narratively reviews studies focused on the impact of management factors during the preslaughter phases (i. e., handling, loading, unloading), and conditions of transport (i. e., stockage density in vehicle), water and food deprivation, and lairage (i. e., duration of waiting and stabling) on physiological, hematological, biochemical, hormonal, oxidant and behavioral responses and meat characteristic variation in camels. The present review includes previously published secondary data such as research articles from reputable journals, books, annual reports from national and international organizations, policy briefs, and other indexed scholarly materials related to the preslaughter stress in camels. Databases included Google Scholar, PubMed, ScienceDirect and Web of Science. After identifying the sources, the search criterion developed was that the literature must use keywords such as welfare, stress, preslaughter stress, thermal stress, heat stress, stress assessment, stress behavioral responses, stress hematological responses, stress hormonal responses, stress pathological responses, oxidant stress, meat alteration and meat safety, and availability of the full-text version. On the other hand, the review papers and articles irrelevant to the topic under study, or presenting no detailed results, were excluded from the review process. The papers were subjected to a primary screening of titles or/and abstracts performed independently by four authors. A total number of 145, 194, 98, and 125 documents were initially collected from Google Scholar, PubMed, ScienceDirect and Web of Science sites, respectively, and were screened for eligibility studies. From the first database, 130 references were related to camel following implementations of various inclusion and exclusion criteria and were selected for full review.

Camel health and welfare

According to Anses [24], animal welfare is defined as the positive mental and physical state linked to the satisfaction of its physiological and behavioral needs, as well as its expectations. This state varies depending on the animal's perception of the situation. Animal welfare has become an important topic, which must be taken into account when assessing legal requirements. Thus, good management practices and good husbandry conditions on a universal scale have stimulated significant research [25], and several studies have established the link between the welfare of farm animals during the handling stages [12,15] before slaughter and the quality and sustainability of their meat. Recently, in their work, Padalino and Faye [26] focused on health management, environmental conditions, handling, and behavioral assessment in dromedaries. Thus, the different authors agree on five freedoms guaranteeing the welfare of these animals, according to sanitary, physiological, zootechnical and psycho-behavioral criteria. The first recommends access to water and feed in appropriate quantities meeting the needs and physiological status of the animal species. For the second freedom, the breeding conditions must not induce psychological suffering to the animal. For the third, the animal must be in physical and thermal comfort. The fourth recommends the absence of pain, lesions and disease of the animal. The fifth freedom consists of the expression of normal behavior of the species. In this context, several research studies have focused on the stress of the dromedary camel under different conditions (disease management, husbandry practices, water and food deprivation, dehydration, loading and stocking density in the truck, transportation, travel time and waiting time at slaughterhouses, harsh environmental conditions and slaughter methods, revealed among the most effective factors that increase stress and, consequently, influence the quality of carcasses and meat [7–9,11,27]. The reactions of the dromedary camel to these stressful situations have been highlighted by the analysis of reliable physiological, biochemical, haematological [7–9], hormonal [10,12,21], behavioral [20], and tissue parameters [11,22]. Camels can be exposed to stress during breeding in traditional or intensive production systems, and during transport on foot or by truck. On the other hand, there is a clear and well-known link between the welfare of the dromedary and the quality of its products, and poor handling of this species could contribute to poor quality of its meat.

Neurophysiology of stress

Once stress is detected, the information is sent to the thalamus and the prefrontal cortex, which analyze the importance of the stressor [28]. Stress stimulates the sympathetic adrenal medullary system and then the hypothalamic-pituitary-adrenal cortex (HPAC) axis to release catecholamines and corticosteroids from the adrenal glands, which is why measuring the concentrations of these hormones in the blood, especially cortisol, is commonly used as a reliable biomarker of stress [29,30]. However, under stressful events, and in order to ensure the body gets readily available energy in an emergency action, mammals produce the catecholamines released first by the sympathetic nerve endings and then by the adrenal medulla, then cortisol which remains active in the body longer than these catecholamines [31]. Thus, for the evaluation of stress, the quantification of cortisol, the most common glucocorticoid molecule (or of its metabolites), has been shown to be the most direct and reliable indicator of the physiological state of an animal, and an index of its response following activation of HPAC axis and an environmental threat of its homeostasis [30,31]. The activation of HPAC axis by stress stimulates the release of corticotrophin-releasing factor by the hypothalamus, which stimulates the adenohypophysis to secrete adrenocorticotropic hormone in blood circulation which in turn stimulates the secretion of cortisol by the adrenal gland [31].

Stress assessment in different biological matrixes

The difficulties in obtaining blood samples and the recognition of the stressful effect of blood sampling are the main drivers of the use of minimally invasive sampling media as biomarkers of adrenal cortex responses to animal stress. In the dromedary camel, biomarkers include in addition to blood [8,9,32–35], urine [12,36], feces [37,38], hair [38] and saliva [39] in different stressful situations such as road transport, dehydration and environmental heat.

Normal values for cortisol levels in serum of healthy camels ranged between 3.6 and 32.6 ng/ml with a mean ± SEM of 10.3 ± 0.6 ng/ml. Saeb et al. [35] reported a serum level between 26 and 40 nmol/L (9.4 and 14.5 ng/mL), and normal values range reported by Faye and Bengoumi [40] were between 3 to 30 ng/ml. According to Hussen and Althagafi [23], serum cortisol levels were significantly higher in female camels (10.8 ± 0.6 ng/ml) than males (7.9 ± 0.9 ng/ml), in non-pregnant (12.1 ± 1.0) than pregnant (10.2 ± 0.8) female camels, and in lactating female camels (12.5 ± 1.0 ng/ml) than non-lactating (9.2 ± 0.7 ng/ml) female camels. During other physiological adaptations, cortisol increased in camels from baseline levels of about 21.9 ± 1.0 ng/mL to over 121.6 ± 5.4 on the day of parturition or increased from 37.1 ± 1.4 ng/mL one day before weaning to 48.0 ± 1.5 and 69.5 ± 1.9 ng/mL at weaning and 3rd day after weaning, respectively [41].

The dromedary camels were defined as stressed animals when their blood cortisol levels were between 40 and 60 ng/ml and were accompanied by obvious signs of fear (restlessness, moaning, kicking, defecation) [42]. However, in addition to the levels of cortisol measured in the blood, those analyzed in the hair and feces could be useful for a reliable retrospective assessment of long-term stress [37,38]. Collection of these samples minimizes stress on the animal and it is easy to transport and store them. In fact, it has been shown in dromedaries that an intravenous injection of adrenocorticotrophic hormone (0.5 mg/animal) increased the level of cortisol in the blood and that of glucocorticoid metabolites in the feces [37]. Thus, twenty-four hours after this injection, the level of cortisol increased from 0.6–10.8 to 10.9–42.2 ng/mL in the blood, and from 286.7 to 2559.7 ng/g in the faeces [37]. Furthermore, in the camel, blood concentrations of cortisol were positively correlated with those analyzed in saliva and/or hair or feces [38]. Cortisol levels in saliva fluctuate within minutes, while the production of feces can take hours to days depending on the species. Furthermore, in the dromedary camel, cortisol levels in blood were higher in females than males and in young animals than adult, and were positively correlated with those analyzed in saliva and/or hair or feces [38,43,44].

Behavioral responses

Dromedaries are generally known for their need for space to display their innate physical activity behavior [45]. After arriving at slaughterhouses following truck transport, camels showed disruptions in certain behaviors related to transport stress, including comfort (lying and standing), ingestion (feeding and rumination), elimination (defecation and urination), and body grooming (rubbing, scratching, and biting) [46]. According to Atkinson [47], animals subjected to road transport stress spent most of their time lying down during and at the end of the journey, and during the recovery period [48]. Camel exhaustion generally depends on their standing or crouching position during transport, and is related to the period and conditions of confinement in the moving means of transport [20,49]. In addition, the loading density of camels is the main concern for the animal during the journey [50], as it is a limiting factor for the animal to lie down in the truck during a long journey, and to be able to get up after a fall.

The frequency of grooming and scratching behavior is considered one of the signs of good health and vitality of animals [51]. In dromedaries, this frequency was reduced or absent during transport, which could be considered the first sign of disease and the main sign of distress, exhaustion and stress [20,52].

Feeding behavior disorders have been observed during transport and stabling and could be explained by the lack of space and social restrictions [52,53]. These disorders could be favored by the lack of watering and shelter or by dehydration induced by transport [54,55]. In addition, after being transported on foot or by trucks, dromedaries often continue to wait in markets or slaughterhouses without eating or drinking, which constitutes a situation of risk of health and behavioral problems in addition to that of transport stress, and which deserves attention to be improved and limited [12,25,56]. A decrease in feed consumption during rest periods following transport, associated with behavioral changes, was reported in dromedaries in a study conducted in Qatar [57]. Additionally, in dromedaries, road transport was responsible for a decrease in the time spent on ingestive behavior [20], as was the case in goats [58]. This effect could be explained at least in part by the prolonged period of dehydration resulting from the long journey, especially in the absence of stops for feeding and watering [59].

Finally, behavioral responses such as slipping, falling, belching, urination, and defecation were observed in dromedaries as stress responses recorded at the end of road transport, during unloading and transport to the stabling area. The frequencies of these responses were positively and significantly correlated with serum cortisol and Malondialdehyde (MDA) levels analyzed in the same animals [7,8]. Indeed, these behavioral responses to stress could be interpreted as pain, agitation, and fear in the presence of conditions that do not respect animal welfare during and after transport and unloading [60,61]. Furthermore, a decrease in elimination behavior by defecation and/or urination during transport of dromedaries was reported by Bekele et al. [59] and Emeash et al. [20], in contrast to transported cattle, which showed an increase in elimination frequency [62,63]. According to Fraser and Broom [16], the frequency of defecation and urination increases at the beginning of the journey under stress, then gradually decreases as the animal adapts and its feces become dry and scanty due to lack of food and water, dehydration and decreased intestinal activity during the journey.

Hematological parameters

Transporting dromedaries on foot had caused a decrease in the number of red blood cells (RBC) which could result from the stress of prolonged walking [20,64,65], while an increase in this count, hematocrit and hemolysis had been noted under the effect of transport in vehicles in camels [66] and goats [67]. According to El Khasmi et al. [7], these parameters were positively correlated with the transport distance. The increase in RBC count and hematocrit observed in dromedary camels 3 hours after rest following transport was lower than that recorded after 10 hours of rest [68]. This increase could be related to body dehydration due to thermoregulation and urination and the associated water deprivation, or to sympathetic activation of the adrenal gland, responsible for an increase in the synthesis and release of catecholamines into the circulation, which stimulates contraction of the spleen, thereby releasing RBC into the circulation [69–72]. Stress induced by handling during transport was able to reduce the leukocyte count resulting in a decrease of the immunity and disruption of the homeostasis in steers [65] and camels [20,68,73]. It should also be noted that camel transportation by truck was responsible for a significant increase in neutrophil-to-lymphocyte ratio (NLR) [7,20], and the values of this parameter were positively correlated with blood cortisol, glucose, and lactate levels under transport stress [21,50,74]. According to Lemrhamed et al. [50], dromedaries showed significantly elevated NLR and hemolysis at the end of a 2-hour transport with a high load density (1.44–1.80 m^2/dromedary). Under these same conditions, this increase persisted 12 to 16 hours after the end of transport [21]. In addition to their association with leukocytosis and neutrophilia (higher NLR), in dromedary camels, blood cortisol levels have also been associated with an increase in the CD4 T cell population, a reduction in the percentage of γδ T cells, a decrease in the expression of CD172a on neutrophils and monocytes, a decrease in the expression of CD14 and CD163 on monocytes, and an increase in the expression of CD45 and MHC I on lymphocytes [23].

Hormonal parameters

According to numerous studies, in camels, high blood cortisol concentrations are a reliable indicator of physical, psychological and physiological stress [7,35,36,75–77]. The response of camels to stressful situations has been investigated in several studies. Indeed, in camels that were trucked approximately 150 km in 2 to 3 hours, serum cortisol levels were significantly higher just after transportation compared to non-transported control camels (172.1 ± 29.6 ng/mL vs. 33.5 ± 3.8 ng/mL) [20]. Other studies have reported elevated cortisol levels in the same species transported by truck [7,35,36,66]. According to El Khasmi et al. [7], highly significant elevations in blood cortisol levels (between 88.32 ± 19.4 and 152.4 ± 25.18 ng/ml) as a function of journey length (between 72 and 170 km) were noted in dromedaries. In a recent study, serum cortisol levels were analyzed in 50 male dromedaries at the slaughterhouse, and they were high (80.2–107.2 ng/ml) to very high (133.7–198.0 ng/ml) in 26, and low in the other 24 camels (13.0–67.9 ng/ml) [23]. Similarly, Sayah et al. [78] measured high to very high serum cortisol levels in male Sahrawi camels at the slaughterhouse. Furthermore, serum samples collected from camels at the slaughterhouse, directly after transport and before slaughter, showed cortisol levels ten times higher than those of serum samples collected from camels in their breeding environment (116.6 ± 15.8 ng/ml vs. 6.8 ± 0.4 ng/ml) [23]. In a recent study conducted at the Casablanca slaughterhouse in Morocco, Lemrhamed et al. [8] observed that the most stressed camels were subjected to a long waiting period before loading (> 24 h), and during loading (> 15 min), unloading (> 5 min), water and food deprivation (> 24 h), and driving to the slaughter room (> 11 min), and they all had elevated serum cortisol concentrations. However, cortisol levels recorded in different studies are highly variable and remain difficult to compare [61].

Additionally, a high loading density in the transport truck, a long waiting period at the slaughterhouses after transport and the slaughter procedure induced a significant increase in blood cortisol levels in the dromedary camel [21,50,74]. Similarly, Sayah et al. [78] reported higher cortisol levels in dromedaries kept in stalls for a short period (874 ± 631 ng/ml) after arrival and before slaughter (1 to 8 h) than in animals kept for a longer period (48 to 96 h) (127 ± 39 ng/ml). The increase in cortisol in dromedaries during the different stages of their handling before slaughter could be due to their presence in an unknown environment and situation [79]. The released cortisol will play the role of an energy regulator meeting the metabolic and physiological needs of the animals [80]. It will induce catabolic activity in peripheral tissues (glycogenolysis, proteolysis and lipolysis) and anabolic activity in the liver (gluconeogenesis and protein synthesis) necessary for stress management [81,82]. In general, an increase in blood cortisol levels is recorded after 15 minutes of exposure to stress, then returns to basal levels after approximately 3 hours [82,83]. The animal could return to normal values after a rest period, but a period of lairage that does not respect good welfare conditions for the dromedary could again increase the concentrations of these hormones [74]. More recently, Sayah et al. [78] evaluated the effect of lairage time on animal stress and meat quality in male dromedaries of the Sahraoui population. The authors found that animals subjected to short-time lairage (from 1 to 8 h) were characterized by high cortisol and plasma glucose levels, compared to those subjected to long-time lairage (from 48 to 96 h). According to Kadim et al. [84], the return to the basal physiological and behavioral state of the dromedary, under conditions of food deprivation but with access to water, was observed after a lairage period of 12 to 24 hours. In dromedaries, in addition to cortisol, other hormones have been favored as indicators of stress. Indeed, road transport [36], a long waiting period at slaughterhouses after transport and the slaughter procedure, have been able to increase blood levels of thyroid hormones [21,74]. On the other hand, the period of handling of farm animals before slaughter is generally marked by deprivation of water and food, which could cause physiological stress marked by body dehydration. Indeed, camels that were severely stressed by dehydration showed a significant increase in cortisol (9.57 ± 0.72 ng/mL vs. 1.39 ± 0.05 ng/mL), noradrenaline, and dopamine, and a significant decrease in adrenaline and serotonin [85] in order to preserve body water, maintain cardiac responsiveness, and produce energy.

Oxidant stress

Increased production of reactive oxygen species (ROS) under oxidative stress (OS) can induce the formation of lipid peroxidation products and oxidized proteins, as well as oxidative damage to DNA and RNA. These ROS are normally eliminated by enzymatic antioxidants like catalase (CAT), glutathione peroxidase (GSH-Px), superoxide dismutass (SOD), etc, and non-enzymatic antioxidants like vitamins C and E, glutathione, melatonin, etc [86]. Indeed, incubation of red blood cells with these vitamins was able to attenuate oxidative alterations of these cells by hydrogen pyroxide and reduce the production of MDA in the incubation medium [87,88].

Under the effect of transport stress, dromedaries showed an imbalance in oxidant-antioxidant status, marked by an increase in SOD activity and a decrease in GSH-Px and tissue ascorbic acid [33,89]. In addition, El Khasmi et al. [7] evaluated the OS related to road transport distance and found that plasma cortisol and MDA levels as well as catalase activity increased progressively and significantly with transport distance. Furthermore, in a survey conducted recently in the slaughterhouse of Casablanca in Morocco, Lemrhamed et al. [8] recorded the duration of the preslaughter operations and the frequency of urination in camels. The authors found that the most stressed camels were submitted to long preslaughter operations and water and food deprivation period, and they all showed a high frequency of urination. Furthermore, these animals showed high circulating levels of MDA, and low CAT and SOD activities [8].

On the other hand, in the muscle (*Musculus abdominis obliquus externus)* of camels with high circulating cortisol levels (80.29–107.21 ng/mL) after transport, MDA levels were higher and CAT activities were lower than in camels with low cortisol levels (13.07–67.9 ng/mL) [9]. Thus, analyzing circulating cortisol levels after transport just before slaughter [7,21,50,66,74], can predict meat quality and bone status in camels [9,90]. Furthermore, in the same species, Barka et al. [90] reported that MDA levels increased significantly while CAT decreased significantly in meat (*Triceps brachii*, *Musculus obliquus*, and *Diaphragma*) when the transport distance before slaughter increased (72 km vs. 160 km).

Camels severely stressed by dehydration showed a significant increase in malondialdehyde and certain antioxidants such as glutathione, retinol, thiamine, and vitamin E [85]. In fact, in this species, Ali et al. [91] investigated the effects of long-term dehydration stress on kidney cortex and medulla with respect to pro-inflammatory markers and oxidative stress, and found a significant increase in the cytokines IL-1β and IL-18 levels, MDA and GSH, and a significant decrease in SOD and CAT. Camel granulosa cells exposed to 45 °C for 2 h also showed an increase in MDA levels associated with a significant increase in the expression of heat shock proteins (HSPs), DNA repair enzymes, and glutathione S-transferase [92].

Finally, exposure of dromedaries to cold-induced stress during the winter season resulted in a significant decrease in blood levels of certain antioxidants such as vitamins A, C, and E, and glutathione associated with an increase in ROS [33,93], suggesting a low antioxidant status of these animals during the winter season [88].

Thermal stress

When faced with environmental heat stress, the dromedary camel showed variations of physiological, biochemical, hematological and hormonal parameters. High ambient temperature increases the ruminant's effort to dissipate body heat, which leads to an increase in heart rate, respiratory rate, core body temperature and water consumption, while food consumption decreases [94–97]. In camels, a decrease in blood glucose in hot climates has been noted in an attempt to reduce their basal metabolic rate to a minimum level. This decrease in glucose utilization could be attributed to glucose consumption by respiratory muscles, decreased food intake, and hyperinsulinemia [98,99]. The increase in heart rate could be an attempt to improve cardiac output, thereby increasing blood flow to the peripheral circulation and facilitating greater heat loss through increased water evaporation at the body surface or the airway mucosa, which helps prevent hyperthermia [100,101].

Regarding the impact of summer heat stress on blood cortisol levels, in dromedaries, a higher cortisolemia (ng/mL) in summer than in winter was observed by Baraka [43] (38.6 ± 5.3 *vs* 28.5 ± 4.8) and Elias and Weil [102] (45.0 ± 11.9 *vs* 8.0 ± 1.3). A similar trend was reported in the same species for corticosteronemia by Zia-ur-Rahman et al. [34]. These authors explained this hyperactivity of the adrenal cortex observed in summer by the stress induced by the external heat load and the dehydration of the body following intense sweat losses, as a major thermolytic pathway. However, other studies, analyzed the values of cortisol in serum, hair and faeces at two distinct periods in 20 male dromedaries aged 3 to 8 years, from semi-extensive farms in the region of Essaouira and intended for slaughter at municipal slaughterhouses in Casablanca, Morocco. These studies reported that cortisol levels in serum (ng/mL), hair and feces (ng/g) were significantly higher in winter than in summer (66.01 ± 13.19 *vs* 25.71 ± 6.71; 0.93 ± 0.26 *vs* 0.61 ± 0.08 and 2.74 ± 0.14 *vs* 1.42 ± 0.35, respectively; $p < 0.05$) [38,103].

Concerning total vitamin D and 25-hydroxyvitamin D, in dromedary, serum levels were significantly higher during the summer season compared to the winter season and showed no significant variation with the season, in the liver, kidney, meat and hump [11,103,104]. According to Shany et al. [105], in dromedary, serum levels of 25-hydroxyvitamin-D (ng/mL) in summer and winter were 443 ± 96 and 276 ± 13, respectively. The increase in the levels of vitamin D and its metabolites in summer could be explained by the strong sunshine stimulating their biosynthesis. Other studies have shown that circulating vitamin D levels in llamas, alpacas and camels [106,107] are not influenced by age, but vary seasonally. These levels were highest during the period from February to July [107]. Variations in circulating vitamin D concentrations in camelids could also be impacted by coat color, month of birth, light intensity and physiological stage [106–109].

During transport, heat stress within the transport vehicle space is recognized as the main threat to animal welfare and health [110]. In fact, in the dromedary camel, a 2-hour road transport by truck during the hot season resulted in a significant increase in rectal temperature (°C), heart rate (beats/min) and respiratory rate (cycles/min) compared to the values measured before transport (40.2 ± 0.4 vs. 38.1 ± 0.3; 58 ± 4 vs. 46 ± 3 and 17 ± 2 vs. 11 ± 2, respectively) [66]. In addition, by comparing the effect of two loading densities in trucks on these physiological parameters, Lemrhamed et al. [50] found that heart rate and respiratory rate were significantly higher at high stocking density (1 camel/1.44–1.80 m^2) than those observed at low stocking density (1 camel/2–3.6 m^2) (62 ± 4 vs. 47 ± 4 and 20 ± 2 vs. 12 ± 1, respectively) which could incriminate thermal stress due to the reduction of surface area for animals' bodies and lack of ventilation. Rectal temperature and heart and respiratory rates, measured just after the camel was transported, approached basal values after 3 hours of rest and returned to their basal values after a 12–16 hours rest period [21,68].

In addition, camels transported by truck in a hot environment showed a significant increase in plasma MDA concentrations up to 24 hours after the end of transport, hyperthermia and increased hematocrit due to water loss through thermolysis, and hemolysis [19,66] suggesting oxidative alterations of the red blood cell plasma membrane [7].

Furthermore, Moussahil et al. [11] recently studied the *antemortem* effect of heat stress associated with high stocking density during transport, on the chemical composition of meat at the 24-hour post-mortem stage in the dromedary camel. The authors observed a significant decrease in pH and catalase activity, and a significant increase in exudate and cooking losses, electrical conductivity, and malondialdehyde and carbonyl contents in meat from animals transported at 29–35 °C with a stocking density of 1 animal/1.74–2.13 m^2 compared to animals transported under moderate temperature and stocking density conditions (21–23 °C and stocking density of 1 animal/3.12–4.31 m^2). Furthermore, dromedary meat exposed to heat (80 °C) was more oxidized than that exposed to 18 °C, showing high contents of malondialdehyde, carbonyls and peroxides, and lower activities of enzymatic antioxidants (CAT, SOD and GSHPx) [27].

Pathological stress

Livestock are exposed to environmental stresses during transport, such as noise, heat, cold, humidity, movement, and unusual social groupings. Furthermore, handling, loading and storage conditions in the vehicle, unloading, and confinement are responsible for distress, injury, and mortality, particularly when transport is not properly managed [111].

According to a recent study, a high incidence of respiratory problems, such as pneumonia, was observed in camels transported long distances in poor conditions [8,112]. This incidence could be explained by the presence of several well-known predisposing risk factors, including inappropriate handling, transportation, mixing, and overcrowding [3]. Transportation remains the main factor leading to stressful conditions and decreased welfare in dromedaries [35], which influences their physiology and behavior [20,55]. Transportation has been mentioned as the main cause of respiratory problems, diarrhea, and colic [18]. Therefore, the resulting pain causes camels suffering from these problems to exhibit abnormal behaviors at the slaughterhouse, associated with higher serum cortisol levels capable of modulating the immune system of these animals [15,23,113,114]. On the other hand, there is a high risk of spreading infectious diseases through various handling of livestock before and during transport [115]. Finally, when animals are exposed to heat stress for a long time, they could experience health problems, infertility, decreased growth, production and immune defense, and cellular and mitochondrial oxidative damage [116]. Thus, it is recommended to improve the transport conditions of these animals in order to preserve their health and welfare during and after transport, not only for them, but also for the welfare of people likely to consume their meat [12]. Table 1 summarizes the mean values of different stress and oxidative stress indicators measured before slaughter in the blood and after slaughter in the meat of camels.

Meat alteration

Different stress conditions before and/or during the slaughter of farm animals significantly influence the post-mortem biochemical transformation of their muscle into meat, by modifying the evolution of its ultimate pH (24 h postmortem stage) (Figure 1).

The camel meat is more prone to quick drip loss and *post-mortem* variations in pH, lipid oxidation rates, and oxidative metabolism due to high myoglobin and polyunsaturated fatty acids concentrations [117–120], thus, it would be more likely to be influenced by the various ante-mortem manipulations of the dromedary.

Table 1. Mean values of different stress and oxidative stress indicators analyzed before slaughter at the blood level and after slaughter at the meat level in camels

Stressors	Samples	Stress biomarkers	Non stressed	Stressed	References
Transport	Blood	Cortisol (ng/mL)	28.58±0.52	152.4±25.18	[44]
		Thyroxine (nM)	154.3±12.6	216.7±24.3	[36]
		Glucose (mM)	5.07±0.28	7.08±0.21	[7]
		Lactate (mM)	9.97±0.31	12.99±0.16	
		Hematocrit (%)	39.17±1.17	43.17±0.98	[66]
		MDA (nM)	1.58±0.38	3.88±0.20	[7]
		CAT (kU/L)	79.13±3.84	60.08±3.18	
	Meat	Glycogen (mmol/kg)	39.4–42.6	34.5–37.5	[36]
		24h *postmortem* pH	5.60±0.11	6.60±0.11	[9]
		Carbonyls (nmol/mg)	1.15±0.11	1.87±0.13	[22]
		Haem pigment (µg/g)	10.18±1.06	13.39±1.18	[9]
		MDA (nmol/kg)	112.74±15.53	157.23±18.41	[22]
		CAT (U/kg)	364.13±19.34	301.53±17.48	
		SOD (µmol/min/ mg)	8.67±0.14	8.67±0.14	
		Exsudate (%)	0.47±0.12	0.76±0.13	
		Drip loss (%)	9.23±1.12	12.56±1.45	[9]
		Cooking loss (%)	21.36±2.64	30.23±3.51	
Loading density	Blood	Hematocrit (%)	33.59±2.18	35.15±3.32	[50]
		NLR	0.91±0.01	1.31±0.11	
		Glucose (mM)	6.50±0.03	7.20±0.02	
		Cortisol (ng/mL)	25.21±0.67	53.33±5.33	
		T_3 (nM)	1.426±0.174	3.569±1.25	
		Thyroxine (nM)	66.50±5.86	110.8±19.93	
	Physiology	Cooking loss (%)	37.7±0.4	40.2±0.4	
		HR (beats/min)	47±4	62±4	
		RR (cycles/min)	12±1	20±2	
Heat	Meat	MDA (nmol/kg)	145±2	281±2	[11]
		Carbonyls (nmol/mg)	1,47±0,01	1,83±0,02	
		CAT (µmol/h/mg)	10,80±0,61	9,00±0,56	
		SOD (µmol/min/ mg)	9,23±0,14	7,86±0,11	
		Water retention (mg/g)	161,46±23,24	112,13±21,33	
		Exsudate (%)	0.43±0.04	0.72±0.06	
		Drip loss (%)	22,63±2,15	28,54±2,34	
		Conductivity (µS/cm/g)	41,59±3,53	52,45±4,37	
Waiting duration in abattoir	Blood	NLR	0.91±0.1	1.31±0.1	[21]
		Cortisol (ng/mL)	25.21±2.67	54.33±5.33	
		T_3 (nM)	1.42±0.17	3.56±0.25	
		Thyroxine (nM)	66.50±6.86	117.8±16.93	
		Glucose (mM)	7.28±0.03	8.14±0.02	

MDA — malondialdehyde, SOD — superoxide dismutase, CAT — catalase, GSHPx — glutathion peroxidase, HR — heart rate, RR — respiratory rate, NLR — neutrophile lymphocyte ratio, T_3 — tri-iodothyronine.

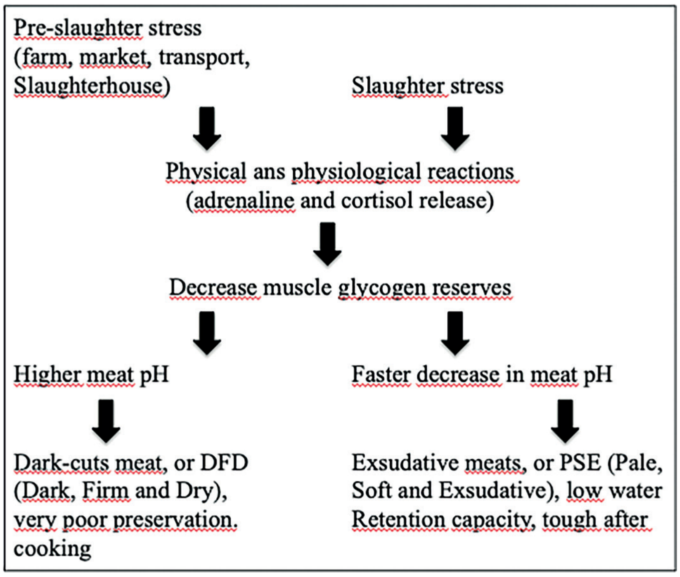

Figure 1. Impact of preslaughter and slaughter stress on the technological and sensory qualities of meat

Long transport distances are stressful factors that could affect the ultimate pH (pHu) (pH at 24 h postmortem) of camel meat. According to Barka et al. [90], the pHu values of muscles (*Triceps brachii*, *Musculus obliquus* and *Diaphragma*) had been higher in camels transported for 160 km compared with camels transported for 72 km only (6.4 ± 0.2 *vs* 5.7 ± 0.2; 6.5 ± 0.2 *vs* 5.6 ± 0.1 and 6.3 ± 0.1 *vs* 5.6 ± 0.2, respectively). High pHu values in camel meat had been associated with high circulating levels of cortisol at slaughter and low post-mortem levels of glycogen in meat [36]. The glycogen levels in camel muscles (*Triceps brachii*, *Musculus obliquus* and *Diaphragma*) (mg/100g) decreased significantly at high distance compared with short one (170 ± 20 *vs* 226 ± 25; 191 ± 21 *vs* 241 ± 27 and 180 ± 23 *vs* 237 ± 25, respectively) [90]. The low glycogen levels might be explained by the duration of the fast [121]. In the dromedary camel, the *antemortem* circulating levels of cortisol were positively correlated with the values of *postmortem* pH, drip loss, cooking loss, dimensional shrinkage and total haem pigment in meat [9]. However, in the same species, although blood levels of 25-hydroxyvitamin D were not influenced by transport stress, the content of this vitamin D metabolite in meat was negatively correlated with dripping loss and cooking loss [122], suggesting a possible role of this vitamin in dromedary meat quality.

At the end of transport, animals' inadequate waiting conditions at the slaughterhouses showed negative effects on the quality of the camel meat. More recently, Sayah et al. [78] evaluated the effect of lairage time on meat quality of male dromedaries of the Sahrawi population. The authors found that animals subjected to long-time lairage (from 48 to 96 h) were characterized by meat with high pH, mineral content, and moisture at 24 h postmortem, compared to those subjected to short-time lairage (from 1 to 8 h), probably due to depletion of glycogen reserves and lactic acid accumulation in the pre- and postslaughter period, and animal fatigue [117,123]. Additionally, Moussahil et al. [22] studied the impact of the durations of different stages prior to slaughter on the physicochemical and biochemical parameters of dromedary meat. The authors found that high duration of transportation (10–11 h), unloading (11–20 min), driving to the slaughterhouse (11–20 min), slaughter (11–20 min) and bleeding (8–9 min) were associated with significantly high exudate content, cooking loss, carbonyl and MDA contents, and significantly low catalase and superoxide dismutase activity in meat. Interrelationship of stress factors occuring before slaughter and their biomarkers and meat quality is presented in Figure 2.

Food safety

The World Organization for Animal Health regards pre-slaughter treatments and handling as important for animal welfare and the nutritional quality of their products [124]. However, handling of camels that does not comply with ethical rules or animal welfare protection laws alters organoleptic, sensory, and technological parameters, which could impact consumer health [84,117]. Moreover, particularly in camel-rearing countries where many diseases are prevalent, animals that have been stressed before

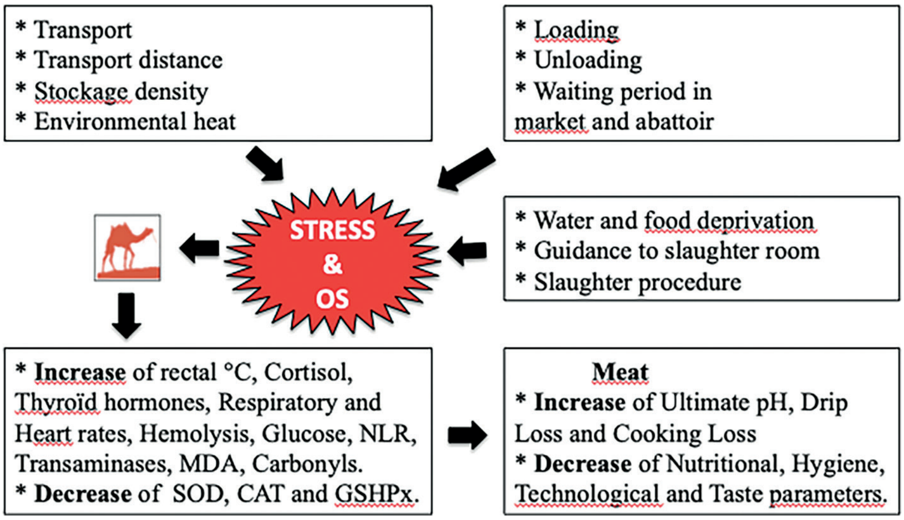

Figure 2. Interrelationship of pre-slaughter stress factors and biomarkers and meat quality (°C — temperature, NLR — neutrophile lymphocyte ratio, MDA — malondialdehyde, SOD — superoxide dismutase, CAT — catalase, GSHPx — glutathion peroxidase)

and during slaughter have pathogen levels ten times higher than normal, and produce poor-quality meat [124]. The sympathetic activity of the adrenal medulla and the adrenocorticotropic axis during stress affects the body's normal functioning, leading to decreased productivity and resistance to infections. Furthermore, the activity of these glands under stress reduces muscle glycogen concentrations, thus significantly reducing meat quality and the shelf life of meat products [61,84]. It should be added that exposing animals to pre-slaughter stress (transport, dehydration, hunger, overcrowding, mental stress, slippery floors, poorly designed premises, poor equipment, excessive noise, and darkness) further weakens their immunity and their ability to resist pathogens, and causes health problems for slaughterhouse employees and consumers [124].

Other factors can affect the nutritional and hygienic quality of meat and, consequently, the health of consumers. Camel slaughter is carried out without stunning, with the legs tied with a rope, in the presence of pain and injuries and without restraint. Camels are slaughtered in a crouching position with their heads held in a caudal position [74], which makes the animals excited and agitated, and reduces the likelihood of having good quality meat [61]. Slaughterers are not trained in animal welfare and food safety and do not have the technical knowledge or experience necessary to carry out their tasks [124].

Examples of guidelines for protecting the animal welfare

Unlike developed countries, very little information is available on camel welfare in relation to mishandling at slaughterhouses in underdeveloped countries. Indeed, measures relating to health controls, animal welfare protection and slaughter procedures have been regulated in Europe (Regulations EC853/2004, 854/2004 and 1099/2009 of the European Commission), Canada (Canadian Food Inspection Agency), the United Kingdom (Department of Environment, Food and Rural Affairs), the United States (United States Department of Agriculture) and New Zealand (National Advisory Committee on Animal Welfare, within the Ministry of Primary Industries) [124]. As an example of guidelines for protecting the welfare of dromedaries during slaughter, "The Australian Animal Welfare Standards and Guidelines" (www.animalwelfarestandards.net.au) is aimed at minimizing their stress, pain, and suffering. These animals are given 24 hours to move around and explore their new environment to acclimatize, reduce stress, and facilitate handling. Feeders and waterers are required for those confined for more than 24 hours. When loading, short, straight alleys and low-incline ramps must be used. Within 24 hours before slaughter, animals are examined by meat safety inspectors to ensure their good health and the quality of their meat, which is fit for human consumption. Just before slaughter, animals are led through a passageway to the slaughterhouse, where they enter a stunning cage and are then slaughtered by severing the main vessels in the neck. The slaughterhouse must meet these standards to obtain Animal Welfare Certification.

Conclusion

The welfare of farm animals and its impact on the quality of their products has long been of interest to scientific researchers in developed countries. However, this topic remains largely unexplored in developing countries, especially in relation to dromedaries, so, no country has conducted any tests or procedures to monitor or control the welfare of camels during transport, sale or slaughter, either through official veterinary services or an animal protection association. Like other domestic animals, dromedaries cannot escape stressful conditions that begin on the farm, at the breeding site, and at the market, and continue with loading, transportation, distance traveled, stocking density during transport, unloading, reception, waiting time, deprivation of water and food, and the slaughter process. This species is more susceptible to these various stress factors and oxidative stress, which can alter camel homeostasis and meat quality, potentially endangering animal health and, consequently, public health and food safety. In Morocco, like in other countries of Africa and the Middle East, the animal welfare standards of the World Organization for Animal Health are often not respected, and are almost non-existent when it comes to dromedaries. Indeed, their transportation and slaughter are not sufficiently regulated by law and are not subject to any official welfare controls. Similarly, since most countries in these regions do not have animal welfare laws, the development of specific laws and regulations addressing this issue remains mandatory, particularly with regard to the transportation, handling, deprivation of water and food, stabling, and slaughter of dromedaries. Furthermore, the vehicles are multipurpose and are neither adapted nor designed for the transport of dromedaries, nor equipped with loading and unloading devices, and offer no protection against harsh thermohygrometric conditions. The animals are also handled roughly by untrained and unqualified operators. Reduction of the time spent on various camel handling operations prior to slaughter, transport means and loading/unloading equipment appropriate for this species are required. Finally, to our knowledge, gaps in current research still exist regarding the topic of pre-slaughter stress/camel welfare/product quality/consumer safety, and no country has conducted tests or procedures to monitor and control camel welfare during transport or slaughter, or to analyze organoleptic and sensory quality under stressful conditions, either through official veterinary services or an animal protection association.

Recommendations

With the aim of addressing the behavioral needs of camels while adopting appropriate and sustainable technologies and practices that respect their welfare, we make the following immediate, medium-term, and long-term recommendations:

Immediate recommendations

- Provide camels with access to a clean water source and feed before slaughter to avoid prolonged periods of hunger and thirst.
- Keep camels in groups to demonstrate their social behaviors.
- Prevent camels from becoming injured, stressed, or overexcited during transport to the slaughterhouse and before slaughter.
- Prevent rough handling of camels by untrained and unskilled operators.
- Avoid prolonged periods of time spent on various camel handling operations before slaughter, such as transport, loading, unloading, and stabling.
- Unload camels as soon as possible after arrival at the slaughterhouse.
- At the end of the transport, unload and move the camels to the stabling area and then to the slaughterhouse, after separating sick or injured ones.
- Never slaughter camels in front of other camels.
- Never leave sharp knives in front of the camels to be slaughtered.
- In the slaughterhouse, thoroughly clean the bleeding trap before slaughtering each camel.
- Encourage respect for camel welfare, their inspection at slaughterhouses and the inspection of their meat by veterinary services to protect consumer health.

Medium-term recommendations:

- Camels must express their feeding behavior, have access to pasture, and be free to walk.
- Camels must have a dedicated area for their handling and observation.
- V-shaped circulation corridors must be used to guide camels after unloading in good conditions.
- Comfortable areas for movement, comfortable rest, and protection from pain, distress, and suffering must be provided, while avoiding poorly designed facilities and allowing animals to recover properly after the transport stress.
- Veterinarians must be regularly present at markets and slaughterhouses to care for camels and ensure their welfare during transport and slaughter.
- Persons handling or transporting camels must have practical experience and/or sufficient training in animal welfare.

