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INFLUENCE OF HEAT TREATMENT TYPE ON THE FAT COMPONENT AND HETEROCYCLIC AROMATIC AMINES FORMATION IN THE CHOPPED POULTRY MEAT PRODUCTS

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Keywords: ω-*3 fatty acids, antioxidants, fat modification, oxidation, vitamins, carcinogens*

Abstract

In this study the influence of heat treatment type on the change in the fatty acid composition, indices and parameters of fat oxidation, the level of heterocyclic amines formation in the chopped poultry ready-to-eat products enriched with ω-3 fatty acids and an antioxidant complex were studied. The composition of ingredients and recipes of ready-to-eat products are developed with consideration of the medical and biological requirements for the diets of oncological patients. These ready-to-eat products feature some antioxidant substances in their composition that can bind free radicals, and provide for a reduction in the risk of carcinogens formation during the manufacturing process. The heat treatment was run in several ways, also called as modes — cooking in a microwave oven (MW), cooking in a convection oven in the "convection" mode with preliminary short-term roasting, steam cooking. For comparison, the conventional, i. e. not enriched food sample was used as a control one. The introduction of ω-3 fatty acids into the food formulation made it possible to change the fatty acid composition towards increasing the proportion of polyunsaturated fatty acids (PUFAs). It was found that the profile of fatty acids was influenced by both the ingredients of the product and the type of its heat treatment. The joint use of a PUFA source and a complex of antioxidants made it possible to obtain a ready-to-eat product with a high level of ω-3; and to ensure the ratio of ω-6 group acids: ω-3 ratio amounts to 1–2:1. Heat treatment of enriched semi-finished products by microwave cooking and by steam cooking showed a lesser effect on the change in the content and composition of polyunsaturated fatty acids — the loss of fatty acids was 1.2% and 2.8%, respectively, while in the "convection" cooking mode with preliminary roasting this loss was equal to 3.5%. It was found that the antioxidant complex in the composition of the food product and gentle heat treatment methods cause less lipid peroxidation and the formation of carcinogenic heterocyclic aromatic amines (HAA) during the food manufacturing process.

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Introduction

In the modern world, among the non-communicable diseases, the oncological diseases now occupy a significant place. Among all carcinogenesis risk factors the share of nutrition is 35% [1, 2]. Quantitative and qualitative inferiority or, on the opposite, the excess of nutrients disrupts the normal metabolism, the functioning of organs and systems, and thus increases the risk of the emergence and development of malignant tumors. Diet is an important component in correcting the nutritional imbalances. The findings from Campbel et.al [3] showed that an increase of vitamin D content in blood serum and a decrease in the ratio of ω6/ω3 fatty acids due to dietary corrections and the inclusion of vitamin supplements into the diet affected the biology of prostate cancer cells in men and caused a decrease in the prostate-specific antigens level (PSA). The patients' diet consisted mainly of fruits, vegetables, turkey, chicken breast and cold-water fish, along with the following food supplements: ω3 PUFAs, curcumin, vitamin D3, and complex of B vitamins [3].

To compose a diet it is feasible to use the specialized and functional food. One of the main requirements for the composition of such food is the presence of antioxidants among the nutrients that can bind free radicals, as well as the absence of carcinogens formed due to the production process. The food products of animal origin are a source of complete proteins and fats, vitamins and minerals and should be included in a complete diet. In this regard, the creation of meat-based food (that could be included in the diet of people suffering from cancer) is one of the efficient ways to correct the structure of their diet.

Taking into account the biomedical requirements for the diet assigned for the cancer patients [4,5] in the Laboratory of "Functional and Specialized Nutrition" of the

V. M. Gorbatov Federal Research Center for Food Systems, recipes for chopped ready-to-eat products from poultry meat were developed. The implementation of medical and biological requirements was carried out through the choice of raw materials, ingredients and biologically active substances that have a beneficial effect on health. A reasonable approach in this case was the optimization of the macro- and micronutrients composition of the product, including the modification of the fat component, as well as the inclusion of ingredients with an antioxidant effect into the composition of the product. This will reduce the intensity of oxidative processes during the heat treatment of the food product and improve lipid metabolism, antioxidative action and vitamin status in patients.

In addition to carcinogens, the main nutritional factors that increase the risk of cancer are an excess of calories, fat, especially animal fat, cholesterol, mono- and disaccharides, a lack of complete proteins, essential amino acids, ω3 PUFAs, dietary fiber, polyphenolic compounds, deficiency of some micronutrients: vitamins C, E, B12, B9, carotenoids, flavonoids [6,7].

Reducing the pressure of carcinogenic risk on the human body and ensuring the optimal and balanced composition of all essential micro- and macronutrients in the diet will help increase the dietary anti-cancer resistance; that is reducing the risk of a tumor development by affecting the endogenous and exogenous reasons or factors of its development.

The current nutritional recommendations provide for reduced consumption of saturated fat by reducing and/or changing the quality of fat in the diet (partial replacement with unsaturated fats) [8].

To prevent the development of malignant neoplasms, nowadays physicians recommend limiting the consumption of red meat. Moreover, the meat of productive livestock animals features a high level of saturated fats; therefore, turkey meat was used as the basic raw material. Turkey lipids are rich in PUFAs, but contain predominantly ω-6 fatty acids.

In order to reduce the content of saturated fats and increase the share of ω-3 PUFA, the fat component of the food product was modified. The important role of ω-3 PUFAs in the correction of lipid metabolism disorders, their beneficial effect on the blood lipid profile has been confirmed and proven by numerous studies. It has been reported in the literature that ω-3 PUFAs provide a positive effect on improving the nutritional status of patients with tumors, increase the immune function of patients, and reduce the level of inflammatory cytokines [9, 10].

While developing modified fat composition of the ready-to-eat products, linseed oil was used as it is a source of ω-3 polyunsaturated fatty acids. Flaxseed oil contains about 44–61% alpha-linolenic acid (ω-3), 1–30% linoleic acid (ω-6), 13–29% oleic acid (ω-9). The share of saturated acids in linseed oil is 9–11%.

Broccoli was used as a source of plant antioxidants. Among other antioxidants, broccoli contains such substances as diindolylmethane, indole-3-carbinol and glucosinolates (glucoraphanin — a precursor of sulforaphane), which are effective for struggle against various types of cancer. Thus, preclinical studies have shown that the antiestrogenic activity of indole-3-carbinol (I3C) and diindolylmethane (DIM) can help reduce the risk of hormone-dependent cancers. It was found that I3C and DIM modulate the expression and activity of biotransformation enzymes that are involved in the metabolism and excretion of many biologically active compounds, including steroid hormones, drugs, carcinogens and toxins [11]. There is confirmed evidence that glucosinolates are powerful compounds that interact with the epigenome to restore the normal epigenetic environment within the malignant cells [12]. Sita et al. [13] reported on the antitumor action of sulforaphane (SFN) used as an auxiliary method together with pre-existing therapies. Several reviewed studies underline the potent activity of SFN to kill tumor cells. In vitro studies have shown the role of sulforaphane in the inhibition of bladder cancer cell lines, cell cycle arrest and induction of apoptosis [13]. As biologically active substances with a proven antioxidant status, we used a complex of vitamins — B9, B12, C, D3. The levels of content and application of the antioxidant complex are set according to the current requirements. According to the requirements, the level of the added vitamins should guarantee their content in the enriched product in amount sufficient to meet at least 15% of the average daily requirement when consuming 100 g of the product, taking into account the inevitable losses of vitamins during the technological process. The antioxidant effectiveness of the vitamin complex in the product has been confirmed by studies. The introduction of antioxidants in the form of a vitamin complex into experimental samples of ready-to-eat products, regardless of the method of their processing, contributed to maintaining the high activity of the enzymatic antioxidant system, which was expressed by a significant increase in superoxide dismutase (SOD) and catalase (CAT) and led to the neutralization of peroxidation products [15].

Certain processes for the manufacture of meat products, including heat treatment, are one of the factors affecting the formation of carcinogens, due to the impact on the fat and protein components. In this regard, it is necessary to recommend those methods of heat treatment that will minimize the risk of their formation.

The fatty acid composition of the fat component is a key element that determines the stability of lipid oxidation during heat treatment. The higher the unsaturation of fatty acids, the more oxidative degradation occurs during heat treatment [16]. In the literature there are numerous results of studying the effect of heat treatment on the change in the fatty acid composition of various types of animal and vegetable fats. PUFAs are the most susceptible to high temperatures, and increasing the amount of ω-3 PUFAs in foods can

increase the food susceptibility to lipid oxidation. During the heat treatment of oils rich in polyunsaturated fatty acids, their oxidation leads to the formation of various radicals, which subsequently can form malondialdehyde [17].

The oxidation of fats is accompanied by a deterioration in their organoleptic properties and the formation of various oxidation products — peroxides at first, and then toxic polymeric compounds. It should be noted that the type and temperature regime of heat treatment plays a key role in managing the direction of oxidative processes.

It is also known that during the heat treatment of high-protein products of animal origin, the potentially carcinogenic compounds are formed, among which heterocyclic aromatic amines (HAA) are that very chemical compounds that have at least one aromatic ring and one amino group in their structure [18]. A row of scientific works prove the relationship between the consumption of products containing HAA and the manifestation of oncological diseases [19]. Therefore, having sufficient information about heterocyclic aromatic amines will help reduce the health risks associated with the above-specified compounds [20].

The process of HAA formation depends on various factors, including temperature, cooking time, fat content, and the presence of precursors. Additional factors that can affect HAA formation are pH, type of meat and ingredients added during cooking such as antioxidants, amino acids, fat, and sugars. Various ways to minimize HAA formation during heat treatment include reformulation of ingredients, modification of heat treatment modes, and the addition of natural and artificial antioxidants [21].

The authors of the article [22] provide data on influence of fat composition on HAA formation. Replacing animal fat with vegetable oils led to HAA level decrease in pork cutlets. At different cooking temperatures, all cutlets with modified fat featured significantly lower amount of MeIQ, 4,8-DiMeIQx.

Taking the above into consideration, it can be assumed that gentle cooking methods and the addition of a complex of antioxidants will minimize the risks of carcinogens during heat treatment.

The purpose of this research is to study the change in the fatty acid composition, fat oxidation parameters, the level of heterocyclic amines formation in ready-to-eat poultry meat products enriched with ω-3 PUFA and with a complex of vitamins during heat treatment, designated for the diets of cancer patients.

Objects and methods

The samples of chopped food products composed of turkey meat, broccoli, rice flour, onion, garlic and enriched with ω-3 fatty acids (linseed oil 2%) with the addition of an antioxidant vitamin complex (vitamins B9, B12, C, D3 intended for functional nutrition of cancer patients) were used as the experimental material. The control sample was composed without the addition of linseed oil and antioxidant vitamin complex. To level the fat content a mixture of vegetable oils (sunflower and rapeseed) was added to the control sample.

The calculated values of the nutritional value of the samples were as follows: protein content 20.3 ± 1.6%; fat content — 7.2 ± 1.5%.

Heat treatment

The prepared semi-finished products were brought to ready-to-eat condition, and raw samples were also left as standards. For the experiment, gentle methods of cooking were chosen — in a microwave oven, by steaming and short-term roasting in a pan, followed by its bringing to complete readiness in a convection oven. In all preparation methods the readiness of the cutlet was determined by its final temperature — 85 °C (microbiological safety criteria) — inside the cutlet.

Microwave cooking

The samples were placed in an NN-C785JF microwave oven (Panasonic, Japan) and cooked at 1,000 W for 8 minutes in two heating cycles of 4 minutes each on each side until a core temperature of 85 °C was reached.