Long-term recommendations:

- Slaughterhouse staff must be trained and public awareness about camel welfare and consumer health must be raised.
- Camel transport drivers must be trained and certified to obtain a mandatory license.
- Facilities that allow for the expression of social behaviors, other behaviors, and good human-animal relations must be provided.
- Guidelines and procedures for unloading, holding, and moving camels at markets and then to slaughterhouses must be developed.
- Vehicles must be suitable and designed for transporting dromedaries, equipped with suitable loading and unloading equipment, and protected against harsh thermohygrometric conditions.
- Procedures for stunning dromedaries before bleeding that comply with halal requirements must be developed.
- Finally, national legislation on the welfare of dromedaries at all stages of the process prior to slaughter, in accordance with international standards, must be developed.

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MEAT SAFETY KNOWLEDGE, ATTITUDE, AND PRACTICES OF POULTRY SLAUGHTERHOUSE WORKERS IN BORDJ BOU ARRERIDJ PROVINCE, ALGERIA

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Abstract

Meat handlers play a crucial role in preventing meat contamination and the spread of meat-borne diseases by following proper hygiene practices. This study aims to assess the level of knowledge, attitudes, and practices (KAPs) of 89 workers in 11 operational poultry slaughterhouses, located in Bordj Bou Arreridj region (Algeria). A cross-sectional study was conducted between February, 2024 and May, 2024, using a structured questionnaire and face-to-face interviews to collect data. The results showed that all respondents (100 %) were male, and all of them lacked formal food safety training, and over one-third of them (35.9 %) had a high level of education. The mean scores for workers' knowledge, attitudes, and practice were 9.90 ± 3.77, 16.21 ± 3.07, and 57 ± 8.7, respectively. The results indicated that most participants (78.7 %) displayed insufficient knowledge, particularly on foodborne pathogens (21.6 %) and foodborne diseases (26.1 %). Although 78.7 % of workers had positive attitudes, 71.9 % of them were using poor practices regarding personal hygiene and obligation of wearing appropriate protective clothes. The level of education significantly associated with the KAP levels ($p = 0.000$, $p = 0.002$, and $p = 0.000$, respectively). In addition, a significant positive correlation was observed between knowledge and attitudes ($r_s = 0.563$, $p < 0.001$), as well as between knowledge and practices ($r_s = 0.389$, $p < 0.001$). These findings indicate that regular practical training is imperative for improving the knowledge, attitudes, and practices (KAPs) of meat handlers regarding meat safety and for preventing meat-borne diseases in the study area.

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Introduction

Foodborne diseases are a significant global issue, affecting approximately 600 million individuals and causing 420,000 deaths each year [1]. These diseases also lead to an estimated US$110 billion in losses in terms of productivity and medical expenses in low- and middle-income countries, thus making them a global health issue and economic burden [2]. Foods of animal origin, particularly meat, are most often considered to be the cause of food poisoning [3].

White meat is an essential part of the human diet since it is an important source of animal protein, vitamins, minerals, and essential amino acids. Due to its nutritional value and reasonable price, it is widely consumed around the world and is considered the most popular meat in Algeria compared to other types of meat [4]. However, it is often contaminated with microorganisms, mainly with bacteria such as *Campylobacter* spp., *Salmonella* spp., *Escherichia coli* O157: H7, *Staphylococcus aureus*, and *Listeria monocytogenes*, causing serious public health problems [5,6]. Furthermore, the sources of contamination by these pathogens are variable; they can originate from sick animals, the production environment, from the meat handlers, and from the contaminated water used during the slaughtering process. [7,8]. Therefore, preventing animal diseases and implementing strict hygiene policies during meat production are both essential for reducing the incidence of meat-borne diseases [8,9].

Meat handlers play a vital role in the cross-contamination of meat. In addition to acting as vectors for numerous pathogens derived from the slaughter environment, such as contaminated tools or the animals themselves, they can serve as reservoirs for various pathogenic bacteria, including *Staphylococcus aureus*, which can colonize their hands and noses [10]. Foodborne outbreaks caused by *Staphylococcus aureus* associated with human-induced contamination pose a threat to consumers' health, particularly those concerning methicillin-resistant *Staphylococcus aureus* (MRSA) [11]. While good hygiene practices, such as proper hand washing with antimicrobial soap and wearing intact clean gloves when handling food, play an important role in preventing the transmission of foodborne pathogens [12,13].

Results of the surveys on the knowledge, attitudes, and practices of food handlers are widely used around the world; they serve as a diagnostic tool that can be used to identify weak points in food safety in order to recommend effective training and solutions to prevent food poisoning [14]. Several studies addressing various topics related to poultry meat safety in poultry slaughterhouses have been conducted in different regions of Algeria [15,16]. However, to our knowledge, no research has focused on the KAP of the workers in slaughterhouses or in other food businesses in the country. Thus, this study aims to assess the knowledge, attitudes, and practices of meat handlers in poultry slaughterhouses regarding meat safety and to examine the impact of participants' socio-demographic characteristics on their KAP level.

Objects and methods

Study design

A cross-sectional study was conducted between February, 2024 and May, 2024 to assess the level of knowledge, attitudes, and practices of the meat handlers, who worked in 11 operational poultry slaughterhouses located in Bordj Bou Arreridj province, northeast Algeria. This province is acknowledged as the third-largest poultry producer in the country, with an annual production exceeding 35,000 tons [17].

Sample group size

First, contact was made with the veterinary services of the Bordj Bou Arreridj province to explain the purpose of the study, identify the location of poultry slaughterhouses, and estimate the number of workers, which was equal to 104. Next, a sample group of 82 people was calculated using the Yamane formula [18]:

$$n = \frac{N}{1 + N(e)2}, \tag{1}$$

where,

n: sample size;

N: population size;

e: level of precision at 95 % confidence interval.

Out of 104 workers, only 89 agreed to participate in this study, representing a response rate of 85.57 %.

Questionnaire

Based on previously published meat safety studies [19,20], a structured questionnaire that consists of four sections was adopted and adjusted in English first, and then it was translated into the local language (Arabic) in order to achieve the aim of this study. The first section included 8 questions related to socio-demographic profiles of the workers (such as gender, age, educational level, work experience, marital status, employment status, and food safety training). The second section examined a workers' knowledge on meat safety, using 20 closed questions focused on personal hygiene, cross contamination, food-borne pathogens, meat-borne illnesses, and time-temperature control. Each question was provided with three optional answers: "true", "false" and "I don't know". The third section aimed to investigate the attitudes of the workers toward good hygiene practices using also 20 statements with three possible choice answers: "agree", "disagree" and "uncertain". Participants' practices were assessed through self-reported practices in the last sections of the questionnaire. This section had 18 questions addressing their hygiene practices and their wearing of protective cloth during the work. Each question required five levels of answers (never, rarely, sometimes, often, and always).

For the knowledge and attitude sections, a score of one point was awarded for each correct answer or statement, whereas the rest of the responses obtained zero. On the other hand, for the practices section, correct responses were scored from 1 (never) to 5 (always), and reversed scoring was employed for items 1, 2, 15, 16, 17, and 18. The total score of each participant was calculated by summing up the correct answers, and the range of scores for Meat Safety Knowledge (MSK), Meat Safety Attitude (MSA), and Meat Safety Practice (MSP) sections was 0–20, 0–20, and 18–90, respectively. Based on the established method [21], the total score was converted to 100 %, and graded as poor (<70 %) or good (≥70 %).

To improve the clarity of the questionnaire, a pre-test was conducted with 15 workers at a slaughterhouse located outside the study area. Based on their feedback, minor modifications were carried out for the final version of the questionnaire. However, the results of this pilot study were not included in the final data pool.

Data collection and statistical Analysis

In order to ensure the accuracy of the responses, a face-to-face interview was carried out by the first author with the assistance of veterinary inspectors during routine slaughterhouse inspections to encourage the workers' participation in this study. First, data was entered on Microsoft Excel and then analyzed through SPSS (Statistical Package for Social Sciences) Version 16. The study provided descriptive statistics (frequency and mean value). Chi-square ($\chi 2$) was used to find the relationship between the sociodemographic profile of the participants and their KAP levels, while Spearman's correlation coefficient was also applied to check the correlation between knowledge, attitudes, and practices scores. A p-value < 0.05 was considered statistically significant for all tests.

Ethical consideration

This research was reviewed and approved by the scientific committee of the Agri-food department, faculty of natural and life sciences at the University of Saad Dahlab Blida1 (Approval No.105/DSA/2023). Moreover, the permission to conduct the study was provided by the veterinary services department of Bordj Bou Arreridj state (Approval No. 1472/IVW/2023). Verbal consent was obtained after a clear explanation of the purpose of the study to all slaughterhouse workers, ensuring them in anonymity and confidentiality of their data in compliance with the World Medical Association's Code of Ethics Declaration of Helsinki [22].

Results

Demographic characteristics of the participants are illustrated in Figure 1. All respondents (100 %) were male and were untrained in food safety, but had their regular health check-up every 6 months, except for 4 people newly recruited. Out of 89 meat handlers involved, more than a third (36 %) were over 40 years old, while none of them was younger than 20 years. The study revealed that one-fourth of respondents (24.7 %) had high-level education and 11.2 % had graduated from a university. Additionally, approximately 44 % of total workers surveyed have less than 5 years of work experience in this sector. Nearly half of respondents (44.9 %) are daily workers, and the majority of them (71.9 %) are married.

Figure 2 summarizes the level of respondents' knowledge categories on meat safety (results for each question were provided as supplemental material). The mean score (SD) for the workers' knowledge was 9.90 (3.77), ranging between 1 and 19 points. However, a high percentage of correct answers (86.2 %) and (68.5 %) were obtained for the questions related to personal hygiene and cross-contamination, respectively. However, the majority of employees possessed poor knowledge of foodborne pathogens (21.6 %) and foodborne diseases (26.1 %). In addition, the workers were least aware on temperature and time control (42.7 %).

Attitude results are summarized in Table 1. The mean score (SD) for the workers' attitude was 16.21 (3.07), ranging between 10 and 20 points. Nearly all the respondents (97.8 %) agreed that washing hands and surfaces before starting work and disinfecting the working knives between meat processing are important practices that reduce the risk of meat contamination. In addition, 94.4 % of workers confirmed that they should use gloves while working, especially when they have injured hands, and they should cover mouths and noses when coughing or sneezing. In contrast, only a few workers disagreed with the following statements: workers can only contaminate meat when they are sick (12.4 %); wearing rings or watches during work increases the risk of meat contamination (18.0 %), and neither smoking (16.9 %), no rubbing hands over face or hair (19.1 %) while working contaminate meat.

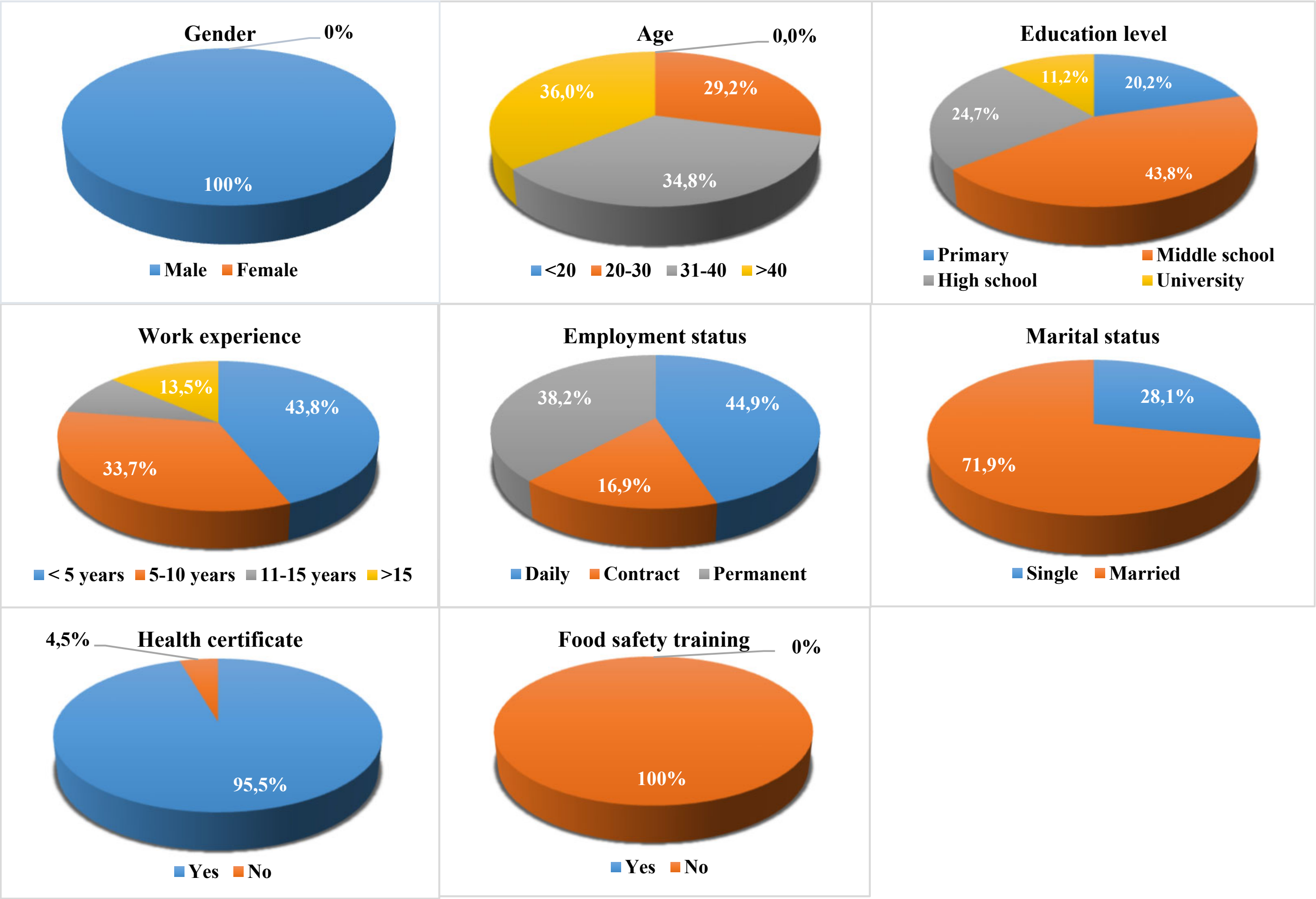

Figure 1. Socio-demographic profiles of a poultry slaughterhouse workers (n = 89)

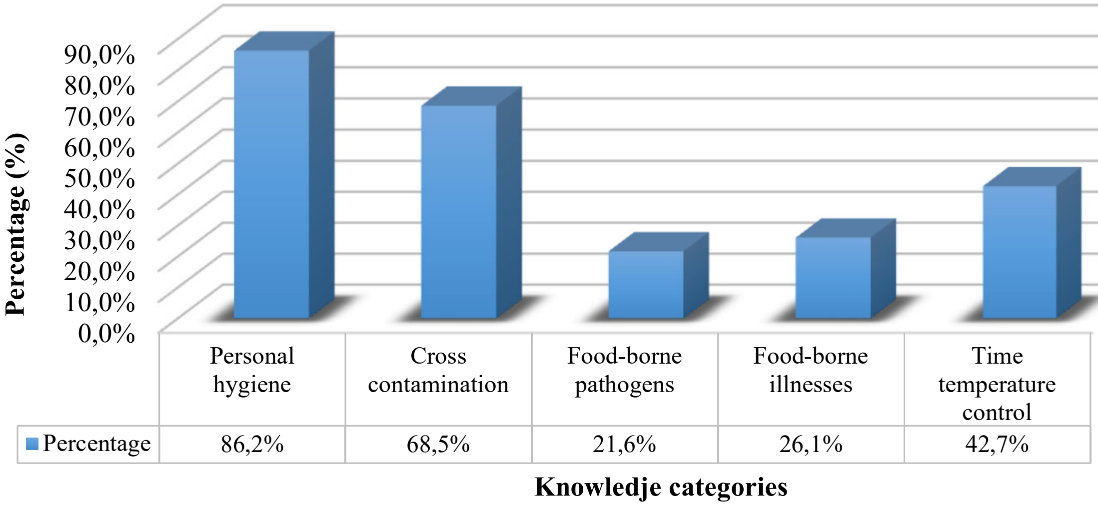

Figure 2. Meat handler's correct responses (%) according to different knowledge categories

Table 1. Summary of responses on meat safety attitude statements of 89 workers in poultry slaughterhouses, Bordj Bou Arreridj province, Algeria

Attitude statements		Responses, n (%)		
		Agree	Disagree	Uncertain
A1	Foodborne illnesses can have harmful effects on a community's health and economy.	78 (87.6)	—	11 (12.4)
A2	Safe meat handling to prevent food-borne illnesses is part of your job responsibilities.	66 (74.2)	11 (12.4)	12 (13.5)
A3	Workers with injured hands must not touch meat without gloves.	84 (94.4)	2 (2.2)	3 (3.4)
A4	Workers can only contaminate meat when they are sick.	51 (57.3)	11 (12.4)	27 (30.3)
A5	Wearing rings or watches during work increases the risk of meat contamination.	58 (65.2)	16 (18)	15 (16.9)
A6	Using gloves is an important practice to improve meat safety.	84 (94.4)	1 (1.1)	4 (4.5)
A7	Wearing apron is an important practice to improve meat safety.	78 (87.6)	7 (7.9)	4 (4.5)
A8	Wearing masks is an important practice to improve meat safety.	77 (86.5)	6 (6.7)	6 (6.7)
A9	Wearing caps is an important practice to improve meat safety.	66 (74.2)	17 (19.1)	6 (6.7)
A10	Proper hands washing before starting work reduces the risk of meat contamination.	87 (97.8)	—	2 (2.2)
A11	Workers should cover their mouths and noses when coughing or sneezing.	84 (94.4)	—	5 (5.6)
A12	Workers should not smoke during work.	58 (65.2)	15 (16.9)	16 (18)
A13	Workers should not rub their hands on face, hair, etc. during work.	47 (52.8)	17 (19.1)	25 (28.1)
A14	Health status of the workers should be evaluated before employment.	77 (86.5)	3 (3.4)	9 (10.1)
A15	Food safety training for workers could improve meat safety and hygiene practices.	71 (79.8)	4 (4.5)	14 (15.7)
A16	Work surfaces and utensils should be properly cleaned before starting work.	87 (97.8)	—	2 (2.2)
A17	Knives should be properly sanitized or changed between meat processing to prevent meat contamination.	87 (97.8)	—	2 (2.2)
A18	Workers should use potable water to wash working surfaces and cutting tools after disinfection.	81 (91)	3 (3.4)	5 (5.6)
A19	Refrigerator temperature should be checked periodically to reduce risk of meat contamination.	81 (91)	—	8 (9)
A20	Improper meat storage can cause health issues for the consumers.	81 (91)	—	8 (9)
	Total	16.21 ± 3.07[a]; (10–20) [b]		

[a] Mean score ± SD. [b] (Min-Max)

According to the self-reported practice results (Table 2), the mean score (SD) for the workers' practice was 57 (8.7), ranging between 40 and 75 points. Only 33.7 % and 60 % of workers do not eat/drink or smoke while working inside the slaughterhouse, respectively. Considering handwashing practices, less than half of respondents reported that they sometimes wash their hands before work (39.3 %), after a rest time (43.8 %), or after smoking, sneezing, or coughing (48.3 %). This study also found that a high percentage of participants always or often washed their hands properly after handling waste (85.4 %) and using the toilet (94.4 %), but 84.5 % of them never or rarely take off their equipment/clothes when using the bathroom. In addition, slightly more than half (51 %) never use masks, and the majority (83.1 %) do not use hair covers while working.

Chi-square (χ^2) test was performed to assess the association between respondents' demographic characteristics and their meat safety KAP levels (Table 3). The results reveal that the majority of participants had a poor level of knowledge (78.7 %) and practice (71.9 %), while 78.7 % had a good level of attitude regarding meat safety. In our study, education level was significantly associated with knowledge level ($\chi^2 = 14.933$; $p = 0.000$), attitude level ($\chi^2 = 9.882$; $p = 0.002$), and practice level ($\chi^2 = 19.615$; $p = 0.000$). On the other hand, work experience showed a significant relationship with attitude level ($\chi^2 = 6.911$; $p = 0.032$). Overall, age, marital status, and professional status were not significantly associated with the KAP levels of practice of safe meat handling.

Table 2. Summary of responses on meat safety practice statements of 89 workers in poultry slaughterhouses, Bordj Bou Arreridj province, Algeria

Practices Statements		Responses, *n* (%)				
		Never	Rarely	Sometimes	Often	Always
P1	Do you eat or drink while working in the slaughterhouse?	30 (33.7)	11 (12.4)	41 (46.1)	4 (4.5)	3 (3.4)
P2	Do you smoke while working in the slaughterhouse?	53 (60)	7 (7.9)	15 (16.9)	8 (9)	6 (6.7)
P3	Do you wash your hands properly before starting work?	4 (04)	8 (9)	35 (39.3)	26 (29.2)	16 (18)
P4	Do you wash your hands before returning to work after a break?	5 (06)	27 (30.3)	39 (43.8)	7 (7.9)	11 (12.4)
P5	Do you wash your hand properly after smoking, sneezing or coughing?	2 (02)	21 (23.6)	43 (48.3)	7 (7.9)	16 (18)
P6	Do you use gloves while working at the slaughterhouse?	11 (12)	21 (23.6)	33 (37.1)	13 (14.6)	11 (12.4)
P7	Do you wash your hands properly before or after using gloves?	22 (25)	27 (30.3)	34 (38.2)	5 (5.6)	1 (1.1)
P8	Do you wash your hands properly after handling waste?	—	—	13 (14.6)	34 (38.2)	42 (47.2)
P9	Do you remove your work equipment/ clothes when using toilets?	55 (62)	20 (22.5)	10 (11.2)	—	4 (4.5)
P10	Do you wash your hands properly after using the toilet?	—	—	5 (5.6)	10 (11.2)	74 (83.1)
P11	Do you wear an apron while working at the slaughterhouse?	8 (09)	14 (15.7)	17 (19.1)	20 (22.5)	30 (33.7)
P12	Do you wash your apron after the workday?	18 (20)	30 (33.7)	30 (33.7)	11 (12.4)	—
P13	Do you use a mask while working at the slaughterhouse?	45 (51)	27 (30.3)	13 (14.6)	3 (3.4)	1 (1.1)
P14	Do you wear a hair cover while working at the slaughterhouse?	74 (83.1)	9 (10.1)	4 (4.5)	1 (1.1)	1 (1.1)
P15	Do you handle meat when suffering from gastroenteritis, coughs or skin diseases?	26 (29)	28 (31.5)	35 (39.3)	—	—
P16	Do you handle meat when you have cuts, wounds, bruises or hand injuries?	23 (26)	20 (22.5)	35 (39.3)	11 (12.4)	—
P17	Do you wear jewelry (rings, watches or other personal items) while working?	47 (53)	9 (10.1)	23 (25.8)	6 (6.7)	4 (4.5)
P18	Do you rub your hands over your face, hair, etc. while working?	12 (13)	13 (14.6)	54 (60.7)	10 (11.2)	—
	Total	57.0 ± 8.7[a]; (40–75) [b]				

[a] Mean score ± SD. [b] (Min-Max)

Table 3. Association between socio-demographic characteristics of respondents and their knowledge, attitude and practice levels on meat safety

	Knowledge level			Attitudes level			Practices level		
	Poor *n* (%)	Good *n* (%)	*p*-Value	Poor *n* (%)	Good *n* (%)	*p*-Value	Poor *n* (%)	Good *n* (%)	*p*-Value
Age (years)									
≤30	22 (22)	4(15.4)	0.514	6 (23.1)	20(76.9)	0.943	20(76.9)	6(23.1)	0.640
31–40	21(80.8)	5(19.2)		5 (19.2)	21(80.8)		17(65.4)	9(34.6)	
>40	27 (73)	10(27)		8 (21.6)	29(78.4)		27(73)	10(27)	
Education level									
Low level	52(91.2)	5(8.8)	0.000*	18(31.6)	39(68.4)	0.002*	50(87.7)	7(12.3)	0.000*
High Level	18(56.3)	14(43.8)		1 (3.1)	31(96.9)		14(43.7)	18(56.3)	
Work experience									
<5 years	31(79.5)	8 (20.5)	0.522	13(33.3)	26(66.7)	0.032*	28(71.8)	11(28.2)	0.967
5–10 years	25(83.3)	5(16.7)		5 (16.7)	25(83.3)		22(73.3)	8(26.7)	
>10 years	14 (70)	6(30)		1 (5)	19(95)		14(70)	6(30)	
Marital status									
Single	19 (76)	6(24)	0.703	6(24)	19(76)	0.703	17(68)	8(32)	0.608
Married	51(79.7)	13(20.3)		13(20.3)	51(79.7)		47(73.4)	17(26.6)	
Employment status									
Daily	30 (75)	10(25)	0.085	13(20.3)	51(79.7)	0.857	26(65)	14(35)	0.385
Contract	15 (100)	0(0)		4(26.7)	11(73.3)		11(73.3)	4(26.7)	
Permanent	25(73.5)	9(26.5)		7(20.6)	27(79.4)		27(79.4)	7(20.6)	
Total	70(78.7)	19(21.3)		19(21.3)	70(78.7)		64(71.9)	25(28.1)	

Note: Low level (primary/ Middle school). High Level (High school / University). *p-value < 0.05 indicates statistical significance.

Based on Spearman's rho (Table 4) significant positive correlation was found between knowledge and attitude ($r_s = 0.563$, $p < 0.001$) as well as between knowledge and practice ($r_s = 0.389$, $p < 0.001$). This study also shows a significant association between the level of education and knowledge ($r_s = 0.564$, $p < 0.001$), attitude ($r_s = 0.220$, $p < 0.05$), and practice ($r_s = 0.566$, $p < 0.001$). However, no correlation was obtained between attitude and practice. In addition, the rest of the socio-demographic characteristics of the respondents provided no significant effect on the KAP score of workers, except for the correlation of work experience to attitude ($r_s = 0.309$, $p < 0.01$).

Discussion

This study is the first carried out in Algeria to assess the level of meat safety knowledge, attitudes, and practices among poultry slaughterhouse workers in the Bordj Bou Arreridj province. Similar figures and trends were observed with regard to gender and lack of food safety training when examining and comparing the socio-

Table 4. Correlation between various socio–demographic variables and KAP score the respondents

Variables	Age	EL	WE	MS	ES	MSK	MSA	MSP
Age	1.000							
EL	–0.002	1.000						
WE	0.549**	–0.017	1.000					
MS	0.655**	0.054	0.393**	1.000				
ES	0.355**	0.047	0.421**	0.175	1.000			
MSK	0.049	0.564**	0.162	–0.008	0.090	1.000		
MSA	0.062	0.220*	0.309**	0.114	0.178	0.563**	1.000	
MSP	0.001	0.566**	–0.020	–0.008	–0.146	0.389**	0.184	1.000

Note: EL = Education level. WE = Work Experience. MS = Marital Status. ES = Employment Status. MSK = Meat Safety Knowledge. MSA = Meat Safety Attitude. MSP = Meat Safety Practice; * Correlation is significant at the 0.05 level (2–tailed); ** Correlation is significant at the 0.01 level (2–tailed).

demographic profile of the participants in this study with the results of the recent meat safety studies carried out in other countries, such as Ethiopia [21], Burkina Faso [23], Kenya [24], and Bangladesh [25], where the male workers (untrained) accounted for 79.1 % (98.9 %), 82.6 % (96.8 %), 96.2 % (92.3 %), and 100 % (100 %), respectively. The predominance of men in this sector can be explained by the fact that slaughterhouses are located in rural areas far from residential areas. The training can improve food handlers' knowledge, attitudes, and practices on food safety [26,27]. The mean age of the workers was 37.2 ± 9.2 years, which is higher than the ages reported by [21] and [20] (29.7 ± 9.6 and 31 ± 9.4, respectively), but lower than the age reported by [24] (41.51 ± 10.95). In this study, a considerable share of the participants possessed a high level of education (high school and university), exceeding one-third share. This phenomenon can be explained by a lack of job opportunities in the Algerian labor market [28].

The mean ± SD of the knowledge score was 9.90 ± 3.77, which was comparably lower than that of meat handlers surveyed in Iran [19] and Ethiopia [21], but higher than that obtained in Bangladesh [20], where the reported averages were 11.7 ± 3.1, 13.12 ± 2.33, and 7.0 ± 1.9, respectively. In addition to the fact that the participants in this study were unaware of the appropriate temperature for storing meat and the effect of freezing on microorganisms, they demonstrated poor knowledge of foodborne diseases and foodborne pathogens such as *Escherichia coli*, *hepatitis A virus,* and *Salmonella*. A study conducted by Elgroud et al. [29] showed that avian salmonella isolated from broiler chicken farms and slaughterhouses in the Constantine province of Algeria may contribute to the emergence of human *Salmonella* strains, which is a disease commonly reported worldwide [30]. However, our study revealed a lack of awareness of this issue among our respondents. These findings, supported by the previous studies showing that meat handlers have less knowledge about foodborne pathogens [20,25,31,32] and foodborne diseases [20,31], could be related to a lack of training, as noted by [31–33], since none of the participants reported having prior formal food safety training.

Attitude is a key factor that can influence the behavior and practices of food handlers in terms of food safety; it reduces the incidence of foodborne diseases [34,35]. According to Zanin et al. [36], attitude is the principal link between knowledge and practices; the workers with adequate knowledge are more likely to put it into practice if they have a proper attitude. Generally, the workers in this study demonstrated positive attitudes toward food safety, with an average score of 16.21 ± 3.07 from a possible 20 points maximum, which is higher than the results obtained by [21,33]. This may be due to the hygiene instructions periodically provided by the veterinarian in order to ensure healthy condition of the meat.

Following proper personal hygiene practices is extremely important to ensure meat safety and prevent foodborne infections and poisoning among the consumers. This study revealed poor food safety practices, with an average score of 57 ± 8.7 out of a possible total of 90 points maximum. The only encouraging results reported by respondents were hand washing after handling waste (85.4 %) and after using the toilet (94.4 %). Interestingly, although workers have a positive attitude toward the meat safety aspects, yet their practices show significant deficiencies. For example, a large proportion of respondents agree that wearing protective clothing such as gloves (94.4 %), aprons (87.6 %), masks (86.5 %), and hairnets (74.2 %) is important for improving meat safety, but only a few of them actually wear such personal gear while working. The same observations were made in the studies conducted by [21,25,37]. Veterinarians and slaughterhouse owners underline that non-compliance with good hygiene practices mainly results from staff shortages and high turnover, which complicates the application of the sanctions against the employees. Similarly, food industry managers in Algeria (95.6 %) [38], Abu Dhabi (87 %) [39], and Turkey (89.3 %) [40] have identified staff turnover as an obstacle to implementing food safety management systems such as HACCP.

As reported by Zelalem et al. [33] and Bahir et al. [14], education plays a key role in growing the meat handlers' awareness and improving their attitudes toward meat safety. In our study, the chi-square test (χ^2) showed that respondents with a higher level of education were significantly more likely to have good knowledge, attitudes, and practices regarding meat safety ($p = 0.000$, $p = 0.002$, and $p = 0.000$, respectively). These results are consistent with those of Adesokan and Raji [41], who demonstrated a significant association between education level and the three variables, suggesting that education also improves food safety practices among meat handlers. Furthermore, our study revealed a positive correlation between knowledge and attitudes ($r_s = 0.563$, $P < 0.001$), as well as between knowledge and practices ($r_s = 0.389$, $P < 0.001$); this indicates that the attitudes and practices of meat handlers are improving as they acquire more knowledge. These results agree with the results reported in studies of [31,33,34,42], which also found a significant positive correlation between KAP levels, unlike the study of Ansari-Lari et al [19], which reported a negative correlation between knowledge and practice, as well as between attitudes and practices.

Limitations

Certain limitations should be acknowledged, including the number of the participants and the geographical area covered (Borj Bou Arreridj province). A larger number of participants from different regions would have made the study more representative. In addition, all participants were males with no food safety training, which limits our ability of better understanding the effect of gender and training on meat safety knowledge, attitudes, and practices of the workers.

Conclusion

This study investigated the levels of knowledge, attitudes, and practices (KAPs) regarding meat safety among poultry slaughterhouse workers in the Bordj Bou Arreridj region of Algeria. The results revealed a lack of knowledge among the participants, particularly concerning foodborne pathogens and diseases. Although most participants surveyed showed positive attitudes toward observing personal hygiene and wearing appropriate protective clothes, their actual practices were suboptimal and still require improvement. Our findings indicated that knowledge improves attitudes and practices related to meat safety among the participants in this study. Additionally, education had a significant and favorable impact on their KAP levels. Accordingly, there is a critical need for regular practical training for the meat handlers in the study area to enhance their meat safety knowledge, attitudes, and practices, and to prevent meat-borne diseases.

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PERCEPTION AND WILLINGNESS TO CONSUME HORSE MEAT IN ALGERIAN SOCIETY

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Abstract
This research aims to assess the perception of horse meat as well as the influence of the factors related to socio-demographic and economic context on its consumption willingness. Data was collected using a self-managed questionnaire via in-person interviews with 102 consumers of horse meat. According to the results, horse meat ranked fifth among consumers' top choices for red meat, behind sheep (85.3%), beef (7.8%), goat (2.9%) and camel (2.9%). A significant percentage of participants (65.7%) expressed a favorable opinion towards horse meat and perceived it to be of good overall quality. The horse meat was primarily consumed for its perceived health and nutritional advantages (46.1%). Horse meat was consumed occasionally only in particular scenarios, especially for some disease remedies like anemia. Except for the price, the analysis of influencing factors revealed that there were no significant effects (p>0.05) on the intake willingness of horse meat based on gender, age, level of education, residence area, or income. This study offers a thorough understanding of the consumer opinions regarding horse meat and may be employed in developing strategies for boosting the acceptance of this meat among the consumers.

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Introduction

The dominance of red meat in diets, especially intensively farmed red meat, has raised alarms, owing to the damages caused to the environment as well as the well-being of all living creatures [1,2]. Thus, the multiple benefits of horse meat make it a game changer for mitigating the above-mentioned challenges [3]. In fact, horses are non-ruminant herbivores that release five times lower volumes of methane into the atmospheric layers in comparison to cattle (117.9 kg CH4/dairy cow/year and 20.7 kg CH4/horse/year), which makes it economical and eco-friendly to produce meat [4,5].

Regardless of its impressive nutritional excellence and other beneficial properties, horse meat consumption remains low across the world [6,7]. Lamri et al. [8] study indicated cultural resistance among Algerian people of Kabylian areas towards eating horse meat as they are totally against using horse meat as a food item. Likewise, the constrained trend of eating horse meat in France was witnessed to undergo a dramatic reduction by Sebbane et al. [9]. Consequently, the global market share of horse meat production remains at a mere 0.25%, with an average consumption of just 0.10 kg per person per year [10].

On the other hand, the term acceptability entails the anticipated attributes of the meat, including its inherent sensory and organoleptic features, nutritional or ecological value, as well as extraneous properties like production management, costs, accessibility, and competitive positioning in the market [11,12]. The previous decade reflected a shift in people's perception of horse meat [13]. In fact, the Food and Agriculture Organization (FAO) reports a gradual rise of 7.57% in horse meat production during 2010–2020 [14], with similar patterns exhibited by Algerian consumers in the recent past. Dramatically, the steady surge in the number of expert horse butchers in Algeria points towards a likely inclination of Algerian communities towards eating horse meat due to its nutritional richness, even among people who disregarded it earlier due to cultural constraints. Moreover, alternative red meats like sheep and beef are becoming increasingly expensive which makes them inaccessible for low-income people, thus increasing the Algerian consumers' inclination towards horse meat available at low prices.

It's worth noting that research on consumer perceptions and factors that affect them has been done more extensively on other animal meats than on horse meat. More specifically, there is a dearth of scientific research on horse meat consumer perception and its influencing factors in Algerian society, which could be of great interest to consumers, producers, and those in charge of economic planning. Consequently, the main goal of this study is to determine the perception as well as to establish the influence of the factors related to socio-demographic and economic context on the consumption willingness of this meat among Algerian consumers.

Objects and methods

The study was carried out according to the guidelines of the Ethical Committee of the Faculty of Natural and Life Sciences of the University of El Oued, Algeria (Protocol number: 22/2023).

The investigation was conducted in three different districts of southeastern Algeria: El Oued, Biskra, and Ouargla. The first district is located at latitude 33°22′16.823″ N and longitude 6°50′52.686″ E, the second is located at latitude 34°51′0″ N and longitude 5°43′59.999″ E, and the third is located at latitude 31°56′60″ N and longitude 5°19′0.001″ E. The climate in these districts was semi-dry, with cold winters, scorching summers, and scarce rainfall, particularly during the coldest months of the year. The communities that residing in these regions were traditionally engaged in agricultural production and raising livestock, which included sheep, camels, and goats.

The participants in this study were interviewed via an organized, independently administered questionnaire to assess various aspects related to horse meat perception and consumption and possible affecting factors that relate to the socio-demographic and economic context. 297 participants were surveyed in person using face-to-face interviews. Besides, after data refining, only 102 surveys (34 for each province) were left usable for analysis due to the exclusion of those who had never eaten horse meat. The study participants were chosen at random with a wide range of demographic characteristics. At first, ten persons from each district were gathered as a focus group to gather information about horse meat consumption opinions. The effectiveness of the questionnaire was tested via a pre-test carried out on a focus group of 15 participants (five from each district). The questionnaire was initially written in French before being translated into Arabic language to make it more accessible. To ensure data quality, a unique code was assigned to every questionnaire to prevent duplicate or repetitive responses from similar respondents. The individuals who participate in the survey must meet the inclusion criteria of being at least 18 years old and having consumed horse meat at least once. After describing the study objectives briefly, volunteer participants were asked to get their verbal consent, and an anonymous questionnaire was utilized to ensure the privacy of respondents.

Open-ended and closed-ended (single-select, multiple-choice) questions are combined in the questionnaire. For fictional and polychromous concerns, which feature a wide range of options for respondents to select from, closed-ended questions served as the best fit. However, open-ended questions were considered the best option for complex inquiries that necessitate further detailed elaboration from respondents beyond predefined categories, enabling them to freely express their thoughts and opinions. The final questionnaire consisted of 25 questions divided into two sections. The first section focused on the socio-demographic characteristics of the respondents, such as gender, age, educational levels, living area, and income, which were explored through a set of five questions. The second section composed of 20 questions was designed to assess the consumers' attitudes regarding horse meat, with questions focused on establishing the possible relationship between the socio-demographic and economic factors and the willingness of the respondents to consume horse meat.

The statistical evaluations were established via SPSS, Version 27.0 software. The descriptive statistics were performed to assess the survey data as frequencies and percentages. The Chi-square or Fisher test was used to examine the statistically substantial effects of the presumed socio-demographic and economic factors on horse meat consumption willingness. In all tests, a *p*-value of less than 0.05 was considered in statistical terms.

Results

Table 1 shows an overview of the demographic traits of the 102 participants. 53.9 % of respondents fell between the 18–30 age bracket, with 23.5 % aged between 31–40 years, and only 2.9 % of respondents' age exceeded 60 years. Regarding the gender group, the survey included 33.3 % (34) female and 66.7 % (68) male respondents. A significant number of respondents (63.7 %) held higher education qualifications and having a university degree. 46.1 % of participants earned an affordable income, besides 53.9 % of them indicated a lower income level. Most of the study participants (68.6 %) lived in rural areas, while, 31.4 % of respondents resided in urban localities.

Table 1. Demographic characteristics of the surveyed respondents

Variable	Groups	Frequencies, n	Proportion, %
Age (years)	18–30	55	53.9
	31–40	24	23.5
	41–50	15	14.7
	51–60	5	4.9
	Above 60	3	2.9
Gender	Male	68	66.7
	Female	34	33.3
Education	Primary	2	2.0
	Secondary	11	10.8
	Tertiary	23	22.5
	University	65	63.7
	None	1	1.0
Income	Acceptable	47	46.1
	Low	55	53.9
Residence	Rural	70	68.6
	Urban	32	31.4

Table 2 summarizes the respondents' opinions regarding horse meat. Evidently, sheep meat has been found to be the most preferred type of red meat, liked by a whopping 85 % of the respondents, leaving behind beef (8 %), goat (3 %), camel (3 %), and horse (1 %) meat. When the respondents were asked to rank their preferences, most of them (83.3 %) ranked horse meat at the last position out of the five meat types surveyed. Alternatively, despite 65.7 % of participants' belief in the favorable quality of horse meat, just 34.3 % expressed their willingness to eat horse meat. The nutritional and health features (46.1 %) and tenderness (32.4 %) were the two key factors that attracted people to horse meat consumption.

Table 2. Consumer beliefs regarding horse meat

Variable	Groups	Frequencies, n	Proportion, %
Which meat variety do you prefer?	Sheep	87	85.3
	Beef	8	7.8
	Goat	3	2.9
	Camel	3	2.9
	Horse	1	1.0
Which rank was taken by the horse meat among the others?	1st	1	1.0
	2nd	3	2.9
	3rd	1	1.0
	4th	12	11.8
	5th	85	83.3
Are you willing to consume the horse meat?	Yes	35	34.3
	No	67	65.7
How do you appreciate the horse meat?	Good	67	65.7
	Not good	35	34.3
What is the main factor driving your decision toward horse meat?	Tenderness	33	32.4
	Color	6	5.9
	Odor	8	7.8
	Flavor	8	7.8
	Nutritional and health attributes	47	46.1

The key potential therapeutic applications of horse meat are described in Table 3. Surprisingly, a large portion of respondents (87.5 %) believed that horse meat could be beneficial in curing certain health conditions, especially because of its high effectiveness in treating anemia. Additionally, the participants also reported the effective usage of horse meat in other treatments like improving growth and treating rheumatism, with significantly fewer proportions (3.4 % for each).

Table 3. Medical uses of horse meat

Use	Frequencies, n	Proportion, %
Immunity enhancement	1	1.1
Treating anemia	77	87.5
Growth improvement	3	3.4
Bone strengthening	2	2.3
Treating jaundice	1	1.1
Increased activity	1	1.1
Treating rheumatism	3	3.4

The consumption willingness of horse meat was examined through various socio-demographic and economic factors (Table 4). According to the findings of the factor analysis, socio-demographic attributes like gender, age, educational qualifications, region of living, and household income had no impact on the likelihood of consuming horse meat ($p > 0.05$). However, the cost factors exhibited a statistical significance as a deciding variable ($\chi^2 = 12.707$), which affected the consumers' willingness.

Table 4. Relationship between horse meat consumption willingness and socio-demographic and economic factors

Variable	Groups	Consumption willingness (Yes) n (%)	Consumption willingness (No) n (%)	Odds Ratio	(χ^2; F)	P-value
Gender	Male	24(35.3)	44(64.7)	1	0.087	0.768
	Female	11(32.4)	23(67.6)	0.876		
Age (years)	18–30	19(34.5)	36(65.5)	1	0.840	0.961
	31–40	8(33.3)	16(66.7)	0.947		
	41–50	6(40.0)	9(60.0)	1.263		
	51–60	1(20.0)	4(80.0)	0.473		
	Above 60	1(33.3)	2(66.7)	0.947		
Education	Secondary	5(45.5)	6(54.5)	1	2.682	0.665
	Tertiary	6(26.1)	17(73.9)	0.423		
	University	24(36.9)	41(63.1)	0.702		
	Primary	0(0.0)	2(100.0)	/		
	None	0(0.0)	1(100.0)	/		
Residence	Rural	23(32.9)	47(67.1)	1	0.210	0.647
	Urban	12(37.5)	20(62.5)	1.226		
Income	Acceptable	16(34.0)	31(66.0)	1	0.003	0.957
	Low	19(34.5)	36(65.5)	1.022		
Price	Cheap	29(48.3)	31(51.7)	1	12.707	0.000
	Expensive	6(14.3)	36(85.7)	0.178		
	No	1(7.1)	13(92.9)	0.122		

Discussion

Despite its health advantages and nutritional value over other red meats, horse meat occupied the last rank in the consumers' preference list and was the least popular type among the survey participants. However, our findings indicated that sheep is the most favored animal for domestic use. This dietary inclination may have arisen from the majority of respondents' perceptions of the better taste and appeal of sheep meat compared to the other meat forms included in our study.

This discovery aligns with Realini et al. [15] study outcomes, emphasizing taste as the key factor influencing consumers' meat preferences. Akin to this, prior research indicates that the primary reasons for consumers' readiness to consume sheep meat relate to its unique taste and texture as opposed to other animal meat forms [16,17]. Furthermore, consistent with our findings, Lamri et al. [8] conducted an online survey to assess meat-eating behaviors and choices in three provinces of the Kabylian areas of Algeria, revealing that horse and camel meats were deemed as less appetizing than chicken, beef, and lamb, respectively. Furthermore, a poll conducted on Canadian customers by Popoola et al. [7] disclosed that 80 % of respondents were unaware of horse meat. Furthermore, Sebbane et al. [9] discovered that horse meat is consumed only in a few instances and infrequently by a small proportion of French people, and according to French national statistics (2021), horse meat consumption impacts only 7 % of French families contributing to merely 0.1 % of meat purchases [18].

Meanwhile most survey participants acknowledged horse meat's good quality, especially because it is packed with healthy nutrients, and can also be utilized to treat certain diseases, a comparable percentage of participants expressed an unacceptability for consuming horse meat. In contrast to other animal meat types studied herein, the very rare consumption of horse meat leads to its occasional consumption. This might be another determinant factor contributing to the dislike of horse meat across survey participants, thus lowering the per person consumption. Food neophobia, which refers to the avoidance of novel food items or unwillingness to taste them, can be justifying consumers' unusual tendencies toward horse meat [19].

Our study clearly shows that the most significant factor influencing customers' decision to eat horse meat corresponds to its health advantages and nutritional value. This finding was in accordance with earlier reports [20,21] that highlighted these traits as the key motivators for red meat eating.

Horse meat has even been touted by scientists as a useful food item and dietary staple, due to its immense chemical, physical, and nutritional features comparable or even superior to other meat types [10,22]. Further, due to its nutritional value, substituting beef with horse meat could result in lower volumes of meat consumption, as consumers may require less horse meat than beef to obtain equivalent nutrients such as iron [3]. Lamy et al. [3] reported that promoting horse meat consumption might be beneficial for populations with limited access to protein-rich sources, especially considering its nutritional excellence and affordability compared to other red meats.

Furthermore, Lamri et al. [8] stated that while deciding about consuming and purchasing red meat, they prioritize its nutritional features (63.5 %), followed by taste (51.9 %) and other attributes (43.1 %) like inquisitiveness and family customs. Furthermore, the earlier study indicated that 60 % of respondents expressed their willingness to purchase greater quantities of meat if supplementary details about its nutritional traits were shared with them.

In our study population, horse meat consumption was predominantly linked with its therapeutic application in curing anemia. Consistent with these results, Stanciu [22] observed that the high mineral and vitamin content of horse meat provides nutritionists with strong justifications for prescribing it to anemic patients. Additionally, Del Bò et al. [23] analysis pointed out that eating horse meat raises the level of polyunsaturated fats (PUFAs) in consumers' red blood cells, specifically modulating PUFAs, which are most beneficial for maintaining nutritional adequacy and the guarding benefits. Additionally, their study demonstrated that horse meat enhances iron content and the omega-3 index by over 7.5 %. Furthermore, Del Bò et al. [23] highlight that being a rich source of iron, even a single serving of horse meat (175 g) fulfills approximately 33 % of the daily required iron intake. The notably lower cholesterol content of horse meat in contrast to other meat forms renders it particularly intriguing from a nutritional perspective [24]. Hence, consuming horse meat can prove to be advantageous for health, particularly among the sufferers of cardiovascular diseases. Interestingly, Pierre [25] highlighted the recommendation of consuming horse meat by the medical fraternity during the 19th and 20th centuries, considering its usefulness in combatting tuberculosis. Additionally, Nurdin [26] documented people's perception of horse meat as a cure for contaminating illnesses (tetanus) in addition to the usability of the fat content of horse meat in treating asthma, burns, and other conditions. Lee et al. [27] proposed the conventional usage of horse bones in managing bone diseases like bone fracture and arthritis, along with the use of its fat content in developing a skin ointment for dealing with various skin issues and healing wounds [27].

This study delved into the effects of socio-demographic factors, like gender, age categories, education qualifications, living regions, and family income, on the willingness to eat horse meat. The findings showed no major variations across the different categories. This finding can be attributed to the unpopularity of this meat form among the participants of our study, which implies that this meat is typically consumed for special purposes, like treating certain illnesses. At the same time, Lamri et al. [8] emphasized that the customs, cultures, lifestyles, and meat consumption patterns of the Algerian public have all contributed to the weak acceptance of horse meat. These findings conflict with other studies [3,9] performed on the horse meat consumers of France, recognizing socio-demographic parameters as significant variables that have a discernible impact on horse meat intake. Prior investigations showed a statistically lower likelihood of horse meat consumption in women as opposed to men.

Regarding the impact of education level, past researchers have observed an inverse correlation between the educational level and the likelihood of buying and eating horse meat. In contrast, younger individuals, who are 18–34 years old exhibited a greater reluctance to eat horse meat rather than other age populations involved in the survey [3]. However, as revealed through a poll held in 2015, the mean quantity of horse meat consumed by households with panelists of 18–44 years old was 18.5 percent and 18.7 percent lesser, respectively, in comparison to the households having subjects from 45 to 64 and above 65 age brackets. Furthermore, earlier findings also revealed a greater likelihood of meat consumption among households earning less than the poverty line [3]. The gender-based variances in preferences noted in preceding studies may be attributed to the fact that women are more emotionally sensitive than men, thus being more likely to absorb adverse information about meat production and consumption mechanisms [28]. This may also account for the modest tendency of horse meat consumption among the women participants of our research in contrast to men. While assessing consumer behaviors, past research has consistently pointed towards the differing perception held by the two genders regarding the significant ethical concerns linked with animal wellbeing and indicated higher animal-friendly attitudes among women instead of men [29,30]. Conversely, the study participants' willingness to consume horse meat remains largely unaffected, yet the participants holding university degrees exhibited a higher willingness to consume horse meat rather than the tertiary or primary degree holders or illiterate individuals, indicating the receptivity of higher educated people to tasting novel delicacies [31]. When customers are well-informed, they prioritize healthy and nutritional components in foods, thus favoring horse meat consumption [32]. A person's responsiveness while contemplating a deciding factor is influenced by the higher degree of education, which further expands their knowledge and information. Our study revealed an intriguing trend of willingness to consume horse meat that was little higher in middle-aged consumers instead of older or younger participants, which can be explained by the notion that the middle-aged participants embrace the responsibilities of household consumption inclusive of maintenance of family health. Their predominant role in daily purchases in comparison to other age groups may increase the suitability of horse meat from their perspective. As a result, the ability to discern the suitability of any product in terms of quality assists the participants of this age group in making logical choices while shopping. In addition, middle-aged individuals are categorized as adults owing to their buying expertise and reasonable approach to decision-making. Furthermore, the modest correlation between respondents' willingness to consume horse meat and their urban location noted in our study may be directly linked to the remarkable abundance of expert butchers trading this meat in urban marketplaces as opposed to rural regions.

Pricing was shown to exert a substantial effect on readiness to consume horse meat in the current study ($\chi^2 = 12.707$; $p < 0.001$). Overall, the consumers were more prepared to consume it if they perceived horse meat as economically priced. This might be attributed to individuals' choice of purchasing goods that do not have a detrimental impact on their household income as well as their purchase parity. This finding echoes the study outcomes of Lamri et al. [8], emphasizing that in the Algerian setting, meat prices continue to be significant determinants for consumers. In fact, the price was rated third among the crucial purchase determinants by the participants of the previous study, indicating freshness and tenderness at the first two ranks. Likewise, while evaluating horse meat intake in French communities, Lamy et al. [3] pinpointed the relatively higher price of horse meat as a potential barrier to growing consumption, particularly for households with lower socioeconomic status (with 16% of the study population citing cost as an obstacle). Additional research focusing on Korean consumers found that the cost of horse meat was a significant factor that influenced consumers' purchase decisions adversely [33].

Additionally, recent research conducted in various nations has emphasized the significance of horse meat pricing for consumers and considered it a significant impediment to the purchase and consumption of this meat form [19,32]. Furthermore, consumers rank price as the second most important factor after taste, which shapes their purchase decisions of meat products [15]. On the contrary, Bernués et al. [34] highlighted the product price as the most critical factor shaping the purchase decisions of consumers. It goes without saying that, irrespective of the differences in the earlier research and their settings, the findings of all were in agreement that the cost of meat had a significant impact on customers' purchase decisions.

Conclusion

Our study highlighted the limited consumption of horse meat in contrast to alternative types of animal production. In the meantime, the enhanced supply and frequency of consumption can be accomplished through communication systems highlighting the health and nutritional aspects of horse meat, accompanied by wider distribution channels ensuring its accessibility in all meat markets at competitive pricing. It would also be beneficial to endorse ready-to-eat horse meat cuisines and their recipes. Additionally, if consumers' opinions of the quality attributes, particularly the favorable hedonic qualities of horse meat are improved, occasional consumers of horse meat may be persuaded to reconsider eating it.

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COMPARATIVE PROTEOMIC STUDY OF MUSCLE TISSUE OF WILD BOARS AND DOMESTIC PIG BREEDS

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Abstract
In the modern conditions, there is a growing consumer interest in products from local (autochthonous) breeds of animals raised in extensive animal husbandry systems. Meat of such animals is often associated with high quality characteristics; however, its molecular foundations remain to be studied insufficiently. Comparative analysis of proteomic profiles of such breeds and their wild ancestor — wild boar — is of particular interest for understanding the fundamental consequences of domestication and selection as well as for revealing key marker proteins determining meat product properties. A comparative proteomic analysis of muscle tissue (M. longissimus dorsi) of wild boar and four pig breeds (Livny breed, Altai meat-type breed, Landrace, Mangalitsa) was carried out to reveal breed-specific molecular patterns associated with key meat quality characteristics. Proteomic profile was studied by two-dimensional electrophoresis (2-DE) and mass spectrometry (MALDI-TOF/TOF). Functional analysis of protein-protein interactions and gene ontology (GO) enrichment were carried out using the STRING database. Twenty one proteins forming a functionally linked network were identified. Significant breed related differences in the composition and modifications of proteins of the contractile apparatus (products of MYL1, MYL2, MYL3, MYL6B, TNNT3, TNNI2 genes), energy metabolism (products of ENO3, ALDOA, CKM, AK1, ATP5F1A genes) and stress response (products of CRYAB, HSPB6 genes) were revealed. The highest degree of proteome transformation was noticed in the Livny breed, which demonstrated a significant similarity with wild boar in terms of several parameters including appearance of atypical myosin light chain MYL6B and a decrease in the level of muscle enolase (products of ENO3 gene). For Mangalitsa, a unique modification of the pattern of expression of myosin light chains and a significant increase in the level of small chaperons were characteristic, which correlates with the conditions of its free-range keeping. Bioinformatics analysis in STRING corroborated statistically significant formation of functional clusters responsible for muscle contraction, metabolism and maintenance of proteostasis. The data obtained suggest that both gene pool (breed) and environmental factors (keeping conditions) exert a complex effect on the proteomic landscape. The revealed protein signatures and their network interactions not only deepen the understanding of the biological foundations of meat quality but also open new prospects for the development of molecular markers in breeding and meat industry aimed at production of products with target properties.

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Introduction

Pork is considered the most consumed meat type in the world. In pork production, the most economically significant characteristics are the growth rate and meat quality. It is these requirements that determined domination of large-scale, highly productive pig husbandry, which led to the fact that four commercial breeds (Landrace, Duroc, Large White (Yorkshire), Pietrain) account for more than 95% of pork production [1]. However, due to the high concern of the society about intensive methods of pig husbandry, consumers more and more often prefer meat from animals that were raised using the extensive method of animal husbandry, which is linked with the possibility of free range for animals, more diverse diets with possibilities of foraging. Extensive pig husbandry is more applicable to local breeds, which differ significantly from highly productive breeds of the traditional production by both productivity and quality indicators of carcasses and meat [2].

In response to this consumer demand together with risks of disappearance of autochthonous breeds, more and more studies are aimed at investigation of a relationship between meat quality characteristics and genetic structure.

Particularly many publications are aimed at the comparison of commercial and local breeds, methods of raising and fattening of pigs. At the first stages, investigations dealt with comparison of physico-chemical properties of meat raw materials. The study by Estévez et al. [3], can serve as a prominent example. It presents the results of the investigation of *M. longissimus dorsi* from free-range Iberian pigs slaughtered at a live weight of 90 kg compared to muscles from commercial pig breeds. The authors present data about a higher content of fat upon the low content of phospholipids, higher content of heme iron and distinct darker (red) color (a^* value) of muscles compared to those of the commercial pigs. *M. longissimus dorsi* from pigs of commercial breeds contained higher proportions of polyunsaturated fatty acids and higher ratio of omega-3 to omega-6. Also, a recent study [4] demonstrated higher moisture content, cooking losses, high a^* and b^* values and content of linoleic acid, linolenic acid and arachidonic acid upon a lower content of crude fat and ash, and L^* value in *M. longissimus dorsi* from Korean native pigs compared to hybrid pigs (Lanrace × Yorkshire x Duroc).

The obtained extensive data about differences in the physico-chemical properties of meat from native and commercial pig breeds formed the basis for conducting deeper molecular investigations that became possible with the development of the modern methods of proteomics and genomics and enable discovering a mechanism of manifestation of meat quality characteristics.

Proteomic differences between native and commercial pig breeds were noticed in a significant number of publications. For example, in [5] significant differences were revealed in expression of protein, synthesis of fatty acids, content of catalase and glutathione peroxidase when comparing the Nero d'Abruzzo with commercial hybrid pigs. Wang et al. [6] presented the results of the proteomic analysis of M. *Longissimus dorsi* from six-month-old pigs of two indigenous Chinese breeds (Tibetan pig and Diannan Small-Ear pig) and two introduced Western pig breeds (Yorkshire and Landrace) using isobaric tag for relative and absolute quantitation (iTRAQ). The authors revealed 288 differentially expressed proteins. Among them, 169 proteins were upregulated and 119 proteins were downregulated. The authors linked differences in muscle growth and formation of muscle fibers between the studied groups with such proteins as aldolase C, enolase 3, phosphoglycerate kinase 1 and 2, troponins (TNNT1, TNNC2, TNNT3), tropomyosins (TPM1, TPM2, TPM3), myosin light (MYL3) and heavy (MYH4) chains. Differences in the ability to deposit lipids are regulated by expression of lipoprotein lipase (LPL); apolipoproteins (APOA1 and APOC3); acyl-CoA dehydrogenase; fatty acid binding protein; acyl-CoA dehydrogenase, C-4 to C-12 straight chain; acetyl-CoA acyltransferase 2; acetyl-CoA acetyltransferase 1; hydroxyacyl-CoA-dehydrogenase and peroxisomal 3,2-trans-enoyl-CoA isomerase.

When comparing tenderloins from Tibetan pigs and Yorkshire pigs, 171 proteins were identified as differentially abundant proteins (DAPs). In tenderloin from Tibetan pigs, upregulated proteins took part mainly in biological pathways of energy production, muscle contraction, immunity and defense, while downregulated proteins in glutathione metabolism [7].

Pan et al. [8] present the results of the transcriptome sequencing of muscle *Longissimus dorsi* from Duroc and Luchuan pigs, which showed differential expression of 3,682 genes. Special attention was paid to MYL2 gene expression in Luchuan pigs, which was significantly higher than that in Duroc pigs at 2 and 8 months of age. The authors link this to the different times at which the maximum growth rate of different pigs appears. With that, 40 genes were associated with biological pathways of growth of skeletal muscles, metabolism of fatty acids and deposition of intramuscular fat.

Kim et al. [9] reported on the results of the complex study of the meat quality characteristics and differences in transcriptome of *M. longissimus dorsi* between the Landrace breed and Jeju native pigs. Phenotypical analysis of meat quality traits revealed that meat from the local breed was characterized by higher content of intramuscular fat and redness. RNA sequencing of muscle samples revealed 427 differentially expressed genes upregulated in Jeju pigs, while 821 genes were upregulated in Landrace pigs. Among candidate genes being indicators of growth and meat quality and also facilitating improvement of indicators of growth and development of pigs, the following key genes were determined: encoding myosins (MYH2, MYH6, MYH7B, MYO5B), growth factors (IGF1, IGFBP5, EGFL6, LINGO1), glycoproteins (SFRP2), matrilin-3, hyaluronan and proteoglycan link protein 1, fibulin-7.

Therefore, data of transcriptomic and proteomic investigations clearly demonstrate molecular differences between pig breeds and prove that genetics and targeted selection exert a profound influence on pork quality traits. Nevertheless, current scientific context has a significant gap: there are no comparative studies of pigs and their common ancestor — wild boar. Proteomic landscape of wild boar (*Sus scrofa*) is to a large extent understudied. Inclusion of the wild phenotype into comparative analysis is a necessary link for understanding the initial muscle proteome not modified by selection, which allows for revealing evolutionary conserved pathways and molecular adaptations that were acquired in the process of domestication and targeted selection.

In this respect, the aim of this work was the comparative proteomic analysis of *M. longissimus dorsi* samples from wild boars, pigs of the commercial breed (Landrace), two local breeds (Altai and Livny) and the introduced breed (Mangalitsa). This study is aimed at revealing universal and unique breed-specific protein patterns associated with growth rate of muscles, transformation of muscle fiber type and metabolic status, which will enable disclosure of molecular mechanisms of meat quality formation that form the base of adaptation to different conditions of keeping and genetic heritage.

Objects and methods

Objects of research were muscle tissue samples of *M. longissimus dorsi* from pigs of two local breeds — Livny (*n* = 6; LV) and Altai meat-type (*n* = 5; AL), commercial pigs (Landrace) (*n* = 5; LD), Mangalitsa breed (*n* = 5; MG) as an introduced breed, as well as wild boar (*n* = 8, WB).

The Livny breed (Livny, Orlov region) is a local Russian meat-fat breed of pigs registered in 1949. Boars of the White Long-Eared, Yorkshire, Large White, and Berkshire breeds were used in its production [10]. At present, only a small population of Livny pigs is kept in one enterprise in the Orlov region. Pigs of the Livny breed achieve slaughter weight for 155–160 days.

The Altai meat-type breed (Altai Republic) is a local Russian meat-type breed registered in 2017. White Large and Landrace pigs and boars of the MAXGRO™ Terminal Line were used in its development. An average yield of lean meat is 58–59 %. Altai pigs grow very fast and achieve body weight of 100 kg for 145–150 days.

The Mangalitsa breed (Krasnoyarsk region) was imported from Hungary to Russia in 2000, raised in free-range conditions with access to pastures.

Wild boars are represented by individuals from different populations (Central European boar *S. scrofa scrofa*, Moscow region; Ussuri boar *S. scrofa ussuricus*, Amur River region).

Livny, Altai and Landrace pigs were kept in conditions of the commercial pig husbandry farm and fed with complete combined feed. After the average live weight of pigs reached 110 ± 10 kg, pigs were transported to the slaughterhouse, were allowed to rest for 12 hours and then slaughtered. Carcasses were chilled at a temperature of 0 °C for 24 hours. Cold carcasses were assessed 24 hours after slaughter. Pieces of *M. longissimus dorsi* with a weight of 500 g (± 10 g) were cut off from each carcass. Samples were obtained, as a minimum, from three pigs at slaughterhouses after conventional processing of carcasses or after shooting in field conditions.

To carry out the experiment, the following reagents were used: acrylamide, methylenebisacrylamide, agarose, Tris, glycine, sodium dodecyl sulfate, ammonium persulfate, Triton X-100, 2-mercaptoethanol, BSA, ampholines pH 3–10 and 5–8, Coomassie Brilliant blue R-250, trypsin (Sigma, USA), amberlite IRA-150L (Amersham Biosciences, Sweden), PageRuler™ Prestained Protein Ladder (Fermentas, USA), urea (Across Organics, Belgium), silver nitrate (Dia-M, Russia).

To perform the proteomic analysis, a minced specimen (100 mg) of muscle tissue was homogenized in 2 ml in the Teflon-glass system in a ysis solution of the following composition: 9M urea, 5 % mercaptoethanol, 2 % Triton X-100, 2 % ampholytes. The homogenate was centrifuged at 800 g for 5 min.

Two-dimensional electrophoresis (2-DE) by O'Farrell was carried out using a chamber Bio-Rad (Bio-Rad, USA) by isoelectric focusing in ampholine-polyacrylamide gel (IEF-PAGE, equilibrium variant), in glass tubes in the first direction and SDS-PAGE in the second direction with slight modifications [11,12,13]: IEF in the first direction was performed in 2.4 mm × 180 mm cylindrical gels until reaching 3650 Volt-hours. Non-equilibrium variant of IEF (with an increase to 2500 Volt/hour) was also used to reveal atypical fractions of troponin. For visualization of proteins, 2DE gels were stained with Coomassie blue R-250 and then with silver nitrate according to [14]. Stained gels were analyzed by scanning (300 points per inch, 48 bit, color,.TIFF) using a scanner Epson Expression 1680 (Epson, Suwa, Nagano, Japan) [15]. Densitometry was carried out using the software ImageMaster 2D Platinum, version 7 (GE Healthcare, Switzerland). No less than three 2-DE were tested for each breed/cross.

For protein identification, individual fractions were cut out from 2-DE gels, cut-out fragments were ground and their trypsinolysis was performed as described earlier [16]. Then, the corresponding sets of peptides were studied by MALDI-TOF MS and MS/MS spectrometry using a MALDI / time-of-flight mass spectrometer Ultraflex (Bruker, Bremen, Germany) with UV laser (336 nm) in a positive ion mode in a range of 500–500–8000 Da with their calibration by known trypsin autolysis peaks. Analysis of the obtained mass spectra (peptide fingerprints) was carried out using the Mascot software (FlexControl 3.3, FlexAnalisis 3.3 and Biotools 3.2), options Peptide Fingerprint (Matrix Science, Boston, Massachusetts, USA), with accuracy of mass measurement MH+ 0.01 %, and was accompanied by the search in the NCBI databases “Proteins of porcine skeletal muscles (*Sus scrofa*)” [17].

Analysis of protein-protein interactions (PPI) and functional enrichment was performed using the STRING database [18,19]. Analysis included all identified proteins (n = 19). A PPI-network was constructed with an average confidence level (score ≥ 0.5). Biological interpretation of the network was carried out based on the gene ontology (GO) enrichment analysis by three directions: biological processes (Biological Process), cellular components (Cellular Component) and molecular functions (Molecular Function). Statistical significance of enrichment was determined with adjustment for false discovery (False Discovery Rate, FDR) with a value of < 0.05.

Statistical processing was carried out using a software package Statistica 10.0. Results are presented as means and standard deviations (Mean ± SD). Statistical significance was calculated with the use of non-parametric Mann–Whitney U test. A probability of 0.05 was chosen as a significant level.

Results

Upon visual clustering using the STRING database, the statistically significant (score > 0.9, PPI enrichment *p*-value: 1.89e-15, local clustering coefficient: 0.692) protein-protein interaction network was distributed into three main clusters: two interlinked heat shock proteins CRYAB and HSPB6, a triangle of interactions of ubiquitin system RPS27A-UBB-KXD1, and a wide network from 14 protein nodes linked by 32 edges (Figure 1).

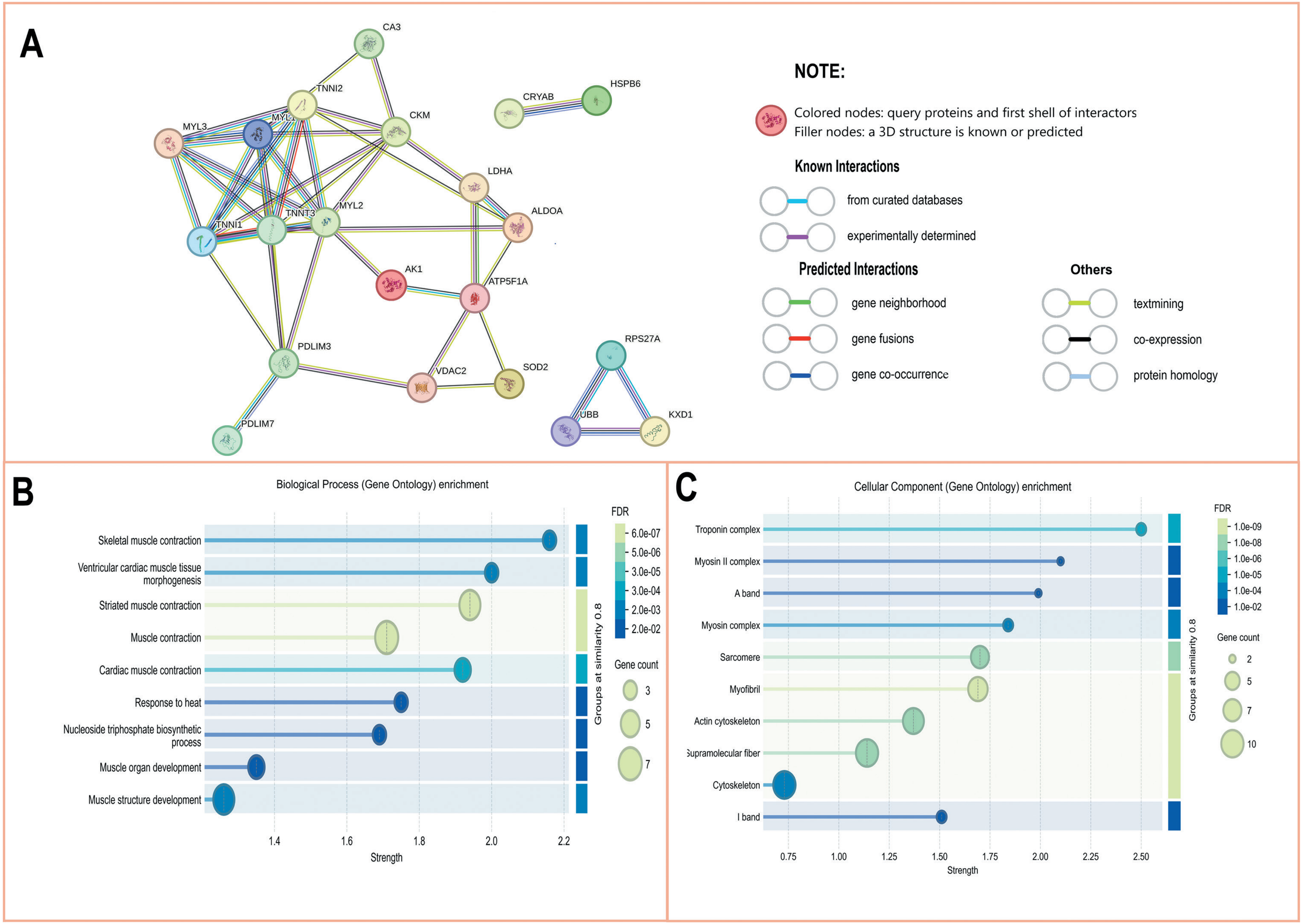

Figure 1. Visualization of protein-protein interactions (A) of identified proteins and distribution by related biological processes (B) and cellular components (C)

The largest cluster of 14 molecules represents a network with a clear functional distribution into key modules reflecting the main biological processes in muscle tissue: contractile apparatus (GO:0006936, GO:0006941, GO:0060048, GO:0003009), energy metabolism (GO:0009142) and mitochondrial functions, including defense against the oxidative stress. The most dense and highly significant module (score > 0.95) is formed by structural and regulatory proteins of myofibrils (GO:0030016, Myofibril), which indicate their close functional and physical relationship in the composition of sarcomeres (GO:0030017, Sarcomere), for example, interaction MYL1-TNNT3 (score 0.967), MYL1-TNNI2 (score 0.951) and TNNI2-TNNT3 (score 0.986). The second key module of the network unites enzymes of glycolysis and the system of generation and buffering of ATP: creatine kinase M-type (CKM) demonstrates multiple links both with contractile proteins (MYL1, score 0.868; TNNT3, score 0.625), and with enzymes of glycolysis (LDHA, score 0.520), which underlines its central role in buffering of ATP and rapid rephosphorylation of ADP near the places of its consumption — myofibrils. In addition, we visualized the interaction between aldolase A (ALDOA), a key enzyme of glycolysis, and lactate dehydrogenase A (LDHA) (score 0.915), which corroborates their cooperation in the anaerobic degradation of glucose. Adenylate kinase 1 (AK1) interacts with mitochondrial ATP syntase (ATP5F1A) (score 0.618) and myosin regulatory chain MYL2 (score 0.509), integrating mitochondrial ATP production with its intracellular turnover and consumption.

PDZ and LIM domain protein 3 (PDLIM3) acts as a link interacting both with contractile elements (MYL1, MYL2, TNNI1, TNNT3), and mitochondrial protein VDAC2. This determines it possible role in the spatial organization of sarcomere (GO:0030017) and location of mitochondria near myofibrils (GO:0030016) for effective ATP supply, which corresponds to its annotation to the component of Z-disc (GO:0030018, Z disc) and actin cytoskeleton (GO:0015629) [20].

Cluster of small chaperons (CRYAB and HSPB6, score 0.603) is involved into the function of the defense system against proteotoxic stress preventing protein denaturation and aggregation (GO:0009408, Response to heat) [21].

Proteins RPS27A (endogenous ribosomal protein), UBB (polyubiquitin, central for proteasome degradation) and KXD1 (interacts with ubiquitin ligases) form a stable functional complex, which central link is the ubiquitin system (score>0.9). RPS27A (GO:0042254, ribosome biogenesis) and UBB (GO:0000209, protein polyubiquitination) are components of this system [22], and KXD1 (GO:0140356, ubiquitin binding) acts as a regulator and adapter linking it with other cellular processes, in particular, with intracellular transport (GO:0006886, intracellular protein transport) through interaction with regulators Rab GTPases (RabGDI), which apparently ensures the directed transfer of ubiquitin substrates [23] and indicates the active processes of targeted degradation of damaged proteins (GO:0006508, proteolysis) and other non-membranous organelles (GO:0043232), regulation of cellular functions. This complex is mostly located in cytosol (GO:0005829) and other non-membranous organelles (GO:0043232), which is a prerequisite for constant renewal of protein pool in the dynamic muscle tissue.

Figure 2 presents two-dimensional electropherograms of *M. longissimus dorsi* from breeds under study, which were stained with Coomassie. First of all, we assessed protein fractions that corresponded to the myosin essential light chains (MLC). It is known that myosin light chains are in the composition of the heads of the myosin molecule as two types of molecules — essential (MYL1) and regulatory (phosphorylated), which ensure the contractile activity of muscle fiber due to changes in a degree of phosphorylation of the regulatory isoform (MYL2). Products of MYL1 gene in vertebrates are presented in a form of two isoforms — long and short (fast type). Each of two heads in the myosin molecule from fast skeletal muscles contains only one MLC isoform (either long or short); therefore, myosin in muscle fibers can exist either as homodimer containing the same MLC in both heads, or as a heterodimer containing different MLC isoforms [24]. Traditionally, essential light chains in domestic pigs are presented as two isoforms - products of MYL1 (fast type, 93%) and MYL3 (slow type, 7%) genes.

Two MYL1 isoforms were revealed for *M. longissimus dorsi*: major, fast type (quantitative content 90%) and minor (10 ± 5%). Among the breeds under study, the ratios of isoforms had insignificant quantitative differences. It was found that the proportion of the myosin long chain (MLC) changes quantitatively depending on the breed. MLC1 (MYL1, fast type, No. 1–3 in Table 1) was the main component, but MYL3 (slow type, No. 7, 8 in Table 1) was also found. An amount of MYL3 for the studied breeds was not higher than 6.5% of the total amount of essential MLC. With that, for *L. Dorsi* of the Landrace breed the slow isoform accounted for 13.9%, while in the Livny and Altai breeds it reached 25%. In the samples from wild boar, two isoforms of the products of the MYL1 gene were detected: major, fast type, as well as fragments of the zone of regulatory myosin light chains and short product of the MYL1 gene.

For *L. Dorsi* of the Mangalitsa breed, part of MYL1/MYL3 molecules (no more than 1%) acquired an unusual property — proteins in the norm have pI 4.8–5.0; with that, the detected fractions showed a shift in pI to a level of 9.50. Apparently, part of molecules of these proteins underwent atypical post-translational modification.

In five out of eight samples of *L. Dorsi* from wild boar, the presence of the additional fraction of myosin essential light chain, MYL6B (No. 9–11 in Table 1), was revealed. It belongs to the smooth muscle or non-muscle type and as a rule, is not detected in skeletal muscle of domestic pigs. This protein for porcine muscle tissue was denoted for now as not characterized, although it is without doubt one of the variants of essential myosin light chains.

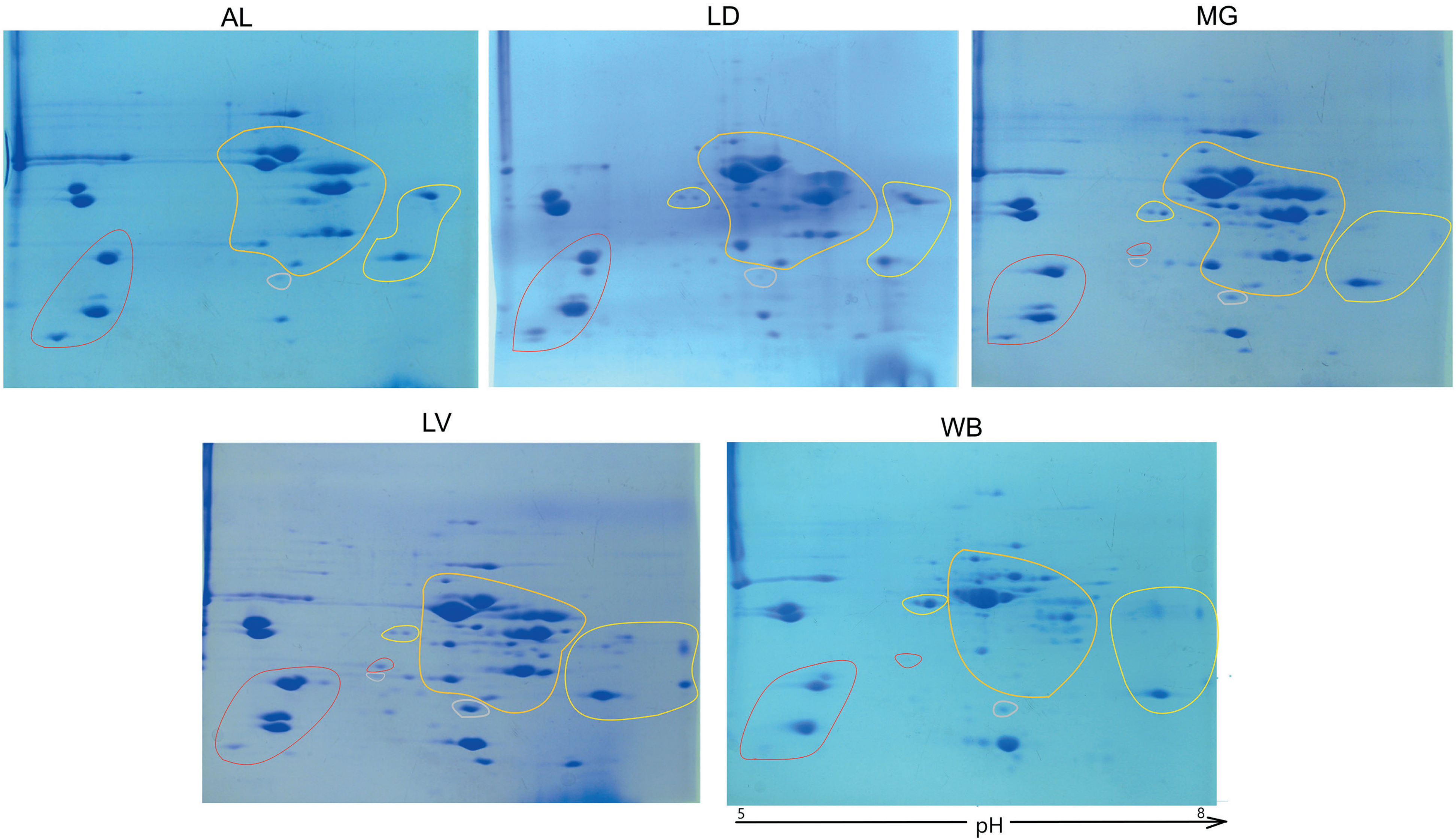

Figure 2. Two-dimensional electropherograms of the *M. longissimus dorsi* samples from the Altai (AL), Landrace (LN), Mangalitsa (MN), Livny (LV) breeds and wild boar (WB). Denotations: Block of fractions characterizing myosin light chains (denoted by red color); block of fractions characterizing troponins (denoted by yellow color); block of fractions of heat shock proteins (denoted by gray color); block of muscle enzymes (denoted by orange color)

With that, the main protein material in the analyzed samples was in the high-molecular-weight fraction, where peptides of the amino acid sequence from 6 to 224 positions out of 224 were determined. However, a minor fraction of the fragment 29–219 was additionally revealed, which was detected in four out of eight samples. In addition, in the major fraction, there is an additional spot shifted by pI to the acid side, apparently due to post-translational modification that has not been identified yet. The presence of the stable fragment indicates that the lifetime of muscle fibers containing MYL6B is less than that of the standard muscle fibers. An increase in the quantity of MYL6B is always linked with a decrease in the quantity of MYL3. It is worth noting that we identified the full-size MYL6B fraction in four out of eight samples, and in one case also its fragment, absolutely similar to what was revealed in the *L. Dorsi* samples from the Livny breed — slow MYL6B isoform accounted for 8.1% on average, which in total accounted for 1/3 of the quantity of MLC in muscle tissue. Therefore, the two-fold decrease in the quantity of shorter (out of two) forms of MYL1 in the Livny breed correlated with replacement of part of long MYL1 isoform with slow type of essential chains MYL3 and MYL6B.