Cooking in a convection oven with pre-roasting

The samples were roasted in a hot frying pan with a Teflon coating on an electric stove, two-burner stove, EPK-27N (LLC "Elinoks", Russia) for 2 minutes on each side. Then they were placed in a steam convection oven Unox XVC304 series Chef Top LI1615AO (UNOX S.r.J, Padova, Italy) and brought to readiness (until 85 °C inside the cutlet) in the convection mode at a temperature of 180 °C. Total cooking time took 27 minutes.

Steam cooking

The samples were placed in an Unox XVC304 Chef Top LI1615AO steam convection oven (UNOX S.r.J, Padova, Italy) and brought to readiness (until 85 °C inside the cutlet) in the "steam" mode — 100%, at a temperature of 110 °C. Total cooking time took 29 minutes.

The final temperature of the samples cooking in the convection oven, both in the "steaming" mode and in the "convection" mode, was measured by inserting a temperature probe into the geometric center of each cutlet. The probe was built-in and connected to the temperature recorder of the steam convection oven. During heat treatment in a microwave oven, a portable RST07951 pro thermometer (with measurement range from –50 to +300 °C) (RST, Sweden) was used to measure the internal temperature.

After cooking, the samples were cooled down to room temperature and weighed to determine the yield of finished products.

Heat treatment losses (L_h) were calculated using the following formula:

$$L_h = (W_r - W_c) \times 100\% / W_r, \% \quad (1)$$

where

W_r — is the weight of the raw meat cutlets;

W_c — is the weight of the cooked meat cutlets.

Further, the samples were prepared for each analysis in accordance with the research procedure and method.

All studies were run at the Laboratory of "Scientific and Methodological Works, Biological and Analytical Research" of the Testing Center of the V. M. Gorbatov Federal Research Center for Food Systems (accreditation certificate No. RA.RU21PP69).

The experimental measurement equipment used in research was calibrated with an assessment of its accuracy and uncertainty in accordance with the requirements of ISO/IEC17025:2017[1].

All chemical reagents (salts and solvents of pure analytical grade) were confirmed by certificates of conformity and quality certificates; supplier is JSC "LenReaktiv" (St. Petersburg, Russia).

All aqueous solutions were prepared with deionized water from a MilliQDirect 8 system (France).

Study of indicators of oxidative stability of lipids

The acid value of the samples was determined by titration of free fatty acids with a solution of potassium hydroxide according to GOST R55480–2013[2].

The peroxide value of the samples was determined by the method according to GOST 34118–2017[3]. The method is based on the reaction of the interaction of the primary products of fat oxidation (peroxides and hydroperoxides) with potassium iodide in an acidic medium, followed by titration with sodium thiosulfate solution and the quantitative determination of released iodine.

Study of fatty acid composition

The composition of fatty acids was determined by gas chromatography according to GOST R55483–2013[4] on the Agilent 7890A automatic gas chromatograph (Agilent Tech., USA) with a flame ionization detector. To determine fatty acids, a Supelco SP 2560 100 m × 0.25 mm × 0.2 mkm chromatographic column (Supelco, USA) was used.

Determination of heterocyclic aromatic amines (HAA)

Instrumental determination and identification of HAA was run by high performance liquid chromatography [23]. The sample analysis was performed on an Agilent 1200 high performance liquid chromatography system (USA) with an Agilent 6410B triple quadrupole mass spectrometer. To determine HAA, a C18 chromatographic column, 4.6 × 50 mm, 1.8 μm (Agilent, USA) was used. The following were used as standard samples:

— standard sample of 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) manufactured by Toronto Research Chemicals (Canada) with a basic substance content of at least 99.0%;
— standard sample of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) manufactured by ChemCruz (USA) with a basic substance content of at least 95.0%.

When choosing the conditions for chromatographic identification, acetonitrile for HPLC produced by Panreac (France) and formic acid Merck (USA) were used.

Statistical processing

All experiments were carried out with three parallel measurements. Quantitative data are presented as the arithmetic mean of three measurements ± standard deviation. Statistical analysis of the experimental data was run by the Kruskal-Wallis test, followed by pairwise comparison by the Mann-Whitney test using the STATISTICA 10.0 software. The significance level for all statistical tests was 5% ($p < 0.05$).

Results and discussion

Losses during ready-to-eat processing of the samples were: 22.0 ± 1.1% when cooked in a microwave oven; 29.0 ± 1.0% when cooking in a convection oven in the "convection" mode with preliminary short roasting and 8.0 ± 1.5% when cooking in a convection oven in the "steam" mode.

Since the fat component of the studied product contains a combination of animal fat and vegetable oils, we analyzed the fatty acid profiles of semi-finished and finished ready-to-eat products cooked in various ways (Table 1).

The fatty acid profile was influenced by both the composition of the product and the type of heat treatment. As can be seen from the above data, the addition of linseed oil instead of a mixture of sunflower and rapeseed significantly changed the ratio of ω-6/ω-3 fatty acids in the experimental semi-finished product by increasing the level of fatty acids of the ω-3 family. The ratio was 1:1.1, which indicates a high nutritional value of the experimental samples and allows considering this meat product as a source of ω-3 fatty acids.

During the heat treatment of samples with various methods until ready, the changes in the distribution of fatty acids was recorded. In quantitative terms, the main saturated fatty acids found in the fat of raw and cooked samples were palmitic acid (C16:0) and stearic acid (C18:0). Heat treatment led to an increase of saturated fatty acids level, both in control and experimental samples. Changes in the quantitative content of ΣSFA were largely caused by an increase in the content of palmitic acid (C16:0). In the steamed test sample, the content of stearic acid did not

[1] ISO/IEC17025:2017 "General requirements for the competence of testing and calibration laboratories". Technical Committee: ISO/CASCO Committee on conformity assessment, 2018. Retrieved from https://www.iso.org/standard/66912.html Accessed December 15, 2022.

[2] GOST R55480–2013 "Meat and meat products. Method for determination of acid value". Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200103311 Accessed December 15, 2022. (In Russian)

[3] GOST 34118–2017 "Meat and meat products. Method for determination of peroxide value". Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/1200146654 Accessed December 15, 2022. (In Russian)

[4] GOST R55483–2013 "Meat and meat products. Determination of fatty acids composition by gas chromatography". Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/1200103852 Accessed December 15, 2022. (In Russian)

Table 1. Fatty acid profiles of raw and cooked poultry cutlets and broccoli

Parameter		Mass fraction, % of the total fatty acids			
		Raw	Microwave	Roasting + "convection"	"Steam cooking" mode
SFA					
Palmitic $C_{16:0}$	contr	10.14 ± 0.16 [a]	11.40 ± 0.19 [b]	15.94 ± 0.17 [c]	12.15 ± 0.18 [d]
	exper	6.27 ± 0.18 [a]	7.96 ± 0.16 [b]	9.70 ± 0.19 [c]	7.04 ± 0.15 [d]
Stearic $C_{18:0}$	contr	3.51 ± 0.14 [a]	3.73 ± 0.07 [b]	5.11 ± 0.14 [c]	3.59 ± 0.04 [a]
	exper	3.08 ± 0.14 [a]	3.39 ± 0.06 [b]	3.29 ± 0.04 [b]	3.01 ± 0.16 [a]
ΣSFA	contr	13.65 ± 0.14 [a]	15.13 ± 0.16 [b]	21.05 ± 0.14 [c]	15.74 ± 0.15 [d]
	exper	9.35 ± 0.13 [a]	11.35 ± 0.12 [b]	12.99 ± 0.13 [c]	10.05 ± 0.14 [d]
MUFA					
Palmitooleic $C_{16:1}$	contr	1.56 ± 0.07 [a]	1.75 ± 0.09 [b]	1.27 ± 0.02 [c]	ND
	exper	ND	ND	ND	ND
Oleic $C_{18:1}$	contr	46.21 ± 0.07 [a]	44.98 ± 0.25 [b]	40.62 ± 0.19 [c]	46.08 ± 0.03 [d]
	exper	40.09 ± 0.26 [a]	36.92 ± 0.18 [b]	35.48 ± 0.23 [c]	38.76 ± 0.27 [d]
Gondoinic $C_{20:1}\omega_9$	contr	0.69 ± 0.09 [a]	0.70 ± 0.07 [a]	0.49 ± 0.08 [b]	1.35 ± 0.19 [c]
	exper	0.62 ± 0.08 [a]	1.25 ± 0.04 [b]	1.09 ± 0.04 [c]	1.17 ± 0.03 [d]
ΣMUFA	contr	48.46 ± 0.09 [a]	47.43 ± 0.21 [b]	42.38 ± 0.16 [c]	47.43 ± 0.15 [b]
	exper	40.71 ± 0.25 [a]	38.17 ± 0.15 [b]	36.57 ± 0.21 [c]	39.93 ± 0.24 [d]
PUFA					
ΣPUFA ω-6	contr	31.84 ± 0.18 [a]	31.29 ± 0.29 [b]	32.04 ± 0.21 [a]	32.07 ± 0.18 [a]
	exper	26.12 ± 0.16 [a]	26.55 ± 0.12 [b]	32.22 ± 0.34 [c]	26.19 ± 0.17 [a]
ΣPUFA ω-3	contr	6.05 ± 0.17 [a]	6.15 ± 0.16 [a]	4.53 ± 0.11 [b]	4.76 ± 0.08 [c]
	exper	23.82 ± 0.21 [a]	23.93 ± 0.17 [a]	18.22 ± 0.17 [b]	23.83 ± 0.06 [a]
ΣPUFA	contr	37.89 ± 0.14 [a]	37.44 ± 0.18 [b]	36.57 ± 0.12 [c]	36.83 ± 0.13 [d]
	exper	49.94 ± 0.19 [a]	50.48 ± 0.18 [a]	50.44 ± 0.27 [a]	50.02 ± 0.27 [a]
ΣPUFA / ΣSFA	contr	2.78 ± 0.03 [a]	2.47 ± 0.05 [b]	1.74 ± 0.03 [c]	2.34 ± 0.04 [d]
	exper	5.34 ± 0.02 [a]	4.45 ± 0.02 [b]	3.88 ± 0.04 [c]	4.98 ± 0.03 [d]
ω-6 / ω-3	contr	5.26 ± 0.03 [a]	5.09 ± 0.02 [b]	7.07 ± 0.03 [c]	6.74 ± 0.02 [d]
	exper	1.10 ± 0.02 [a]	1.11 ± 0.03 [a]	1.77 ± 0.03 [b]	1.10 ± 0.04 [a]

Note: Different letters (a, b, c, d) denote a statistically significant difference between samples cooked by different methods at $p < 0.05$.

change significantly. ΣSFA of microwave-cooked sample increased by 10.8%; in the sample cooked by steam convection oven in the "convection" mode with preliminary roasting — by 54.2%; in the sample cooked a steam convection oven in the "steam" mode — by 15.3%. In the test samples, the increase in the percentage of ΣSFA in the test samples was as follows: in the microwave — 21.4%; in the "convection" mode with preliminary roasting — 38.9%; in the "steaming" mode — 7.5%.

The monounsaturated fatty acid profiles of the control and experimental samples were different and were represented mainly by oleic (C18:1) and gondoic (C20:1) acids before and after cooking.

Heat treatment led to a decrease in monounsaturated fatty acids amount in control and experimental samples for all methods of cooking. The decrease of ΣMUFA percentage in control samples after heat treatment was: microwave cooking — 2.1%; "convection" cooking mode with preliminary roasting — 12.6%; "steaming" cooking mode — 2.1%, in experimental samples — 6.2%, 10.2% and 1.9%, respectively. At the same time, the content of ΣMUFA decreased mainly because of decrease of oleic acid mass fraction, both in control and experimental samples in all methods of cooking.

In our experiment, the heat treatment led to a slight decrease in the amount of polyunsaturated fatty acids in control samples. ΣPUFA in microwave-cooked controls decreased by 1.2%; in the "convection" mode with preliminary roasting — by 3.5%; in the "steam" mode — by 2.8%. In the experimental samples, ΣPUFA did not change significantly in all methods of cooking.