With regard to the regulatory myosin light chain (MYL2, No. 4–6 in Table 1), significant changes in the samples of the analyzed breeds were also noted. According to the results of the computer densitometry, the quantity of MYL2 in *M. L. Dorsi* from Livny and Mangalitsa pigs increased by 5.5 times compared to other breeds. This calcium regulated contractile protein in muscle tissue is presented in a form of two closely located fractions. For *L. Dorsi* from Landrace, phosphorylation at the amino acid position 15S was found in one of the fractions, which determines the mechanism of contraction. For domestic pig, this modification was confirmed for the first time, although in humans this variant was revealed long before with experimental support [25,26].

Among identified proteins, there were also other representatives of slow and fast isoforms of proteins, in particular, a complex of fractions of troponin T of slow skeletal muscles, which in domestic pigs is represented as a set from a range of fractions differing by pI and weight. With that, the main quantity of these fractions belongs to higher-molecular-weight ones. Due to various types of post-translational modifications, this polymorphic protein is represented in a form of three fractions differing by pH, which ratio in the skeletal muscle of domestic pigs is 10/70/20% according to the results of computer densitometry.

Two representatives of the troponin family, troponin T (TNNT3, No. 12 in Table 1) and troponin I (TNNI2, No. 13–15 in Table 1), which belong to the fast type, had quantitative differences. Troponin I in the Altai and Landrace breeds was found in lower quantities than in other breeds. These genes express a whole family of various isoforms, which quantity is quite stable in muscle tissues. To reveal the presence of isoforms, we used the non-equilibrium variant of IEF with an increase to 2500 Volt/hour and not 3600 as in the equilibrium variant. As a result, the presence of additional fractions was found for *L. Dorsi* of the Livny breed: a fragment of 4.5 LIM domain-containing protein and troponin I (No. 20, 21 in Table 1), which belongs to the slow type and appeared as a fragment in a large quantity and without N-terminal part.

For the samples of wild boar and Mangalitsa, a clear link with a decrease in the quantity of slow isoforms was not revealed; the disappearance of the alkaline isoform was mainly noted. With that, it is possible to note that in the samples containing maximum quantity of *MYL6B* the redistribution of isoform by quantity took place. They became equal, which possibly was determined by changes in the ratio of muscle fibers of fast and slow types. Also, the fraction of cytoplasmic malate dehydrogenase was identified. It was found in trace quantities in all samples from wild boar.

Changes in the composition and distribution of heat shock proteins were noticed. αB crystallin (No. 23 in Table 1) is actively expressed in tissues with a high level of oxidative metabolism, such as skeletal and cardiac muscles [27], and performs a broad range of important functions. Its increased expression was found in red fibers (slow type) and is induced by various stress factors (temperature jumps and other extreme conditions), the presence of deep oxidative stress caused by disorder of mitochondrial metabolism. The importance of αβ-crystallin for a network of intermediate filaments is well known. αβ-crystallin can inhibit the assembly of intermediate filaments in cytoskeletal fractions, linked with intermediate filaments, thereby regulating interactions of intermediate filaments with other cellular proteins. It was assumed that down-regulation of αβ- crystalline facilitates proteolytic degradation of actin and myosin, which leads to more tender meat. For *L. Dorsi* from the Livny breed, Mangalitsa and wild boar, its quantity increased up to three times compared to that from Landrace and Altai breeds of pigs. The ratio of the crystalline and MYL6B levels allows for assuming a correlation of these indicators. In addition, an increase in the content of heat shock protein HSPB6 (No. 24 in Table 1) was revealed, which was acetylated by the N-terminal part in *L. Dorsi* from the Livny breed and Mangalitsa. Also in three samples of wild boar, the quantity of HSPB6 was higher compared to that in the Livny and Landrace breeds. This protein is similar in structure and function with αβ-crystallin and, apparently, an increased level is also related to changes in the proportion of fast and slow muscle fibers.

Variability in the content of muscle enzymes was observed. In *L. Dorsi* from Mangalitsa, Landrace and four samples from wild boar, the ratios of ATP synthase subunit alpha (ATP5F1A, No. 25 in Table 1) were at a close level. With that, its content was increased in three samples from wild boar and the samples from the Livny breed, which can be linked with an increase in the quantity of mitochondria in muscle cells. For the Livny breed, a fluctuation in the quantity of aldolase A (No. 35–36 in Table 1) was also observed, which was more dense in *L. Dorsi* from the Altai breed (by more than 1.35 times ($p < 0.05$) compared to other pigs).

Table 1. Identified protein fractions from domestic pig and wild boar characterizing breed differences

No.	Name of protein (symbol of gene)	Number in database	S/M/C*	MM/ pI (exp.)**	MM/ pI (calc.)**
	Myosin light chains				
1	Myosin light chain 1 (MYL1)***(1)	A0A5G2QVS9	154/23/70	23,0/9,50	20,9/4,86
2	Myosin light chain 1 (MYL1)***(2) + Acetyl (N-term)	A0A287BJF1	116/27/75	17,0/4,75	20,6/4,94
3	Myosin light chain 1 (MYL1)***(2) + Acetyl (N-term)	A0A287BJF1	198/20/72	17,0/4,75	20,6/4,94
4	MYL2 (MYL2)***(3)	Q8MHY0	288/49/93	19,0/4,85	18,9/4,8
5	MYL2 (MYL2)***(3) + Phospho (15S)	Q8MHY0	286/45/95	19,0/4,85	18,9/4,86
6	MYL2 (MYL2)***(2)	Q8MHY0	389/44/93	19,0/4,85	18,9/4,86
7	Myosin light chain 3 (MYL3) + Acetyl (Protein N-term	F1SNW4	200/29/80	22,0/5,00	21,7/5,00
8	Myosin light chain 3 (MYL3) ***(1)	F1SNW4	164/33/69	22,0/5,00	21,7/5,00
9	Fragment Uncharacterized protein (MYL6B)***(1)	F1SM01	207/23/82	22,0/5,20	24,0/5,53
10	Mixture of Uncharacterized protein (MYL6B)***(1) and Carbonic anhydrase 3 (CA3)***(1)	F1SM01 Q5S1S4	326/30/92 75/10/50	24,0/5,80	24,0/5,53 29,4/7,72
11	Mixture of Uncharacterized protein (MYL6B) and Carbonic anhydrase 3 (CA3)***(1)	F1SM01 Q5S1S4	374/33/92 106/6/32	24,0/5,80	24,0/5,53 29,4/7,72
	Troponins				
12	Troponin T, fast skeletal muscle (TNNT3)***(2)	Q75NG7	231/57/79	30,0/9,50	30,7/8,69
13	Troponin I (TNNI2) + Acetyl (Protein N-term)	Q4JH15	254/24/64	21.0/9,30	21,3/9,02
14	Troponin I (TNNI2) + Acetyl (Protein N-term)	Q4JH15	398/44/86	21.0/9,30	21,3/9,02
15	Troponin I (TNNI2)+ Acetyl (Protein N-term) with fragment PDZ and LIM domain 7 (PDLIM7)	Q4JH15 A0A287AZ96	285/34/73 48/7/33	21.0/9,30	21,3/9,02 25,2/9,65
16	Mixture of Troponin I1, slow skeletal type (TNNI1) and Myosin light chain 3 (MYL3)	A0A287BG25 F1SNW4	67/9/32 41/7/28	23,0/9,50	24,8/9,77 21.7/5.00
17	Mixture of Four and a half LIM domains (FHL1)***(1) and Troponin T, fast skeletal muscle (TNNT3)***(1)	A0A5K1U1D3 Q75NG7	69/4/7 197/4/26	30,0/9,50	40,7/9,24 30,7/8,69
18	Mixture of Isoform 2 of Troponin T, slow skeletal muscle (TNNT1); L-lactate dehydrogenase A chain (LDHA) and Voltage-dependent anion-selective channel protein 2 (VDAC2)	Q75ZZ6-2 P00339 F1S2F6	201/33/73 88/15/55 70/12/57	30,0/7,40	30,0/6,41 36,6/8,18 31,6/7,48
19	Mixture of Isoform 2 of Troponin T, slow skeletal muscle (TNNT1) + Acetyl (Protein N-term); L-lactate dehydrogenase A chain (LDHA) and Voltage-dependent anion-selective channel protein 2 (VDAC2) + Acetyl (Protein N-term); fragment Creatine kinase (CKM)	Q75ZZ6-2 P00339 F1S2F6 A0A287AMP3	267/37/79 49/9/35 56/10/53 41/10/36	30,0/7,40	30,0/6,41 36,6/8,18 31,6/7,48
20	Troponin T, fast skeletal muscle (TNNT3)***(2) with C-term fragment Four and a half LIM domains 1 (FHL1)***(1)	Q75NG7 A0A5K1UHX7	189/30/55 108/20/48	30,0/8,40	30,7/8,69 40,1/8,82
21	Four and a half LIM domains 1 (FHL1)***(1)	A0A5K1UHX7	309/58/74	29,0/8,70	40,1/8,82
22	Troponin I (TNNI1)	B3VCE8	399/52/92	22,0/9,00	21,6/9,61
	Heat shock proteins				
23	Alpha-crystallin B chain (CRYAB) + Acetyl (Protein N-term)	Q7M2W6	322/27/99	20,0/7,60	20,1/6,76
24	Heat shock protein family B (small) member (HSPB6) + Acetyl (Protein N-term)	A0A287AQR8	254/15	17,5/6,00	17,4/5,95
	Muscle enzymes				
25	ATP synthase subunit alpha (ATP5F1A)	F1RPS8	399/44/66	52,0/7,40	58,2/8,95
26	Mixture of Adenylate kinase isoenzyme 1 (AK1) and C-term fragment of Superoxide dismutase [Mn], mitochondrial dismutase (SOD2)	P00571 A0A287A8A8	375/36/86 315/8/68	20,5/7,90	21,6/8,38 48,5/10,68
27	Mixture of Adenylate kinase isoenzyme 1 (AK1) and C-term fragment of Superoxide dismutase [Mn], mitochondrial dismutase (SOD2)***(2)	P00571 A0A287A8A8	22/144/70 359/17/39	20,5/7,90	21,6/8,38 48,5/10,68
28	Mixture of Adenylate kinase isoenzyme 1 (AK1) and fragment PDZ and LIM domain protein 3 (PDLIM3)***(1)	P00571 Q6QGC0	268/31/82 119/05/21	21,5/7,95	21,6/8,38 39,5/6,79
29	Mixture of Adenylate kinase isoenzyme 1 (AK1) and Superoxide dismutase (SOD2)***(1)	P00571 A0A287A4Z2	304/36/86 164/19/76	20,5/7,90	21,6/8,38 27,1/8,52
30	Adenylate kinase isoenzyme 1 (AK1)	P00571	509/44/92	21,5/7,95	21,6/8,38
31	Creatine kinase M-type (CKM)***(1)	Q5XLD3	389/42/84	43,0/7,40	43,0/6,61
32	Creatine kinase M-type (CKM)***(1)	Q5XLD3	467/47/88	43,0/7,40	43,0/6,61
33	2-phospho-D-glycerate hydro-lyase (ENO3)***(1)	A0A5Г2P7P6	806/54/85	45,0/7,70	46,9/8,05
34	2-phospho-D-glycerate hydro-lyase (ENO3)***(3)	A0A5Г2P7P6	817/57/84	45,0/7,70	46,9/8,05
35	Fructose-bisphosphate aldolase (ALDOA)***(1)	A0A286ZYX8	551/44/85	40,0/8,60	39,8/8,49
36	Fructose-bisphosphate aldolase (ALDOA)	A0A286ZYX8	522/44/84	40,0/8,60	39,8/8,49
37	Mixture of Acyl-CoA-binding protein (DBI), Cardiac phospholamban (PLN)***(1), C-term fragment of Adenylate kinase isoenzyme 1 (AK1)***(1)	A0A5S6I0G9 P61013 A0A286ZQ79	82/8/62 134/6/42 92/3/13	10,0/6,80	9,9/8,07 6,1/9,15 29.1/9.08
38	Ubiquitin B (UBB)***(2), Ubiquitin-60S ribosomal protein L40 (UBA52)***(2) Ubiquitin carboxyl extension protein 80 (RPS27A)***(2)	A7U5U2 P63053 A0A287AZA7	325/31/62 288/31/73 277/31/36	8,0/6,50	25,7/6,86 14,7/9,87 22,8/9,69
39	Ubiquitin B (UBB)***(2)	A7U5U2	354/16	8,0/6,50	25,7/6,86

* S/M/C — the Mascot Score is an indicator of conformity or “scorecard”; match peptides are the number of matched peptides; coverage is the percentage of the complete amino acid sequence of the protein using the identified peptides.

** MM/pI (exp.) are the obtained estimates according to electrophoretic mobility in the DE, MM/pI (calc.) are the estimates made using the data on the amino acid sequence, taking into account signal peptide removal, but with no consideration of other post-synthetic modifications using the ExPASy Compute pI/Mwtool software.

*** msms — indication of confirmatory identification by tandem mass spectrometry, the number of sequenced tryptic peptides is indicated in parentheses.

For *L. Dorsi* from the Altai, Livny, Landrace and Mangalitsa breeds, a mixture of adenylate kinase 1 and C-terminal fragment of mitochondrial superoxide dismutase (No. 26 in Table 1) was found and its quantity was 2–3 times higher than that in wild boar. Presumably, the fraction of adenylate kinase 1 has two isoforms, one of which is modified and its quantity is changed depending on the breed. Adenylate kinase catalyzes the interconversion of various adenosine phosphates (ATP, ADP and AMP) and plays an important role in cellular energy homeostasis.

The fraction of muscle enolase (ENO3) in muscle tissue is one of the major in all analyzed samples. In domestic pigs it is comparable in quantity with muscle creatine phosphokinase (Figure 1, No. 31–34 in Table 1), except for the Livny breed and wild boar, where the quantity reduced practically three times (by 16 % when norming by the quantity of muscle creatine phosphokinase), which can be linked to reduced glycogen synthesis. In the Altai, Mangalitsa and Livny breeds, modified fraction of ENO3 was 21.5 % of the major fraction. In the samples of *L. Dorsi* from wild boar, the quantity of ENO3 varied — in five samples the quantity reduced up to two times, while in three samples it reduced even more.

Upon identification of the ENO3 fraction in the Livny breed, the amino acid replacement 298Q/K — glutamine with lysine — was revealed. In the samples of the Altai breed, 298Q (glutamine) was present in this position. The sequence corresponded to Landrace. In the Uniprot database, the information about amino acid replacements in protein is absent, and it is denoted as a canonical form 298Q like in the database Sus_scrofa scrofa_20210319 (47259 sequences; 25814658 residues). In one sample from wild boar, the results of identification (No. 48) showed that it is muscle enolase ENO3; however, the spectrum of tryptic peptides contained a mass peak corresponding to the variant 298Q, and tandem mass spectrometry of the peak with m/z 1050.5 showed (like in the Livny breed) that the variant 298K is also present. Therefore, this animal was heterozygous and possibly the occurrence of these variants in wild animals is different from domestic breeds/ hybrids, which are more homogeneous in terms of genome.

In the Livny breed, Mangalitsa breed and wild boar, the presence of the ubiquitin fraction (No. 38,39 in Table 1) was revealed. This protein was discovered in 1975 [28]. Mammals (including humans) have four different genes encoding ubiquitin — UBB, UBC, UBA52 and RPS27A. Each of these genes codes a single copy of ubiquitin in the composition of polyprotein (polypeptide consisting of the precursors of several proteins that subsequently separate as a result of restricted proteolysis). The product of the UBA52 gene is initially synthesized as ubiquitin "sewn" to ribosomal protein L40, and the product of the RPS27A gene as ubiquitin "sewn" to S27A. Ubiquitin contains 74 amino acid residues, the calculated parameters of mass/pI is 8.45/6.56. All revealed peptides were exactly within its amino acid sequence. One of the functions of ubiquitin is lipid binding and formation of fat layers in muscle tissue. Apparently, an increased quantity of ubiquitin can be realized through the enhanced formation of fat layers.

Discussion

Table 2 presents qualitative differences in changes in proteins between studied domestic pig breeds and wild boar.

When performing investigations of the muscle tissue samples from breeds/hybrids of domestic pigs, it was found that they clearly differ by the composition of different variants of fast and slow types of muscle tissue. Myosin light chains are traditionally linked with the growth of muscles. In pigs, muscle growth and differences in muscle fibers can be regulated by MYL3 [29]; with that, MYL3 transcripts were found in abundance in Pietrain and Polish Landrace [30]. We revealed in our work an increase in expression of MYL3 in the samples of tissues from the Altai and Livny breeds as well as in Landrace.

Puig-Oliveras et al. [31] showed differential expression of MYL6B in muscles when comparing groups with high and low level of backcrossing of Iberian x Landrace by the peculiarities of the fatty acid composition. High expression of myosin light chains (MYL2, MYL3 and MYL6B) was revealed in normally stained part of m. *biceps femoris*, in cardiac muscle [32]. A high level of expression of MYL2 gene

Table 2. Summary table of the obtained results on changes in protein fractions

Fractions	Altai breed	Landrace breed	Mangalitsa breed	Livny breed	Wild boar
Myosin light chains	**↑↑↑ MYL3;**	**↑↑ MYL3**	**MYL1/MYL3 pI shift to 9.5**	**↓↓↓ MYL1; ↑↑MYL2; ↑↑↑ MYL3; appearance of MYL6B**	**Replacement of MYL3 with MYL6B isoform;**
Troponins	**↓↓ TNNT3, TNNI2**	**↓↓ TNNT3, TNNI2**		**Formation of fragment FHL1; appearance of TNNI1**	
Heat shock proteins			**↑↑↑ CRYAB, HSPB6**	**↑↑↑ CRYAB, HSPB6**	**↑↑↑ CRYAB**
Muscle enzymes	**↑↑ATP5F1A ↑↑AK1+SOD2**	**↑↑ATP5F1A ↑↑AK1+SOD2**	**↑↑ATP5F1A ↑↑AK1+SOD2 ↑↑UBB**	**↑↑↑ ATP5F1A ↑↑AK1+SOD2 ↓↓ ENO3+a.з. 298Q/K ↑↑↑ UBB +a.з. 68M/H**	**↓↓↓ ENO3, +a.з. 298Q/K ↑ UBB**

was established in skeletal muscles of Duroc and Large White pigs compared to the local breed Piau. The authors indicated the relation of the gene to the growth of mammalian skeletal muscles [8]. We revealed appearance of the MYL6B fractions in *M. Dorsi* from pigs of the Livny breed and replacement of a part of MYL3 fractions with MYL6B isoform in the samples from wild boar.

A decrease in the MYL1 expression upon an increase in MYL2 in pigs of the Livny breed as well as the shift of the isoelectric point of MYL1/MYL3 fractions in Mangalitsa can be associated with the increased formation of intermuscular and intramuscular fat, which classifies them as fat-type breeds. This is confirmed by the results of [33]. Based on the results of the revealed different patterns of MYL1 expression, the authors characterized the local breed Lantang as fatty and commercial breed Landrace as lean.

The quantity of troponin T is regarded as a good indicator of meat tenderness [34]. TNNI1 plays an important role in muscle development and determines quality characteristics of meat, which was demonstrated in [35] on meat samples from pigs of the Mongcai breed. Appearance of slow troponin I in the muscle tissue samples from Livny pigs can also be linked with an increased content of intramuscular fat [36]. TNNI1 is assigned to the sex-specific biomarker upon low level of intramuscular fat [37] and is linked with type I fiber in Large White pigs [38]. It can be assumed that in the Livny breed there are certain differences in the FHL1 gene, which encodes 4.5 LIM domain-containing protein responsible for the development of skeletal and cardiac muscles [33,39]. These differences lead to the accelerated decomposition of this protein and changes in the development of muscle fibers as defects in this gene result in the development of a range of myopathies [40].

The content of HSPB6 can decrease during rigor mortis and is usually associated with beef tenderness [41,42]. Its increased content in Mangalitsa and Livny pigs can be an indicator of higher toughness of meat, which is balanced by increased fatness.

It is interesting that the ATP5F1A gene is linked with increased female and male fertility in Swallow-bellied Mangalitsa [43] and, correspondingly, its expression was increased in all analyzed pig breeds except for wild boar.

An increase in the expression of the mixture of adenylate kinase 1 (AK1) and C-terminal fragment of mitochondrial superoxide dismutase (SOD2) was revealed in muscle tissue of the Altai, Livny, Landrace and Mangalitsa breeds despite data about their reduced content in lean commercial breeds [44] and high expression in combination with an increased content of ENO3 in fat-type local breeds [33].

The presence of polymorphisms in the ENO3 gene can be linked with a percentage of fat, average thickness of backfat, meat marbling and amount of intramuscular fat in two different populations of domestic pigs [45]. For Mangalitsa and wild boar, the revealed combination of the reduced level of ENO3 with the amino acid replacement 298Q/K can facilitate better survival of animals, in particular, due to specific features of the development of fat layer upon temperature fluctuations [46].

An increased expression of protein ubiquitin (UBB) in our study was shown for Livny pigs, Mangalitsa and wild boar, which can be related to the characteristics of semen [47] and is linked with the regulation of the transcriptional network of the innate immunity [48]. In addition, the ubiquitin — proteasome system influences muscle homeostasis and development of fat layers [49].

Predictions of the protein interaction network demonstrated that differences in the growth of muscle and muscle fibers between Chinese local breeds and introduced Western breeds are regulated by genes ENO3, TNNT3 and MYL3. Gene TNNT3 also was described as a candidate gene for meat quality in Tunchang pigs [50].

Conclusion

Identified proteins represent the coherent functional system and are included in the functional clusters of muscle work: from the contractile apparatus (troponin, myosin) to the systems of its energy supply (glycolysis, creatine kinase system, oxidative phosphorylation) and defense against the accompanying metabolic stress (antioxidant system), including for maintenance of the long-term integrity of muscle fibers in conditions of high functional load.

Compared to the Altai and Landrace breeds, pigs of the Livny and Mangalitsa breeds are more similar to wild boar by a broad range of traits. For pigs of the Livny breed changes touch all analyzed blocks of protein fractions. Of particular interest is the appearance of the myosin fractions MYL6B, hydrolase ENO3 with the amino acid replacement 298Q/K upon an increase in the content of alpha-crystallin CRYAB, which is typical of wild boar.

The revealed similarity of the proteomic profile between pigs of the Livny breed and wild boar, apparently, is a consequence of their genetic closeness, determined by crossbreeding of local long-eared pigs, which theoretically could crossbreed with wild boar. Keeping the Mangalitsa pigs in the free-range conditions upon lowered environmental temperatures determine the characteristic changes in the protein profile of muscle tissue, namely, modification of the expression of myosin light chains and an increase in the level of alpha-crystallin B-chain (CRYAB), which is approximated to the living conditions of wild boar.

The revealed systemic interrelations form a significant basis for the following deepened comparative analysis of proteomic landscapes and understanding of molecular evolution between different pig breeds and wild boar. Atypical proteins (MYL6B, variants ENO3 and UBB) are promising targets for deep molecular genetic analysis aimed at establishing their contribution to quality and functional-technological properties of meat raw materials.

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Review article

EFFECT OF ESSENTIAL OILS IN MEAT

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Abstract
Consumer demand for safer and healthier food products has driven the meat industry to seek natural alternatives to synthetic preservatives. Essential oils (EOs), volatile compounds derived from plants, have emerged as promising natural preservatives due to their strong antimicrobial and antioxidant activities. This review provides a comprehensive analysis of the effectiveness of EOs in extending the shelf life of meat and meat-based products. It discusses the composition and major bioactive compounds such as terpenes, terpenoids, and phenylpropanoids, which play key roles in preservation. Moreover, critical factors influencing EO effectiveness, including concentration, interactions with food matrices, and application methods, such as direct incorporation, vapor-phase diffusion, and encapsulation technologies, are thoroughly examined. The mechanisms of EO action, including disruption of microbial cell membranes, generation of reactive oxygen species (ROS), enzyme inhibition, and DNA damage, are described in detail. The antioxidant activity of EOs is also discussed, including their ability to scavenge free radicals, chelate metals, and deactivate singlet oxygen. This review emphasizes the potential of EOs as natural preservatives that support food safety and quality in line with consumer preferences for clean-label products with minimal synthetic additives.

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Introduction

The global meat market has increased over the past fifty years because of its role as a primary source of animal protein with essential amino acids that are important for the human body [1]. Protein is an integral criterion for the quality of meat and meat products. Meat products are products of further processing of meat derived from muscles and/or animal fat and are produced with the use of additives to improve sensory quality and product volume [2]. Basic technologies in the meat processing industry include curing, seasoning, filling, smoking, cutting, and mixing [2]. Meat is vulnerable to spoilage and has short shelf life due to high nutrient and moisture content with almost neutral pH being a suitable medium for microbial growth [3]. In addition, meat especially with high fat content, is very susceptible to oxidation. This process can reduce the quality of a product by producing an unpleasant aroma, changes in taste, and reduced nutritional value [4]. Lipid oxidation in meat is influenced by various factors, such as light exposure, storage temperature, acidity level, and fatty acid composition. In addition, proteins in meat can also undergo oxidation, which has an impact on color changes and decreased nutritional content [5].

As a result, many meat industries are using chemical preservatives to extend shelf life and avoid deterioration of meat and meat products. These chemical preservatives play a role as antimicrobial agents to inhibit microbial growth, thus maintaining the quality of products. Antimicrobial compounds are commonly used in meat products such as chloride, sulfides, organic acids, nitrites [6,7]. Other chemical preservatives applied in food include butylated hydroxyanisole (BHA) and butylated hydroxyl toluene (BHT) [8]. However, nowadays, the awareness of people about benefits of consuming healthier food is increasing. They tend to prefer natural preservatives compared to chemical preservatives [9]. Furthermore, artificial or chemical preservatives might have adverse effects on consumers' health associated with toxicity effects [10]. Therefore, the use of natural preservatives is more preferred and has been developed in many food industries.

Specifically, the application of herbs, spices, and essential oils in food has been developed over the years. Some studies reported about the effect of essential oils and extracts on food as antioxidants and antimicrobials [11]. Also, plant essential oils have abilities as antiviral and antifungal agents and are regarded as good sources of novel antimicrobial substances [12]. Several studies have been conducted to know the effect of plant essential oils in meat and meat products. Ibrahim et al. [13] studied the use of basil essential oil in minced pork which proved to have a good effect on microbial inhibition and sensory properties. Other plant essential oils that have good potential as preservatives include oregano oil with antibacterial, antifungal, and antioxidant activities [14]; cinnamon oil with antiseptic, antipyretic, and anti-inflammatory activities [15]; garlic oil with the ability to regulate and strengthen the function of the immune system [16]; black pepper oil with anticancer, antifungal, antiseptic and antioxidant activities [17]. Contrary to chemical preservatives, essential oils offer tremendous beneficial effects in food, especially meat and meat products. Given these conditions, a comprehensive study is needed regarding the effectiveness of essential oils as natural preservatives in meat products. Therefore, this review is compiled to examine a degree to which essential oils contribute to extending the shelf life of meat and to understand the mechanism of their active compounds in inhibiting microbial growth and lipid oxidation. A deeper understanding of the preservative potential of essential oils is expected to facilitate the adoption of safer and more sustainable preservation strategies in the food industry, aligning with the growing consumer demand for products with minimum synthetic additives.

Objects and methods

Information was collected from several databases: Scopus, PubMed, ScienceDirect, ResearchGate, and Google Scholar. The keywords used as a search strategy were essential oil, meat, meat product, meat preservation, meat contamination, and oxidation. The information collected from journals was published from 1987 until 2025 (159 references were selected for this review); language: studies published in English. Inclusion criteria were relevance articles on essential oils application, including bioactive compounds, meat and meat products, essential oil mechanism, meat deterioration. Exclusion criteria in this review were unrelated topics: studies not focusing on the application of essential oils on meat and meat products and no access to the full text articles.

Key bioactive compounds in essential oils

Essential oils, also known as volatile or aromatic oils, are concentrated, hydrophobic liquids consisting of volatile aromatic compounds extracted from plants [18]. These oils, often referred to as essences, are complex mixtures of various volatile constituents biosynthesized by living organisms and extracted from the plant matrix through methods such as water, steam, and dry distillation, or mechanical processes like expression in citrus fruits [18,19]. The composition of essential oils is highly variable and is influenced by factors such as plant species, geographical location, and the extraction method used [20]. They are primarily composed of volatile compounds of terpenoid origin, including monoterpenes and sesquiterpenes, which may appear as hydrocarbons or their oxygenated derivatives, such as alcohols, aldehydes, ketones, esters, and phenols. Non-terpenoid compounds such as phenylpropanoids and fatty acids also contribute to the complexity of essential oils [20].

Essential oils (EOs) are concentrated liquids containing volatile compounds extracted from various plant parts, such as flowers, leaves, seeds, bark, and roots — using specific extraction techniques, with steam distillation being the most common method due to its ability to preserve volatile constituents [21,22]. In plants, EOs play essential roles in aroma production, communication, and defense mechanisms [21], while industrially they are widely utilized in medicine, cosmetics, perfumery, and the food industry because of their diverse biological activities, including antioxidant, antimicrobial, anti-inflammatory, anticancer, and antiallergic properties [23,24]. Their lipophilic nature and small molecular size allow EOs to penetrate biological membranes easily, enabling them to exert various therapeutic effects [21].

The bioactivity of essential oils is primarily attributed to the presence of specific organic compounds, including terpenes, terpenoids, phenylpropanoids, and others. There are several key bioactive compounds in EOs:

- **Terpenes and Terpenoids:** These are the most prevalent bioactive compounds in essential oils. They include a variety of structures that impart diverse biological activities such as antimicrobial, anti-inflammatory, and antioxidant properties. Some well-known terpenes include limonene, α-pinene, and thymol [23,24];
- **Phenylpropanoids:** These compounds have been recognized for their anticancer potential. Compounds such as eugenol and geranyl acetate are notable for their antioxidative and antimicrobial properties, making them significant in both therapeutic and food preservation contexts [25,26];
- **Thymol:** Found in many essential oils, thymol exhibits multiple bioactivities, including anti-inflammatory effects. It acts by modulating molecular pathways such as NF-κB and JAK/STAT, and also shows potential in therapeutic formulations due to its antimicrobial powers [27].

Bioactive compounds encompass a wide array of secondary metabolites synthesized by plants and other organisms beyond just the commonly recognized few. While it is common to discuss primary bioactive compounds such as flavonoids, phenolic acids, and carotenoids, there are many more diverse types of bioactive compounds found in various sources. For example, guava leaves are rich in a range of bioactive compounds including tannins, terpenes, alkaloids, and others, which exhibit significant synergistic effects in enzyme inhibition, as well as antimicrobial and anti-inflammatory activities [28]. Ficus fruits also contain a diverse range of biologically active compounds such as carotenoids, flavonoids, phenols, and vitamin C, contributing to their functional properties and health impacts [29].

Moreover, the secondary metabolites derived from *Fomitopsis betulina*, which include terpenoids, phenols, and other classes, exhibit pharmacological activities such as anti-cancer, anti-inflammatory, antimicrobial, antiviral, and anti-malarial effects [30]. Additionally, compounds from marine organisms identified over a span of several decades amount to thousands of chemical substances, highlighting the extensive variety inherent in marine bioactive compounds [31]. In agricultural by-products and plants, various bioactive compounds such as flavonoids, phenolic acids, and α- and β-acids are identified, indicating that the range of bioactive constituents extends well beyond the commonly referenced trio of polyphenols, terpenoids, and alkaloids [32].

There are several applications and benefits of EOs:

- **Food Preservation:** Essential oils are increasingly being used as natural additives for extending the shelf-life of food products. Due to their potent antibacterial and antioxidant properties, they serve as a natural alternative to synthetic preservatives. Composite films incorporating EOs enhance the phenolic stability and shelf-life of food items [33,34];
- **Biotechnological and Industrial Uses:** Essential oils possess characteristics that make them useful in bioactive films and packaging materials, offering a dual role in preservation and environmental sustainability. For example, films made with oregano essential oil have shown high antimicrobial activity, suggesting potential in smart packaging solutions [35].
- **Medicinal Uses:** Essential oils have traditional uses in medicine, supported by scientific findings of bioactive properties like anti-inflammatory, anticancer, and more. They are explored for various therapeutic applications in pharmaceuticals and other health-related fields [24,26].

Factors influencing the effectiveness of essential oils

The efficacy of essential oils (EOs) as preservatives in meat products is modulated by several key factors. In particular, the concentration of the oil used, the composition of the food matrix, and the mode of application all play critical roles in determining antimicrobial and antioxidant outcomes. Below, we review how each of these factors can influence the effectiveness of EOs in meat systems.

Concentration and dosage

The concentration of EOs necessary to inhibit pathogens is a critical factor in their antimicrobial effectiveness. The minimum inhibitory concentration (MIC) of an EO against foodborne microorganisms serves as a baseline for its potency [36]. MIC assays, such as broth dilution methods, identify the lowest concentration that prevents microbial growth in vitro, which informs the dosage for preservative effects. However, translating MIC values from laboratory settings to real food systems is not easy, as studies indicate that EOs effective in vitro may require higher concentrations in actual meat products due to the complex meat matrix and storage conditions that can reduce EO activity [37].

Moreover, EOs are potent flavor agents, and their concentrations can significantly affect the sensory attributes of meat. Even at sub-inhibitory levels, EOs can impart distinct aromas and tastes, but higher concentrations near the MIC can negatively impact consumer acceptability [37]. For example, adding approximately 1.5 % (w/w) of clove or lemongrass oil to ground beef, close to the MIC for *Listeria*, led to a significant decrease in sensory scores for taste and overall acceptability [38]. Concentrations above 3 % were deemed unacceptable in terms of flavor and caused noticeable color changes [38]. This highlights the challenge of balancing antimicrobial efficacy with flavor, as excessive EO can lead to off-flavors and odors perceived as spoilage [37]. Therefore, producers must identify the lowest effective EO dose that inhibits pathogens while remaining below sensory detection thresholds. Combining EOs with other preservation methods, such as mild heating or acids, can help achieve microbial control while minimizing sensory impact [37].

Interaction with food components

The performance of EOs in meat products is significantly influenced by the composition of meat, particularly its fat, protein, and moisture content. EOs, being hydrophobic, tend to partition into lipid-rich phases. This leads to reduced concentrations of active compounds in high-fat meats, which can bind or absorb EOs, diminishing their bioavailability and requiring higher initial doses for antimicrobial effects [36]. Conversely, higher moisture content facilitates EO dispersion and enhances contact with microbes [36].

This phenomenon has been observed in various food systems, such as the reduced antibacterial activity of cinnamon and clove oils against *Listeria* in whole milk due to milk fat binding, while these oils were more effective in skim milk [36]. Similarly, EOs inhibited bacteria more effectively in low-fat cheese compared to full-fat cheese [36]. Essential oils require some moisture to diffuse and contact microbes, indicating that very low-moisture conditions can limit their effectiveness. The fat-protein matrix of meat can act as a sink for lipophilic EO compounds, reducing their active portion, while the water phase is crucial for their antimicrobial and antioxidant functions. Therefore, producers must consider the specific meat composition, as a high-fat sausage may need more EO or different delivery methods than a low-fat product to achieve similar preservative effects [36].

Additionally, the food matrix impacts the antioxidant efficacy of EOs. While many EOs can slow lipid oxidation in meats, their effectiveness depends on their distribution in the lipid phase. If EOs are tightly bound to proteins or trapped away from fats, their ability to scavenge free radicals is reduced. However, if EOs dissolve preferentially in fat, they can protect it from oxidation, provided they remain stable and well-dispersed. High water activity can promote uniform EO dispersion and potentially generate oil-in-water microemulsions that carry antioxidants to lipid oxidation sites. Thus, the intrinsic properties of meat and their interactions with EO compounds are critical for the efficacy of EOs as preservatives [36]. Successful EO application often requires tailoring formulations to the specific food matrix or employing strategies to mitigate matrix binding effects.

Mode of application

The application of essential oils (EOs) to meat products significantly influences their effectiveness and practicality, with two common methods: direct incorporation and vapor-phase diffusion. Direct incorporation involves mixing the EO with meat or adding it to marinades, casings, or edible coatings, ensuring immediate proximity to spoilage or pathogenic microbes. This method can inhibit microbial growth and oxidative spoilage but may lead to rapid loss of volatile compounds and undesirable flavor profiles, particularly in mildly flavored meats [39]. Additionally, some EOs may react with meat components during processing, potentially reducing their effectiveness.

In contrast, vapor-phase application introduces an EO as a volatile vapor in the packaging environment, allowing for diffusion onto the meat surface without complete absorption. This method can be achieved through impregnated packaging materials, EO-infused sachets, or active coatings that release the oil slowly. Vapor-phase application provides uniform surface coverage, effectively targeting surface-growing molds or bacteria [36,40]. It also minimizes sensory impact on the meat. Exposing refrigerated chicken breasts to EO vapors significantly slowed microbial growth and lipid oxidation without detectable sensory changes [41]. However, vapor-phase methods are generally more effective against surface contamination and may not address bacteria deep within solid meat products. The choice between direct and vapor application depends on the type of meat product, target microbes, and packaging conditions.

In recent years, encapsulation techniques have become innovative methods for delivering essential oils (EOs) in meat systems, merging direct and controlled-release applications. Encapsulation protects EO molecules by entrapping them in carriers like emulsions, liposomes, or biopolymer matrices, which control their release. For instance, nanoemulsions, which are oil-in-water emulsions with droplet sizes in the nanometer range, can be mixed into meat batters or sprayed onto surfaces [41]. These tiny droplets, stabilized by food-grade emulsifiers, enhance EO distribution and reduce rapid evaporation, improving contact with microorganisms while minimizing strong aroma intensity [41].

Liposomes, another encapsulation strategy, consist of phospholipid bilayer vesicles that can carry hydrophobic EO components in their lipid phase, allowing for better stability and a more controlled antimicrobial effect [42]. Encapsulation addresses challenges like volatility and poor water solubility of EOs, enabling reduced effective doses and gradual release during storage, which extends shelf life [43]. Encapsulation has shown promising results in preserving meat quality. For example, thyme essential oil in nanoliposomes demonstrated a strong bacteriostatic effect against *Salmonella enteritidis* in chicken meat without negatively impacting sensory qualities [42] Similarly, *Zataria multiflora* essential oil in nano-liposomal form exhibited enhanced antioxidant and antimicrobial activity in beef patties compared to free oil [44]**.** Other systems, such as biopolymer nanoparticles and solid lipid nanoparticles, have also been explored for protecting EOs and modulating their release [45].

Overall, encapsulated EOs allow for controlled release, maintaining inhibitory concentrations longer and reducing initial aroma bursts [45]. This approach aligns with vapor-phase concepts, where EOs can be encapsulated in coatings that release them into the package atmosphere over time. Advances in delivery methods, from vapor-phase systems to nanoencapsulation, enable food scientists to optimize antimicrobial efficacy while preserving meat quality [36]. By selecting or combining application methods, the benefits of EOs can be maximized to enhance meat product safety and shelf life.

Antimicrobial mechanisms of essential oils

Cell membrane disruption

Essential oils interact with the lipids in the cell membranes of microorganisms due to their hydrophobic nature. This interaction disrupts the cell membrane, leading to leakage of cytoplasmic contents and cell death [46,47]. Furthermore, the hydrophobic components of EOs can penetrate the lipid bilayer, causing structural and functional damage to the membrane [46,48].

Reactive oxygen species (ROS) generation

EOs can induce the production of ROS within microbial cells. The oxidative stress caused by ROS can damage cellular components, including proteins, lipids, and DNA, leading to cell death [47].

Enzymatic inhibition and DNA damage

EOs can inhibit essential enzymes and cause DNA degradation, further contributing to their antimicrobial effects [47].

Antioxidant mechanisms of essential oils

Free radical scavenging

Essential oils are composed of compounds that have the ability to neutralize free radicals, thereby preventing oxidative injury to food products. This process serves in delaying lipid oxidation and improving food quality [49–51]. The free radical is neutralized through the acceptance of a hydrogen atom from the antioxidant compound, leading to the creation of a more stable molecule that is defined by a low standard reduction potential [52]. The resonance delocalization in the ring structure of phenolic substance determines the stability of antioxidant radicals [53]. Examples of compounds that possess the ability to neutralize free radicals include carotenoids, ascorbic acid, flavonoids, lignans, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA) [54]. Nonetheless, the effectiveness of antioxidants is influenced by various factors, including the delocalization of antioxidant radicals, pH levels, and reduction potential. The transfer of hydrogen from the antioxidant to the free radicals in food exhibits thermodynamic stability when the bond dissociation energy in the antioxidant is low [55]. The capacity of antioxidants to donate hydrogen increases as their reduction potential decreases [53].