Polyunsaturated fatty acids of the ω-3 group reacted differently to heat treatment in control and experimental samples. Cooking on the "convection" mode with preroasting led to a decrease in the concentration of ΣPUFA ω-3 in the control samples by 25% in the test samples — by 23.5%. When cooking in a steam convection oven in the "steaming" mode, the percentage of ΣPUFA ω-3 in the control sample decreased by 21.3%, while in the experimental sample it did not change significantly. The mass fraction of ΣPUFA of the ω-3 group did not change significantly when cooking the cutlet in a microwave oven, both in the control and in the experimental sample. The use of the "convection" mode with preliminary roasting significantly changed the ratio of ω-6 and ω-3 acids because of a significant decrease in their content in reference to the raw sample and samples cooked by other methods.

The addition of an antioxidant complex allowed ensuring a high level of added ω-3 fatty acids in the experimental sample after heat treatment. At the same time, the greatest safety was ensured with the use of microwave heating and steam cooking. Also, the presence of broccoli in the composition of the product could beneficially affect the high preservation of ω-3 fatty acids, both in the experimental and control samples. Cheng L. J. et al. [24] reported on the ability of various vegetables and, to a greater extent, broccoli to protect fats from oxidation and retained 99% of ω-3 fatty acids (eicosapentaenoic and docosahexaenoic acids) from their initial amount after 4 weeks of their storage at 40 °C in dry oil emulsions by adding the vegetable puree to tuna oil. Gheysen et al. [25] also found that broccoli in the emulsified state was found to be highly effective in protecting of ω-3 fatty acids against the oxidation in microalgae biomass.

Thus, the addition of linseed oil to the semi-finished turkey made it possible to achieve high values of the amount of PUFA by increasing the content of ω-3 fatty acids, improve the ratio of PUFA/SFA, as well as the ratio of ω-6/ω-3 fatty acids, which is one of the most important indicators of the nutritional value of lipids. In the control sample, the ω-6/ω-3 values ranged from 5.09 to 7.07 depending on the method of cooking. The highest value on those acids was observed for the sample cooked in the "convection" mode with preliminary roasting. The values of the ratio ω-6/ω-3 in the test samples were significantly lower than in the control ones, which is associated with the introduction of additional ω-3 fatty acids into the test samples and ranged from 1.1 to 1.77. Cooking in a microwave oven and in a steam convection oven in the "steaming" mode did not significantly affect the ratio ω-6/ω-3 in the test samples, and cooking in the "convection" mode with preliminary roasting led to an increase of this value.

Literature data on studies of the effect of heat treatment on the fatty acid profile of various types of meat and meat products, including poultry meat, are very contradictory.

Similar to our results in the studies of Wereńska M. et al. [26] showed an increase in ∑SFA of fatty acid profile of goose meat exposed to various heat treatment methods, while all considered types of heat treatment caused a significant decrease in the proportion of monounsaturated fatty acids (C16:1), however, in their work they did not consider the method of microwave cooking.

At the same time, studies by Nudda et al. [27] and Maranesi et al. [28] showed that none of the cooking methods affected the concentration of ∑MUFA in lamb samples.

Most studies declare that PUFAs are the most prone to oxidative degradation. Heat treatment reduces their concentration in the finished product; however, on the contrary, there are many studies that do not agree with these results. For example, according to Kouba et al. [29] and Gerber et al. [30] PUFAs play a role of structural lipids in muscle and are less susceptible to changes during cooking, and their proportional increase is expected in meat after heating, as observed in our study.

At the same time, Jiang et al. [31] and Campo et al. [32] also believe that unsaturated fatty acids, especially PUFAs, are less affected by cooking than SFAs; and explain this by the fact that PUFAs are more integrated into the membrane structure, while SFAs are more concentrated in the triglyceride fraction. The proportional change in fatty acid composition can be explained by the loss of fat during cooking, which affects mainly adipose tissue triglycerides with a relatively higher amount of SFA rather than unsaturated fatty acids.

In the studies of A. Nudda et al. the increase in PUFA content in cooked meat was recorded, partly due to an increase in linolenic acid (C18:3ω3) (+25.4%) and mainly due to an increase in the concentration of very long chain PUFA, especially EPA and DHA (+51.0%) [27].

In a study by Campo et al, no significant differences were found in either individual ω-3 fatty acids or ∑PUFA ω-3 in various lamb cooking methods like stewing, grilling, and roasting [32].

The heating processes of pork and lamb meat in the study by Janiszewski et al. [33], did not always lead to statistically significant changes in FA profile, and in many cases the values of the most commonly used indicators of the beneficial properties of fat remained unchanged, for example, the ratio ω-6/ω-3 remained below 4÷5, which corresponds to recommendations of EFSA (2012) and FAO/WHO (2008) [34,35].

Jiang et al. [31] found that grilling did not significantly affect the total content of fatty acid in beef steaks and the levels of most fatty acids, but the level of linolenic acid (C18:3ω3) increased.

Opposite results were obtained by Maranesi et al. In their study, lamb meat microwaved and roasted on a preheated electric grill showed decrease of ∑PUFA ω-3 and ∑PUFA ω-6 [28].

Janiszewski et al [33] found that the grilling process had almost no significant effect on the FA profile in lamb. In both raw and roasted lamb leg a similar ratio of individual acids was found, as well as equal proportional shares of SFA, MUFA and PUFA. At the same time, heat treatment of pork led to significant changes in its fatty acid composition and caused a significant increase in SFA and a decrease in individual PUFAs, especially PUFAs of the ω-3 group — eicosapentaenoic, docosapentaenoic and docosahexaenoic acids.

Campo et al. [32] found no significant difference in fatty acid profile between the raw and grilled lamb. The explanation for these results was based on the short grilling time and the fact that its final temperature was not very high (75 °C).

According to [36], heating reduced the PUFA/SFA ratio in beef, but did not change the ω-6/ω-3 ratio.

Emektar et al [37] showed in their studies that heat treatment of vegetable fats resulted in significant degradation of unsaturated fatty acids, mainly PUFAs. Oleic acid was practically stable in sunflower and soybean oils at all

temperatures, while it decomposed significantly in olive oil under the same conditions. High temperatures and PUFA content increase the rate of oxidation and produce more degradation products, like 2-dienals.

Mitrea et al [38] and colleagues reported that heating of liquid vegetable oils (sunflower, rapeseed and corn) up to 180 °C led to significant changes in the percentage of saturated fatty acids, as well as increased the acid and peroxide numbers, while the percentage of mono- and polyunsaturated fatty acids decreased.

Lipid oxidation

The resistance to fatty component oxidation in the samples, containing various types of vegetable oils was evaluated by changing the acid and peroxide numbers. The risk of the formation of peroxidation products increases along with an increase of unsaturated fatty acids content, while the presence of antioxidants can reduce it [39,40]. The chemical composition of products provided a significant influence on the rate of thermal oxidation of fat, which is explained, in particular, by significant amount of antioxidants in some of them. Thus, the proteins included in the products are capable to provide antioxidant effect; some substances, formed as a result of melanoidin formation, have an oxidation-reducing effect and can interrupt the chain of oxidative transformations.

While comparing the influence of different methods of cooking the enriched semi-finished products on the formation of oxidation products, it was found that no matter what methods of heat treatment in the finished product was used, the level of lipid oxidation products significantly increased in comparison with the parameters of the raw product. Heat treatment in the "convection" mode with pre-roasting showed the lowest oxidative stability of lipids, as evidenced by the higher values of lipid oxidation, both in the control and in the experimental sample compared to the other cooking methods, which was confirmed by the loss of polyunsaturated ω-3 fatty acids. At the same time, cooking in a steam convection oven in the "steaming" mode did not provide a significant effect on the peroxide value and acid value, both in control and experimental samples, and the parameters of samples cooked in a microwave oven took an intermediate position.

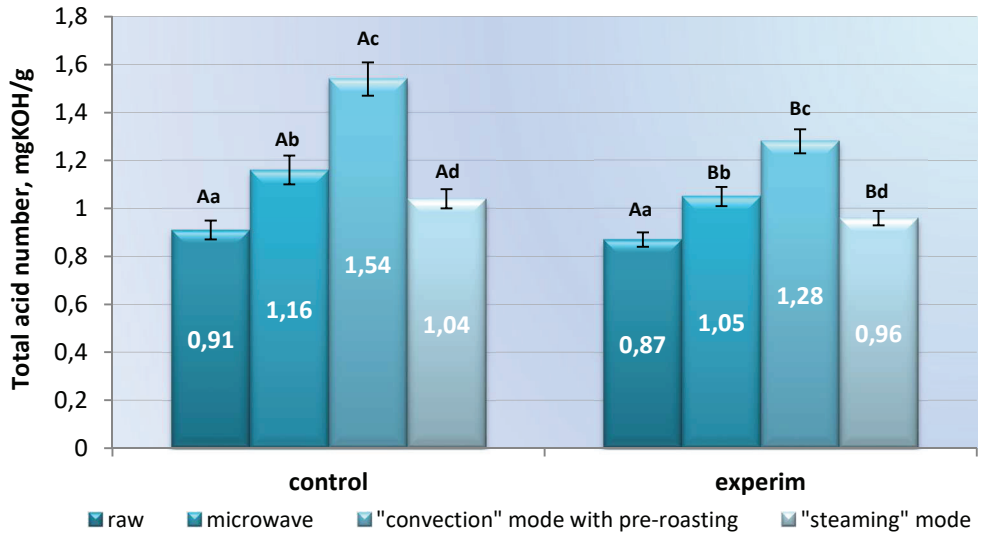
The influence of processing methods on the content of free fatty acids, expressed as a change in acid value, is shown below in the Figure 1.

The parameters of acid value in the cooked test samples in comparison with the raw sample increased: after microwave cooking by 20.7%; when cooking in the "convection" mode with preliminary roasting — by 47.1%; when cooking in a steam convection oven in the "steaming" mode — by 10.3%, while in control samples it increased by 27.5%, 69.2% and 14.3%, respectively.

The different dynamics of changes in acid values in the experimental samples and control samples may be associated with a different amount of included unsaturated fatty acids, which are more susceptible to oxidation, as well as a different content of antioxidant substances.

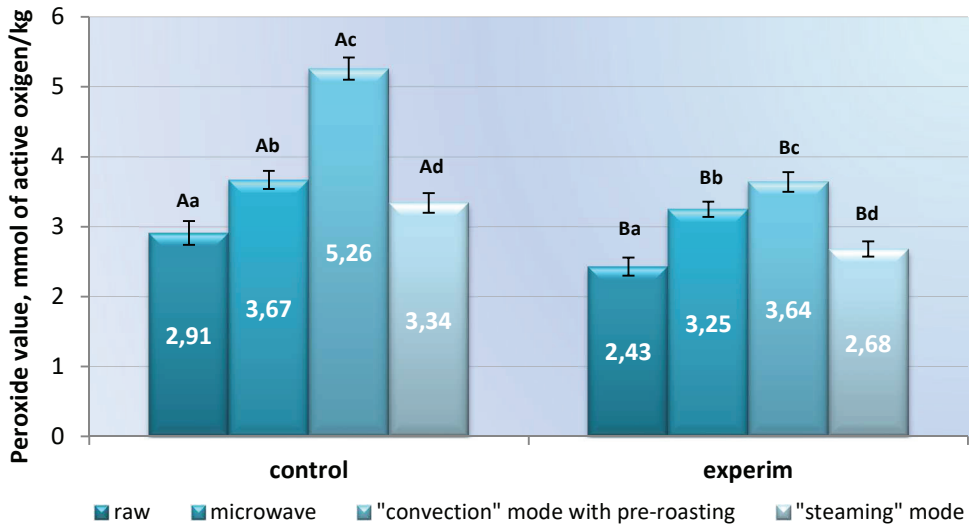
Free fatty acids are considered to be a good indicator of fat lipolysis, either due to the lipase and/or microbial lipase enzymes contained, or due to the effect of oxidation during heat treatment [41,42]. Sobral et al [43] reported that oven and microwave cooking equally increased lipid oxidation in chicken burgers, as evidenced by the loss of free amino acids and ω-6 PUFAs prone to oxidation. At the same time, the addition of oregano to burgers as a natural antioxidant significantly reduced lipid oxidation and maintained the content of ω-6 PUFA [43]. Scientists [44] showed that the cooking method (boiling in water, roasting with fat and without fat) provided a significant influence on the composition of fatty acids in the ready-to-eat products from mallard duck meat. The most favorable ratio ω-6/ω-3 was found in oil-fried products. The least changes in lipid oxidation was recorded in the process of roasting the skin-off products.

The peroxide values of the samples are shown below in the Figure 2.



Note: Different lowercase letters (a, b, c, d) denote a statistically significant difference between the samples cooked in different ways at $p < 0.05$; different capital letters (A, B) denote a statistically significant difference between control samples and experimental samples at $p < 0.05$.

Figure 1. The influence of various heat treatment methods on the acid value of ready-to-eat products



Note: Different lowercase letters (a, b, c, d) mean a statistically significant difference between samples prepared in different ways at $p < 0.05$; different capital letters (A, B) mean a statistically significant difference between control and experimental samples at $p < 0.05$.

Figure 2. The influence of various methods of heat treatment on the peroxide number of the ready-to-eat products

The peroxide values, similar to the acid value in the experimental finished samples, in comparison with the raw ones, increased: when cooked in a microwave oven — by 33.7%; when cooking in the "convection" mode with preliminary roasting — by 49.8%; when cooking in a steam convection oven in the "steaming" mode — by 10.3%, while in control samples it increased by 26.1%, 80% and 14.8%, respectively.

Heterocyclic amines

As the potentially hazardous compounds can accumulate during the technological processing of products, in particular xenobiotics of endogenous origin, i. e. heterocyclic aromatic amines, it is important to study the conditions for their formation during the heat treatment of ready-to-eat products, as well as the choice of a cooking method that minimizes HAA formation.

Table 2. Effect of cooking methods on mutagenic HAA content

Cooking methods		HAA, ng/g of the ready product		ΣHAA, ng/g of the ready product
		MeIQx	PhIP	
Microwave	contr.	2.15 ± 0.86^{a}	5.67 ± 2.27^{a}	7.82 ± 3.13^{a}
	exper	LoQ	$0.72 \pm 0.29^{*a}$	$0.72 \pm 0.29^{*a}$
"Convection" mode with pre-roasting	contr.	3.12 ± 1.25^{a}	8.13 ± 3.25^{a}	11.25 ± 4.5^{a}
	exper	1.73 ± 0.69^{a}	4.32 ± 1.73^{b}	6.05 ± 2.42^{b}
"Steaming" mode	contr.	2.21 ± 0.88^{a}	5.53 ± 2.21^{a}	7.74 ± 3.0^{a}
	exper	$0.2 \pm 0.08^{*b}$	$0.65 \pm 0.26^{*a}$	$0.85 \pm 0.34^{*a}$

Notes: * — statistically significant difference ($p < 0.05$) of the test samples relative to the control; a, b — different letters denote a statistically significant difference ($p < 0.05$) between samples cooked by different methods.

The scientific literature reports that MeIQx occurs more frequently than other HAAs in cooked meat products [45,46]. In our work, MeIQx was found in all the studied samples, except for the experimental sample cooked in a microwave oven. At the same time, the concentration of MeIQx in the test samples was significantly lower than in the control samples for all studied methods of heat treatment: in the "convection" mode with preliminary roasting — by 44.5%; in the steam convection oven in the "steaming" mode — by 91%. Convection cooking with pre-roasting resulted to the highest concentration of MeIQx in the samples compared to other cooking methods.

PhIP was found in all studied samples. Its maximum concentration was observed in the samples prepared in the "convection" mode with preliminary roasting. At the same time, in the experimental samples with the added antioxidant complex, the content of PhIP was significantly lower than in the control ones. The inhibitory effect of the antioxidant complex on PhIP amounted to 87%, 47%, and 88% in poultry cutlets with broccoli cooked in a microwave oven in the "convection" mode with pre-roasting and in a steam convection oven in the "steaming" mode, respectively.

Based on the obtained results, it can be concluded that the antioxidant vitamin complex added into the test samples provided an inhibitory effect on HAA formation, which is confirmed by some foreign studies, according to which the introduction of various antioxidants into the product reduces the level of dangerous HAA formation.

Comparison of various methods of cooking the semi-finished products until their readiness showed that cooking in microwave and by steaming forms less HAA compared to the convection method with pre-roasting.

Our results are consistent with data from Jamali et al., who state that the amount of HAA is directly proportional to losses during the meat cooking [47].

Keşkekoğlu et al [48] in their study of cooked beef and chicken meatballs reported that HAA formation depended on both the type of meat and method of the cooking. At the same time, the addition of a natural antioxidant (e. g. pomegranate seed extract) to minced meat significantly reduced the concentration of HAA in the finished product [48].

The amount of total HAA was significantly reduced in beef cutlets cooked at 200 °C for 3 minutes after the addition of water-soluble vitamins [49]. The decrease of total HAA content in marinated pork cooked for 1 hour was achieved by adding α-tocopherol [50].

The study [51] came to the conclusion that addition of L-ascorbic acid and α-tocopherol reduced mutagenicity in pork sausages [51].

The addition of oregano, as a natural antioxidant, significantly reduced the concentration of HAA at various cooking methods and at temperatures of 175 °C, 195 °C, and 225 °C [52].

Suleman R. et al noted that grilling lamb cutlets led to the higher total HAA than cooking them with both infrared and overheated steam [53,54].

Conclusion

Based on the requirements of the diet composed for the cancer patients, a recipe for ready-to-eat products from turkey has been developed for its inclusion into the complex therapy of patients. The addition of ω-3 fatty acids into the product composition made it possible to change the fatty acid composition towards the increasing of PUFA proportion. In order to enrich the diet of patients and to reduce oxidative processes during heat treatment and storage of the ready products, a complex of vitamins featuring an antioxidant effect was added to the recipe. The study of the influence of various methods of cooking the products until ready-to-eat condition on the composition of fatty acids and their oxidation showed that, depending on the composition of the product and the method of its heat treatment, the ratio of fatty acids and the intensity of oxidative processes varied. The addition of an antioxidant vitamin complex increased the survivability of added ω-3 fatty acids during the heat treatment of the product in a microwave and by steam cooking. Also, gentle heat treatment methods and application of antioxidants cause the formation of HAA in the product to a lesser extent, but it was not possible to completely block their formation. Taking into consideration the obtained results, for the cooking of a meat-based oncoprophylactic product, it is recommended to cook meat with a minimum heat load, i. e. on steam or with a minimum cooking time in a microwave oven. More research is needed for further investigation of the inhibition of HAA formation in ready-to-eat food products due to addition of the antioxidants.

REFERENCES

1. Mayne, S. T., Playdon, M.C., Rock, C.L. (2016). Diet, nutrition, and cancer: past, present and future. *Nature Reviews Clinical Oncology,* 13(8), 504–515. https://doi.org/10.1038/nrclinonc.2016.24
2. Shin, S., Fu, J., Shin, W.K., Huang, D., Min, S., Kang, D. (2023). Association of food groups and dietary pattern with breast cancer risk: A systematic review and meta-analysis. *Clinical Nutrition*, 42(3), 282–297. https://doi.org/10.1016/j.clnu.2023.01.003
3. Campbell, R. A., Li, J., Malone, L., Levy, D. A. (2021). Correlative analysis of vitamin D and omega-3 fatty acid intake in men on active surveillance for prostate cancer. *Urology*, 155, 110–116. https://doi.org/10.1016/j.urology.2021.04.050
4. Aslanova, M.A., Derevitskaya, O.K., Soldatova, N.E., Dydykin, A.S., Bero, A.L. (2020) Medico-biological requirements for food products for the prevention of malignant neoplasms. *Meat Branch*, 8(212), 30–33. https://doi.org/10.33465/2308–2941–2020–08–30–33 (In Russian)
5. Aslanova, M.A., Derevitskaya, O.K., Dydykin, A.S., Bero, A.L., Soldatova, N.E. (2020). Medico-biological requirements for meat-based functional products with regard to the metabolic direction and consumer characteristics. *Vsyo o Myase*, 5S, 40–43. https://doi.org/10.21323/2071–2499–2020–5S-40–43 (In Russian)
6. Clemente, G., Gallo, M., Giorgini, M. (2018). Modalities for assessing the nutritional status in patients with diabetes and cancer. *Diabetes Research and Clinical Practice*, 142, 162–172. https://doi.org/10.1016/j.diabres.2018.05.039
7. Sharafetdinov, Kh. Kh., Kaganov, B.S., Voznyy, E.K., Plotnikova, O.A., Bulygina, T.V. (2014). Nutrition in oncological diseases: tendencies and perspectives. *Nutrition*, 4(2), 60–76. https://doi.org/10.20953/2224–5448–2014–2–60–76 (In Russian)
8. Frolova, Yu.V., Kochetkova, A.A., Sobolev, R.V., Vorobyeva, V.M., Kodentsova, V.M. (2021). Oleogels as prospective nutritional ingredients of lipid nature. *Voprosy Pitaniia*, 90(4), 64–73. https://doi.org/10.33029/0042–8833–2021–90–4–64–73 (In Russian)
9. Cheng, M., Zhang, S., Ning, C., Huo, Q. (2021). Omega-3 Fatty Acids Supplementation Improve Nutritional Status and Inflammatory Response in Patients With Lung Cancer: A Randomized Clinical Trial. *Frontiers Nutrition*, 30(8), Article 686752. https://doi.org/10.3389/fnut.2021.686752
10. Story, M.J. (2021). Zinc, ω-3 polyunsaturated fatty acids and vitamin D: An essential combination for prevention and treatment of cancers. *Biochimie*, 181, 100–122. https://doi.org/10.1016/j.biochi.2020.11.019
11. Williams, D.E., Rumbel, H.P. (2017). Indole-3-Carbinol. Retrieved from https://lpi.oregonstate.edu/mic/dietary-factors/phytochemicals/indole-3-carbinol. Accessed July 30, 2022.
12. Mitsiogianni, M., Amery, T., Franco, R., Zoumpourlis, V., Pappa, A., Panayiotidis, M.I. (2018). From chemo-prevention to epigenetic regulation: The role of isothiocyanates in skin cancer prevention. *Pharmacology and Therapeutics*, 190, 187–201. https://doi.org/10.1016/j.pharmthera.2018.06.001
13. Sita, G., Hrelia, P., Graziosi, A., Morroni, F. (2018). Sulforaphane from cruciferous vegetables: Recent advances to improve glioblastoma treatment. *Nutrients*, 10(11), Article 1755. https://doi.org/10.3390/nu10111755
14. Abbaoui, B., Lucas, C.R., Riedl, K.M., Clinton, S.K., Mortazavi, A. (2018). Cruciferous vegetables, isothiocyanates, and bladder cancer prevention. *Molecular Nutrition and Food Research*, 62(18), Article e1800079. https://doi.org/ 10.1002/mnfr.201800079
15. Aslanova, M.A., Derevitskaya, O.K., Bero, A.L., Soldatova N. E. (2022). Antioxidant activity of functional ready-to-eat products for cancer patients. *Journal of Hygienic Engineering and Design*, 41, 226–231.
16. Wereńska, M., Wołoszyn, J., Okruszek, A., Marcinkowska, W., Haraf G. (2023). The effects of sous-vide, microwave cooking and stewing of goose meat on fatty acid profile and lipid indices. *Poultry Science*, 102(2), Article 102337. https://doi.org/10.1016/j.psj.2022.102337
17. Zhuang, Y., Dong, J., He, X., Wang, J., Li, C., Dong L. et al. (2022). Impact of heating temperature and fatty acid type on the formation of lipid oxidation products during thermal processing. *Frontiers in Nutrition*, 9, Article 913297. https://doi.org/10.3389/fnut.2022.913297
18. Khan, I.A., Khan, A., Zou, Y., Zongshuai, Z., Xu, W., Wang, D. et al. (2022). Heterocyclic amines in cooked meat products, shortcomings during evaluation, factors influencing formation, risk assessment and mitigation strategies. *Meat Science*, 184, Article 108693. https://doi.org/10.1016/j.meatsci.2021.108693
19. Sugimura, T., Wakabayashi, K., Nakagama, H., Nagao, M. (2004). Heterocyclic amines Mutagens/carcinogens produced during cooking of meat and fish. *Cancer Science*, 95(4), 290–299. https://doi.org/10.1111/j.1349–7006.2004.tb03205.x
20. Oz, E., Oz, F. (2022). Mutagenic and/or carcinogenic compounds in meat and meat products: Heterocyclic aromatic amines perspective. *Theory and Practice of Meat Processing*, 7(2), 112–117. https://doi.org/10.21323/2414–438X-2022–7–2–112–117
21. Olalekan Adeyeye, S.A., Ashaolu, T.J. (2021). Heterocyclic amine formation and mitigation in processed meat and meat products: A mini-review. *Journal of Food Protection*, 84(11), 1868–1877. https://doi.org/10.4315/JFP-20–471