Metal chelating

The activation energy in the initiation stage is lowered when metal is present during oxidation. In particular, metal catalyzes the production of hydroxyl radicals by hydrogen peroxide and removes the hydrogen from food radicals [56]. Metal chelating prevents oxidation by preventing the development of insoluble metal complexes, metal redox cycling, and steric barrier between metals and dietary ingredients [57]. Ascorbic acid, polyphenols, lignans, and amino acids are among the antioxidant substances that have been identified as metal chelators [58].

Singlet oxygen quenching

The quenching process may involve both physical and chemical mechanisms. Singlet oxygen undergoes deactivation and conversion into ground-state triplet oxygen via physical quenching, facilitating the transfer of charge or energy [59]. A single-state charge transfer complex is created when the quencher donates an electron to the singlet oxygen. The complicated transitions to the triplet state occur via the intersystem crossing mechanism. Ultimately, a quencher and triplet oxygen are linked to the triplet state charge transfer complex. However, the chemical quenching method produces oxidation products through a quencher oxidation reaction [60].

Mechanisms of meat deterioration

Microbial spoilage

Growth of spoilage and pathogenic microorganisms

Fresh meat is highly susceptible to microbial growth due to its nutrient-rich and high-water content. While internal tissues of living animals are sterile, contamination occurs during slaughter and processing [61]. Bacteria from equipment, handlers, and the environment colonize the meat surface, leading to spoilage, which is primarily driven by microbial contamination rather than autolytic enzyme activity or chemical oxidation [62]. Pathogenic bacteria, such as *Salmonella* and *Escherichia coli*, may not show obvious spoilage signs but pose food safety risks, while spoilage microorganisms like *Pseudomonas* spp. actively deteriorate meat quality without necessarily causing illness.

The growth of spoilage and pathogenic microbes in meat is influenced by intrinsic factors such as pH, water activity (a_w), and nutrient composition. Post-mortem meat pH typically ranges from 5.5 to 5.8, allowing many spoilage bacteria to thrive despite their preference for near-neutral pH. The high water activity of fresh meat (approximately 0.99) supports microbial proliferation, as most bacteria require $a_w > 0.90$ [63]. Extrinsic factors, including temperature, humidity, and oxygen availability, also significantly affect microbial growth rates. For example, improper storage temperatures above 5 °C can accelerate bacterial growth, leading to spoilage within days [61]. In contrast, strict cold storage (0–4 °C) can extend shelf-life by delaying bacterial growth. Additionally, humidity and packaging conditions influence microbial survival, with high humidity favoring bacteria and oxygen-rich environments promoting aerobic spoilage. In conclusion, meat spoilage and pathogen growth are determined by initial contamination and growth conditions, emphasizing the need to control factors like temperature, pH, and moisture to suppress microbial proliferation [61,63].

Common spoilage bacteria and their impact on meat quality

Microorganisms can spoil meat, with specific bacterial groups being primarily responsible for quality defects. Studies indicate that in chilled fresh meats stored aerobically, *Pseudomonas* and *Brochothrix* are often the main spoilers [61]. *Enterobacteriaceae* also frequently appear, particularly in processed or temperature-abused meats. Each group affects meat quality through changes in odor, texture, and appearance.

— *Pseudomonas* spp.: This Gram-negative, aerobic genus thrives at refrigeration temperatures and is a leading cause of spoilage in raw meat stored in air. They metabolize meat nutrients, producing volatile compounds that create off-odors described as “fruity” or “putrid” [64]. High populations lead to dense colonies and a slimy film on the meat, resulting in a wet and soft texture. Certain strains can discolor meat, such as *P. fluorescens*, which may create a greenish sheen [61]. Overall, *Pseudomonas* spoilage is marked by sticky surfaces, strong off-odors, and loss of color.

— *Brochothrix thermosphacta*: This Gram-positive, nonsporeforming rod is a major spoilage organism in both aerobic and anaerobic conditions [65]. It ferments sugars and amino acids, producing off-odors like “buttery” or “sour dairy” [65]. While it does not produce heavy slime, it can contribute to surface tackiness and discoloration [61]. *B. thermosphacta* is tolerant of low-oxygen conditions and often indicates advanced spoilage, particularly when accompanied by characteristic odors.

— *Enterobacteriaceae*: This family includes facultative anaerobic Gram-negative rods that can spoil meat, often contaminating it during slaughter or processing. They are typically outcompeted by pseudomonads in fresh meat but become significant in minced or vacuum-packaged products. Their spoilage is evident through strong decompositional odors and discoloration. Some members produce colored pigments or slime, contributing to surface stickiness [61]. Their presence is associated with unacceptable smells and compromised meat quality, and some strains pose food safety risks due to their pathogenic nature.

Role of moisture and storage conditions in microbial contamination

— **Moisture availability (water activity):** Moisture is a critical factor in microbial spoilage, as bacteria require water to grow, and the high water content of meat renders it inherently perishable. Water activity (a_w) measures the availability of free water for microbial use;

fresh beef, with an a_w close to 0.99, offers near-optimal conditions for bacterial proliferation [63]. Consequently, when other environmental conditions are favorable, bacteria can multiply rapidly on meat surfaces. Controlling a_w has long been a fundamental preservation strategy, involving methods such as drying, salting, or the addition of curing salts to bind water. Lowering a_w effectively inhibits microbial growth [61], emphasizing the importance of moisture control. High-moisture meat or storage under high-humidity conditions accelerates microbial activity, whereas reducing available moisture can significantly prolong shelf-life. For example, meats stored in environments with reduced relative humidity may develop a dry surface "crust" that resists bacterial colonization although this may alter the product's appearance. This highlights the central role of a_w in limiting microbial spoilage. In summary, meat products with higher levels of free moisture are substantially more susceptible to microbial contamination and deterioration than drier counterparts.

— **Storage conditions and cross-contamination:** Proper storage conditions are essential to prevent microbial spoilage of meat. Among these, temperature is the most critical factor: maintaining meat at refrigerated or frozen temperatures significantly slows bacterial growth, whereas improper storage commonly referred to as temperature abuse can accelerate spoilage. Even psychrotrophic spoilage bacteria exhibit markedly faster growth at moderately elevated temperatures (8–10 °C compared to 0–4 °C), leading to a substantial reduction in shelf-life by several days [61]. When meat is left at ambient temperature, mesophilic bacteria including pathogenic microorganisms can proliferate rapidly, reaching hazardous levels within hours and posing both spoilage and food safety risks. The storage atmosphere also plays a key role: aerobic conditions promote the growth of organisms such as *Pseudomonas* (as previously discussed), whereas vacuum or modified-atmosphere packaging can inhibit aerobic microbes but may permit the growth of anaerobes or lactic acid bacteria. Therefore, selecting appropriate packaging and gas composition is crucial for targeting specific spoilage organisms. For instance, high-CO_2 modified atmospheres are effective in suppressing *Pseudomonas* growth and can thereby extend the shelf-life of meat [62]. However, elevated carbon dioxide or reduced oxygen levels do not eliminate spoilage; instead, they shift the dominant microbial population toward species that tolerate such environments (e. g., *Brochothrix* or lactic acid bacteria), which typically cause different spoilage manifestations, such as souring rather than putrid odors.

Storage of meat is crucial to prevent cross-contamination, which can lead to spoilage or pathogenic microbes from external sources. Human handling and equipment in processing plants are major vectors of contamination, with unclean gloves, coughing, and movement between areas being major causes. Inadequate sanitation can lead to bacteria from one carcass or surface transferring to others. The processing environment often harbors resilient microbes, such as *Brochothrix thermosphacta*, which can form biofilms on surfaces, protecting bacteria and allowing them to persist [65]. Interventions like refrigeration, humidity control, sanitation, and natural preservatives are vital for maintaining meat product quality [63,61]. By managing water activity, storage conditions, and preventing contamination, the shelf-life of meat can be significantly extended, and microbial spoilage can be slowed.

Oxidation in meat product

Meat and meat products are susceptible to bacterial and chemical degradation due to their high nutritional content. The primary factor contributing to nonmicrobial deterioration in meat and meat products is oxidation. Generally, the process of oxidation leads to the removal of electrons when meat interacts with oxygen [66]. The occurrence of lipids and proteins in meat renders them susceptible to oxidation, especially since natural antioxidants diminish rapidly after slaughter [67]. Moreover, the degree of its susceptibility is affected by the types of muscle, breeds of animals, and spices [68]. The methods utilized for processing and preservation significantly impact the degree of oxidation, encompassing elements like irradiation, cooking, freezing, chilling, additives, and packaging [66].

Lipid oxidation

Lipid oxidation occurs in the muscles, starting at the stage of slaughter and persisting through the processing of animals, influenced by environmental factors and the limited antioxidant capacity of meat [69]. Lipid oxidation is acknowledged for leading to undesirable flavors, changes in color, shortened shelf life, unpleasant odors, and potential toxicity in meat products [70]. Despite these negative outcomes, lipid oxidation is considered preventable and strongly affects the nutritional integrity of meat and derived products [71]. Moreover, it contributes to the progressive decline of sensory qualities, which are crucial for consumer acceptance [72]. Among food constituents, lipids are one of the least stable components and are highly prone to oxidative degradation [68]. Oxidation in lipids may proceed through three principal pathways: photo-oxidation, enzymatic oxidation, and autoxidation [73]. The mechanism involves unsaturated fatty acids reacting with oxygen in a free radical chain reaction, producing hydroperoxides as the primary oxidative products [74]. Unlike other lipid oxidation products, hydroperoxide does not play a role in the development of odor and aroma. Nonetheless, this compound leads to the formation of a secondary compound due to its high instability, resulting in the production of esters, aldehydes, hydrocarbons, alcohols, and acids [75]. The extent of lipid oxidative stability is affected by several factors, including heat and light exposure, oxygen availability, the balance of antioxidants and pro-oxidants, and the degree of unsaturation of fatty acids [71].

Furthermore, the constituents found in muscle tissue can function as catalysts, including myoglobin, iron, ascorbic acid, and hydrogen peroxide.

Photo-oxidation

Photo-oxidation occurs especially as a result of ultraviolet (UV) radiation and in the presence of sensitizers, leads to a radical reaction that results in the formation of hydroperoxides. This process differs when it occurs without sensitizers and light [76]. This mechanism differs from the initiation that took place in autoxidation. Hydroperoxides are generated through an alternative pathway rather than the free radical mechanism outlined in autoxidation [77]. The procedure of photo-oxidation involves multiple essential phases. At the outset, light energy is captured, resulting in the excitation of the singlet sensitizer. Following this, the resulting reactions can be classified into three separate pathways:

- The production of singlet oxygen resulting from the interaction between an excited triplet sensitizer and molecular oxygen [78]. Following this, singlet oxygen reacts with the double bonds found in unsaturated fatty acids, resulting in the production of hydroperoxide when alkyl radicals are not present [52].
- The generation of superoxide radical anion occurs when an excited sensitizer reacts with triplet oxygen via electron transfer, leading to the abstraction of hydrogen from unsaturated fatty acids, which subsequently initiates lipid oxidation. Furthermore, the interaction between superoxide radical anion and hydrogen peroxide resulted in the generation of hydroxyl radical and singlet oxygen. The products have the potential to induce lipid oxidation through interactions with fatty acids. The metal can facilitate this process [53].
- The abstraction of hydrogen can lead to the formation of alkyl radicals in unsaturated fatty acids through the action of an excited triplet sensitizer [52]. The subsequent reaction involves an alkyl radical that interacts with oxygen, resulting in the formation of a peroxy radical. This process can trigger lipid oxidation by abstracting hydrogen from neighboring fatty acids [78].

Enzymatic oxidation

Lipoxygenase performs a crucial role in enzymatic oxidation, enabling the transfer of oxygen to the hydrocarbon chain of fatty acids. The reaction produces hydroperoxides and peroxides that feature conjugated double bonds [76]. The concentration of lipoxygenase influences the rate of lipid oxidation; increased levels of the enzyme accelerate oxidative reactions. The extraction of hydrogen from the methylene group of polyunsaturated fatty acids occurs during the enzymatic oxidation process, resulting in the formation of a conjugated diene system that subsequently interacts with molecular oxygen. The chemical reaction of a peroxy radical with hydrogen linked to another unsaturated fatty acid yields an alkyl radical and a conjugated hydroperoxy diene [79].

Autoxidation

Catalytic agents such as free radicals, temperature variations, pH conditions, and the presence of metal ions play a crucial role in influencing radical-driven reactions in food systems [79]. There are three primary stages of lipid oxidation: initiation, propagation, and termination [66]. The primary mechanism for lipid oxidation in meat and meat products is autoxidation, which occurs when oxygen interacts with unsaturated fatty acids [79]. During the initiation stage, reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radicals, and superoxide anions are produced, leading to the activation of oxygen molecules. This activation is often enhanced by catalytic substances in combination with external factors like heat or light exposure. In the propagation step, oxygen reacts with alkyl fatty acids to produce peroxyl radicals (ROO*). These radicals subsequently interact with unsaturated fatty acids to generate lipid hydroperoxides (ROOH). Due to their unstable nature, hydroperoxides degrade into volatile compounds, which are associated with undesirable off-flavors [80], as well as alterations in color, protein function, and overall stability [81]. The alkyl radical will combine with oxygen during the propagation step to create radicals like peroxyl radicals. After that, the previously generated radical might stabilize and take hydrogen from another weak molecule to create a lipid hydroperoxide. Before termination occurs (two R* merge and terminate), the propagation process may take place numerous times [82].

Protein oxidation

The oxidation of protein presents a considerable issue in meat, as it directly affects the quality of a product. Nonetheless, the complex mechanisms governing protein oxidation have not been well investigated. Oxidative modifications in proteins occur along the amino acid sequence [83]. The range of reactive oxygen species (ROS) responsible for protein oxidation includes both radical forms such as superoxide (O_2*), hydroxyl (OH), thiyl (RS), and peroxyl radicals (ROO*), as well as non-radical compounds, such as hydrogen peroxide (H_2O_2) and lipid hydroperoxides (ROOH), along with reactive aldehydes [84]. Several ROS, including superoxide anions, hydroxyl and peroxyl radicals, peroxynitrite, and hydrogen peroxide are capable of initiating oxidative damage to proteins [67]. These reactive species can be generated through multiple pathways, such as exposure to ionizing radiation, redox reactions mediated by enzymes, or metal-catalyzed reactions [85]. Protein oxidation leads to alterations in protein conformation, particularly affecting its secondary and tertiary structures. This occurs when oxidizing agents interact with the protein backbone [67]. The modification of protein is acknowledged as a crucial element affecting its properties, including solubility and gelation, which subsequently impacts the physical quality of meat, such as cooking loss and hardness [86].

At the molecular level, protein oxidation typically begins at the carbon atom of amino acid residues, producing relatively stable protein-centered radicals that subsequently react with molecular oxygen to form alkyl peroxyl radicals. These peroxyl radicals may either degrade into imines, which upon hydrolysis cause cleavage of the protein backbone, or interact with nearby molecules to produce hydroperoxides [85]. Protein oxidation begins with hydrogen abstraction, resulting in the generation of protein radicals that subsequently interact with molecular oxygen [67]. Oxidation induces modifications to proteins encompassing cross-linking and fragmentation of the protein [85].

Factors affecting lipid oxidation in meat products

The process of lipid oxidation in meat encompasses intricate reactions involving both substrates and catalysts, along with various mechanisms at play. The elements influencing lipid oxidation include the composition of meat, which is an intrinsic factor, as well as the methods of processing and storage, which are considered extrinsic factors [87]. The progression and stability of lipid oxidation are strongly influenced by the balance between pro-oxidant and antioxidant agents present in the system [87]. Among the intrinsic components of meat, fatty acids represent the most critical factor determining oxidative susceptibility. However, other catalytic contributors such as transition metals, heme proteins, intrinsic antioxidant compounds, and pro-oxidant enzymes also play an important role in driving these reactions. In addition, extrinsic factors related to the animal itself, including genetic background, feeding regimen, production system, and even the specific muscle type, can alter meat composition and, consequently, modulate its oxidative behavior [68]. Furthermore, the existence of light or oxygen significantly affects the process of oxidation. The storage conditions significantly influence the rate of oxidation [77]. Essentially, every stage in the processing of muscle can facilitate oxidation, including cutting, deboning, and cooking [88].

Fatty acids

The quantity of fat and the composition of fatty acids are critical elements in the progression of lipid oxidation [89]. The level of fat unsaturation exhibits an exponential correlation with oxidation stability [74]. The presence of additional double bonds increases the susceptibility of meat to oxidation reactions [90], with (E)-isomers exhibiting greater vulnerability compared to trans isomers. Furthermore, an increase in the chain length of fatty acids will lead to a rise in oxidation [91].

Metals and heme protein

The catalytic role of metals, whether present in free form or bound within heme proteins, is a major factor driving oxidative reactions in meat [78]. Within muscle tissue, iron primarily occurs in myoglobin and hemoglobin, as well as within the active centers of specific enzymes. Among these, myoglobin and hemoglobin are the most abundant heme proteins in meat, and their degradation can release iron into the system [78]. The amount of heme protein varies depending on both the muscle type and animal species, and this concentration is a key determinant of oxidative stability. Heme proteins function as catalysts that accelerate the generation of reactive oxygen species. Their interaction with lipids is mutually reinforcing, as lipid peroxidation can enhance heme protein oxidation and vice versa [70]. Moreover, acidic conditions further promote the susceptibility of heme proteins to oxidation [78]. During lipid oxidation, aldehydes are produced, which destabilize the redox state of heme proteins and stimulate oxidation processes mediated by oxy-heme proteins [70].

Prooxidant enzymes

Meat contains various enzymes that have the potential to facilitate oxidation processes. The presence of polyunsaturated lipids leads to the production of active catalysts by those enzymes [77]. Lipoxygenase serves as the primary enzyme responsible for lipid oxidation, functioning by abstracting hydrogen from the allylic methylene position of polyunsaturated fatty acids [92]. This enzyme is involved in the initial phase of the process. Myeloperoxidase is another enzyme that facilitates lipid oxidation in meat tissue. This enzyme is present when meat comes into contact with blood during the slaughter process. The interaction between H_2O_2 and chloride results in the formation of hypochlorous acid, which subsequently reacts with $O_2{\bullet}^-$, leading to an increase in •OH levels.

Endogenous antioxidants

Various antioxidant substances present in meat influence the speed of lipid oxidation. The compounds serve to shield meat from the influence of free radicals or catalysts that promote oxidation. The primary categories of antioxidants are classified into three main groups: vitamins, peptides, and enzymes. The capacity of these compounds to neutralize radicals is critically evaluated in the oxidation process [53]. Vitamin E is the primary vitamin found in animal tissue. The protective mechanism involves a significantly higher rate of lipid radical attack on α-tocopherol compared to lipids. Consequently, it safeguards unsaturated fatty acids from the assault of free radicals. In the oxidation process, α-tocopherol transfers a hydrogen atom lipid peroxy radical, effectively scavenging the peroxy radicals and resulting in the formation of a tocoperoxy radical [53].

Peptides

Peptides play a role in oxidative stability through mechanisms such as radical scavenging, metal chelation, and the reduction of hydroperoxides. The antioxidant activity of the peptide is significantly influenced by the amino acids present in its chemical structure [87]. A variety of peptides exhibiting antioxidant properties include leucine, valine, tyrosine, histidine, methionine, and tryptophan within their amino acid sequences. The peptide containing tyrosine or tryptophan functions in the oxidation process by scavenging free radicals, whereas histidine operates by chelating metals like copper or iron [93]. Moreover, the predominant dipeptides include carnosine and anserine, which function by scavenging free radicals and chelating metals [93].

Enzymes

The main enzymes that play a role in preventing oxidation are catalase, glutathione peroxidase, and superoxide dismutase [92]. They impede oxidation via multiple mechanisms. For example, the role of superoxide dismutase includes the removal of $O_2\bullet^-$ and the generation of oxygen and H_2O_2 [93].

Storage condition

The primary factor affecting lipid oxidation is the storage condition, which includes both time and temperature. The oxidation process is enhanced by both time and temperature, with an increase in oxidation observed as the temperature rises [53]. Variations in temperature encourage oxidation by facilitating the formation of ice crystals, which in turn leads to the release of prooxidants and promotes oxidation due to cellular damage [94]. Moreover, meat subjected to freezing and thawing periods is susceptible to oxidation [92]. Furthermore, extended storage durations facilitate the release of iron from heme-protein, leading to reactions during both the initiation and propagation phases [92].

Effects of essential oils in meat products

In recent years, essential oils (EOs) have gained increasing attention as natural preservatives in meat and meat products due to their antimicrobial and antioxidant properties. This section presents a comparative review of selected research articles that evaluate the application of essential oils and their nano-based delivery systems on different types of meat, including beef, pork, camel, and ostrich. The studies highlight their effects on physicochemical parameters such as lipid oxidation (e. g., TBARS and peroxide values), microbial spoilage, sensory quality, and shelf-life extension.

Table 1 summarizes various essential oils (EOs) that have been evaluated for their application in meat products, highlighting their active compounds, the type of meat tested, and their mechanisms of action. For instance, thymol and carvacrol, the key components of basil and thyme essential oils, were effective in preventing fat oxidation in minced pork by scavenging free radicals responsible for rancidity [96]. Similarly, combination of rosemary, thyme, and clove EOs could retard lipid oxidation and inhibit microbial growth in mechanically deboned chicken meat proteins due to the ability of phenolic compounds to disrupt bacterial membranes [96]. On the other hand, Shahbazi et al. investigated the effects of thyme and cinnamon EOs on ground beef and showed that compounds, such as cinnamaldehyde, benzaldehyde, limonene, linalool, and eugenol not only inhibited microbial growth but also enhanced sensory properties such as aroma and flavor [98].

Several essential oils (EOs) have been extensively studied for their preservative effects in meat products, particularly due to their antimicrobial and antioxidant properties. For instance, blue mint bush EO, which contains carvacrol, thymol, and p-cymene, demonstrated strong antimicrobial activity in chicken meatballs by disrupting microbial cell walls, thus prolonging shelf life [98]. Similarly, rosemary EO, rich in carnosol, carnosic acid, and rosmanol, inhibited microbial growth and lipid oxidation in chicken meatballs, improving both safety and sensory quality [99]. In cooked pork sausages, nutmeg EO showed potential to slow down lipid oxidation and inhibit microbial growth due to the action of its compounds such as borneol, geraniol, and linalool, while also enhancing aroma [101].

Clove EO, with eugenol as the main compound, exhibited dual antioxidant and antimicrobial effects in Chinese bacon. It scavenged free radicals and reduced lipid oxidation markers such as malondialdehyde (MDA) and peroxide value (POV). Microencapsulation using β-CD-MOFs further improved its stability and efficacy [103]. Likewise, cinnamon EO (cinnamaldehyde) disrupted microbial membranes and extended the microbial shelf life in various meat types, including pork and chicken [104]. In beef *Mentha pulegium* EO inhibited a broad spectrum of microbes and lowered TBARS and TVB-N values, indicators of oxidative spoilage. Its nanoencapsulated form (16 ppm) significantly enhanced bioavailability and stability [105]. Meanwhile, Litsea EO, composed of citral and d-limonene, showed strong antibacterial activity in duck meat by damaging bacterial cell walls. Its nanoemulsion form also improved solubility and stability, resulting in a more effective preservation effect [106]

Essential oils derived from ajwain (Carum copticum) and cardamom, formulated into nanoemulsions, have demonstrated significant antimicrobial and antioxidant activities in processed meat products such as Mortadella sausage during cold storage. These nanoemulsions improved the sensory quality and texture of the product while extending its shelf life by inhibiting the growth of *Staphylococcus aureus* and enhancing protein stability and moisture retention [112]. Additionally, the use of *Thymus fedtschenkoi Ronniger* essential oil combined with thymoquinone in a starch-based coating exhibited high efficacy in suppressing the growth of *Listeria monocytogenes* and *Salmonella enteritidis* on chicken fillets over 12 days of storage. Active compounds such as thymol and carvacrol act by disrupting bacterial cell membranes, increasing permeability, and inhibiting vital enzyme activities, thereby reducing microbial viability [109]. These findings highlight the potential of essential oils, particularly when

Table 1. The effect of essential oils in meat products

Type of EO	Key compounds	Type of meat	Mechanism of action	Reference
Basil or thyme EO	Thymol, carvacrol	Minced pork meat	Prevents fat oxidation	[95]
Rosemary, thyme, clove	Cymol, α-pinene, eucalyptol	Mechanically deboned chicken meat protein	Retard oxidation and inhibit microbial activity	[96]
Thyme and cinnamon	cinnamaldehyde, benzaldehyde, limonene, linalool and eugenol	Ground beef	Inhibit microbial growth and improve sensory qualities	[97]
Blue mint bush	Carvacrol, thymol, p-cymene	Chicken meatball	Inhibits microbial growth	[98]
Rosemary	Carnosol, carnosic acid, rosmanol, rosmadiol	Chicken meatball	Inhibits microbial growth, reduces lipid oxidation, improves sensory quality	[99]
Thyme	Thymol	Dried meat (jerky)	Inhibits microbial growth	[100]
Nutmeg	Borneol, geraniol, linalool	Cooked pork sausage	Slows lipid oxidation, inhibits microbial growth, improves aroma quality	[101]
Oregano	Carvacrol and thymol	Dried meat (jerky)	Inhibits microbial growth, improves sensory properties	[102]
Thyme	Thymol, Carvacrol, p-cymene, γ-terpinene	Pork, turkey, broiler, beef, and processed meats (e. g. salami)	Disruption of cell membranes, leakage of intracellular contents, inhibition of respiration and energy metabolism	[37]
Clove	Eugenol	Chinese bacon (preserved meat product)	Scavenges free radicals, reduces lipid oxidation	[103]
Cinnamon	Cinnamaldehyde	Pork filet, pork bacon, chicken filets, chicken skin, salmon, scampi	Inhibits microbial growth by disrupting microbial cell membranes	[104]
Oregano	Carvacrol, Thymol	Pork filet, salmon	Disrupts bacterial cell walls and cytoplasmic membranes	[104]
Thyme	Carvacrol, Thymol	Pork filet, scampi	Affects membrane permeability, interferes with microbial enzyme systems; extends microbial shelf life	[104]
Pennyroyal	Pulegone, Menthone, Isomenthone (typical for *M. pulegium*)	Beef meat	Inhibits growth of aerobic mesophilic bacteria, lactic acid bacteria, *Pseudomonas*, molds, and yeasts.	[105]
Litsea	Citral, d-limonene	Duck meat	Destroys bacterial cell wall (notably against *Staphylococcus aureus*), enhances water solubility, reduces volatility, and improves stability, enhances preservation effect	[106]
Myrtle	1,8-Cineole, Myrtenyl acetate, α-Pinene, Linalool	Ostrich meat	Reduces lipid oxidation, protein oxidation, stabilizes pH, and preserves phenolic content. Improves flavor and acceptability, and enhances stability and efficacy.	[107]
Artemisia afra	carotenes, xanthophylls, flavonoids)	Broiler chicken	Improves slaughter weight, meat pH, water-holding capacity, and intramuscular fat. Enhances fatty acid profile. Stabilizes oxidation post-mortem.	[108]
Calamansi	D-limonene (52.64 %), α-pinene (17.29 %)	Chicken breast	Cell wall damage, cell membrane disruption, intracellular content leakage, inhibition of virulence genes, inhibition of biofilm formation and metabolic activity, is effective in preservation, and maintains sensory quality during cold storage	[109]
Pimenta	Eugenol, methyleugenol, β-caryophyllene	Turkey (breast meat and skin)	Disrupts cell membranes, reducing viability; exhibits antimicrobial synergy with peracetic acid (PAA), reducing spoilage organisms such as *Pseudomonas*, lactic acid bacteria, total aerobic bacteria, and psychrophilic bacteria	[110]
Ajwain	Thymol (~50 %)	Mortadella sausage	Disrupts bacterial cell membranes, enhances shelf-life and color, reduces fat and improves protein level	[111]
Cardamom	1,8-cineole, α-terpinyl acetate, linalool	Mortadella sausage	Antioxidant and antimicrobial properties, enhances sensory profile (taste, aroma), nanoemulsion increases bioavailability and product acceptability	[111]
Thymus fedtschenkoi Ronniger	Thymol, Carvacrol, Thymoquinone	Chicken fillets	Disrupts bacterial cell membranes, increases permeability, inhibits enzyme activity, and reduces cell viability	[112]

integrated with nanotechnology and biopolymer-based systems, as natural and innovative solutions to enhance the microbial safety and sensory quality of meat products during storage.

Conclusion

Essential oils are effective natural preservatives for meat products due to their antimicrobial and antioxidant properties. Their efficacy is influenced by concentration, food composition, and application method. Encapsulation techniques improve EO stability and reduce sensory impact. With proper formulation, EOs offer a clean-label solution to extend shelf life and enhance meat product safety, aligning with current consumer demands.

The references below are arranged in the order of their mention in the text.

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STUDY OF MEIQX AND PHIP ACCUMULATION IN PORK PATTIES DURING THEIR SEQUENTIAL HEAT TREATMENT

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Abstract

Heterocyclic aromatic amines, such as MeIQx and PhIP, are carcinogenic and mutagenic compounds formed in meat products during high-temperature cooking. Despite their proven harmful effects, they are not regulated in food products, as home-cooked products are at the highest risk of HAA formation. Therefore, research on effective ways to reduce their formation during home cooking is urgently needed. One promising approach is the use of vegetable oils, due to their high content of vitamin E, known for its antioxidant properties. However, the maintenance of these oils' inhibitory properties against HAA formation during repeated use is poorly understood. The aim of this study was to determine whether the inhibition of HAA formation by vegetable oils may be maintained during the sequential frying of multiple meat products. Three types of vegetable oils were used in the study: olive oil, high-oleic sunflower oil, and medium-oleic sunflower oil. Four pork patties were sequentially fried for each oil at a temperature of 160 °C until the center of the patty reached 72 °C. HAA content was determined using HPLC–MS/MS, and the fatty acid composition and vitamin E content of the oils were also examined. The data obtained showed no statistically significant accumulation of HAA from the first to the fourth patty fried in the same oil. This indicates that the antioxidant activity of the oils is maintained even after repeated use. However, significant differences were found between the different types of oils. The lowest content of both MeIQx (0.54 ng/g) and PhIP (1.75 ng/g) was recorded in patties fried in medium oleic sunflower oil, and the highest content was recorded in olive oil (0.89 ng/g and 2.37 ng/g, respectively). A clear negative correlation was established between the vitamin E content in the oils and the level of HAA formation. A negative correlation with the total proportion of unsaturated fatty acids was also noted. The results of the study demonstrate that vegetable oils, particularly medium-oleic sunflower oil with a high vitamin E content, effectively inhibit HAA formation during the heat treatment of meat products and maintain this ability during sequential frying of up to four products. This confirms the key role of antioxidants in inhibiting the Maillard reaction leading to HAA formation.

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Introduction

Almost any animal product requires heat treatment before consumption. Heat treatment imparts a more pleasant flavor and aroma, makes its nutrients more bioavailable, and makes the product safer from a microbiological standpoint. However, heat treatment also produces compounds that are potentially harmful to the human body. These compounds include heterocyclic aromatic amines (HAA), which are formed primarily in animal-based foods during heat treatment, especially high-temperature processes such as frying, baking, etc. [1,2]. In general, the formation of HAA is influenced by many factors. These include the extent of heat treatment, its duration, the type of raw materials used, and non-meat ingredients included in the product [3].

Despite the fact that HAA have proven carcinogenic and mutagenic effects [4], they are currently not standardized or controlled in food products in any country. This is partly due to the fact that homemade foods are more at risk of their formation, and it is impossible to control such products. Nevertheless, the global scientific community is actively working to find ways to reduce the amount of HAA formed in food products during heat treatment [5].

The main areas of research to find ways to reduce the amount of HAA formed include temperature modifications and the addition of plant-based ingredients containing components with high antioxidant activity (e. g., vitamin E) to meat product recipes, immediately before heat treatment, or prior to heat treatment (e. g., marinating).

The effect of ingredients containing components with high antioxidant activity on the formation of HAA was demonstrated in [6]. It was found that the addition of blueberry, cherry, acerola, and grape seed extracts to fish resulted in a reduction in HAA, such as norharman and PhIP, by 94.85 and 71.15 % relative to the control sample, respectively, while the amounts of IQ, 8-MeIQx, and 4,8-DiMeIQx decreased to concentrations below the detection limit of the method. Components included in garlic, namely diallyl disulfide, also significantly reduce the amount of HAA in the product. The addition of a nanoemulsion of diallyl disulfide with soy protein resulted in a reduction in the amounts of MeIQx, PhIP, and harman in fried pork by 52 %, 77 %, and 49 %, respectively [7].

The results of a study [8] investigating the effect of rosemary extract and pure vitamin E on the formation of IQ, MeIQ, MeIQx, DiMeIQx, and PhIP in minced beef products showed that the addition of vitamin E to the product formulation reduced the amount of formed HAA by 70–80 %, while rosemary extract reduced the amount of HAA by 12–87 %.

The effect of vegetable oils on the formation of HAA was most clearly demonstrated in a study [9], in which lard (control sample) and a mixture of lard with sunflower oil, olive oil, or pomegranate oil (test samples) were added to lean pork. The results of the study showed that partial replacement of animal fat with vegetable oil may lead to a reduction in the amount of HAA in the product by 83–100 %. The greatest effect was observed with pomegranate oil.

Some plant proteins exhibit an effect similar to that of vegetable oils in HAA formation. For example, adding soy protein isolate to pork resulted in a 69 %, 79 %, and 100 % reduction in MeIQx, 4,8-MeIQx, and IQx, respectively [10]. Carbohydrates also influence HAA formation. For example, study [11] examined the effect of adding corn, potato, and cassava starch to beef patties. The results showed that adding corn or cassava starch to the product before heat treatment reduced the amount of HAA formed in the product, while potato starch, conversely, increased their amount. Corn starch demonstrated the greatest inhibitory effect on HAA formation, with reductions observed in this study reaching 55 %.

Study [12] demonstrated the effect of adding carrot, tomato, and lettuce extracts to beef patties. Carrot extract demonstrated the greatest inhibitory effect, reducing the total HAA content in the product by almost 33 %. It also reduced each of the 12 analytes determined. Tomato extract demonstrated lower inhibitory properties, reducing the total HAA content in the product relative to the control sample by approximately 20 %. Furthermore, tomato extract did not reduce every analyte, slightly increasing some. The lowest HAA reduction was observed with lettuce extract, reducing the total HAA content relative to the control sample by approximately 8 %. As with tomato extract, lettuce extract did not reduce every analyte determined, but more importantly, adding lettuce extract to beef patties significantly increased the content of some HAA. Similarly, Zhang et al. [13] studied the effect of celery, carrot, and sweet potato extracts on HAA formation in mackerel cooked at different temperatures (180, 210, and 240 °C). In this case, the greatest effectiveness was observed with the addition of celery extract. The total HAA content in the product containing it was more than 12 times lower than in the control sample. Sweet potato reduced the total HAA content by almost 10 times, and carrots reduced it by almost 5 times.

Considering the “harmfulness” of meat, which has been actively discussed in the media in developed countries in recent years, the idea that plant-based products are safer than meat is being actively promoted. The authors of [14] studied the carcinogen content in beef patties and patties made from plant-based ingredients (soy, corn, and rice). They found that overall, plant-based patties produced lower levels of HAA than beef patties, although the levels of PhIP in beef and soy patties were almost the same. However, plant-based products produced significantly higher levels of PAHs than meat products.

The antioxidant activity of compounds in non-meat ingredients directly influences the amount of HAA formed during heat treatment. However, Zhang et al. [15] used a completely new approach to heat treatment of food products to increase their antioxidant capacity. The authors treated meat raw materials with plasma-activated water. This treatment of meat raw materials resulted in a reduction of the total amount of HAA in fried patties by up to 11 %.

Returning to the use of plant components to reduce the amount of HAA formed, it is worth noting the significant reduction achieved by the authors of the study [16]. In this study, an aqueous suspension of mandarin peels of varying concentrations was added to the frying of rabbit meat. According to the results obtained, the addition of mandarin peels during frying reduced the amount of HAA formed by 54–95 %.

It is important that, despite their good performance in inhibiting HAA formation in meat products, the addition of non-meat ingredients may negatively impact the development of flavor and aroma during heat treatment. For example, the authors [17] investigated the inhibitory properties of capers, oregano, wine, and green tea. Having established that the selected ingredients significantly impacted HAA formation in the product to varying degrees, they decided to evaluate the extent to which these ingredients might affect sensory perception. According to the presented results, capers and oregano had a little effect on the flavor and aroma of the products, while samples containing green tea and wine differed significantly from the “standard samples” and were the least appealing [18].

The study [19] examined the effect of the type of oil used for frying on the amount of HAA formed. Eight types of oils (seven vegetable oils and butter) were examined. According to the data presented, all vegetable oils used reduced the amount of HAA formed. The greatest reduction was observed in samples containing avocado oil, while butter, conversely, led to an increase in the amount of HAA formed. For all seven vegetable oils, the degree of HAA reduction correlated negatively with their vitamin E content; i. e., the more vitamin E in the oil, the less HAA formed when frying meat products in it.

The authors of the study [20] demonstrated that the antioxidant capacity of oils can be increased by adding antioxidants of various origins. Adinandra leaf extract, mixed with tea polyphenols, sesamol, or rosemary extract, was added to soybean oil before frying chicken breast. According to research results, adding only adinandra leaf extract to the oil resulted in a 12–38 % reduction in the total HAA content in the product. Meanwhile, a mixture of adinandra with tea polyphenols, sesamol, and rosemary extract resulted in a 34–61 %, 16–45 %, and 25–48 % reduction in HAA content, respectively.

Not all substances may exhibit the same inhibitory or catalytic effect under different conditions. This is well demonstrated in [21], which presents the effect of treating squid surimi with gallic acid before heat treatment on the MeIQx content. Squid meat treated with gallic acid was fried at temperatures of 100, 120, 140, 160, 180, 200, and 220 °C. According to the results, a significant reduction in MeIQx due to gallic acid was observed with heat treatment from 120 to 180 °C. At temperatures of 100 and 220 °C, the amount of MeIQx in the test sample was slightly lower than in the control, while at 200 °C, more MeIQx was formed in the test sample.

This review demonstrates that there are numerous ways to reduce the amount of HAA in fried products, ranging from adding non-meat ingredients to treating meat with plasma-activated water. However, information regarding the likelihood of HAA formation during repeated frying of minced meat products in the same oil is very limited. Therefore, research aimed at studying the accumulation of HAA during sequential frying of products made from minced meat products is highly relevant. The aim of this study was to determine whether the inhibitory properties of vegetable oils on HAA formation are maintained during sequential frying of several minced meat products.

Objects and methods

To make the patties, we used pork (*M. longissimus dorsi*), C1 grade chicken eggs, and pasteurized reconstituted cow's milk with a fat content of 3.2 %. The pork was chopped, removing visible fat and connective tissue, and then minced in a household meat grinder with a mesh diameter of 3–5 mm. Eggs were cracked into a 500 ml cup and then mixed with a whisk for 3 minutes until smooth. The ingredients were mixed at a meat: milk: egg ratio of 7:2:1, then mixed by hand until smooth. The resulting minced meat was formed into round patties, each weighing 50.0 ± 2.0 g and measuring approximately 6 cm in diameter and approximately 3 cm in height.

High-oleic sunflower oil, medium-oleic sunflower oil, and olive oil were used for cooking. These oils were chosen as the most commonly used for home cooking.

The heat treatment was carried out in a 15.5 cm diameter nonstick frying pan placed on RCT basic laboratory heater with an attached immersion thermometer (IKA, Germany). 50 ml of oil was added to the pan and heated to a constant temperature of 160 °C. Once the temperature was reached, a patty was placed in the pan and fried until the center of the patty reached a constant temperature of 72 °C. The patties were flipped every 2 minutes. Once the temperature reached, the patty was fried for an additional 2 minutes, then removed from the pan and placed in a plastic container with a lid, where it cooled to room temperature. After the first sample, three more were fried in the same oil and heat treated under the same conditions. Considering that after frying the fourth patty, there was almost no vegetable oil left in the pan, it was decided to fry four patties in each of the selected oils.

After cooling to room temperature, the patties were ground using Buchi B-400 laboratory homogenizer (Buchi, Switzerland) and then stored in plastic containers in a freezer at a temperature no higher than –18 °C until analyzed for HAA content (MeIQx and PhIP). HAA was quantified according to [22].

Vegetable oils were analyzed for fatty acid composition according to GOST 31663-2021[1] using Agilent 7890A gas chromatograph with a flame ionization detector (Agilent, USA), and vitamin E content was determined according to GOST 32307-2013[2] using Dionex Ultimate 3000 liquid chromatograph with a UV detector (Thermo Fisher Scientific, USA).

STATISTICA 10 software was used to calculate HAA content in the samples. Results were presented as mean ± SD. Statistical significance was calculated using analysis of variance (ANOVA) with Tukey's test. A probability of 0.05 was chosen as the significance level.

Results and discussion

Table 1 shows the results of determining the vitamin E content in oils.

Table 1. Vitamin E content in oils, mg/100 g

Oil type	Vitamin E content
Olive oil	**13.8**
High-oleic sunflower oil	**42.4**
Medium-oleic sunflower oil	**46.1**

Table 2 shows the results of the FA content in vegetable oils.

Table 3 and Table 4 show the results of the HAA content analysis in the samples.

The letters a, b show the significant difference between the obtained results of the average content of MeIQx in the batch.

The letters a, b show the significant difference between the obtained results of the average content of PhIP in the batch.

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[1] GOST 31663-2021 "Vegetable oils and animal fats. Determination of methyl esters of fatty acids by gaz chromatography method". Retrieved from https://internet-law.ru/gosts/gost/56502/ Accessed August 18, 2025

[2] GOST 32307-2013 "Meat and meat products. Determination of fat-soluble vitamins by high performance liquid chromatography". Retrieved from https://docs.cntd.ru/document/1200107182 Accessed August 18, 2025

Table 2. Fatty acid composition of vegetable oils, %

Fatty acid	Olive oil	High-oleic sunflower oil	Medium-oleic sunflower oil
Myristic C14:0	less than 0.1	less than 0.1	less than 0.1
Palmitic C16:0	10.8	2.9	5.0
Palmitoleic C16:1	0.6	less than 0.1	less than 0.1
Margaric C17:0	less than 0.1	less than 0.1	less than 0.1
Heptadecenoic C17:1	less than 0.1	less than 0.1	less than 0.1
Stearic C18:0	3.6	3.2	4.0
Oleic C18:1	56.2	82.4	57.9
Linoleic C18:2	28.0	10.1	32.0
Linolenic C18:3	0.3	0.2	less than 0.1
Arachidic C20:0	0.4	0.3	0.2
Gondoic C20:1ω9	less than 0.1	0.2	0.1
Behenic C22:0	less than 0.1	0.8	0.8
Lignoceric C24:0	less than 0.1	less than 0.1	less than 0.1
∑ unsaturated fatty acids	84.5	92.9	90.0

Table 3. MeIQx content in the samples, ng/g

Vegetable oil used	Sample				Average content in a batch
	1	2	3	4	
Olive oil	0.88 ± 0.09	0.85 ± 0.10	0.96 ± 0.06	0.85 ± 0.11	0.89
High-oleic sunflower oil	0.56 ± 0.06	0.61 ± 0.07	0.61 ± 0.04	0.60 ± 0.08	0.60a
Medium-oleic sunflower oil	0.55 ± 0.04	0.49 ± 0.04	0.53 ± 0.06	0.58 ± 0.05	0.54ab

Note: Results are presented as the mean of three parallel measurements ± standard deviation. The standard deviation indicates the precision of the method.

Table 4. PhIP content in the samples, ng/g

Vegetable oil used	Sample				Average content in a batch
	1	2	3	4	
Olive oil	2.35 ± 0.22	2.38 ± 0.18	2.42 ± 0.18	2.31 ± 0.24	2.37
High-oleic sunflower oil	1.84 ± 0.31	1.79 ± 0.15	1.75 ± 0.13	1.78 ± 0.20	1.79a
Medium-oleic sunflower oil	1.72 ± 0.07	1.76 ± 0.12	1.75 ± 0.09	1.75 ± 0.15	1.75ab

Note: Results are presented as the mean of three parallel measurements ± standard deviation. The standard deviation indicates the precision of the method.

The results showed that HAAs do not accumulate in the products during sequential frying in the same oil. A slight difference in the obtained MeIQx and PhIP concentrations is observed, but it is statistically insignificant and is most likely due to an error in the determination method. Moreover, no difference was observed even between the first samples, which were fried in the original volume of oil, and the fourth samples, which were fried in a pan with significantly less oil. Thus, this circumstance may indicate that the oils do not lose their antioxidant activity during heat treatment when frying up to four samples of minced pork products in them sequentially.

However, statistically significant differences in the average MeIQx and PhIP content were observed in all three batches. This difference was most pronounced between the patties fried in olive oil and those fried in two types of sunflower oil. The amount of MeIQx in patties fried in olive oil was approximately 1.5 and 1.6 times higher than in patties fried in high-oleic sunflower oil and medium-oleic sunflower oil, respectively. A similar pattern was observed for the amount of PhIP: in patties fried in olive oil, its content was approximately 1.3 and 1.4 times higher than in patties fried in high-oleic and medium-oleic sunflower oils, respectively. The difference in the average MeIQx and PhIP content in patties fried in different sunflower oils was also statistically significant. Among the three selected types of vegetable oils, the lowest amount of HAA was formed in patties fried in medium-oleic sunflower oil.

Overall, the difference in HAA content negatively correlates with the vitamin E content of the oils used. Olive oil has the lowest vitamin E content, while the samples fried in it produced the highest amounts of HAA. Medium-oleic sunflower oil contains slightly more vitamin E than high-oleic sunflower oil, and the formation of HAA in patties fried in medium-oleic sunflower oil was correspondingly slightly lower than in patties fried in high-oleic sunflower oil. The strong negative correlation found between the vitamin E content in the oil and the level of HAA formed in the patties is fully consistent with the generally accepted mechanism of antioxidant action. Vitamin E, by inhibiting radical lipid oxidation reactions and suppressing peroxidation, indirectly prevents the formation of active intermediates (such as aldehydes), which are key participants in the Maillard reaction and subsequent stages of HAA formation. Thus, the higher vitamin E content in sunflower oils provides more effective protection against oxidation during frying, which leads to a decrease in the formation of MeIQx and PhIP. However, the obtained results do not agree with the results obtained in [19], in which the amount of HAA in steaks fried in sunflower and olive oils was at approximately the same level, and in steaks fried in high-oleic sunflower oil, less HAA was formed than in steaks fried in medium-oleic sunflower oil. The significant difference in HAA content between olive and sunflower oils may probably be explained by the fact that in [19], piece products were subjected to heat treatment, while in the present study, the raw material was ground, and in products made from ground meat, HAA are formed more intensively than in products made from whole pieces. This is due to the larger surface area in contact with atmospheric oxygen and the heating surface, a higher yield of reactive precursors (creatinine, free amino acids, sugars) from destroyed muscle cells, and also, possibly, the presence of additional catalytic components released during grinding. Nadeem et al. [23] conducted a large review of works devoted to methods for reducing HAA in meat products. A large number of works have shown that the addition of plant components to meat products allows for a decrease in the amount of formed HAA, however, the authors of the work [24] cited in this review emphasize that when marinating meat raw materials with plant ingredients, they also extract from the meat raw materials both precursors and inhibitors of the reaction of HAA formation. Work [24] may indirectly confirm the differences in the results of the present work with [19], since when grinding the raw materials, as was already noted earlier, a greater number of HAA precursors are released. That raw material grinding plays a positive role in the formation of HAA can be seen by comparing the obtained results with those of [25], where the authors added a source of omega-3 acids, flaxseed oil, to ground products. This resulted in a significant reduction in HAA relative to control samples. The present study did not aim to test the pre-added oil. However, based on the fact that the HAA content did not increase from sample to sample during sequential frying of products in the same oil, it can be concluded that grinding the raw material allows the vegetable oil to better penetrate the products and, accordingly, better exhibit its inhibitory properties.

The fatty acid composition of the oils (Table 2) also shows significant differences. Olive oil has the lowest total unsaturated fatty acid content (84.5 %) compared to sunflower oils (92.9 % and 90.0 %). Oleic acid (C18:1, 56.2 %) accounts for the majority of the unsaturated fatty acid content in olive oil, while for high-oleic and medium-oleic sunflower oils, these are oleic acid (82.4 % and 57.9 %) and linoleic acid (C18:2, 10.1% and 32.0 %), respectively. The HAA content in the studied samples also negatively correlates with the amount of unsaturated fatty acids in the oils. Olive oil has the lowest amount, while sunflower oils contain approximately the same amount, as does vitamin E. However, there is no reliable information on the effect of unsaturated FAs on the HAA formation. On the one hand, some studies argue that unsaturated FAs are more prone to oxidation upon heating, and their oxidation products (e. g., reactive aldehydes) may act as precursors or catalysts in the formation of HAA. When discussing the catalytic properties of unsaturated FAs, it is important to emphasize that it is their oxidation products that lead to an increase in the amount of HAA. A study [26] demonstrated a direct proportional relationship between the amount of PhIP formed and the content of propanal, hexanal, and other aldehydes formed as a result of fatty acid oxidation in the samples. It was demonstrated in [27] that aldehydes and other fatty acid oxidation products lead to an increase in HAA. According to the results, the higher the degree of fat oxidation and, accordingly, the more oxidation products are formed, the greater the amounts of PhIP formed in model systems of phenylalanine and creatine/creatinine, the main precursors of PhIP in meat. This is also confirmed by the research of Zhao et al. [28], which showed that when *Flos sophorae immaturus* extract is added to the product, due to its antioxidant properties, fewer oxidation products, essentially aldehydes, and, accordingly, fewer HAA are observed in the samples by reaction with thiobarbituric acid. A large review article [29] is devoted to the instability and strong oxidizability of unsaturated fatty acids (especially omega-3). Moreover, their oxidation actively occurs not only at elevated temperatures, but also under normal storage conditions of products containing them. Thus, the use of sources of unsaturated fatty acids in order to reduce the amounts of HAA during the preparation of meat products may not be a very good idea, since they will already contain some amounts of FA oxidation products. However, earlier Aslanova et al. [25] showed that the addition of flaxseed oil as a source of omega-3 FA resulted in a significant decrease in the HAA content in the samples, much greater in percentage terms than the use of flaxseed oil for frying [19], despite its relatively high vitamin E content, which may be due to the fact that the smoke point of flaxseed oil is quite low. In general, the influence of fat oxidation products on the reaction of HAA formation was discussed in detail in a review paper [30]. Aldehydes are the main products in the lipid oxidation reaction, and as a result of their oligomerization and cyclization, a large number of heterocyclic structures are formed in food products, which subsequently participate in the formation of carcinogenic and mutagenic HAA. Thus, sources of unsaturated fatty acids, since such acids are most prone to oxidation, should, in theory, lead to an increase in the amounts of HAA formed.

On the other hand, as shown in some studies, in addition to the antioxidant activity of unsaturated fatty acids themselves, their oxidation products may participate in competing reactions leading to the formation of compounds with antioxidant activity, or physically block highly reactive intermediates or functional groups in the reaction of HAA formation. The review paper [31] states that polyunsaturated fatty acids, due to their antioxidant activity, prevent the formation of HAA. This is confirmed by the work [32], the results of which show a significant decrease in HAA in the product due to the use of olive oil.

The inhibitory properties of unsaturated fatty acids in the reaction of HAA formation have also been proven in [33], where the authors marinated raw meat in propolis extract, a source of acids such as oleic, linoleic and linolenic. As a result of using propolis extract, it was possible to achieve a reduction of some HAA by up to 100 %. The inhibitory properties of polyunsaturated fatty acids are shown in [34]. Unlike most studies, the experiment was not conducted with raw meat. Instead, the authors experimentally reproduced the reactions occurring in meat products during heat treatment, which demonstrated the inhibitory properties of unsaturated fatty acids in isolation.

Therefore, it is difficult to assess the influence of unsaturated fatty acids on the amount of HAA formed in the samples studied in this study. However, the presence of unsaturated fatty acids in the product is important, as at high concentrations, unsaturated fatty acids may reduce the ability of HAA to form DNA adducts, which, accordingly, reduces the likelihood of tumors in the body from consuming products containing carcinogens [35]. In the oils used in this study, oleic acid accounted for the largest proportion of their composition. According to [27], the amount of oleic acid positively correlates with the amount of MeIQx and DiMeIQx formed in the product. However, based on the data obtained during this study, a relationship between oleic acid content and the amount of HAA cannot be established.

In this study, a negative correlation was observed between the total unsaturated FA content and HAA level, which is similar to the vitamin E content. Therefore, it is not possible to clearly separate the contributions of unsaturated FAs and vitamin E to the inhibition of HAA formation. The high unsaturated FA content of sunflower oils is likely an additional factor contributing to their antioxidant capacity, but vitamin E plays a key regulatory role in inhibiting adverse reactions.

This study demonstrates that the choice of vegetable oil for frying is a significant factor influencing HAA formation in meat products. Sunflower oils, especially medium-oleic oils, exhibit more pronounced inhibitory properties compared to olive oil due to their higher vitamin E content and fatty acid composition. A key finding is the stability of these inhibitory properties even when using the same volume of oil sequentially for frying up to four products.

Conclusion

The data obtained in this study showed that vegetable oils, most likely due to their high vitamin E content, maintain their inhibitory properties against HAA formation in meat products during heat treatment. The results showed that up to four patties can be fried in a single oil without increasing the HAA content. This study provides further confirmation that vitamin E, due to its antioxidant properties, plays a significant role in reducing the HAA content in meat products during heat treatment. HAA content also negatively correlates with the unsaturated fatty acid content of the oils used. However, it is impossible to say with certainty whether they inhibit HAA formation, as numerous studies currently exist demonstrating both the inhibitory properties of unsaturated fatty acids and, conversely, their catalytic properties. This study also once again demonstrates the risk of HAA formation in meat products during home heat treatment, and that this risk can be managed.

The practical significance of this study lies in confirming the feasibility of using vegetable oils to reduce HAA formation during heat treatment of meat products at home. The results of the study can be used to develop recommendations for the safe preparation of meat products. The data obtained expand our understanding of the mechanisms of HAA formation during heat treatment of meat products and confirm the important role of the antioxidant properties of vegetable oils in reducing the formation of potentially harmful compounds.

Although oils maintain their effectiveness in reducing HAA formation, further research is needed in related areas. First, it is necessary to study the maintenance of antioxidant properties in a larger number of oils, both those commonly used in heat treatment of meat products at home and those rarely used, such as canola, corn, avocado, and pomegranate oils. Second, it is important to study the accumulation of not only HAA but also other compounds potentially or proven to be harmful to the human body, such as acrylamide, acrolein, and PAHs. Third, it is of interest to study the effect of the degree of purification (refining) of oil on its antioxidant capacity and ability to inhibit HAA formation. Finally, sensory analysis of products prepared using oils after repeated heating is necessary to provide a comprehensive consumer assessment.

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QUALITY ATTRIBUTES OF LAMB SATAY AND THEIR CORRELATION WITH CONSUMER PREFERENCE IN TEGAL REGENCY, INDONESIA

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Abstract

Sheep commodities holds significant potential for the development of the livestock sector in Tegal Regency, especially for lamb satay, which is a local culinary icon. However, the red meat industry faces food safety issues that affect consumer acceptance and satisfaction. This study aims to evaluate the quality of lamb satay from various producers in Tegal Regency and its correlation with consumer satisfaction. Samples of satay and sheep meat were collected from three selected producers. Laboratory analysis included microbiological analysis, nutritional content analysis, and physicochemical properties analysis, each with three replicates. Consumer satisfaction was measured using a questionnaire given to 32 consumers, consisting of 15, 8, and 9 respondents for producers A, B, and C, respectively. The results showed no statistically significant differences ($p > 0.05$) in microbiological quality, including TPC, E. coli, and S. aureus. Nutritional and physicochemical parameters such as protein, fat, ash, water, carbohydrates, pH, and water activity also showed no differences between producers, indicating consistent quality. A total of 18 fatty acids were identified in lamb satay. A positive relationship ($p < 0.01$) was found between consumer satisfaction and protein, moisture, ash, pH, and water activity levels, while fat and carbohydrates showed a negative correlation ($p > 0.01$). In addition, all sensory attributes had a significant effect ($p < 0.01$) on consumer satisfaction. In conclusion, lamb satay in Tegal Regency has consistent quality among producers. Higher levels of moisture, ash, pH, water activity, and sensory attributes increase consumer satisfaction, while higher fat and carbohydrate content decrease it.

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Introduction

Sheep commodity holds significant potential for the development of the livestock sector in Indonesia, particularly in Tegal Regency. This region is among the regencies with the highest sheep population in Central Java. This is also supported by ideal geographical conditions, such as the availability of abundant forage land, a favorable climate, and the presence of a community with a long-standing tradition of sheep farming. According to data from the Central Statistics Agency or Badan Pusat Statistik (BPS), sheep population in Tegal Regency reached 189,094 heads in 2023 [1]. The sheep commodity plays an important role in supporting the local economy, both as a source of income and as raw material for processed culinary products, especially the traditional lamb satay of Tegal Regency. With its abundant sheep population, Tegal Regency has great potential to develop local culinary products based on lamb meat.

Tegal lamb satay has become a culinary icon that represents a deeply rooted local tradition. The uniqueness of this satay lies in its main ingredient, which is young lamb meat known as "balibul" (under five months old), due to its more tender texture compared to older sheep. Lamb from younger sheep is more tender than that from older ones due to differences in muscle fiber size and structure [2]. The satay-making process generally involves grilling over charcoal at a temperature of approximately 200–220 °C. The culture of consuming lamb satay in Tegal is rooted not only in flavor preferences but also in the social habits of the community. This dish is often served at various events, ranging from family gatherings to business meetings.

Lamb meat is a popular source of protein in Indonesia and has the high nutritional value. According to previous studies, lamb meat has approximately 19 % protein [3], and 4.6–5.5 % fat [4]. These favorable nutritional contents make lamb meat an important source of nutrients in the dietary patterns of the population. The quality of lamb meat generally consists of three main aspects, namely physical, chemical, and sensory characteristics. Physical and chemical attributes such as pH, water activity (a_w), fat content, protein, moisture, and other components affect meat tenderness, juiciness, and flavor.

Chemical and nutritional attributes, such as fat content in food, provide a complex sensory experience and play a critical role in influencing consumer preferences [5]. The presence and amount of fat significantly affect key sensory attributes such as juiciness, texture, mouthfeel, and overall flavor, all of which are central to consumer satisfaction and product acceptance [6]. Based on previous research, fat is closely associated with desirable sensory qualities such as creaminess, richness, and palatability, which enhance the overall enjoyment of meat products. These positive sensory effects make fat an important component in many meat products [7]. However, recent dietary trends have shifted toward healthier food choices, with increasing emphasis on reducing fat consumption. This shift is driven by mounting evidence linking excessive fat intake with negative health outcomes, including obesity, cardiovascular diseases, and even cancer [8]. The interplay between different types of dietary fat, metabolic responses, and disease mechanisms is complex, suggesting that fat content in food must be carefully managed, not only to preserve sensory quality but also to promote better health outcomes.

In addition to nutritional and sensory aspects, the safety of lamb meat is another essential factor affecting its overall quality and consumer trust. *Escherichia coli, Salmonella* spp., and *Staphylococcus aureus* are common indicators of potential food safety hazards [9]. Such microbial contamination impacts food safety, consumer health, and acceptance of products [10]. Food safety issues in the red meat industry pose a major threat to public health and has become an increasing global concern [11]. Such contamination can trigger outbreaks of foodborne zoonotic diseases. Most serious food safety cases affect consumer health and often result in product recalls and the removal of contaminated meat products from the food supply chain. Food safety problems are most frequently caused by microbiological factors, especially pathogenic bacteria [12]. These issues can also influence consumer satisfaction with meat and meat-based products.

Several studies on the quality of processed lamb meat have been conducted, namely analyses of proximate composition and sensory characteristics of lamb sausage [13], as well as of the physicochemical, microbiological, and organoleptic characteristics of fermented sausages from premium IPB lamb with varying proportions of jack bean flour [14]. However, information and studies regarding the quality of lamb meat and processed lamb satay products, particularly in Tegal Regency, remain very limited. Therefore, this study aims to comprehensively evaluate the microbiological, nutritional, physicochemical, and sensory quality of lamb satay in Tegal Regency and analyze their correlation with consumer satisfaction, thereby contributing to the improvement of local food product development strategies.

Objects and methods

Sample and sampling technique

The study was conducted in Tegal Regency using a survey method and purposive sampling approach. Lamb satay producers were deliberately selected based on specific criteria, namely their willingness to participate in the study and having operated their business for at least one year. A total of three different producers were selected and interviewed, originating from three different districts, including Adiwerna (Producer A), Lebaksiu (Producer B), and Slawi (Producer C). Interviews were conducted with each satay producer to obtain detailed information on their production workflow, including production steps, grilling practices, the type of sheep used, and the seasoning formulations. Additionally, samples of satay and raw lamb meat were collected from each of these producers. Samples were collected from thin-tailed sheep (*domba ekor tipis*), which is the most commonly used breed among satay producers in Tegal Regency.

The raw lamb meat and lamb satay samples were transported to the laboratory in a cooling box at a controlled temperature of ±4 °C to ensure safe and aseptic conditions during transport. Analyses were conducted immediately upon arrival to minimize quality changes. Satay samples from producers A, B, and C (Figure 1) were analyzed for microbiological parameters, nutritional composition, and physicochemical properties.

Consumer satisfaction survey

Questionnaire was designed to measure consumers' perceptions, preferences, and satisfaction levels regarding the lamb satay they consume. This questionnaire was ad-


(a) (b) (c)

Figure 1. Lamb satay from producer A (a), producer B (b), and producer C (c) (Source: personal documentation, 2024)

ministered to 32 consumers, consisting of 9, 8, and 15 respondents for producers A, B, and C, respectively. These respondents were consumers who had purchased and consumed satay products from each producer. Respondents were selected using purposive sampling with specific criteria, namely willing to complete the questionnaire, at least 17 years of age, currently and previously having consumed satay from one of the selected sellers at least once, and being in good health at the time of completing the questionnaire. In addition, consumer demographic data including age, gender, education level, occupation, monthly income, and region of origin, were also recorded as supplementary information for the study [15].

The sensory evaluation of the satay products was conducted directly at the producers' location by consumers who purchased and consumed the products. Consumer satisfaction was assessed based on five main attributes including flavor, aroma, texture, color, and product piece size, immediately after consumption, using the 5-point Likert scale, where 1 = very dissatisfied, 2 = dissatisfied, 3 = neutral, 4 = satisfied, and 5 = very satisfied. Each sensory attribute and the overall satisfaction score were rated accordingly.

Microbiological analysis

Microbiological analysis included total plate count (TPC), *Escherichia coli* (*E. coli*), and *Staphylococcus aureus* (*S. aureus*), following the Indonesian National Standard (SNI) methods [16]. The analysis consisted of two main stages, namely dilution and inoculation. In the dilution stage, a 10^{-1} dilution was prepared by mixing 25 g of the sample with 225 mL of Buffered Peptone Water (BPW, Oxoid Ltd., UK), which served as both the solvent and diluent. In the inoculation stage, 1 mL of the diluted sample from the 10^{-2}, 10^{-3}, and 10^{-4} dilutions was transferred into sterile petri dishes containing 20 mL of Plate Count Agar (PCA, Oxoid Ltd., UK) for TPC analysis. Additionally, 1 mL of the diluted sample from the 10^{-1}, 10^{-2}, and 10^{-3} dilutions was poured into petri dishes along with 20 mL of molten agar media. Eosin Methylene Blue Agar (EMBA, Oxoid Ltd., UK) was used for *E. coli* and Baird-Parker Agar (BPA, Oxoid Ltd., UK) for *S. aureus*. Homogenization was performed using a figure-eight motion, and the plates were left to set. The inoculated plates were then incubated in an inverted position at 34–36 °C for 24–48 hours.

Nutritional and physicochemical content

Nutritional analysis included the content of moisture, protein, fat, ash, and carbohydrates, conducted in accordance with standard procedures indicated in [17], while fatty acids were determined according to [18]. The moisture content was determined by oven drying (WTB Binder GmbH, Germany), ash content by dry combustion in a muffle furnace Vulcan 3–550 (Dentsply Sirona, USA), protein content was analyzed using the Kjeldahl method, while the fat content was determined by Soxhlet extraction with petroleum ether solvent, and the carbohydrate content was calculated using the difference method. Fatty acid analysis was carried out by converting triglycerides and free fatty acids in the samples into fatty acid methyl esters (FAMEs), which were then analyzed using gas chromatography-mass spectrometry (GC–MS) Thermo Trace 1310 (Thermo Scientific, USA).

Physicochemical analyses of the samples were conducted based on methods indicated in [17], including measurements of pH and water activity (a_w). The pH was measured three times for each sample using a portable pH meter Ionix PC60 (Apera Instruments, USA) equipped with a penetration probe and automatic temperature compensation. Prior to measurement, the instrument was calibrated using two buffer solutions with pH 4.0 and pH 7.0. The electrode was then inserted into the sample, and the pH value was recorded directly from the display. Water activity was measured using an a_w meter Novasina MS1 (LabMaster-aw neo, Switzerland) by activating the device, allowing the reading to stabilize, and recording the water activity value automatically.

Statistical analysis

Lamb satay samples from each producer were analyzed in the laboratory in three repetitions, except for the fatty acid analysis, which was performed in a single repetition. The raw lamb samples were also analyzed in a single repetition. Consumer demographic profile was analyzed using descriptive analysis. Data from the laboratory were analyzed using one-way analysis of variance (ANOVA) to determine differences in lamb satay quality among producers. Consumer perception data were analyzed using Spearman's correlation analysis to assess the relationship between consumer satisfaction and laboratory quality parameters. Principal component analysis (PCA) was also used to reduce data dimensions and identify patterns or groups among producers based on objective quality parameters and consumer perceptions. Furthermore, multiple regression analysis was conducted to evaluate the influence of sensory attributes (flavor, aroma, texture, color, and product piece size) on consumer satisfaction. Statistical analysis was performed using R-studio 2025.05.1–513 (Boston, USA).

Results and discussion

Consumer demographic profile

Understanding consumer demographics is essential for identifying the factors that influence food preferences and purchasing behavior. Variations in age, gender, education, occupation, and income levels can shape consumers' perceptions of product quality and their willingness to buy ready-to-eat meat products [19]. The demographic profile of the respondents is presented in Table 1.

Most of the satay consumers in this study were from Tegal Regency (75 %) and were predominantly male (68.75 %). The age group of 21–30 years represented the largest segment (53.13 %), indicating that satay consumers were mainly young and within the productive age group.

This finding is consistent with the previous study [20], which demonstrated that individuals of productive age tend to show higher consumption levels toward developing products, including ready-to-eat foods. The majority of respondents also held a bachelor's degree (53.13 %), suggesting that consumers possessed adequate knowledge and awareness regarding food quality and safety.

Table 1. Lamb satay consumer demographics

Parameter	Frequency (n)	Percentage (%)
Region		
Tegal	24	75.00
Purwakarta	2	6.25
Rembang	1	3.13
South Halmahera	1	3.13
Pemalang	2	6.25
Demak	1	3.13
Bogor	1	3.13
Gender		
Female	10	31.25
Male	22	68.75
Age (years)		
> 50	6	18.75
41–50	3	9.38
31–40	6	18.75
21–30	17	53.13
Education Level		
High school or below	11	34.38
Bachelor's degree	17	53.13
Graduate degree or higher	4	12.50
Occupation		
Civil servant	8	25.00
Entrepreneur	15	46.88
Self-employed	4	12.50
Other	5	15.63
Monthly Income		
< IDR 2 million (> USD 120)	3	9.38
IDR 2–4 million (USD 120–240)	15	46.88
IDR 5–8 million (USD 300–480)	5	15.63
> IDR 8 million (> USD 480)	9	28.13

Note: IDR = Indonesian Rupiah, USD = United States Dollar.

In terms of occupation and income, nearly half of the respondents were self-employed (46.88 %) with a monthly income ranging from IDR2–4 million (46.88 %). This condition reflected that satay consumers generally came from the middle-income group, who maintained a relatively stable purchasing power for ready-to-eat meat products. According to [21], economic factors and social characteristics such as age and education level play a significant role in determining consumer preferences for meat. In the context of this study, most satay consumers were young, educated individuals and were able to evaluate products not only based on price but also by considering aspects of quality, flavor, and overall consumption experience.

Total microbes and pathogenic bacteria of lamb satay

Microbiological analysis was conducted to assess the quality and safety of lamb satay produced by three different producers. The raw lamb used as the main ingredient for satay was also analyzed to provide information on its raw material quality. This analysis was considered crucial to ensure that the products meet food safety standards, as meat products are highly susceptible to contamination by pathogenic microorganisms, such as *S. aureus* [22] and *E. coli*, which may pose risks to consumer health [23]. The measurements were performed by counting the number of colonies grown on selective media, and the results were expressed in Log CFU/g.

Table 2. Microbiological load of raw lamb used for satay

Parameter	Producers		
	A	B	C
Total plate count (Log CFU/g)	5.95	5.92	5.91
E. coli (Log CFU/g)	2.02	2.22	2.14
S. aureus (Log CFU/g)	2.19	2.16	2.29

Note: Log CFU/g = $\log_{10}$ of colony forming units per gram; Data are presented as mean.

Microbiological analysis of raw lamb used for satay production (Table 2) showed that total plate count (TPC) ranged from 5.91 to 5.95 log CFU/g, indicating that the microbial load of the raw meat was still within the acceptable limit for raw meat, which is 10^5 CFU/g or 5 log CFU/g according to international guidelines [23]. The presence of Escherichia coli was detected at levels between 2.02 and 2.22 log CFU/g, while Staphylococcus aureus ranged from 2.16 to 2.29 log CFU/g. These results indicate a slight level of microbial contamination, which may have originated from handling during slaughtering, transportation, or storage. The acceptable limits for E. coli and S. aureus in raw meat should be below 10^2 CFU/g or 2 log CFU/g [24,25]. In addition, the total microbial count and the presence of pathogens decreased after the grilling process, as shown in Table 3. This reduction is likely attributed to the exposure to high temperatures during grilling, which can destroy microbial cell structures and reduce their viability.

The results of microbiological analysis of lamb satay show that there are no significant differences ($p > 0.05$) in all microbiological parameters between the three different producers. This indicates that the products from producers have similar microbiological quality and meet equivalent

Table 3. Microbiological load of lamb satay from different producers

Parameter	Producers			p-value
	A	B	C	
Total plate count, Log CFU/g	3.65 ± 0.06	3.74 ± 0.11	3.56 ± 0.09	0,142
E. coli, Log CFU/g	1.12 ± 0.10	1.39 ± 0.21	1.26 ± 0.07	0,139
S. aureus, Log CFU/g	1.62 ± 0.26	1.60 ± 0.05	1.49 ± 0.15	0,632

Note: Log CFU/g = $\log_{10}$ of colony forming units per gram; Data are presented as mean ± standard deviation.

food safety standards. The absence of significant differences in TPC values suggests comparable levels of cleanliness and sanitation in their production facilities. Similarly, the statistically non-significant levels of *E. coli* and *S. aureus* presence indicate that the risk of pathogen contamination in products from the three different producers is relatively the same.

The TPC of lamb satay samples in Tegal Regency was below the acceptable limit recommended by Good Manufacturing Practice (GMP) guidelines, specifically ≤5 log CFU/g [24]. The presence of S. aureus was also within the satisfactory limit for ready-to-eat food products, including cooked meat products, which is <2 log CFU/g (100 CFU/g) [26]. However, E. coli counts exceeded the recommended satisfactory limit of <1 log CFU/g (<10 CFU/g) for such products [26]. This suggested potential for cross-contamination or inadequate pre or post-cooking hygiene practices, such as the use of cutting tools, serving containers, or insufficient sanitation [27]. Although it does not directly indicate a high risk of disease, the presence of *E. coli* above the satisfactory limit remains an important indicator in food safety evaluation, particularly for ready-to-eat products like lamb satay. Therefore, enhanced sanitation oversight and education on hygienic practices are essential for both producers and sellers to minimize the risk of microbial contamination.

Nutritional and physicochemical content of lamb satay

The analysis of the nutritional content (fatty acids and proximate composition) as well as physicochemical properties in food products is essential to comprehensively assess the quality and characteristics of the product. The fatty acid profile and proximate composition affect sensory quality and provide an overview of the main nutritional content, which serves as a quality indicator [28]. Physicochemical properties contribute to the stability of the product during storage and processing [29].

The results showed that the three samples of lamb satay from different producers contained 18 fatty acids (Table 4). The fatty acid profile of the lamb satay samples showed variations among producers, particularly in the proportions of saturated and unsaturated fatty acids. These variations may reflect differences in meat sources, feed composition, and fat metabolism among the animals used by each producer. The fatty acid composition was dominated by saturated fatty acids (SFAs), followed by monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). Animal fats, such as those found in lamb satay, are generally dominated by three main fatty acids, namely palmitic acid (C16:0) and stearic acid (C18:0), which belong to the SFA group, and oleic acid (C18:1n9c), which belongs to the MUFA group. The combination of these three fatty acids contributes to a characteristic property known as plasticity. This property allows animal fats to retain their solid form at room temperature, while becoming soft and malleable at higher temperatures, such as during grilling [30].

Table 4. Fatty acid content of lamb satay from different producers

Fatty acid	Content, %w/w		
	A	B	C
Saturated fatty acids (SFAs)			
Butyric acid (C4:0)	0.07	0.11	0.06
Caproic acid (C6:0)	0.07	0.06	0.04
Caprilic acid (C8:0)	0.11	0.09	0.18
Capric acid (C10:0)	0.29	0.21	0.28
Lauric Acid (C12:0)	0.96	0.52	0.91
Myristic Acid (C14:0)	3.80	2.79	3.04
Pentadecanoic Acid (C15:0)	0.49	0.73	0.42
Palmitic Acid (C16:0)	20.25	20.86	24.74
Heptadecanoic Acid (C17:0)	1.15	1.65	1.47
Stearic Acid (C18:0)	16.62	26.20	23.28
Arachidic Acid (C20:0)	0.09	0.17	0.13
Monounsaturated fatty acids (MUFAs)			
Myristoleic Acid (C14:1)	0.06	0.28	0.04
Palmitoleic Acid (C16:1)	1.53	1.36	1.65
Cis-10-Heptadecanoic Acid (C17:1)	0.36	0.29	0.38
Elaidic Acid (C18:1n9t)	2.50	1.21	3.22
Oleic Acid (C18:1n9c)	25.93	18.02	32.00
Polyunsaturated fatty acids (PUFAs)			
Linoleic Acid (C18:2n6c)	0.94	0.39	1.33
Linolenic Acid (C18:3n3)	0.12	0.03	0.05
Total fatty acids	75.34	74.96	93.22
Total saturated fatty acids (SFAs)	43.90	53.39	54.55
Total unsaturated fatty acids (UFAs)	31.44	21.58	38.67
Total polyunsaturated fatty acids (PUFAs)	1.06	0.42	1.38
Total monounsaturated fatty acids (MUFAs)	30.38	21.16	37.29
Ratio SFAs/UFAs	1.39	2.47	1.41

Note: Fatty acid contents are expressed in% w/w (percentage weight per weight).

The three major fatty acids commonly found in animal fats (palmitic acid, stearic acid, and oleic acid) have also been investigated for their potential health benefits in humans. Oleic acid is known to play a role in lowering low-density lipoprotein (LDL) cholesterol and increasing high-density lipoprotein (HDL) cholesterol levels, both of which are important indicators of heart health [31]. Previous studies have also found that stearic acid shows potential as an anticancer agent through its mechanism of inhibiting key control points in the cell cycle and its ability to selectively induce apoptosis in malignant breast cancer cells without affecting normal cells. These findings suggest that stearic acid may play a role in preventing cancer cell proliferation [32].

Palmitic acid acts as an energy source and an important component in the formation of cell membranes and the body's lipid metabolism. However, excessive palmitic acid has been linked to an increased risk of metabolic syndrome, cardiovascular disease, cancer, neurodegenerative disorders, and chronic inflammatory conditions through mechanisms involving disruption of molecular signaling pathways [33]. However, the fatty acid analysis of raw lamb meat was not carried out in this study. The fatty acid analysis focused on determining the total fatty acid content in the lamb satay product. For future studies, it is recommended to include fatty acid profiling of both raw and grilled samples to better understand the effect of the grilling process on lipid composition and nutritional quality. Additionally, the proximate composition and physicochemical parameters were also analyzed to find variations among different raw lamb and satay producers.

Table 5. Nutritional and physicochemical quality of raw lamb used for satay

Parameter	Producers		
	A	B	C
Protein (%)	18.39 ± 0.25	21.94 ± 0.08	19.91 ± 0.19
Fat (%)	4.73 ± 0.17	3.70 ± 0.14	6.05 ± 0.03
Ash (%)	1.02 ± 0.03	1.25 ± 0.03	1.23 ± 0.01
Moisture (%)	74.07 ± 0.12	71.57 ± 0.19	71.37 ± 0.38
Carbohydrate (%)	1.80 ± 0.28	1.55 ± 0.10	1.46 ± 0.23
pH	5.76 ± 0.03	5.86 ± 0.04	5.87 ± 0.01
Water activity	0.97 ± 0.01	0.95 ± 0.01	0.96 ± 0.01

Note: Data are presented as mean ± standard deviation.

The nutritional and physicochemical characteristics of raw lamb used for satay are shown in Table 5. Compared to the raw satay samples (Table 5), several compositional changes were observed after grilling (Table 6). In general, the moisture content decreased, while the fat content increased after grilling. The reduction in moisture is mainly attributed to water evaporation due to high grilling temperatures. The decrease in water content during barbecuing occurs as a result of the high temperature, which causes shrinkage of the myofibrillar proteins and the perimysial connective tissue, thereby reducing the water-holding capacity of meat [34]. This phenomenon is also related to an increase in the fat content observed in the lamb satay after grilling in the present study, as the loss of moisture leads to a relative concentration of lipids in the final product. According to [35], the water content in charcoal-grilled lamb patties decreased significantly, while the protein and fat contents increased at each cooking interval.

Table 6. Nutritional and physicochemical parameters of lamb satay from different producers

Parameter	Producers			*p*-value
	A	B	C	
Protein (%)	17.98 ± 0.37	18.10 ± 1.64	19.55 ± 2.85	0.425
Fat (%)	28.26 ± 3.71	30.40 ± 1.76	27.42 ± 1.44	0.155
Ash (%)	0.94 ± 0.16	0.68 ± 0.07	0.95 ± 0.15	0.071
Moisture (%)	50.46 ± 3.07	47.83 ± 1.80	50.40 ± 0.32	0.365
Carbohydrate (%)	2.41 ± 1.44	2.97 ± 2.09	1.07 ± 0.77	0.362
pH	6.15 ± 0.03	6.11 ± 0.05	6.16 ± 0.03	0.272
Water activity	0.85 ± 0.02	0.84 ± 0.01	0.84 ± 0.01	0.751

Note: Data are presented as mean ± standard deviation.

The results also show that parameters of nutritional content (Table 6), namely protein, fat, ash, moisture, and carbohydrate content, had no significant differences between the three different producers ($p > 0.05$). These findings indicate that the lamb satay products from the three producers had relatively uniform nutritional compositions, reflecting consistency in raw material selection, such as the type of lamb used, as well as in basic processing techniques including the meat cut types and grilling methods applied. This consistency is important for ensuring product quality stability in the market, including attributes such as flavor, aroma, texture, and juiciness, which collectively determine the overall eating experience and consumer satisfaction [36]. It also plays a role in maintaining consumer preferences for distinctive sensory and organoleptic characteristics, as well as preserving the identity of Tegal lamb satay as a widely recognized regional culinary icon. Sensory attributes have consistently been shown to serve as strong predictors of consumer purchase intention and repeat buying behavior [37].

The physicochemical parameters (pH and water activity) showed no significant differences among the three producers ($p > 0.05$). Despite the lack of significance, this uniformity indicates that similar handling and processing practices were applied, contributing to product consistency. The pH is a key determinant of meat quality, influencing attributes such as tenderness, juiciness, color, and shelf life. The relatively stable pH values among samples helped maintain water holding capacity (WHC) and supported favorable sensory characteristics [38]. Meanwhile, the water activity values ranged from 0.84 to 0.85, indicating sufficient free water availability to support microbial growth. Although slightly below the threshold for pathogenic bacteria (≥0.91), these values fall within the optimal range for yeast (0.87–0.91) and mold (0.80–0.87) [39].

Correlation of lamb satay quality and consumer satisfaction

The correlation analysis between lamb satay quality attributes (nutritional and physicochemical) and consumer satisfaction was conducted to identify specific quality parameters that could influence sensory characteristics and could subsequently affect consumer acceptance or satisfaction [40]. The evaluation included chemical and physical characteristics that potentially influenced the flavor, aroma, and texture of the meat, which were then correlated with the satisfaction scores of each consumer from the respective producers. The results were expected to provide an overview of the key factors influencing consumer preferences, serving as a guide for improving product quality and competitiveness.