22. Lu, F., Kuhnle, G. K., Cheng, Q. (2017). Vegetable oil as fat replacer inhibits formation of heterocyclic amines and polycyclic aromatic hydrocarbons in reduced fat pork cutlets. *Food Control*, 81, 113–125. https://doi.org/10.1016/j.foodcont.2017.05.043
23. Utyanov, D.A., Kulikovskii, A.V., Knyazeva, A.S., Kurzova, A.A., Ivankin, A.N. (2021). Methodical approach for determination of the heterocyclic aromatic amines in meat products using HPLC–MS/MS. *Theory and Practice of Meat Processing,* 6(2), 118–127. https://doi.org/10.21323/2414-438X-2021-6-2-118-127
24. Cheng, L.J., Sanguansri, L., Hlaing, M.M., Singh, T., Shrestha, P., Augustin, M.A. (2022). Use of vegetables for enhancing oxidative stability of omega-3 oils in the powdered state. *Food Chemistry*, 370, Article 131340. https://doi.org/10.1016/j.foodchem.2021.131340
25. Gheysen, L., Durnez, N., Devaere, J., Bernaerts, T., Van Loey, A., De Cooman, L. et al. (2020). Oxidative stability of vegetable purees enriched with n-3-LC-PUFA microalgal biomass: Impact of type of vegetable. *International Journal of Food Science and Technology*, 55(2), 751–759. https://doi.org/10.1111/ijfs.14378
26. Wereńska, M., Haraf, G., Wołoszyn, J., Goluch, Z., Okruszek, A., Teleszko, M. (2021). Fatty acid profile and health lipid indicies of goose meat in relation to various types of heat treatment. *Poultry Science*, 100(8), Article 101237. https://doi.org/10.1016/j.psj.2021.101237
27. Nudda, A., Battacone, G., Boe, R., Manca, M.G., Rassu, S.P.G., Pulina, G. (2013). Influence of outdoor and indoor rearing system of suckling lambs on fatty acid profile and lipid oxidation of raw and cooked meat. *Italian Journal of Animal Science*, 12(4), 459–467. https://doi.org/10.4081/ijas.2013.e74
28. Maranesi, M., Bochicchio, D., Montellato, L., Zaghini, A., Pagliuca, G., Badiani, A. (2005). Effect of microwave cooking or broiling on selected nutrient contents, fatty acid patterns and true retention values in separable lean from lamb rib-loins, with emphasis on conjugated linoleic acid. *Food Chemistry*, 90(1–2), 207–218. https://doi.org/10.1016/j.foodchem.2004.03.043
29. Kouba, M., Benatmane, F., Blochet, J.E., Mourot, J. (2008). Effect of a linseed diet on lipid oxidation, fatty acid composition of muscle, perirenal fat, and raw and cooked rabbit meat. *Meat Science*, 80(3), 829–834. https://doi.org/10.1016/j.meatsci.2008.03.029
30. Gerber, N., Scheeder, M.R.L., Wenk, C. (2009). The influence of cooking and fat trimming on the actual nutrient intake from meat. *Meat Science*, 81(1), 148–154. https://doi.org/10.1016/j.meatsci.2008.07.012
31. Jiang, T., Busboom, J.R., Nelson, M.L., O'Fallon, J., Ringkob, T.P., Joos, D. et al. (2010). Effect of sampling fat location and cooking on fatty acid composition of beef steaks. *Meat Science*, 84(1), 86–92. https://doi.org/10.1016/j.meatsci.2009.08.025
32. Campo, M.M., Muela, E., Olleta, J.L., Moreno, L.A., Santaliestra-Pasias A.M., Mesana, M.I. et al. (2013). Influence of cooking method on the nutrient composition of Spanish light lamb. *Journal of Food Composition and Analysis*, 31(2), 185–190. https://doi.org/10.1016/j.jfca.2013.05.010
33. Janiszewski, P., Grześkowiak, E., Lisiak, D., Borys, B., Borzuta, K., Pospiech, E. et al. (2016). The influence of thermal processing on the fatty acid profile of pork and lamb meat fed diet with increased levels of unsaturated fatty acids. *Meat Science*, 111, 161–167. https://doi.org/10.1016/j.meatsci.2015.09.006
34. EFSA. (2012). Scientific opinion on the tolerable upper intake level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). EFSA panel on dietetic products, nutrition, and allergies (NDA). *EFSA Journal*, 10(7), Article 2815. https://doi.org/10.2903/j.efsa.2012.2815
35. FAO/WHO (November 10–14, 2008). *Interim summary of conclusions and dietary recommendations on total fat & fatty acids*. Joint FAO/WHO Expert Consultation on Fats and Fatty Acids in Human Nutrition, WHO, Geneva.
36. Alfaia, C.M.M., Alves, S.P., Lopes, A.F., Fernandes, M.J.E., Costa, A.S.H., Fontes, C.M. G.A. et al. (2010). Effect of cooking methods on fatty acids, conjugated isomers of linoleic acid and nutritional quality of beef intramuscular fat. *Meat Science*, 84(4), 769–777. https://doi.org/10.1016/j.meatsci.2009.11.014
37. Emektar, K., Kantekin-Erdogan, M.N., Tekin, A. (2022). Furan formation in some vegetable oils during heat treatments. *Food Chemistry*, 386, Article 132744. https://doi.org/10.1016/j.foodchem.2022.132744
38. Mitrea, L., Teleky, B.-E., Leopold, L.-F., Nemes, S.-A., Plamada, D., Dulf, F.V. et al. (2022). The physicochemical properties of five vegetable oils exposed at high temperature for a short-time-interval. *Journal of Food Composition and Analysis,* 106, Article 104305. https://doi.org/10.1016/j.jfca.2021.104305
39. Burri, S.C.M., Ekholm, A., Bleive, U., Püssa, T., Jensen, M., Hellström, J. et al. (2020). Lipid oxidation inhibition capacity of plant extracts and powders in a processed meat model system. *Meat Science*, 162, Article 108033. https://doi.org/10.1016/j.meatsci.2019.108033
40. Peiretti, P.G., Gai, F., Zorzi, M., Aigotti, R., Medana C. (2020). The effect of blueberry pomace on the oxidative stability and cooking properties of pork cutlets during chilled storage. *Journal of Food Processing and Preservation*, 44(7), Article e14520. https://doi.org/10.1111/jfpp.14520
41. Soriano, A., Cruz, B., Gómez, L., Mariscal, C., Ruiz, A.G. (2006). Proteolysis, physicochemical characteristics and free fatty acid composition of dry sausages made with deer (Cervus elaphus) or wild boar (Sus scrofa) meat: A preliminary study. *Food Chemistry*, 96(2), 173–184. https://doi.org/10.1016/j.foodchem.2005.02.019
42. Abdel-Naeem, H.H.S., Sallam, K.I., Zaki, H.M.B.A. (2021). Effect of different cooking methods of rabbit meat on topographical changes, physicochemical characteristics, fatty acids profile, microbial quality and sensory attributes. *Meat Science*, 181, Article 108612. https://doi.org/10.1016/j.meatsci.2021.108612
43. Sobral, M.M.C., Casal, S., Faria, M.A., Cunha, S.C., Ferreira, I.M.P.L.V.O. (2020). Influence of ready-to-eat practices on protein and lipid oxidation of chicken meat burgers during cooking and in vitro gastrointestinal digestion. *Food and Chemical Toxicology*, 141, Article 111401. https://doi.org/10.1016/j.fct.2020.111401
44. Krempa, A., Czerniejewska-Surma, B., Surma, O., Plust, D., Zapletal, P. (2019). Effect of cooking methods on sensory and lipid quality of mallard duck meat. *European Poultry Science*, 83, 1–13. https://doi.org/10.1399/eps.2019.261
45. Zhang, L., Wang, Q., Wang, Z., Chen, Q., Sun, F., Xu, M. et al. (2022). Influence of different ratios of sucrose and green tea leaves on heterocyclic aromatic amine formation and quality characteristics of smoked chicken drumsticks. *Food Control*, 133(Part A), Article 108613. https://doi.org/10.1016/j.foodcont.2021.108613
46. Oz, F., Kotan, G. (2016). Effects of different cooking methods and fat levels on the formation of heterocyclic aromatic amines in various fishes. *Food Control*, 67, 216–224. https://doi.org/10.1016/j.foodcont.2016.03.013
47. Jamali, M.A., Zhang, Y., Teng, H., Li, S., Wang, F., Peng, Z. (2016). Inhibitory effect of rosa rugosa tea extract on the formation of heterocyclic amines in meat cutlets at different temperatures. *Molecules*, 21(2), Article 173. https://doi.org/10.3390/molecules21020173
48. Keşkekoğlu, H., Üren, A. (2014). Inhibitory effects of pomegranate seed extract on the formation of heterocyclic aromatic amines in beef and chicken meatballs after cooking by four different methods. *Meat Science*, 96(4), Article 1446–1451. https://doi.org/10.1016/j.meatsci.2013.12.004
49. Wong, D., Cheng, K.-W., Wang, M. (2012). Inhibition of heterocyclic amine formation by water-soluble vitamins in Maillard reaction model systems and beef cutlets. *Food Chemistry*, 133(3), 760–766. https://doi.org/10.1016/j.foodchem.2012.01.089
50. Lan, C.M., Kao, T.H., Chen, B.H. (2004). Effects of heating time and antioxidants on the formation of heterocyclic amines in marinated foods. *Journal of Chromatography B*, 802(1), 27–37. https://doi.org/10.1016/j.jchromb.2003.09.025
51. Pourazrang, H., Moazzami, A.A., Bazzaz B. S.F. (2002). Inhibition of mutagenic N-nitroso compound formation in sausage samples by using L-ascorbic acid and α-tocopherol. *Meat Science*, 62(4), 479–483. https://doi.org/10.1016/S0309-1740(02)00042-6
52. Khan, I.A., Liu, D., Yao, M., Memon, A., Huang, J., Huang, M. (2019). Inhibitory effect of Chrysanthemum morifolium flower extract on the formation of heterocyclic amines in goat meat cutlets cooked by various cooking methods and temperatures. *Meat Science*, 147, 70–81. https://doi.org/10.1016/j.meatsci.2018.08.028
53. Suleman, R., Wang, Z., Aadil R. M., Hui T., Hopkins D. L., Zhang D. (2020). Effect of cooking on the nutritive quality, sensory properties and safety of lamb meat: Current challenges and future prospects. *Meat Science*, 167, Article 108172. https://doi.org/10.1016/j.meatsci.2020.108172
54. Suleman R., Hui T., Wang Z., Liu H., Zhang D. (2020). Comparative analysis of charcoal grilling, infrared grilling and superheated steam roasting on the colour, textural quality and heterocyclic aromatic amines of lamb cutlets. *International Journal of Food Science and Technology*, 55, 1057–1068. https://doi.org/10.1111/ijfs.14388