Table 7. Average satisfaction of consumer toward lamb satay from three different producers

Producers	Number of respondent/consumer (n)	Average consumer satisfaction
A	9	4.22
B	8	3.64
C	15	4.30

Consumer satisfaction with lamb satay varied among producers (Table 7). Overall, all producers were rated within the “High” category on a 1–5 Likert scale, although a clear difference in mean scores was evident between producer B and the other two producers. This variation can be an indication of differences in consumer satisfaction based on the quality of lamb satay, which were subsequently analyzed through correlation analysis and principal component analysis (PCA). The PCA method was appropriate for this study as it involved multiple quality assessment attributes that were intercorrelated, thereby facilitating the identification of the main factors influencing consumer satisfaction [41].

Table 8. Correlation levels of lamb satay quality and consumer satisfaction

Variable	Coefficient (ρ)	Correlation	*p*-value
Protein	0.2145	No correlation	0.2384
Fat	–0.5941	Negative	0.0003**
Ash	0.5941	Positive	0.0003**
Moisture	0.5014	Positive	0.0033**
Carbohydrate	–0.5941	Negative	0.0003**
pH	0.5010	Positive	0.0034**
Water activity	0.5010	Positive	0.0034**

Note: ** indicate highly significant correlation ($p < 0.01$)

The Spearman correlation analysis (Table 8) showed a significant positive correlation ($p < 0.01$) between moisture content, pH, and water activity with consumer satisfaction levels. The coefficient values indicated that higher levels of these variables were significantly associated with increased consumer or panelist acceptance. In contrast, protein content showed a non-significant correlation ($p > 0.01$). Conversely, fat and carbohydrate contents exhibited a significant negative correlation ($p < 0.01$) with consumer satisfaction scores, meaning that higher fat and carbohydrate levels tended to result in lower consumer satisfaction scores.

Principal component analysis (PCA) was also performed to reduce data dimensionality and identify the main physicochemical attributes associated with consumer satisfaction levels. The biplot visualization (Figure 2) showed that samples with very high satisfaction levels were located close to the vectors of variables such as moisture, ash, pH, and water activity. In contrast, samples with low to moderate satisfaction levels were positioned closer to the vectors of fat and carbohydrate variables.

Table 9. Variable loadings on the first three dimensions of PCA

Variable	Loading value	
	Dim 1	Dim 2
Protein	0.260	–0.606
Moisture	0.433	0.096
Fat	–0.430	0.129
Ash	0.434	0.080
Carbohydrate	–0.354	0.441
pH	0.301	0.547
Water activity	0.393	0.328

Note: Dim = Dimension, represents the principal components (PC1, PC2).

Additionally, the loading values (Table 9) indicated that the vectors of ash (0.434), moisture (0.433), and fat (–0.430) contributed the most to Dim 1, whereas pH (0.547) and carbohydrate (0.441) were the dominant contributors to Dim 2. Interestingly, the protein vector pointed toward the lower-right quadrant, in a different direction from most of the other vectors. This distribution pattern suggests potential differences in how each attribute is associated with the underlying components represented by the two dimensions.

The results of the Spearman correlation and PCA analyses consistently showed that consumers tended to prefer lamb satay with higher moisture, ash, pH, and water activity levels, as well as lower fat and carbohydrate contents. Optimal moisture and water activity play an important role in maintaining the tenderness, juiciness, and texture of meat products, thereby directly influencing consumer evaluations of sensory attributes [42]. A stable pH value

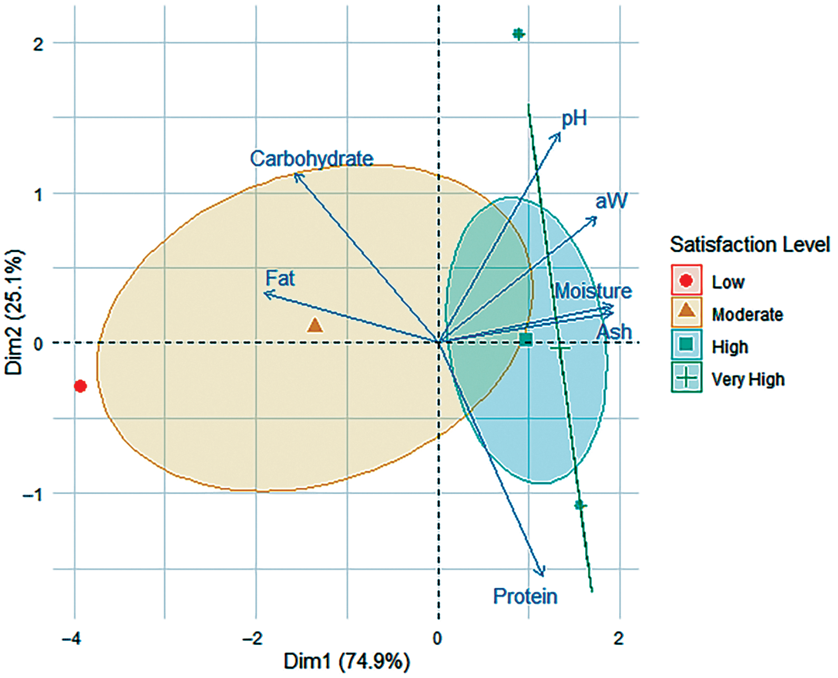

Figure 2. PCA biplot of lamb satay quality attributes and consumer satisfaction

is also believed to have an effect on the texture [43], reduce flavor changes during storage, and maintain the sensory stability of the product [38]. In addition, ash content showed a strong positive correlation, which is assumed to reflect the presence of minerals or other compositional elements that enhanced the perception of sensory attributes. Previous studies reported that ash or certain mineral contents played an important role in shaping flavor [44], and enhancing panelists' perception of quality [45].

Although protein content tended to increase when intramuscular fat was low and water content was high [46], our results showed no significant correlation between protein levels and consumer satisfaction. Interestingly, products with higher fat content were associated with lower consumer satisfaction, suggesting that fat level played a more critical role than protein in influencing consumer preferences, possibly due to perceptions of greasiness. These findings were consistent with previous studies indicating that consumers tended to prefer products with lower levels of fat and carbohydrates, presumably because lamb satay with higher fat content was perceived as too greasy and high in calories [47]. This preference aligned with current consumer trends that favored healthier, lower-calorie options. Supporting evidence includes a study reporting that Polish consumers preferred low-fat cooked meat products and tended to reject meat containing intramuscular fat, as it was perceived to be high in calories and cholesterol [48]. Moreover, excessive fat consumption, commonly observed in Western diets, has been associated with an increased risk of obesity and type-2 diabetes, partly due to ectopic lipid accumulation in skeletal muscle tissue [49]. These findings emphasized the importance of balancing chemical composition to influence sensory quality and consumer acceptance, suggesting that product formulation should consider an optimal balance of chemical, nutritional, and physical characteristics to effectively meet consumer preferences.

The pattern of correlation among variables observed in this study showed relatively consistent and similar trends. This was likely influenced by the limited sample size and the interdependence among the analyzed variables, which resulted in a nearly uniform direction of correlation. Therefore, further studies with a more comprehensive research design and a larger sample size are needed to obtain a more representative and in-depth understanding.

Influence of lamb satay sensory quality on consumer satisfaction

Based on the previous analysis in this study (correlation and PCA), it was necessary to evaluate the influence of sensory quality attributes of lamb satay, such as flavor, aroma, color, texture, and cut size, to understand the factors affecting consumer satisfaction. These sensory attributes play a crucial role in determining consumer acceptance and preference for a product [50]. Furthermore, these attributes contribute significantly to the overall level of consumer satisfaction.

Table 10. Multiple linear regression of the lamb satay sensory attributes on consumer satisfaction

Variable	Coefficient (β)	Standard error (SE)	p-value
Color	**20.944**	**5.519**	**0.001****
Aroma	**14.894**	**4.569**	**0.003****
Texture	**19.562**	**3.287**	**0.000****
Flavor	**21.892**	**3.998**	**0.000****
Piece size	**22.267**	**3.087**	**0.000****

Note: ** indicates highly significant effect ($p < 0.01$).

The regression analysis results (Table 10) showed that all sensory attributes, such as color, aroma, texture, flavor, and product piece size, had a significant influence on consumer satisfaction. This is indicated by p-values all below 0.01, demonstrating a very strong relationship. These findings suggest that sensory quality greatly affects the acceptance and satisfaction of consumers toward lamb satay. The highest coefficient (β) values were observed sequentially for piece size, flavor, color, texture, and aroma.

Based on Figure 3, the regression model demonstrates excellent predictive performance for consumer satisfaction. The graph compares the actual consumer satisfaction values on the Y-axis with the predicted values from the model on the X-axis. The red dashed line represents the condition of a perfect prediction, where the predicted value is exactly equal to the actual value. The close proximity of most data points (blue dots) to the red dashed line indicates that the constructed regression model was highly accurate and reliable.

These findings support the perspective that sensory quality is a key factor influencing a level of consumer acceptance and satisfaction of food products [51]. Sensory responses were also strongly influenced by the chemical composition of the product, which determined essential attributes such as flavor and texture [37]. Previous study also stated that the flavor attribute had the greatest influence on consumer liking of game meat products, followed by texture [52]. Innovations, such as fermentation in processed meat products (sausages), enhance both flavor and safety [53]. Therefore, lamb satay producers were strongly advised to pay close attention to the management of sensory attributes or quality throughout the production and product development process. This included the selection of high-quality raw materials, standardization of seasoning formulations, and strict control over cooking techniques (such as temperature and grilling duration) to ensure optimal sensory quality [54]. By prioritizing innovation in these sensory aspects, producers would not only be able to meet consumer expectations but also potentially create a sustainable competitive advantage and build strong brand loyalty in the market. It should be noted that in this study, no specific measurements of the dimensions or average weight of the satay pieces were conducted. Therefore, the evaluation of the "product piece size" was entirely based on

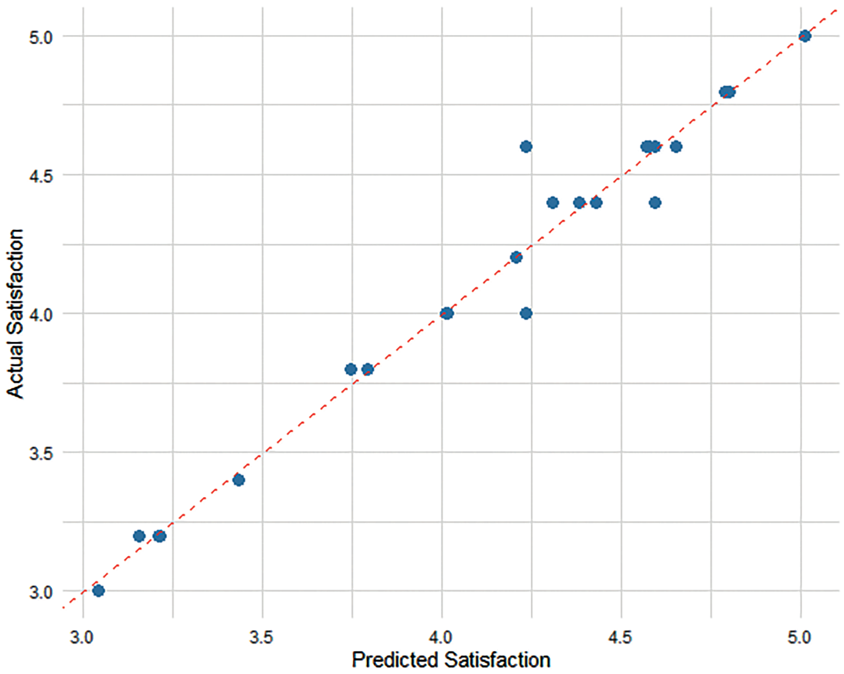

Figure 3. Actual and predicted consumer satisfaction plot

consumers' subjective perception of the appropriateness of the served pieces, as reflected in the 5-point Likert scale. This approach was intended to capture consumers' direct impressions of portion size as a sensory attribute influencing their overall satisfaction.

Conclusion

This study found that the quality of lamb satay from various producers in Tegal Regency showed uniformity in microbiological aspects, nutritional content, and physicochemical characteristics. Total of 18 fatty acids were successfully identified in the product. However, the contamination level of Escherichia coli exceeded the safety threshold for ready-to-eat food. This study also revealed that the physicochemical quality of lamb satay was strongly correlated with consumer satisfaction levels. Higher levels of moisture, ash, water activity, and pH were associated with increased consumer satisfaction and acceptance. Conversely, high fat and carbohydrate contents reduced consumer preference. These findings align with current consumer trends favoring healthier food products. In addition, sensory quality or attributes play a crucial role in shaping consumer satisfaction and acceptance of lamb satay. Based on the findings of this study, it is recommended that lamb satay producers in Tegal Regency enhance the hygiene of their production processes to minimize Escherichia coli contamination and optimize the balance of physicochemical composition in order to improve sensory quality as well as consumer acceptance and satisfaction.

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TESTING OF METHODS FOR DETECTING *SALMONELLA* IN THE AIR OF POULTRY PROCESSING PLANTS

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Keywords: *pathogens, air, poultry, methods, PCR, Salmonella*

Abstract

The aim of the study is to compare the effectiveness of microbiological and PCR methods for detecting Salmonella in the air at various technological sites in four poultry processing plants and in one poultry farming enterprise. The objects of the study were air samples collected on two nutrient agars: non-selective PCA agar and selective XLD agar. Microbiological and PCR methods were used. Air samples collected on PCA agar were cultured in the BPW (enrichment stage). The culture liquid obtained in this way was used in the isolation of Salmonella by the microbiological and PCR methods. The identification of colonies typical of Salmonella isolated by the microbiological method was carried out by mass spectroscopy. The conducted study demonstrated the indisputable advantages of the PCR method (after enriching air samples in BPW) over the classic microbiological method without enrichment for monitoring Salmonella in the air of poultry processing plants. The PCR method has a higher sensitivity and detection speed, allowing the pathogen to be detected even at low concentrations in a sample. This is especially important for monitoring areas with a potentially low microbial load, such as the final washing of broiler chicken carcasses. The microbiological method without the enrichment stage showed low detection of Salmonella in the study of 66.6 % of air samples (false negative results were obtained) of poultry processing plants and 80 % of air samples taken at the poultry farming enterprise. Increasing its sensitivity to a level comparable to the PCR method is possible only with the introduction of an additional enrichment step in a liquid non-selective nutrient medium, for example, in buffered peptone water. Thus, for prompt and reliable control of Salmonella contamination in the air, it is advisable to use the PCR method as the most rapid and sensitive tool, ensuring high reliability of results even with minimal bacterial contamination, and the microbiological method with sample enrichment as a relatively slow but reliable "golden" standard method.

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Introduction

At enterprises producing beef, pork and poultry meat, the air is acknowledged as a factor facilitating contamination of food products. In particular, slaughter shops are potentially critical as animals and poultry are the main source of microbial contamination of the air [1–3]. Analyses of the air at various sites of technological operations on slaughtering animals and poultry show similar trends in the microbial counts despite quantitative differences with lower levels before slaughter and higher values in the slaughter process both in "clean" (for example, at the site of accumulation of carcasses that are ready to chilling) and in "dirty" (bleeding) sites [4]. Processing of poultry meat includes the multistep sequence of operations, including slaughter, which consists in hanging, stunning, neck cutting and bleeding; dressing, which includes scalding, defeathering, head pulling, hock cutting, venting, evisceration, crop removal, neck cracking and cutting of neck flap; inside and outside washing of carcasses, decontamination and reprocessing; inspection of carcasses after slaughter, carcass chilling before cutting and packaging as described in [5] and GOST R 52469-2019[1].

As food safety has received high priority [6] and the industry has found itself under pressure to supply foods with minimal processing [7], fresher taste and appearance, with lower content of preservatives [8] and long shelf-life, it is necessary to apply an intervention strategy to control vectors of food contamination [9]. At food enterprises, bioaerosols can also be a potential factor influencing food safety and quality [10,11]. Practically all microorganisms in bioaerosols are easily transferred by air flows; however, their multiplication in the air is unusual due to the lack of moisture and nutrients. Despite sensitivity to the environmental conditions, foodborne pathogens can survive in the air combined, for example, with dust particles [12].

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[1] GOST R 52469-2019 "Poultry processing industry. Poultry processing. Terms and definitions". Retrieved from https://docs.cntd.ru/document/1200167787. Accessed October 26, 2025

Due to the constant air movement in food enterprises, the control of the air environment is difficult, but the correct location of intake and exhaust air ducts, door openings and technological equipment optimizes the movement of air flows [13,14]. Periodical monitoring of the level of microorganisms in the air facilitates revealing potential sources of food contamination [15,16].

Air samples are analyzed using various methods: microscopic, microbiological, biochemical or molecular [17,18]. A choice of a method for air analysis for one or another indicator will depend on sensitivity and specificity of a method as well as on a method of air sampling [19,20]. It is necessary to choose a method for investigation before performing the procedure of air sampling.

Traditionally, culture-based methods are used in the food industry to quantify microorganisms in the air [21]. To reveal microbiota of the air environment, it is preferable to use different nutrient media to detect as many microbial species as possible [22,23]. A limitation of the culture-based microbiological method is the fact that with this approach only part of microbial population can be detected. For example, it is impossible to detect viable but non-culturable (VBNC) microorganisms using this method [24]. Despite this disadvantage, the microbiological method is a "golden" standard in the food microbiology.

Using phase contrast microscopy, it is possible to see both culturable and non-culturable forms of microorganisms in an air sample [25].

Molecular methods, such as 16S rDNA amplification by polymerase chain reaction (PCR) with the following sequencing and DNA-DNA hybridization, allow for increasing sensitivity and specificity with the simultaneous reduction of time necessary for analysis. For example, quantitative PCR (qPCR), which is successfully used in medical investigations for assessment of the total and species-specific airborne bacterial load, can also be used to control the air environment in food enterprises. Sensitivity of qPCR is higher by several orders of magnitude than that of the microbiological methods. Moreover, it is able to amplify DNA of VBNC cells [26]. At present, molecular methods are not used in the food industry as routine methods for air monitoring in facilities of food enterprises despite their advantages.

The aim of this research is to compare effectiveness of the microbiological and PCR methods for detection of *Salmonella* in the air at different technological sites of poultry processing plants.

Objects and methods

Objects of the research were air samples taken at different sites of the technological process in four poultry processing plants with complete or incomplete cycle of poultry slaughter and processing, as well as lairage zones.

- Enterprise No. 1: air samples were taken at the sites of raw material preparation ($n=2$), species pre-packing, production of sausage products ($n=2$), production of semi-finished products near the exit of products from a quick freezer, production of semi-finished products near the conveyer belt with nuggets after deep fat frying ($n=2$), production of semi-finished products near the deep fat fryer, production of semi-finished products near the packaging machine ($n=2$), packaging of finished products (frankfurt-type sausages), near the packaging machine, packaging of the finished products, near the heat shrink equipment, container washer, storage chamber for finished products;
- Enterprise No. 2: air samples were taken at the sites of evisceration, chilling, forming and packaging of semi-finished products, packaging of breaded culinary frozen products ($n=2$), cutting of semi-finished products in pieces ($n=2$), pre-packaging of dry auxiliary ingredients, container washer;
- Enterprise No. 3: air samples were taken at the sites of poultry hanging, bleeding, scalding and defeathering, evisceration and venting, spray cabinet, near the machine for leg processing, near the site of location of the machine for head pulling, air-water droplet spraying, Maestro, deboning of the thigh fillet, bath for by-products, container washing, machine deboning of breasts, accumulation of carcasses, final washing, carcass packaging;
- Enterprise No. 4: air samples were taken in the lairage zone, in the tunnel, in the gallery, location of the deboning cone, at the site of the line for further processing of thighs/breasts, on the line of modified atmosphere packaging (MAP), on the line of vacuum-packaging, in the buffer zone, on container washer, near the Morris water chiller, at the site of further processing of by-products. This is a poultry processing plant which carries out turkey slaughter and produces semi-finished products in pieces from turkey meat.

Air sampling was carried out during the working process using an air sampler Airwel (ALLIANCE BIO EXPERTISE, France) on the solid nutrient media: non-selective PCA (Plate Count Agar) (State Research Center for Applied Microbiology and Biotechnology (SRC AMB), Russia) and selective medium XLD (Xylose Lysine Deoxycholate) (SRC AMB, Russia). The volume of air samples was no less than 200 liters.

To detect *Salmonella*, two methods were used: microbiological and PCR.

Microbiological analysis of air samples collected on the non-selective PCA medium (SRC AMB) was carried out with enrichment in the buffered peptone medium (BPW) (SRC AMB, Russia) and of those collected on XLD agar without enrichment (SRC AMB, Russia). XLD agar was placed into an incubator (Binder BD240) (Binder, Germany) immediately after air sampling and incubated at a temperature of 37 °C for 24 hours. PCA agar with air samples was aseptically transferred from Petri dishes to homogenization bags, 100 cm^3 of BPW was added and homogenization was carried out. Then, incubation was carried out at a temperature of 37 °C for 24 hours.

After incubation, colonies characteristic of *Salmonella* were taken from XLD agar for the following identification.

In parallel, after incubation, 1 cm^3 of culture liquid was taken from BPW and transferred into liquid selective nutrient media: RV (Rappaport-Vassiliadis Broth) (SRC AMB, Russia) and MKTT (Müller-Kauffmann Tetrathionate Broth) (SRC AMB, Russia). Cultures were incubated at temperatures of 41.5 °C and 37 °C, respectively, for 24 hours. After that, cultures were transferred from each medium to the surface of XLD agar and incubation was carried out at a temperature of 37 °C for 24 hours. Colonies characteristic of *Salmonella* were transferred on TSA, incubated at a temperature of 37 °C for 24 hours and subjected to identification.

Identification was carried out by time-of-flight mass spectrometry (MALDI-TOF-MS) using a mass spectrometer Autof MS1000 (Autobio Diagnostics, China). To this end, bacterial mass of colonies was placed on a target plate and dried at room temperature. Then, 1.2 µl of formic acid was placed for 10 min. into each well with dried bacterial mass and dried, 1.2 µl of HCCA matrix (α-ciano-4- hydroxycinnamic acid, 99 %) was applied and dried again. The MALDI target was placed into the instrument and the equipment for microbial identification was started up using the software FlexControl (acquisition of spectra). Over several minutes, the software of the apparatus compared the obtained bacterial mass spectra of unknown microorganisms with spectra of identified microorganisms contained in the database of the instrument. During comparison based on the correlation of the obtained peaks and their intensity, matching scores were calculated: if the value was lower than 6.0, a result was considered unreliable and was not used in the subsequent work. A result was considered reliable and was taken into account at values of 6.0–9.0 at the genus level and 9.0–9.5 at the species level.

In parallel, bacteria of the genus *Salmonella* were detected by PCR from air samples after enrichment of PCA in BPW. DNA was isolated from 1 cm^3 of BPW using a set of reagents for the extraction of nucleic acids from animal biological samples (VECTOR BEST, Russia) with the Auto-Pure 4800 automatic nucleic acid extraction and purification instrument (Allsheng, China). PCR was performed on the instrument Fluorite (Xi'an TianLong Science and Technology Co., Ltd, China) using a kit for detection of *Salmonella* DNA "Reagent kit for detection of *Salmonella* spp. DNA by real-time PCR" (VECTOR BEST, Russia) according to manufacturer's instruction manual. Interpretation of the results was performed automatically by VECTOR BEST software (2025). All positive results were confirmed by the microbiological method[2].

Results and discussion

Fifty two air samples were taken at the enterprises for slaughter and processing of broiler chickens ($n=3$) and turkey ($n=1$). The presence of *Salmonella* in them was determined using two methods described in the methods of research.

Salmonella were not detected by any of the used methods in the air samples taken at the following sites of enterprise No. 1: raw material preparation ($n=2$); species pre-packing; production of sausage products ($n=2$); production of semi-finished products, near the exit of products from a quick freezer; production of semi-finished products near the conveyer belt with nuggets after a deep fat fryer ($n=2$), near the deep fat fryer and packaging machine ($n=2$); packaging of finished products (frankfurt-type sausages), near the packaging machine, near heat shrink equipment; container washer; storage chamber for finished products.

Salmonella were absent in all air samples taken at different sites of enterprise No. 2: evisceration of carcasses; chilling of carcasses; cutting carcasses into semi-finished products ($n=2$); forming and packaging of semi-finished products; packaging of breaded culinary frozen products ($n=2$); prepackaging of dry auxiliary ingredients; container washer;

Also, *Salmonella* were absent in all air samples taken at enterprise No.4: in the lairage zone; in the tunnel; in the gallery; at the sites of location of the deboning cone and the line for further processing of thighs/breasts; on the line of modified atmosphere packaging (MAP); on the line of vacuum-packaging, in the buffer zone, on container washer, near the Morris water chiller, at the site of further processing of by-products.

In the air samples taken at enterprise No. 3, *Salmonella* were absent, but not at all sites. *Salmonella*-free sites included deboning of the thigh fillet and breasts, location of the bath for by-products; container washing; accumulator of carcasses and their package; the "dirty" zone, where machines for leg processing and head pulling from broiler chicken carcasses were located; application of air-water droplet spraying.

However *Salmonella* were detected in the air samples at several sites of enterprise No.3: carcass hanging, bleeding, scalding and defeathering of broiler chicken carcasses, evisceration and venting, at the site of location of Maestro/pan conveyor and final washing of carcasses. Table 1. presents data obtained by different methods of analysis.

Table 1. Results of *Salmonella* detection in the air samples taken at poultry processing enterprise No.3 and analyzed by different methods

Sites at enterprise No.3	Presence of *Salmonella* spp.		
	Microbiological method		PCR method
	after enrichment	without enrichment	
Hanging	detected	detected	detected
Bleeding	detected	not detected	detected
Scalding / defeathering	detected	detected	detected
Evisceration / venting	detected	not detected	detected
Maestro/ pan conveyor	detected	not detected	detected
Final washing	detected	not detected	detected

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[2] GOST 31659-2012 (ISO 6579:2002) "Food products. Method for detection of Salmonella bacteria". Retrieved from https://docs.cntd.ru/document/1200098239. Accessed October 26, 2025.

Salmonella were detected in all six samples (100 %) by the PCR method and microbiological method after enrichment of the air samples. Without enrichment, *Salmonella* were detected only in two samples (at the sites of hanging and scalding / defeathering) out of six.

Following the aim of the research and based on the results obtained, including those presented in Table 1, we can conclude that PCR and microbiological methods used with the pre-enrichment of the air samples in BPW are significantly superior. With such an approach *Salmonella* were detected in 100 % of analyzed samples. Comparative analysis of two approaches of the microbiological method revealed an importance of the enrichment stage when detecting *Salmonella* in the air. Presumably, the microbiological method without enrichment of the air samples is not effective due to the low level of air contamination with *Salmonella*, which can vary depending on a processing object (live poultry, carcasses, meat).

It is possible that the reason for not detecting *Salmonella* in the air samples at enterprise No.1 even with the use of the sensitive PCR method is the fact that this enterprise does not perform poultry slaughter, which is a process with a high risk of contamination of the air environment. Processing of chilled or frozen raw materials (dressing, forming, frying and packaging), which is carried out at this enterprise carry insignificant risk of active entry of *Salmonella* into the air. In case of their transfer to the air, they can be undetected depending on the sensitivity of the method.

At enterprise No. 2, air samples were taken at the sites with a high risk of contamination, namely evisceration and chilling of carcasses, as well as at the sites with medium and low risk of contamination — production and packaging of semi-finished products, respectively. The targeted sampling at the first two sites was determined by the fact that poultry intestine is the main reservoir of *Salmonella* [27], which creates an increased probability of their entry into the air during technological operations. Air samples at the sites of production and packaging of semi-finished products were used to assess the sensitivity of the methods. Comparative analysis of air samples taken at these critically important points allows for more objective assessment of the effectiveness of PCR and microbiological methods in the conditions of the real contamination load. However, *Salmonella* were absent in the air samples taken at all sites, including at the site of evisceration. This is possible when the integrity of the gastro-intestinal tract is not violated and, as a consequence, there is a low level of air contamination, which is lower than sensitivity even of the PCR method.

For valid comparative assessment of the methods for *Salmonella* detection, it was necessary to ensure conditions at which the concentration of targeted microorganisms in samples would reliably exceed the threshold of the sensitivity of methods. This is what determined the extended sampling at enterprise No. 3, which included all sites of the cycle of poultry slaughter and primary processing (poultry hanging, poultry bleeding, carcass scalding, carcass defeathering) contrary to enterprises No.1 and No. 2. The choice of these zones was not accidental, as they are characterized by the maximum microbial load, including *Salmonella*, which is confirmed by both the data of monitoring of the objects of the production environment (not presented in the paper) and the results of the analysis of the air by foreign colleagues [28]. Therefore, sampling at zones with undoubtedly high contamination enabled creating a representative model for the comparative analysis of effectiveness of the PCR and microbiological methods. *Salmonella* were detected in the air samples taken in these zones as well as in the zones of carcass evisceration, pan conveyor, application of air-water droplet spraying and final washing. In [29], an increase in the percent of broiler carcass contamination (from 10 to 40 %) was observed during evisceration and spray washing after evisceration; with that, *Salmonella* counts increased from 3.9 to 5.1 log CFU/carcass. Apparently, detection of *Salmonella* on broiler chicken carcasses after washing can also be explained by the presence of flagella in *Salmonella*– a key factor of adhesion to the skin of broilers [30].

Comparative analysis revealed key technological operations that present a risk of air contamination. This is indicated by the detection of *Salmonella* in the air at the evisceration site and, what is especially indicative, after final washing of carcasses, which was confirmed by the used methods. At the same time, at the sites of leg processing, head pulling and production of semi-finished products (enterprise No. 3), neither PCR nor the microbiological method revealed *Salmonella* in the air. The most significant for comparison of the methods for *Salmonella* detection is divergence of the results at the identical sites (evisceration and chilling) of enterprises No. 2 and No. 3. At enterprise No. 3, *Salmonella* in the air of these zones were detected, while at enterprise No. 2 they were not. This contradiction revealed by both methods indicates that a level of air contamination depends not only on the type of operations but also on other factors. For example, Ferguson et al. [31] stated that effectiveness of sanitary measures and initial contamination of raw materials can be such factors. The air represents the main hazard, being the main route of transfer of contaminants [32] and acting as a key vector for bioaerosols — suspended particles containing bacteria, mold spores and yeasts. Settling on products and equipment surfaces, bioaerosols create a direct risk of microbiological contamination [31]. Contamination at the stage of poultry processing can occur from multiple sources: production environment of a shop, poultry itself, equipment and personnel. These contaminants can have physical, chemical or biological nature [33].

At enterprise No. 4, which like enterprise No. 3 is a poultry processing plant of the whole cycle of slaughter and processing, but only of turkeys, *Salmonella* were not detected in any air samples by either the microbiological method (with and without enrichment) or by PCR. Consistent negative results obtained by both methods prompt to propose several assumptions about reasons for *Salmonella* absence in the air of this enterprise that were corroborated upon the detailed analysis of the enterprise. The absence of *Salmonella* in the air of this enterprise was a consequence of the complex of measures, such as prevention at a level of raw materials, when the strict veterinary control is carried out, and work is performed with certified enterprises supplying *Salmonella*-free poultry; technological solutions that consist in automation of slaughter and evisceration processes minimizing formation of aerosols, as well as the use of effective ventilation system supplied with bactericidal lamps; organizational and hygienic measures in a form of the verified procedure of sanitary treatment of equipment, clear zoning and logistics of flows of raw materials, finished products and personnel. Therefore, the complex approach at enterprise No. 4 allowed for creating conditions under which a level of air contamination with *Salmonella* was lower that the limit of detection even for a highly sensitive method such as PCR, which is corroborated by consistent results of both methods and emphasizes an importance of preventive measures.

In addition, analysis was carried out for air samples ($n=22$) taken randomly in one of the facilities of the poultry feeding station where pre-slaughter holding of broiler chickens is performed. From this enterprise, broiler chickens are sent to poultry processing enterprise No.3, where *Salmonella* were detected in the air samples. The results are presented in Table 2.

Table 2. Results of *Salmonella* detection in the air samples of the facility of the poultry feeding station, where pre-slaughter holding of broiler carcasses is performed

Air samples	Presence of Salmonella spp.		
	Microbiological method		PCR after enrichment
	after enrichment	without enrichment	
1.	not detected	not detected	detected
2.	not detected	not detected	not detected
3.	not detected	not detected	not detected
4.	not detected	not detected	not detected
5.	not detected	not detected	not detected
6.	detected	not detected	detected
7.	detected	not detected	detected
8.	detected	not detected	detected
9.	not detected	not detected	not detected
10.	not detected	not detected	not detected
11.	detected	detected	detected

Data on air contamination with *Salmonella* were obtained for the facility where poultry were kept before slaughter. Similar to the previous investigation, the results obtained by the microbiological and PCR method after sample enrichment had the 100 % agreement of the results. With that, for one sample, the 100 % agreement was obtained for all three approaches. These approaches detected *Salmonella* in 45.5 % of the analyzed air samples. However, detection rate of *Salmonella* in the air samples by the microbiological method without enrichment was no more than 9.1 %.

As a result of comparative analysis of the methods (PCR and microbiological method) and approaches (with and without enrichment) for *Salmonella* detection in the air of poultry processing plants and one poultry farming enterprise, key differences in their effectiveness were established. The PCR method demonstrated 100 % detection rate of *Salmonella* after enrichment of the air samples, which is in agreement with other studies indicating that enrichment of samples in BPW for 18–24 hours with the following detection of *Salmonella* by the PCR method can improve their detection [34,35]. This method enables detecting both viable and injured microbial cells. Contrary to this method, the microbiological method can detect only viable and culturable *Salmonella* cells both without enrichment, for example in areas with a high level of contamination, and with enrichment even in areas with a low level of contamination. The study confirmed that the main risks of air contamination occur at the stages of slaughter and primary processing at the hanging sites where due to stress poultry make intense movements and feathers, down hair and dust from their surfaces contaminated with *Salmonella* enter the air; at the sites of scalding and defeathering due to formation of bioarosols; at the sites of evisceration due to the rupture of the intestine and at the sites of final washing due to cross-contamination via air flows. From poultry farming enterprises, however, birds come for slaughter being already contaminated with *Salmonella*.

Thus, the differences in the results obtained on the contamination of the air between enterprises were determined not by the errors of these methods but by the different levels of biosecurity at these enterprises. Therefore, the differentiated approach can be recommended to effectively monitor the air in production facilities of poultry processing plants: the PCR method with the pre-enrichment of air samples for the operative control of the air at all sites especially after sanitary treatment and the microbiological method with enrichment within the framework of the production control as well as for confirmation of positive results obtained by the PCR method and acquisition of the native culture for serotyping of *Salmonella* circulating in an enterprise. According to an opinion of several authors, traditional culture-based methods have limitations compared, for example, with the method of high-precision sequencing CRISPR-SeroSeq as they are based on isolation of several colonies and consequently underestimate the diversity of serovars. Combination of selective pre-enrichment with the molecular method in the environmental samples demonstrated comparable isolation of serovars in comparison with the traditional enrichment reducing at the same time the isolation process by 24 hours [36]. However, when monitoring pathogens in the air, it is necessary to assess viability of microorganisms to reveal whether they present a threat to human or poultry health. It is recommended to combine methods, both culture-dependent and culture-independent (for example, PCR), to prevent false negative results of detection of pathogens [37]. Control of the air environment, especially at poultry farming enterprises, is of utmost importance. Contrary to common beliefs about oral transmission of the microorganism, transfer of *Salmonella*, in particular *S.* Enteritidis, can occur by the airborne route, and an impetus of this event can be induced molting of poultry [38]. Molecular methods detect microorganisms irrespective of their viability, which leads to overestimating their concentration in the air [39]. When using the microbiological method, it is recommended to combine several selective nutrient media, for example, Brilliant Green agar, Modified Lysine Iron agar and XLT4. With that, it is important to choose an effective method for air sampling, which can ensure a sensitive alternative to the traditional method for detection of this pathogen in the poultry environment [40].

Conclusion

The performed research demonstrated undeniable advantages of the PCR method (samples after enrichment) compared to the classic microbiological method without enrichment for *Salmonella* monitoring in the air environment of poultry processing plants. The PCR method has higher sensitivity and speed of detection, making it possible to detect the pathogen even at a low concentration in a sample. This is especially important for the control at the stages of potentially low microbial load, such as the site of final washing of broiler chicken carcasses. The microbiological method without the enrichment stage showed low detection rate. False negative results were obtained in 66.6 % of air samples when analyzing the air in the poultry processing plants and 80 % of air samples taken in the poultry farming enterprise. Increasing its sensitivity up to a level comparable with the PCR method is possible only upon introduction of the additional stage of enrichment in the liquid non-selective nutrient medium. Therefore, for rapid and reliable control of contamination of the air environment with *Salmonella*, it is advisable to use the PCR method as the most rapid and sensitive tool that ensures high reliability of results even at minimal bacterial contamination and the microbiological method with enrichment of samples as a relatively slow but reliable "golden" standard method.

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ANTIOXIDANT POTENTIAL OF PROTEIN HYDROLYSATES FROM POULTRY BY-PRODUCTS OBTAINED BY MICROBIAL FERMENTATION

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Abstract

Protein hydrolysates and bioactive peptides are promising components with antioxidant properties. This study aimed to evaluate the antioxidant activity of protein hydrolysates obtained from microbial fermentation of broiler chicken gizzards using a concentration of bifidobacteria and propionic acid bacteria incorporated into whey. The total antioxidant capacity was determined by the FRAP method, the antiradical activity was determined using the DPPH assay with the detection of the IC_{50} *index to assess the antioxidant potential. The results showed that the FRAP antioxidant activity of the experimental hydrolysate sample obtained by fermentation using bifidobacteria was 30 % lower than that of other samples. However, this sample exhibited the greatest free radical scavenging effect, with an* IC_{50} *of 1.363 mg/g. The content of free amino acids and peptides was also determined by UHPLC combined with mass spectrometry. The properties of peptides were identified by the in silico method using the BioPep and PeptideRanker databases. The research results showed an increase in the content of free amino acids in hydrolysates during microbial fermentation. The content of a bioactive peptide with antioxidant properties — VW, as well as several peptides with potentially high antioxidant properties, was revealed. The results obtained show the prospects for obtaining protein hydrolysates from poultry by-products by their microbial fermentation, as well as the need for further deeper studies of peptides with potential antioxidant properties.*

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Introduction

A major challenge facing the food industry is the need to extend the shelf life of foods susceptible to oxidative spoilage. Oxidative spoilage is a complex process that degrades the sensory characteristics of products (including taste, aroma, and texture) and reduces nutritional value, which can negatively impact consumer health. Furthermore, toxic metabolites can accumulate during this process, which also poses potential health risks. On an industrial scale, it is often simpler and more cost-effective to use synthetic preservatives and antioxidants, as these substances effectively slow oxidation processes and extend the shelf life of products. However, in light of increasing consumer demands for food quality and safety, manufacturers are increasingly forced to rethink their approaches and shift their focus to natural food additives [1,2]. Natural antioxidants, in particular, play a key role in slowing the rate of oxidative reactions, including lipid oxidation, which in turn leads to a reduction in hydrogen peroxide and free radical levels in foods [3–5]. In recent years, active peptides, which have high potential as natural antioxidants, have attracted particular attention from researchers [6,7].

These biologically active components not only help extend the shelf life and enhance the stability of food products, but also possess a number of additional beneficial properties. This makes them particularly attractive for use in diets and in industrial food processing settings [8,9].

However, obtaining pure active peptides requires complex technological approaches to extraction and purification, which significantly increases their final cost. At the same time, numerous studies confirm the antioxidant properties of protein hydrolysates containing antioxidant peptides obtained from various types of protein raw materials. Other advantages of protein hydrolysates compared to purified peptides have been noted, such as the formation of oligopeptides as a result of absorption [10,11]. In recent years, research on protein hydrolysates containing bioactive peptides as functional and technological additives for food production has attracted the attention of food scientists all over the world [12,13]. Many studies corroborate the potential of using protein hydrolysates containing antioxidant peptides as food preservatives. The effectiveness of protein hydrolysates in reducing fat oxidation in food systems has been experimentally proven. Studies of various food products containing carp hydrolysates have shown reductions in free fatty acids, peroxide value, and malondialdehyde levels, which generally contributes to improved shelf life of high-fat food systems [14,15].

Peptides have a lower molecular weight than proteins, and therefore are easily digestible and more bioavailable, and exhibit higher biological activity. Protein cleavage is carried out due to hydrolysis caused by the action of catalysts (acids, alkalis or enzymes). As a result, the cleavage products of the protein molecule are sequentially formed — first polypeptides, peptides, and then amino acids [16].

Enzymatic protein hydrolysis is the most widely used process to produce bioactive peptides, which are important components in a variety of fields, from medicine to the food industry [16–18].

This method offers a gentler and more environmentally friendly approach compared to traditional chemical processing of protein raw materials. Unlike chemical methods, which often involve the use of toxic reagents and can have a negative impact on the environment, enzymatic hydrolysis utilizes natural enzymes, minimizing the risk of harming the ecosystem.

However, modern research points to the significant advantages and promising potential of microbial fermentation, particularly using bacteria with a well-developed proteolytic system [19–21].

Certain microbial strains not only can effectively break down proteins into peptides but also have an additional impact on the quality of the final product. Microbial fermentation can facilitate the formation of new bioactive peptides with unique functional properties and also influence the amino acid composition, which can improve the nutritional properties of the resulting product [22,23]. Microbial fermentation enables the precise engineering of peptides with targeted functions, including antimicrobial and antioxidant activity, by harnessing bacterial metabolism. To fully realize this potential, further research is needed to refine the fermentation conditions.

Numerous studies highlight the significant potential of meat raw materials for producing protein hydrolysates and bioactive peptides, which can serve as functional components in diets, contributing to improved health [24,25].

With the active participation of microbial endo- and exopeptidases, protein proteolysis occurs, leading to the release and accumulation of bioactive peptides in the substrate. Under specific conditions, microbial fermentation produces significant quantities of short peptides and free amino acids, which have multiple beneficial effects on the body [26].

In this case, the activity of peptides as a whole depends on the sequence of amino acid residues, electronic properties and the degree of hydrophobicity. Scientific publications have noted a wider range of biological activity of protein hydrolysates obtained from several food proteins [27], as well as during the enzymatic processing of by-products [28].

Transforming animal and poultry by-products into protein hydrolysates offers a dual benefit: unlocking a high-value protein source and reducing agricultural waste. These products, often perceived as waste, actually contain significant amounts of amino acids and bioactive molecules, making them an important component in the development of new processing technologies and applications in the food and feed industries. Research shows that hydrolysates obtained from liver and heart exhibit not only high antioxidant activity but also pronounced antimicrobial activity [11]. This opens up new opportunities for their use as functional ingredients in various products, helping to extend shelf life and improve consumer safety. Activation of these hydrolysates can significantly improve the organoleptic and nutritional characteristics of final products, making them an important element in sustainable development strategies for the agricultural industry and processing [14]. Therefore, the development of technologies aimed at the rational and efficient use of by-products obtained from farm animals and poultry is an important scientific task with practical significance.

This process takes into account the interests of both producers seeking to optimize their resources and reduce losses, and consumers interested in high-quality and healthy products [11,29].

Protein hydrolysates containing bioactive peptides exhibit a diverse range of functional and physiological effects that impact both the human body and food systems. In terms of their effects on the human body, these hydrolysates exhibit a significant variety of properties, including immunomodulatory, anticancer, antihypertensive, antioxidant, anti-inflammatory, mineral-binding, opioid, antilipid, anti-aging, and osteoprotective effects [16–18,24,26,28]. Each of these effects is based on specific mechanisms of interaction between bioactive peptides and cellular receptors, enzymes, and other molecules in the body, allowing hydrolysates to be used as functional supplements for the prevention and treatment of various diseases.

The activity of protein hydrolysates in food systems is also worth noting. They exhibit antioxidant and antimicrobial properties, making them useful for improving food preservation and extending their shelf life.

Their antioxidant activity helps neutralize free radicals, which in turn prevents oxidative processes in food materials, preventing their spoilage. Antimicrobial properties help combat pathogenic microflora, ensuring the safety and quality of food products. By virtue of their bioactive peptides, protein hydrolysates offer dual utility: they provide documented health benefits while enhancing functional properties, paving the way for their adoption in nutraceutical, pharmaceutical, and next-generation food products [8,13,30,31].

The main goal of this study is to identify the antioxidant properties of protein hydrolysates obtained by microbial fermentation of broiler chicken stomachs with the addition of bifidobacteria and propionic acid bacteria concentrates in whey.

Objects and methods

Objects

This study characterized protein hydrolysates (PH) derived from gizzards of ROSS-308 broiler chickens (41 days old) via microbial fermentation. Hydrolysis was conducted in a soft cheese whey medium under three conditions: with a concentrate of *Bifidobacterium longum* B379M (PH-B), with a concentrate of *Propionibacterium freudenreichii shermanii* KM 186 (PH-P), and a control without bacterial addition (PH-C). The bacterial concentrates (Propionix, Moscow, Russia) had concentrations of viable bacterial cells of 10^{11}–10^{12} CFU/cm^3 (bifidobacteria) and 10^{10}–10^{11} CFU/cm^3 (propionibacteria). Optimized fermentation parameters presented in a previous study were used to obtain hydrolysates [32]. The hydrolysate production technology is shown in Figure 1.

Determination of antioxidant activity of protein hydrolysate

The Ferric-reducing antioxidant power (FRAP) assay of hydrolysate samples was determined in ethanol extracts. In order to prepare sample extracts, a sample was mixed with 96 % ethanol in a ratio of 1:15 (g:ml), homogenized using an automatic homogenizer S10 (Stegler, China) for 2 min at 8000 rpm, infused for 60 min at 22 ± 2 °C and filtered through a paper pleated filter.

The total antioxidant capacity of alcohol extracts was determined by the FRAP method on an SF-2000 spectrophotometer (OKB Spektr, Russia) in accordance with the procedure [33]. In order to prepare the FRAP reagent, 0.3 M acetate buffer (pH 3.6), was mixed with 10 mM solution of the photometric reagent TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) (Acros Organics, China), by dissolving it in 40 mM hydrochloric acid and 20 mM aqueous iron (III) chloride (PanReac AppliChem, Spain) in ratios of 10:1:1, respectively. In order to measure the FRAP assay of the extract, 1.45 ml of freshly prepared FRAP reagent and 50 µl of the sample diluted with distilled water depending on the activity, or distilled water for measuring the control sample, were added to the tube. The reaction mixture was incubated for 30 min at 37 °C in the dark, after which the optical density was recorded at a wavelength of 594 nm. The FRAP assay of the samples was calculated according to the calibration curve ($R2 = 0.9987$), which was constructed using quercetin (Sigma-Aldrich, India) in the concentration range of 140 µM — 300 µM, and expressed in µmol-equiv. quercetin/g sample.

The DPPH radical scavenging activity (RSA) of the protein hydrolysates was determined according to [34]. Briefly, 0.5 g of each sample was subjected to extraction in 20 cm^3 of 95 % ethanol for six hours at 20 °C. Subsequently, 1 ml of the extract was combined with 1 cm^3 of an ethanolic DPPH solution and left to react in the dark for 30 minutes. The absorbance of the resulting mixture was measured at 517 nm using a Jenway 6404 UV/Vis spectrophotometer (Jenway, UK).

The radical scavenging efficiency (RSA) was calculated as a percentage using the formula:

$$RSA_{DPPH} = \left[1 - \frac{A_i - A_k}{A_0}\right] \cdot 100\%, \qquad (1)$$

where: A_k is the value for test sample solution mixed with DPPH solution; A_i is the value for test sample solution mixed with 95 % ethanol; A_0 is the value for *DPPH* solution mixed with 95 % ethanol.

The IC_{50} value was also determined. This indicator characterizes the concentration of the substance that binds 50 % of the formed DPPH radicals. The IC_{50} value was determined from a calibration plot of RSA_{DPPH} values (%) for various concentrations of protein hydrolysates (from 0 to 0.05 mg/ml).

Determination of free amino acids in protein hydrolysates

Free amino acids were determined using liquid chromatography on an Agilent 1260 Infinity LC system (Agilent Technologies, USA). Samples were prepared by liquid extraction of the hydrolysate in a 20 % trichloroacetic acid solution, and the resulting homogenate was then adjusted to pH 2.2 using acidified saline buffer. The mixture was then centrifuged BKC-TL4IV (Biobase, China) (20 minutes at

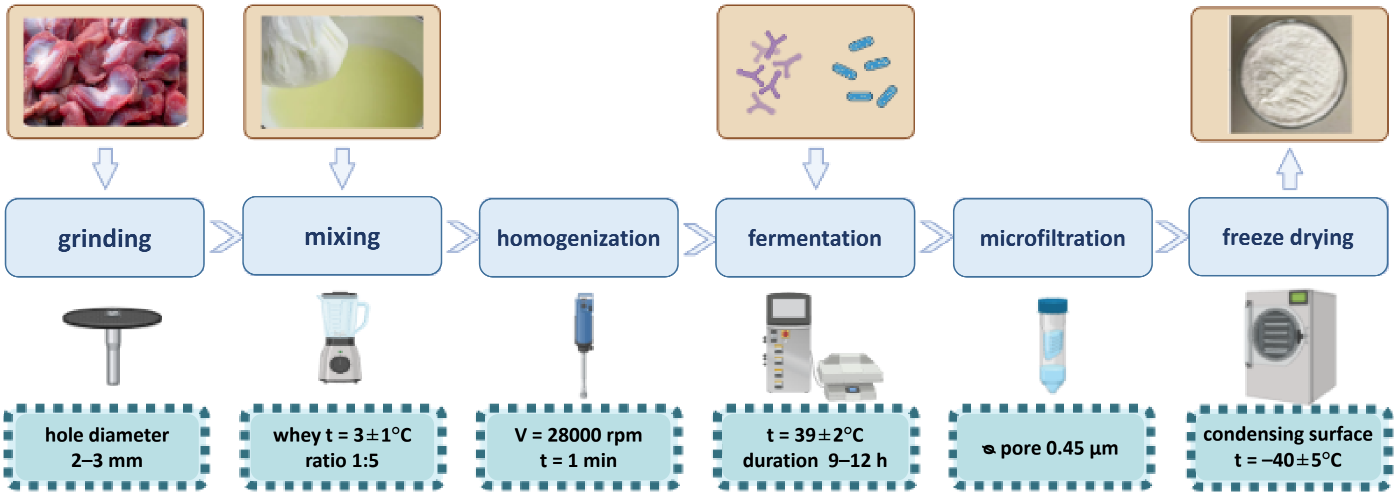


Figure 1. Technology flow chart for protein hydrolysate production (Created with BioRender.com)

4 °C with an RCF of 10,000 × g), and the supernatant formed after centrifugation was filtered into a vial. Chromatographic separation was performed on a C18 PA column (3.5 µm, 4.6 × 150 mm, ZORBAX) using a mixture of acetonitrile, methanol, and water in a ratio of 45 : 45 : 10 as mobile phase A and mobile phase B consisting of Na_2HPO_2 (1.42 g) and $Na_2B_2O_2$ (2.1 g), with pH 8.2. Orthophthalaldehyde for primary amino acids and 9-fluoromethyl chloroformate for secondary amino acids were used as derivatizing agents. Amino acid standards from Sigma Aldrich were used.

The content of free amino acids was expressed as milligrams per 100 g of liquid hydrolysates.

UPLC-ESI-Q-TOF-MS analysis and sequencing of biopeptides

The peptide separation was conducted on an Agilent Technologies 1290 Infinity UHPLC system [35,36], configured with an AdvanceBio Peptide Mapping column (2.1 × 250 mm, 2.7 µm) and a ZORBAX Extend-C18 guard column. The mobile phases consisted of 0.1 % formic acid in H_2O (A) and acetonitrile (B), delivered at a flow rate of 0.2 mL/min with a 10 µL injection volume. A 184-minute linear gradient was employed: 2 % B for 5 min, ramping to 43 % B over 165 min, then to 100 % B over 1 min, holding at 100 % B for 6 min, and finally returning to 2 % B over 7 min.

The UHPLC system was coupled to an Agilent 6545XT AdvanceBio LC/Q-TOF mass spectrometer equipped with a DuoJet Stream ESI source operating in positive ion mode. Key source parameters were: capillary voltage, 4000 V; nozzle voltage, 500 V; drying gas, 13 L/min at 325 °C; and nebulizer pressure, 35 psi. The ion funnel settings were 150 V (high-pressure) and 65 V (low-pressure). Data was acquired in full-scan MS (150–2100 m/z) and data-dependent MS/MS modes. The mass axis was calibrated internally using purine and HP0921. Nitrogen served as the collision gas.

Analytes were identified based on mass fragmentation using MSDIAL software (v.5.1) [36,37], applying mass accuracies of 0.01 Da for MS1 and 0.05 Da for MS2, which yielded over 300 compounds from the aqueous and ethanolic hydrolysate extracts. Individual peptide quantification was performed against a Leytragin® standard curve (1–1000 ng/mL) [38], with results expressed as mg Leytragin equivalents per 100 g of hydrolysate.

Quantification of the major peptides was performed using Leytragin® calibration curves, with a regression coefficient >0.990. The identified peptides were analyzed using the BioPep [39] and PeptideRanker [40] databases.

Statistical analysis

The experimental data were derived from five replicate samples, with each replicate subjected to three analytical measurements. The results are expressed as the mean value of the five replicates plus or minus the standard deviation. To assess statistical significance, the data were subjected to one-way ANOVA and Tukey's honest significant difference (HSD) test, implemented via a publicly accessible web application [41], using a significance threshold of $p \leq 0.05$.

Results and discussion

Antioxidant activity of protein hydrolysate

When assessing the antioxidant activity of protein hydrolysates, the following methods are mainly used: DPPH• radical absorption and iron-reducing antioxidant capacity (FRAP).

Methods for determining the antioxidant activity of hydrolysates (peptides) based on electron transfer include the ability of antioxidants to reduce iron and the activity of scavenging DPPH radicals [42]. FRAP analysis shows the reducing potential of antioxidant compounds through interaction with the ferric complex and tripyridyltriazine. According to Wong et al. [43], the antioxidant activity of peptides is mediated by functional groups — including phenolic hydroxyl, sulfhydryl, and imidazole — which directly scavenge free radicals and chelate prooxidant metal ions, thereby terminating oxidative chain reactions.

The results presented in Table 1 showed that the antioxidant activity of the FRAP hydrolysate sample obtained by fermentation with propionic acid bacteria was not statistically different from the control hydrolysate sample (PH-C). However, the level of antioxidant activity in the hydrolysate sample obtained by fermentation with bifidobacteria was approximately 30 % lower than that of the other samples.

The results show (Table 1) that the sample of the hydrolysate fermented with bifidobacteria at a concentration of 1.363 mg/g has the greatest radical scavenging effect.

The calculated IC_{50} value for the DPPH peptide EPEVLR of 2.03 mg/ml in the studies of Lin et al. (2025) [44] was comparable to the values for the commercial antioxidant glutathione, which allowed the authors to conclude that it has significant antioxidant efficacy.

Table 1. Antioxidant properties of protein hydrolysates

Sample	FRAP, µmol-equiv. quercetin/g	DPPH, IC50 mg/g
PH-C	235 ± 7.02a	2.994 ± 0.015b
PH-P	242 ± 5.87a	1.597 ± 0.011b
PH-B	190 ± 4.8a	1.363 ± 0.009b

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

Most antioxidant peptides contain from 4 to 16 amino acids and have a molecular weight of about 400–2000 Da [45]. It was noted that low molecular weight peptides have higher electron-donating properties, therefore the FRAP of peptides decreases with increasing molecular weight. The experimental results confirm that the ultrafiltered sheep placenta peptide fraction < 3 kDa exhibited the highest FRAP activity [46].

In this case, the type of amino acid plays an important role: aromatic amino acids neutralize radicals by donating protons; hydrophobic amino acids can increase the amount of peptides at the water-lipid interface and scavenge free radicals from the lipid phase; carbonyl and amino groups in the side chain of acidic amino acids act as metal ion chelators [47].

High antioxidant activity was found in quinoa peptides consisting of amino acid residues such as arginine, histidine, aspartate, glycine, and glutamate [48].

Hydrolysate samples with a large number of short-chain peptides have a higher reducing capacity due to better contact of proton-donor groups with the metal.

The results obtained by Liu et al. [45] showed that as the meat matured and the amount of small peptides increases, FRAP activity increases. The authors attribute the reducing capacity of antioxidant peptides to their more accessible structure, where exposed functional groups of amino acid residues can readily react with oxidizing agents [12].

The antioxidant activity of hydrolysates may be due to changes in protein structure that occur as a result of enzymatic hydrolysis. The ability of amino acids to interact with oxidants leads to changes in their structure and, consequently, to changes in the properties of residual groups.

Free amino acids in protein hydrolysates

The bioavailability and nutritional value of the human diet depend on the presence of free amino acids, which are absorbed directly in the small intestine, unlike native proteins. Their breakdown occurs through enzymatic hydrolysis in the digestive tract. This property ensures their rapid participation in protein synthesis and other metabolic functions in the body.

Analysis of free amino acids revealed an increase in their content in both the control and experimental hydrolysate samples after fermentation (Figure 2).

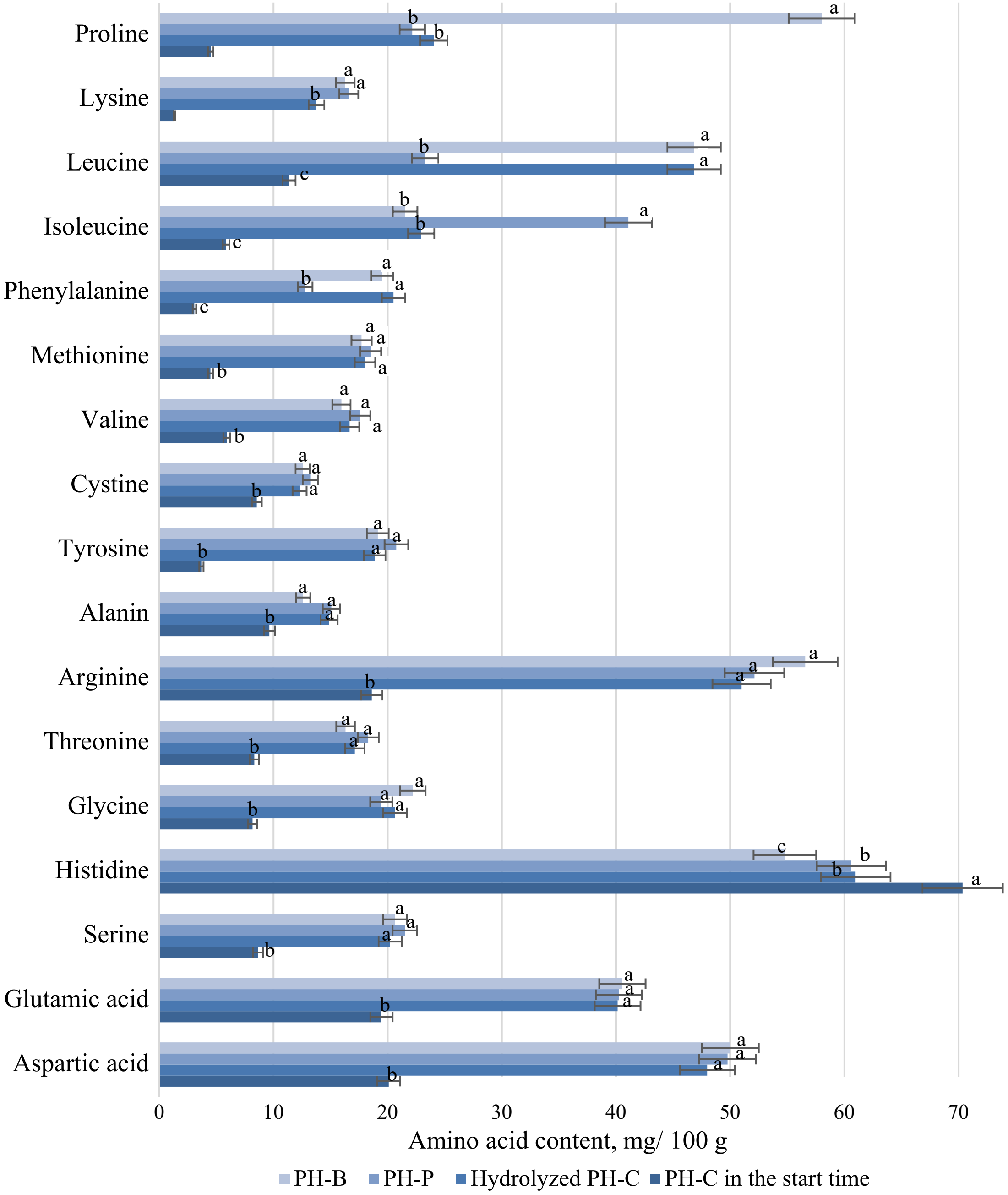


Figure 2. The content of free amino acids in protein hydrolysates. Means in a row without a common superscript letter differ ($P < 0.05$) as analyzed by one-way ANOVA and the TUKEY test ($n = 5$).

It is particularly noteworthy that in the hydrolysates from the gizzard of broiler chickens fermented using propionic acid bacteria and bifidobacteria, a significant increase in the level of essential amino acids was recorded compared to the control sample at the initial stage (from 212.14 mg/100 g to 463.15 mg/100 g and 501.31 mg/100 g, respectively). In the experimental samples, a decrease in the histidine content by 12–23 % relative to the initial values was also observed. Significant differences in the amino acid content were also noted in all experimental samples compared to the control sample before fermentation. The presented results demonstrate that the bacteria used exhibit proteolytic activity against proteins of the muscular and connective tissue of the gizzard. The effective action of exopeptidases on proteins is noteworthy. The significant accumulation of individual free amino acids confirms that probiotic microorganisms produce them during metabolism. Based on the findings in [49], the abundant free amino acids — tyrosine, methionine, and lysine — are likely key contributors to the observed antioxidant activity in the studied hydrolysates.

Fermentation of black soldier fly larvae paste by *L. paracasei* was shown by Zhang et al. [29] to markedly enhance the free amino acid profile, with levels of serine, valine, isoleucine, aspartic acid, glutamic acid, and histidine increasing by over 100 % compared to the control. Furthermore, over 90 % of the resulting small peptides were rich in hydrophobic amino acids, a feature linked to their antioxidant potential [29]. This aligns with the established principle that antioxidant capacity in hydrolysates arises from a synergy of hydrophobic and hydrophilic residues [50].

Specifically, the presence of key hydrophobic amino acids, such as proline, alanine, valine, leucine, and isoleucine, is a critical design consideration for synthetic antioxidant peptides [51].

The antioxidant contribution is further amplified as amino acids, such as tyrosine, methionine, proline, histidine, lysine, and tryptophan, are intrinsically bioactive, exerting antioxidant effects both within peptide sequences and in their free form [52].

According to our research, a significant accumulation of the amino acid proline was observed in the hydrolysate obtained by fermentation of by-products by bifidobacteria. It is also worth noting that the hydrolysate obtained by fermenting with propionic acid bacteria contained approximately twice as much isoleucine after 12 hours of fermentation compared to the control.

Sulfur-containing amino acids, which can act as antioxidants on their own, are particularly noteworthy. Therefore, peptides containing these amino acids in very short chains may possess high antioxidant potential. Cysteine has proton-donating properties, while essential amino acids can chelate metal ions [53].

During the fermentation process, the hydrolysates we obtained released a significant amount of sulfur-containing amino acids, which may also have influenced the enhancement of the antioxidant properties of the hydrolysates.

According to Wang et al. [54], the antioxidant potency of the VKVGNEF and MEAPPHI peptides stems from their abundance of hydrophobic amino acids — such as proline, valine, and methionine. These residues are strategically distributed along the peptide chains and are notably positioned at the terminal ends, a configuration that enhances their activity. The mechanism for effective free radical scavenging is primarily mediated by phenylalanine at the C-terminus of VKVGNEF, which scavenges oxidative chains via hydrogen atom transfer.

Many authors have studied the effect of the amino acid composition of hydrolysates and peptides on the antioxidant activity and the mechanisms of such action (Table 2).

High antioxidant activity was demonstrated in soy hydrolysates against oil oxidation during several frying cycles of chips. The authors attribute the oxidative stability of palm kernel oil after 8 and 12 frying cycles to its low content of hydroperoxides, carbonyl and volatile compounds, unique structural features, and high short-chain peptide

Table 2. Mechanisms of manifestation of antioxidant properties of amino acids

Amino acid	Mechanism of action	Source
histidine	scavenges free radicals through the imidazole ring	[55]
proline	due to the low ionization potential of the pyrrolidine ring, as a proton/hydrogen donor, it can quench singlet oxygen	[56]
cysteine, lysine, histidine, methionine, tryptophan, and tyrosine	effectively scavenge free radicals	[57]
sulfur-containing amino acids (cysteine and methionine)	due to the easy oxidation of sulfhydryl groups by free radicals, they protect normal cells from damage	[58]
cysteine, histidine, aspartic acid, and glutamic acid	promote metal chelation as an important part of free radical inhibition	[56,59]
hydrophobic amino acids	could easily pass through the cell membrane lipid bilayer to destroy a reactive oxygen species in cells	[60–63]
	might increase the affinity and reactivity of peptides to the cell membranes and contribute to the accessibility of peptides to lipid-soluble reactive oxygen species to terminate lipid peroxidation	[64]
aromatic amino acids (tryptophan, tyrosine, histidine, and phenylalanine)	phenolic, indole and imidazole groups act as hydrogen radical donors for electron-deficient free radicals	[64–66]

content. The mechanism for this activity of the hydrolysate during soy fermentation with pepsin was revealed by analyzing the results of SDS-PAGE and tandem mass spectrometry. Furthermore, the scientists note the high content of amino acids with electron-donating capacity — tryptophan, histidine, and methionine [67].

UPLC-ESI-Q-TOF-MS analysis and sequencing of biopeptides

As a result of determining the peptides (Figure 3) using high-performance liquid chromatography combined with mass spectrometry, it was established that their total number in hydrolysates is more than 300.

In silico studies play a crucial role in assessing the activity of protein hydrolysates, providing powerful tools for analyzing and predicting their functional properties at the molecular level.

These approaches, based on computational models and algorithms, allow researchers to process and interpret large volumes of bioinformatics data, making them indispensable in modern scientific research. One of the key aspects of using *in silico* methods is the ability to rapidly screen and identify potential bioactive peptides derived from protein sources. Combining molecular modeling, structural biology, and computational analysis enables effective prediction of the activities of such peptides, including antioxidant, antimicrobial, and anti-inflammatory properties.

This significantly accelerates the discovery and development of new functional supplements based on protein hydrolysates, which is relevant in the face of growing consumer demand for products with improved nutritional properties.

In order to identify bioactive peptides, as well as to predict their biological activity *in silico*, the BioPep database was used, as recommended by many scientists [68,69].

The PeptideRanker system is recommended for assessing the probability of peptide activity by conditionally assigning scores from 0 to 1, where “1” and “0” represent the highest and lowest probability, respectively [40].

Peptide profiling (Table 3) revealed a diverse range of physiologically active sequences.

Notably, the antioxidant peptide VW was present at a significantly elevated concentration in the PH-P sample relative to the control.

We also detected several other peptides with putative antioxidant functions, including HHY, SQLPLHR, GHHS, PTHHFHVALL, and AVHHMVW, although these were found in statistically insignificant quantities (BioPep database). Furthermore, analysis using the PeptideRanker database (Table 4) identified additional high-potential bioactive peptides, among which PHHSSASCCLW, PPHM, and HGVCWIY were also predicted to possess antioxidant activity.

Means in a row without a common superscript letter differ ($P < 0.05$) as analyzed by one-way ANOVA and the TUKEY test.

Table 3. Characterization of the identified peptides

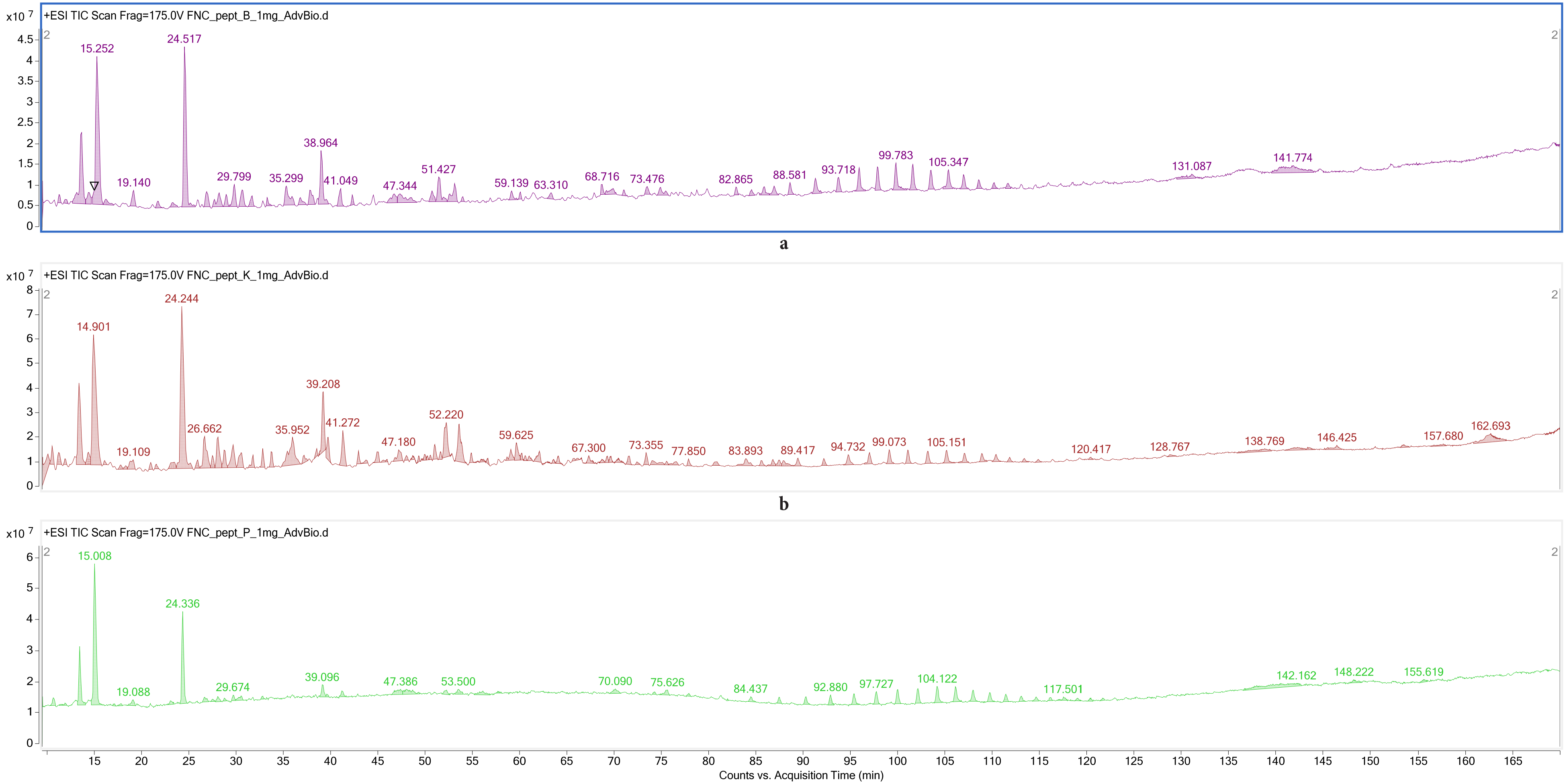
Sequence	Activity (according to BioPep)	Content, mg/100 g PH		
		PH-B	PH-C	PH-P
TR	dipeptidyl peptidase IV inhibitor	0.2 ± 0.01^{e}	0.5 ± 0.01^{d}	9.0 ± 0.03^{d}
SY	ACE inhibitor dipeptidyl peptidase IV inhibitor	10.6 ± 0.03^{b}	20.3 ± 0.08^{b}	9.8 ± 0.06^{c}
VW	ACE inhibitor Antioxidative dipeptidyl peptidase IV inhibitor alpha-glucosidase inhibitor	1.4 ± 0.01^{c}	7.5 ± 0.02^{c}	20.1 ± 0.17^{b}
PPP	ACE inhibitor	16.1 ± 0.11^{a}	15.2 ± 0.09^{a}	35.7 ± 0.26^{a}
SW	dipeptidyl peptidase IV inhibitor	1.1 ± 0.03^{d}	3.0 ± 0.07^{d}	7.2 ± 0.07^{e}

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

Table 4. Peptides with high potential biological activity according to the PeptideRanker database

Sequence	Probability that the peptide will be active (according to PeptideRanker)	Content, mg/100 g PH		
		PH-B	PH-C	PH-P
KEPPPGM	0.752229	$0,8 \pm 0.01^{e}$	$4,2 \pm 0.01^{e}$	$1,7 \pm 0.01^{e}$
HGVCWIY	0.759981	$6,2 \pm 0.02^{c}$	$0,2 \pm 0.01^{e}$	$4,1 \pm 0.01^{e}$
PGTHPLLVF	0.761792	$3,4 \pm 0.02^{c}$	$0,7 \pm 0.01^{e}$	$0,2 \pm 0.01^{e}$
SGAPM	0.770648	$157,0 \pm 1.31^{a}$	$10,4 \pm 1.29^{a}$	$14,4 \pm 0.02^{c}$
PAVVSCLPGPL	0.771864	$8,5 \pm 0.02^{c}$	$0,4 \pm 0.01^{e}$	$6,2 \pm 0.01^{e}$
PPPGV	0.776567	$0,2 \pm 0.01^{e}$	$6,2 \pm 0.02^{c}$	$0,4 \pm 0.01^{e}$
HGSPGHGWVL	0.780265	$0,2 \pm 0.01^{e}$	$11,5 \pm 0.02^{c}$	$6,8 \pm 0.02^{c}$
GRGHIWGQM	0.782054	$0,2 \pm 0.01^{e}$	$12,6 \pm 0.02^{c}$	$0,2 \pm 0.01^{e}$
PHHSSASCCLW	0.785109	$16,0 \pm 0.02^{c}$	$0,1 \pm 0.01^{e}$	$10,1 \pm 0.01^{e}$
ICIMAPIAF	0.786922	$0,2 \pm 0.01^{e}$	$4,3 \pm 0.02^{c}$	$24,8 \pm 0.06^{c}$
VGICIYCL	0.791613	$0,4 \pm 0.01^{e}$	$8,2 \pm 0.02^{c}$	$0,4 \pm 0.01^{e}$
VICFFSVW	0.823681	$6,2 \pm 0.02^{c}$	$0,1 \pm 0.01^{e}$	$4,1 \pm 0.01^{e}$
GLGGAWAF	0.83727	$0,1 \pm 0.01^{e}$	$3,1 \pm 0.02^{c}$	$0,4 \pm 0.01^{e}$
KVPPPRPPL	0.837789	$3,5 \pm 0.02^{c}$	$0,1 \pm 0.01^{e}$	$0,2 \pm 0.01^{e}$
GSAPCPG	0.860877	$6,4 \pm 0.02^{c}$	$14,1 \pm 0.02^{c}$	$0,1 \pm 0.01^{e}$
PGGPGPAM	0.872244	$0,1 \pm 0.01^{e}$	$2,2 \pm 0.01^{c}$	$0,1 \pm 0.01^{e}$
IHPF	0.888252	$0,2 \pm 0.01^{e}$	$4,9 \pm 0.02^{c}$	$0,6 \pm 0.01^{e}$
PPHM	0.899505	$9,6 \pm 0.02^{c}$	$1,8 \pm 0.01^{e}$	$0,1 \pm 0.01^{e}$
PCSIF	0.899527	$7,5 \pm 0.02^{c}$	$16,5 \pm 0.09^{b}$	$0,2 \pm 0.01^{e}$
GCTF	0.906178	$12,3 \pm 0.02^{c}$	$3,6 \pm 0.02^{c}$	$10,3 \pm 0.02^{e}$
QPPQPALAGLVF	0.906993	$0,8 \pm 0.01^{e}$	$3,5 \pm 0.01^{b}$	$15,8 \pm 0.02^{c}$
VAPWIMM	0.927034	$12,6 \pm 0.09^{b}$	$0,1 \pm 0.01^{e}$	$0,2 \pm 0.01^{e}$
PFGAFCNVW	0.93261	$6,4 \pm 0.02^{c}$	$0,2 \pm 0.01^{e}$	$0,2 \pm 0.01^{e}$
IVCWLPAF	0.937182	$0,1 \pm 0.01^{e}$	$10,3 \pm 0.02^{c}$	$0,1 \pm 0.01^{e}$
GGPPPPPPHPG	0.960782	$8,6 \pm 0.02^{c}$	$0,2 \pm 0.01^{e}$	$6,1 \pm 0.01^{e}$
PPPHPFPVALL	0.961163	$12,1 \pm 0.02^{c}$	$0,2 \pm 0.01^{e}$	$0,2 \pm 0.01^{e}$
GFPFPGIHW	0.975569	$0,1 \pm 0.01^{e}$	$10,1 \pm 0.02^{c}$	$1,9 \pm 0.01^{e}$

Values are means ± SEM, $n = 5$ per treatment group.



a

b

c

Figure 3. Chromatograms obtained during the research of protein hydrolysates: a) PH-B, b) PH-C, c) PH-P

The hydrolysate obtained by fermentation of raw materials with bifidobacteria contained the lowest content of a peptide with measured antioxidant activity. However, it contains a large number of peptides with high potential antioxidant activity; which, apparently, affects the formation of a high antiradical activity of DPPH (76.5 % for the 1 % protein hydrolysate solution).

Li et al. [70] determined that H, P, C, Y, W, F, and M are involved in the prevention of lipid peroxidation [70] and in the transfer of electrons and protons. The authors attributed the enhanced DPPH radical scavenging activity in the 5–10 kDa fraction to a high proportion of hydrophobic residues, specifically aliphatic (Val, Ile, Leu) and aromatic (Phe, Tyr) amino acids, a finding that aligns with our data. We further posit that leucine and proline within the active peptides facilitate interactions with radical species through hydrophobic forces. This is consistent with prior research [58], which has documented the broad bioactivity of tryptophan-containing peptides.

These peptides possess antioxidant activity, allowing them to protect cells from oxidative stress, and also act as angiotensin-converting enzyme (ACE) inhibitors, which may help regulate blood pressure. Furthermore, they exhibit antidiabetic properties, helping to normalize blood sugar levels and improve metabolism. Thus, tryptophan-containing peptides represent a promising group of compounds for the development of functional foods and therapeutic agents. Peptides with tryptophan at the C-terminus, identified in experimental samples of hydrolysates, have a high capacity to reduce trivalent iron ions.

Numerous researchers in food science and biochemistry have identified bioactive peptides with antioxidant properties obtained through whey hydrolysis, confirming the high value of this source as an ingredient for the development of functional foods [28,59].

These studies open new horizons in understanding the mechanisms of antioxidant action and their potential use in the human diet. Furthermore, modern scientific research is actively pursuing studies aimed at analyzing the bioactive peptides formed in raw meat during natural maturation and fermentation, which can significantly improve their nutritional properties and functionality in food products [71,72].

Significant research focus is being directed toward the byproducts of enzymatic hydrolysis, presenting a promising route for valorizing resources that were once considered inaccessible [42,73].

Concurrently, microbial fermentation of livestock and poultry organs is emerging as a key area of interest. This approach has proven effective, as demonstrated by the fermentation of broiler chicken stomachs to generate a protein hydrolysate containing peptides with both documented and potential antioxidant properties. These results highlight the importance and need for further research aimed at identifying and thoroughly characterizing the discovered peptides. A detailed analysis of their properties could facilitate the development of new functional additives that could improve human health and extend the shelf life of food products, a significant step toward creating a nutritious and safe diet.

Conclusion

The results of studies conducted to evaluate the properties of protein hydrolysates obtained by microbial fermentation demonstrate a high level of antioxidant activity in these compounds.

The results showed that the FRAP antioxidant activity of the experimental hydrolysate sample obtained by fermentation using bifidobacteria was 30 % lower than that of other samples. However, this sample exhibited the greatest free radical scavenging effect, with an IC_{50} of 1.363 mg/g.

A significant accumulation of the amino acid proline was observed in the hydrolysate obtained by fermentation of by-products by bifidobacteria. It is also worth noting that the hydrolysate obtained by fermenting with propionic acid bacteria contained approximately twice as much isoleucine after 12 hours of fermentation compared to the control.

During the fermentation process, the hydrolysates we obtained released a significant amount of sulfur-containing amino acids, which may also have influenced the enhancement of the antioxidant properties of the hydrolysates.

These data are consistent with the results of peptide analysis, highlighting the link between the fermentation process and the properties of the resulting hydrolysates. The microbial species used demonstrated pronounced proteolytic activity, which can be characterized by the significant accumulation of free amino acids and peptides in the resulting hydrolysates.

During the analysis of the hydrolysates, peptides with established antioxidant activity and peptides with potential antioxidant properties were identified, opening new horizons for further research. These findings validate the scientific potential for refining the production and application of these peptides.

Consequently, the study confirms microbial fermentation as an effective method for generating protein hydrolysates and underscores the necessity of further characterizing the detected peptides. Such research is a significant contribution to food science, paving the way for novel functional products with enhanced antioxidant activity and superior nutritional profiles, thus creating new opportunities for safe, healthy food ingredients.

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