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EFFECT OF CHICKEN BONE PASTE ON THE PHYSICO-CHEMICAL AND FUNCTIONAL-TECHNOLOGICAL PROPERTIES OF PÂTÉ MASS

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Keywords: *meat bone paste, pâté mass, technology, chemical composition, nutritional value, fine grinding*

Abstract

The article describes the development of priority directions for improving the economic efficiency of poultry farming, as well as a complex of prospective scientifically substantiated measures that ensure dynamic development of the industry in modern conditions. The technology for obtaining chicken meat-bone paste from chicken bones by fine grinding is described in this article. Experimental samples of pate masses were developed with the addition of chicken meat-bone paste from 5 to 25% instead of poultry meat in the recipe. The influence of the degree of addition of chicken meat-bone paste on the chemical composition, functional-technological and structural-mechanical properties of pâté masses was studied. The addition of chicken meat-bone paste to pâté masses leads to an increase in the ash content from 1.3% in the control sample to 2.74% in the sample with 25% meat-bone paste. With an increase in the amount of meat-bone paste, there is a tendency towards a decrease in the fat content, but the product is enriched with minerals, and its energy value increases. The trend of increasing protein content is observed. Thus, the protein content in the control sample was 16.46%, and with the addition of 25% chicken meat-bone paste, it increased to 17.11%. The water-binding capacity (WBC) index in the experimental samples with the addition of meat-bone paste increased by 11.09% compared to the control sample. The addition of chicken meat-bone paste up to 25% leads to a slight decrease in WBC. Increasing the percentage of replacement of poultry meat with chicken meat-bone paste up to 20% leads to an increase in WHC (from 69.6 to 72.6%). It has been found that the maximum values of functional-technological properties of pâté mass are achieved when adding 20% chicken meat-bone paste, further increase in the content of chicken meat-bone paste leads to the appearance of looseness in the pâté mass and a decrease in the yield during thermal processing.

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Introduction

The meat and meat product market represents a vital segment of the global food market, both in terms of capacity and number of participants. Its leading role is determined not only by the volumes of production and consumption of meat and its processed products but also by their value as the primary source of animal-origin protein. In 2022, meat production worldwide exceeded 340 million tons including 135 million tons of poultry meat. Poultry meat volumes increased from 125 million tons in 2019 to 135 million tons in 2022 [1]. The chicken meat market was the largest segment of the animal-based protein market, accounting for 266.7 billion dollars or 83.5% of the total volume in 2019 [2]. Poultry meat production in Kazakhstan was 282 thousand tons in 2021 and was expected to reach 320 thousand tons in 2022.

Growing consumption of chicken in increasingly diverse and ready-to-eat forms has led to an excess of by-products in recent years. Processing chicken results in a large amount of by-products that can be used as a source of protein [3]. In deep processing of poultry, in addition to the most valuable parts (breasts and thighs), parts with significantly lower muscle tissue content are obtained — carcasses, wings, necks, and others.

During the processing of chicken, a large amount of waste is generated, some of which contain significant amounts of valuable nutrients. One such waste product is chicken bones, which are mainly underutilized or used in a limited capacity in animal feed production [4]. The effective utilization of bones is crucial to prevent environmental pollution. The utilization of bones is one of the mandatory and extremely important tasks for most modern meat pro-

cessing plants. The main reason for its emergence is the constant and active growth of waste from production in the meat industry as a whole. Therefore, the rational and efficient processing of bones becomes an ideal solution to a whole range of problems in the industry [5].

During the mechanical deboning of poultry meat, two products are obtained: meat mass (mechanically separated meat) and bone residue. The meat mass is a finely ground, pasty, viscous mass ranging in color from light pink to dark red (depending on the raw material being processed), with no off-odor. The bone residue is a coarsely ground meat-bone mass ranging in color from light pink to dark red (depending on the type of raw material being processed), with no off-odor [6].

The valuable bio-potential of secondary meat and bone raw materials is determined by their high concentration of biologically active substances, such as collagen, amino acids, fatty acids, calcium, phosphorus, and others. The content of mineralized collagen in this raw material can reach 50% or more of its weight. Therefore, it is expedient to use it in functional food additives of osteotropic and gerodietic direction, which should include low-molecular-weight proteins of collagen origin and minerals [7].

Chicken bones account for about 25% of the total weight of a whole chicken and are an important source of animal protein and a byproduct of chicken processing. Dried chicken bones contain 12.0–35.0% protein, mostly collagen. Chicken bone is high in calcium and is good for bone growth. Chicken bones contain approximately 19% protein, 9% fat, and 15% ash [8, 9]. The elemental composition of bone tissue is characterized by the following data (in %): CaO — 52; MgO — 1.2; P_2O_5–40.3; Na_2O — 1.1, K_2O — 0.2; Cl — 0.1; F — 0.1; CO_2–5.0 [10, 11].

Typically, the use of bones in the food industry is limited due to their coarse and gritty texture, and bones are still primarily used as animal feed or plant fertilizers due to their low cost. To increase the added value of animal bones, some of them are used for intensive processing, including the production of protein hydrolysates from chicken bones [12], and the extraction of gelatin using pre-alkaline treatment [13].

Ultrasound pretreatment is used for enzymatic extraction of protein from chicken bones [14]. In [15], enzymatic hydrolysis is used to obtain collagen from bones of spent hens, and fermentable collagen has wider prospects for the use in functional nutrition and medicine, as well as being an effective way for complete complex utilization of chicken bones. Budnik and Peshuk (2021) developed boiled sausages using 5% to 20% bone paste instead of beef and determined that the optimal amount is 10%, with histological studies revealing fine-grained and homogeneous microstructures with large vacuoles filled with fat [16].

Because of the rapid growth of chicken farming and the need for sustainable and renewable high-quality protein sources, as well as the importance of resource utilization, food security, and environmental principles, the effective utilization of chicken bone waste in food production and processing remain a pressing issue that requires urgent attention. Therefore, the comprehensive and high-value utilization of by-products has attracted increasing attention and become a relevant topic in applied research in recent years. The above makes it possible to assert that the development of theoretical foundations and practical application of deep zero-waste processing of poultry bones and its use in new technologies of functional meat products for enriching them with valuable macro- and microelements and food nutrients are currently relevant issues [17, 18].

The aim of this study is to investigate the effect of adding chicken bone paste on the physicochemical and functional-technological properties of pâté masses.

Materials and Methods

The objects of the research were chicken meat-bone paste obtained by finely grinding chicken bones and pâté masses with the addition of chicken meat-bone paste.

Chicken meat-bone paste production

In the first stage of the research, a technology for obtaining chicken meat-bone paste was developed. The technological scheme for obtaining chicken meat-bone paste is presented in Figure 1.

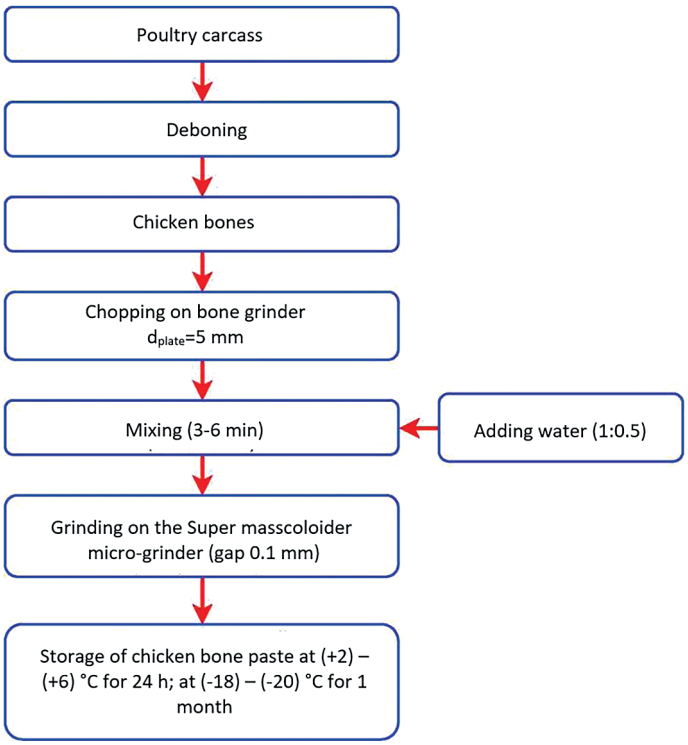


Figure 1. Process flowchart of chicken bone paste production

Samples for the study were bones from the neck, breast, legs, wings, and carcasses of chickens obtained after slaughter. The raw materials were subjected to initial processing at the first stage, except for the neck part. Next, the bones were pre-frozen for 1 hour at a temperature ranging from –18 to –20 °C in freezers. After freezing, the chicken bones were ground using a grinder with a mesh diameter of 5 mm. The samples of ground meat-bone mince were

mixed in a mixer with gradual addition of ice water from 25 to 50% of the mass of the mince. Mixing was carried out for 3 to 6 minutes until the complete binding of water and ground bone mass. The obtained samples of bone mass were further ground using a microgrinder "Supermasscolloider" (Masuko Sangyo Co., Japan) with a gap between the grinding wheels of 0.1 mm. This resulted in the formation of chicken meat-bone paste with a uniform, homogenous consistency.

Development of experimental samples of pâté with chicken meat-bone paste

At the next stage, experimental samples of pâté with the addition of meat-bone paste from 5 to 25% instead of poultry meat were developed (Table 1). As a control sample, we used the production method and recipe of meat pâté, the recipe of which includes poultry meat, beef liver, pork fat, onion, carrot, parsley (dry root), broth, and spices. Experimental samples were developed with the addition of chicken meat and bone paste.

Table 1. Formulation of pâté mass

Ingredient	Control	Experimental samples with the addition of chicken meat and bone paste				
		V1	V2	V3	V4	V5
Chicken meat	60.70	55.70	50.70	45.70	40.70	35.70
Beef liver	17.60	17.60	17.60	17.60	17.60	17.60
Chicken bone paste	0	5	10	15	20	25
Pork fat	5.30	5.30	5.30	5.30	5.30	5.30
Onion	6.30	6.30	6.30	6.30	6.30	6.30
Carrots	5.70	5.70	5.70	5.70	5.70	5.70
Parsley (dry root)	0.60	0.60	0.60	0.60	0.60	0.60
Ground black pepper	0.05	0.05	0.05	0.05	0.05	0.05
Common salt	1.05	1.05	1.05	1.05	1.05	1.05
Broth	2.70	2.70	2.70	2.70	2.70	2.70
Total	100	100	100	100	100	100

The determination of the overall chemical composition was carried out by the method of single sample of the investigated sample. The method involves the sequential determination of the moisture, fat, ash, and protein content in a single sample of the product, using a device for the accelerated determination of moisture and fat content in meat and dairy products.

The moisture content was determined according to ISO 1442:1997[1] by drying a sample of the meat product at 150 °C temperature for 60 min. The fat content was determined according to ISO 1443:1973[2] by using of a Soxhlet apparatus for fat extraction, and the calculation of fat content based on the weight of the extracted fat and the weight of the initial sample. The ash content was determined according to ISO 936:1998[3] by weighing the analyzed sample before and after incineration to determine the weight of the ash. The protein content was determined by calculation.

The determination of the water binding capacity was based on the extraction of moisture by the test sample under light pressure, sorption of the released water by filter paper, and determination of the amount of separated moisture by the size of the spot it left on the filter paper.

To determine the water-holding (WHS) and fat-holding capacities (FHC), the standard method was used using a milk butyrometer. The evaluation of the water-holding capacity is based on the difference between the moisture content in the meat mixture and the amount of moisture released during thermal processing. The fat-holding capacity of the meat mixture is determined as the difference between the fat content in the sample and the amount of fat released during thermal processing.

Water-holding capacity (*WHC*, %) was determined by the formula:

$$WHC = W - MGC, \quad (1)$$

moisture generating capacity (*MGC*, %)

$$MGC = a \cdot n \cdot m^{-1} \cdot 100, \quad (2)$$

where

W — total mass fraction of moisture in the sample,%;
a — butyrometer division value; *a* = 0,01 cm³;
n — number of divisions;
m — sample weight, g.

Fat-holding capacity (*FHC*, %) was determined by the formula:

$$FHC = g_1 \cdot g_2^{-1} \cdot 100, \quad (3)$$

where

g_1 — mass fraction of fat in the sample after heat treatment, %;
g_2 — the same before heat treatment, %.

Mass fraction of fat in the sample (*g*, %)

$$g = [10^4 \cdot \alpha \cdot (n_1 - n_2) \cdot m_1]/m_2, \quad (4)$$

where

α — coefficient characterizing such a fat content in the solvent, which changes the refractive index by 0.0001%;
n_1 — refractive index of a pure solvent;
n_2 — refractive index of the test solution;
m_1 — weight 4,3 cm³
α — monobromonaphthalene, g;
m_2 — sample weight, g.

The active acidity (pH) of the medium was determined according to ST RK ISO 2917–2009[4] using a pH-150MI device (LLC "Izmeritelnaya Tekhnika", Russia), by immersing two electrodes into a solution and fixing the pH value on the scale of the device. The solution (aqueous extract) was prepared from the crushed product with water (in a ratio

[1] ISO 1442:1997 "Meat and meat products — Determination of moisture content" Technical Committee: ISO/TC34/SC6 Meat, poultry, fish, eggs and their products, 1997.

[2] ISO 1443:1973 "Meat and meat products — Determination of total fat content" Technical Committee: ISO/TC34/SC6 Meat, poultry, fish, eggs and their products, 1973.

[3] ISO 936:1998 "Meat and meat products — Determination of total ash" Technical Committee: ISO/TC34/SC6 Meat, poultry, fish, eggs and their products, 1998.

[4] ST RK ISO 2917–2009. "Meat and meat products. pH determination. Control method". Astana: State Standard of the Republic of Kazakhstan, 2010. — 16 p.

of 1:10). The pH was measured after 30 minutes of standing at a temperature of 20 °C.

Statistical analysis

Statistical analysis was performed using Statistica 12.0 (STATISTICA, 2014; StatSoft Inc., Tulsa, OK, USA). After checking normal distribution and variance homogeneity (Shapiro–Wilk), the differences between samples were evaluated using oneway ANOVA. The Tukey HSD test was used for means comparisons. Differences were considered to be statistically significant at $p \leq 0.05$. Data are presented as mean values ± standard deviation (SD).

Results and discussion

At the subsequent stage, the chemical composition of pâté masses with the addition of meat-bone paste ranging from 5 to 25% in the formulation was investigated. The results are presented in Table 2.

Table 2 shows that the addition of chicken meat-bone paste to the composition of pâté masses leads to an increase in the ash content from 1.3% in the control sample to 2.74% in the sample with 25% meat-bone paste. A slight decrease in the moisture content was noted in the samples.

Increasing the amount of meat-bone paste in the formulation of pâté masses tends to decrease the mass fraction of fat, but also results in an enrichment of the product with mineral substances and an increase in its energy value.

In [19], it was noted that a decrease in the fat content was observed in meat sausages with the addition of mechanically deboned poultry meat.

Other authors have found that the addition of mechanically deboned poultry meat in an amount of 20% to the formulation of sausages leads to a decrease in the moisture content, while the addition of cooked chicken skin did not affect the moisture content, but increased the ash and protein content [20].

In [21], it was noted that increasing the content of mechanically deboned poultry meat in meat sausages leads to a product with the higher moisture content and lower protein content.

There is a tendency towards an increase in the protein content. For instance, the protein content in the control sample was 16.46%, while with the addition of 25% chicken meat-bone paste, it increased to 17.11%.

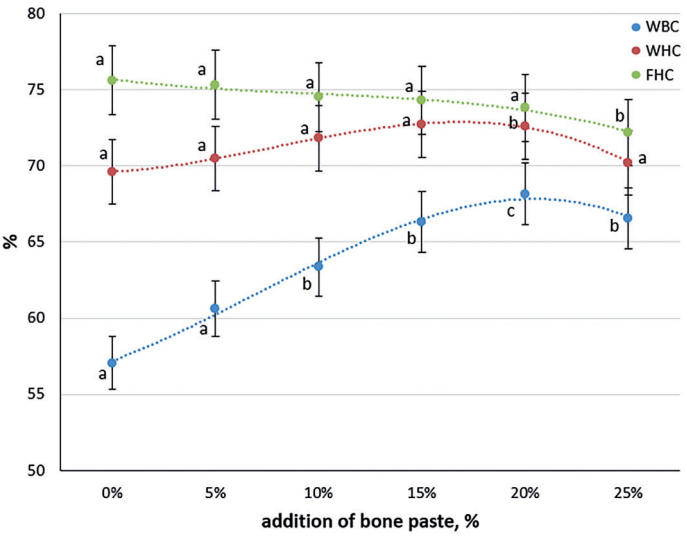
The results obtained indicate that the introduction of meat-bone paste into the recipe instead of meat improves the content of total protein, which influences its amino acid composition, and hence the level of biological value.

Chemical composition analysis showed that pâté mass with meat-bone paste has a sufficiently high content of protein and fat, and its main feature is a high content of mineral substances. Thus, the addition of meat-bone paste to the recipe of meat pâté leads to an increase in the protein and ash content, and a decrease in the fat content.

Changes in functional and technological properties and pH of pâté masses with the addition of chicken meat-bone paste

The obtained pâté masses were evaluated for their functional and technological properties, specifically, their water-binding capacity (WBC), water- and fat-holding capacities (WHC and FHC, respectively), and pH were determined. The functional and technological properties were analyzed to establish the dependencies of WBC, WHC, FHC, pH on the amount of added chicken meat-bone paste.

The analysis of the data for the water-binding capacity (WBC) (Figure 2) shows that this parameter increased by 11.09% (from 57.06 to 68.15%) in the experimental samples with the addition of chicken bone paste compared to the control sample. It was found that adding up to 25% of chicken bone paste leads to a slight decrease in WBC. A low water-binding capacity affects the loss of moisture and soluble substances during heat treatment.



[a–f] means with different uppercase letters differing significantly among different samples of pâtés ($p < 0.05$)

Figure 2. Variation of WBC, WHC and FHC of pâté mass depending on the addition of chicken bone paste (n, %)

Table 2. Chemical composition of pâté masses

Parameter	Control	Experimental samples with the addition of chicken meat and bone paste				
		V1	V2	V3	V4	V5
Moisture, %	62.54 ± 0.81[a]	62.43 ± 0.82[a]	62.34 ± 0.71[a]	62.26 ± 0.88[a]	62.14 ± 0.83[a]	62.01 ± 0.86[a]
Protein, %	16.46 ± 0.21[a]	16.59 ± 0.16[a]	16.74 ± 0.18[a]	16.86 ± 0.20[a]	16.98 ± 0.19[a]	17.11 ± 0.18[a]
Fat,%	19.7 ± 0.06[b]	19.40 ± 0.04[b]	19.13 ± 0.06[b]	18.79 ± 0.05[b]	18.48 ± 0.07[a]	18.14 ± 0.05[a]
Ash, %	1.3 ± 0.19[a]	1.58 ± 0.23[b]	1.79 ± 0.13[c]	2.09 ± 0.18[d]	2.40 ± 0.14[e]	2.74 ± 0.17[f]
Energy value, kcal/100g	243.14	240.96	239.13	236.55	234.24	231.7

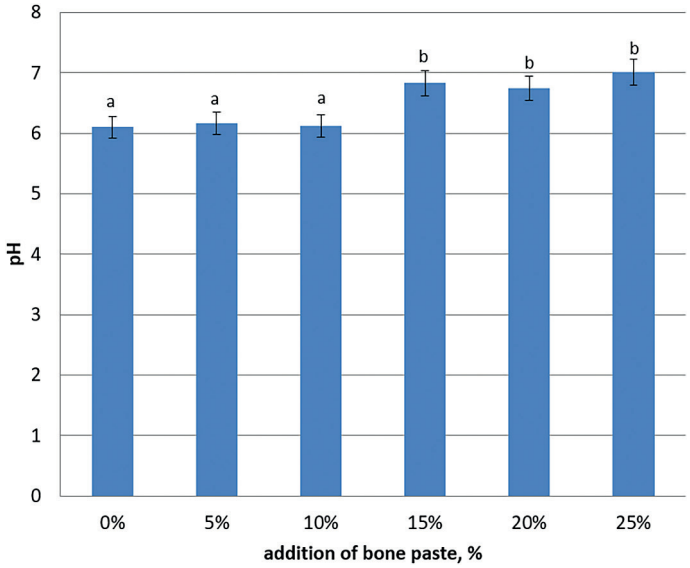
[a–f] means within the same row with different uppercase letters differing significantly among different samples of pâtés ($p < 0.05$)

Increasing the percentage of poultry meat substitution with chicken bone paste up to 20% leads to an increase in the water holding capacity (WHC) from 69.6 to 72.6%. However, adding more than 20% results in a decrease to 70.2%. The improvement in both water-holding and binding capacity can be attributed to the increase in proteins (collagen) in the pâté mixture, which is capable of swelling and has good water-holding properties. Collagen fibrils mainly undergo swelling and softening due to the presence of free water. The increased water content in collagen reduces the temperature required for its coagulation, thereby promoting water retention in the pâté mixture and improving its texture [22,23].

The value of the fat-holding capacity (FHC) decreases with the addition of chicken meat and bone paste from 75.6% in the control sample to 72.2% when 25% is added. The addition of chicken meat-bone paste does not lead to a significant decrease in the fat-binding capacity, as collagen fibers weakly retain fat components in the interprotein spaces.

Based on the conducted research, it has been established that the maximum values of the functional and technological properties of the pâté mass are achieved when adding 20% chicken meat bone paste. Further increase in the content of chicken meat bone paste leads to the appearance of looseness in the pâté mass and a decrease in the yield during thermal processing. Thus, the most recommended option is to add 20% chicken meat bone paste instead of poultry meat in the recipe of meat pâté.

There was no significant difference in the pH value between the experimental and control samples. Although the pH value of the experimental sample was slightly shifted towards alkaline (Figure 3).



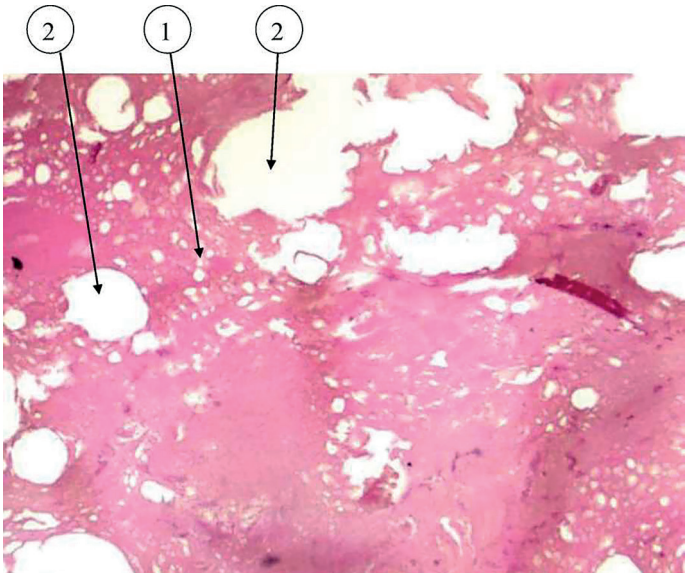
[a–f] means with different uppercase letters differing significantly among different samples of pâtés ($p < 0.05$)

Figure 3. Variation of pH of pâté mass depending on the addition of chicken bone paste (n, %)

The addition of mechanically deboned chicken hydrolyzate to the sausage recipe lowers the pH. The results indicate that hydrolysates of mechanically deboned chicken meat can potentially improve the physicochemical properties of sausages in meat production [24].

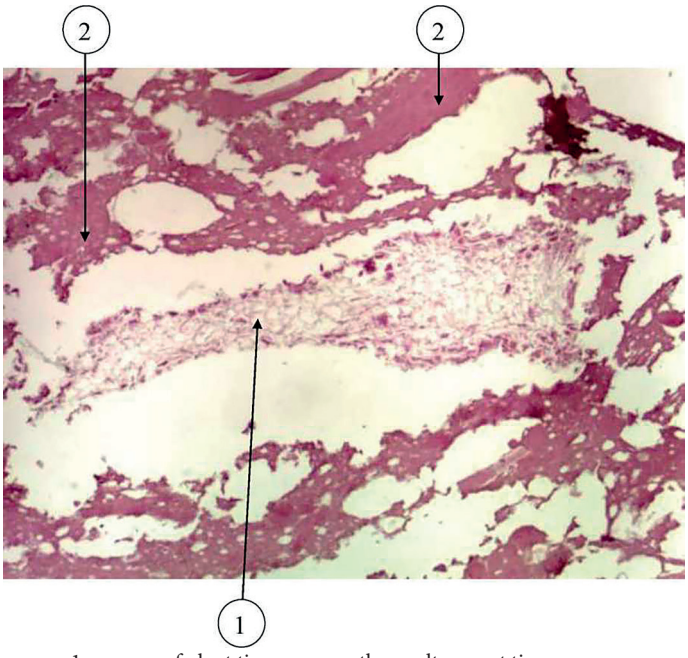
The impact of replacing poultry meat with chicken meat and bone paste on the physico-chemical and functional-technological properties of pâté masses was studied, and an optimal level of 20% chicken meat and bone paste addition was determined.

Pâtés are characterized by fine raw material grinding, which affects the product's consistency. Histological studies were conducted to determine the degree of influence of the introduced chicken meat and bone paste on the recipe of meat pâtés. Figures 4 and 5 depict control samples of meat pâté.



1 — with small vacuoles, 2 — with large vacuoles. Xe × 400.

Figure 4. Microstructure of control pâté mass



1 — areas of plant tissue among the poultry meat tissue mass,
2 — individual fragments of poultry meat muscle fibers and a structureless mass with many vacuoles of different sizes. X ×400.

Figure 5. Microscopic structure of the control sample

Microscopic examination of the control sample of meat pâté did not reveal any fragments of dense connective tissue, but showed masses consisting of fragments of muscle fibers, epithelial cells of the liver with sparsely

located nuclear elements, and elements of loose connective tissue. In addition, there were particles of plant tissue with preserved nuclear elements among the tissues of the poultry meat, and particles of plant tissue with a characteristic histological structure and pronounced nuclear elements on the periphery of the plant ingredient fragment in the pâté. The structure was fairly homogeneous, with some areas being less dense due to the presence of a large number of vacuoles, and other areas being denser, representing homogeneous masses of pâté with inclusions of small vacuoles.

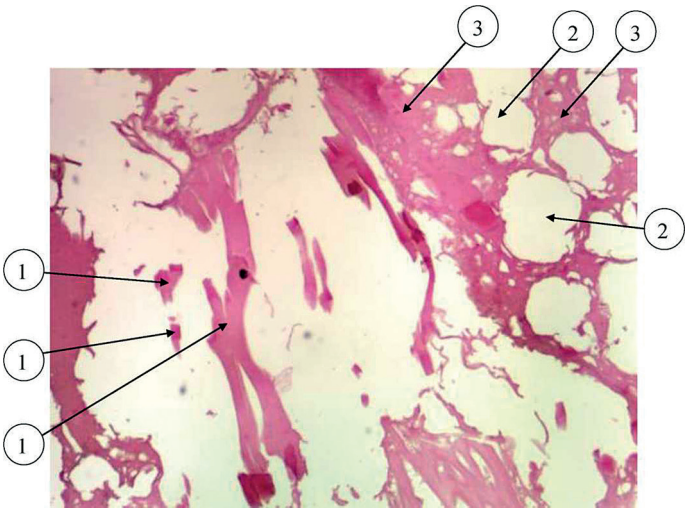
The experimental meat pâté sample contains cross-sectioned muscle fibers and vacuoles in the finely structured mass of the pâté. Microscopic examination of the pâté structure reveals predominantly small particles of various tissues of the poultry meat (small fragments of muscle fibers, fibers of loose connective tissue, and particles of dense connective tissue) and plant-origin tissues. There are also densely packed particles of poultry meat tissues with vacuoles and inclusions of plant tissue particles. The sample contains pieces formed by fat cells. The particle distribution in the experimental sample is relatively uniform throughout the product volume compared to the control sample (Figures 6 and 7). Unlike the control pâté sample, the experimental pâté sample contains inclusions of dense connective tissue upon microscopic examination of the microstructure.

Gezgin et al. [25] developed a technology and recipe for sausages with the addition of mechanically deboned poultry meat. In samples of thermally processed chicken sausage, a higher amount of bone and cartilage tissue was detected during histological analysis.

Nagdalian et al. [26] conducted a quantitative analysis of the microstructure characteristics of sausage with added mechanically separated poultry meat, and determined the characteristics of bone and cartilage inclusions in the samples. Multiple round voids with a diameter of 50–150 μm can be observed in the microphotographs of all samples. Botka-Petrak et al. [27] analyzed mechanically deboned meat from broiler chickens and found that connective and muscle tissue were the main components, while diffusely scattered cartilage tissue was low in content. Cartilage and a small amount of bone tissue were observed in the samples.

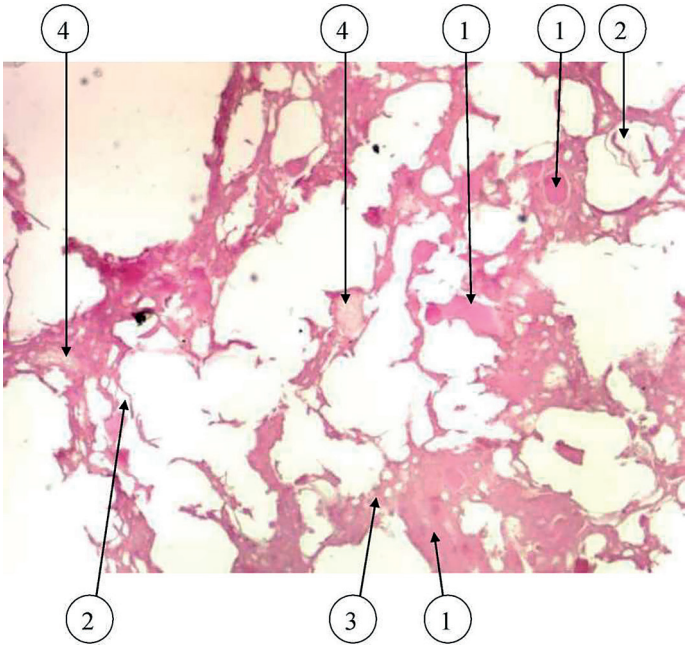
Antipova et al. [28] have developed a method for the rational use of the bone remains of poultry. The resulting mass of the bone residue consists of pieces of bone up to 3 cm in size and a small amount of fleshy tissues (about 5%). Histomorphological studies confirm the presence of a strengthened structure — bone tissue. It has been established that the mass fraction of protein in the bone residue is 25%, fat — 18.9% by weight of the raw material.

Therefore, the structure of the experimental sample with the addition of chicken meat and bone paste differs from the control sample in the presence of dense connective tissue inclusions and particles of bone tissue.



1 — transversely longitudinally dissected muscle fibers, 2 — vacuoles, 3 — homogeneous pate mass. X × 400

Figure 6. Microscopic structure of the experimental sample of pâté mass



1 — small fragments of muscle fibers, 2 — connective tissue fibers, 3 — particles of bone tissue, 4 — tissue of plant origin. X × 400

Figure 7. Microscopic structure of the experimental sample of pâté mass

Conclusion

Thus, as a result of the conducted experimental studies, a comprehensive processing of chicken meat-bone raw materials into fine-dispersed meat-bone paste has been proposed and the effect of its addition level (from 5% to 25%) on the physico-chemical, functional-technological properties, and microstructure of pâté masses has been studied. The nutritional value, functional-technological and structural-mechanical properties, pH, microscopic structure of pâté masses were determined, and the possibility of using chicken meat-bone paste instead of the main raw material in the production of meat pâtés was demonstrated. As a result of the studies, it was established that the maximum values of functional-technological properties of the pâté mass are achieved with the addition of 20% chicken meat-

bone paste. Further increase in the content of chicken meat-bone paste leads to the appearance of looseness in the pâté mass and a decrease in the output during thermal processing. The most recommended approach is to add 20% chicken meat-bone paste as a replacement for chicken meat in the recipe for meat pâté. A comparative analysis of the chemical composition showed that the addition of chicken meat-bone paste increases the ash and protein content of the pâté mass while decreasing the fat and moisture. Histological analysis revealed that the experimental meat pâté sample had transversely sectioned muscle fibers and vacuoles in the fine structure of the pâté mass. In contrast to the control pâté sample, the experimental pâté sample showed inclusions of dense connective tissue upon microstructural examination. Based on the conducted research and obtained results, the chicken meat-bone paste is a viable option for the use in the recipe of meat products as a replacement for the primary raw material.

REFERENCES

1. Production of meat worldwide from 2016 to 2022, by type. Retrieved from https://www.statista.com/statistics/237632/production-of-meat-worldwide-since-1990/ Accessed February 25, 2023
2. Ivanchenko, A.V. (2021). Forecast of production in the poultry meat market. *Services in Russia and Abroad*, 15(2(94)), 121–131. https://doi.org/10.24412/1995-042X-2021-2-121-131 (In Russian)
3. Ananey-Obiri, D., Matthews, L., Tahergorabi, R. (2020). Chicken processing by-product: A source of protein for fat uptake reduction in deep-fried chicken. *Food Hydrocolloids*, 101, Article 105500. https://doi.org/10.1016/j.foodhyd.2019.105500
4. Bee, S.-L., Mariatti, M., Ahmad, N., Yahaya, B.H., Hamid, Z.A. (2019). Effect of the calcination temperature on the properties of natural hydroxyapatite derived from chicken bone wastes. *Materials Today: Proceedings*, 16(Part 4), 1876–1885. https://doi.org/10.1016/j.matpr.2019.06.064
5. Londoño-Zapata, L., Franco-Cardona, S., Restrepo-Manotas, S., Gomez-Narvaez, F., Suarez-Restrepo, L., Nuñez-Andrade, H. et al. (2022). Valorization of the by-products of poultry industry (bones) by enzymatic hydrolysis and glycation to obtain antioxidants compounds. *Waste and Biomass Valorization*, 13, 4469–4480. https://doi.org/10.1007/s12649-022-01801-1
6. Trindade, M.A., de Felício, P.E., Castillo, C.J.C. (2004). Mechanically separated meat of broiler breeder and white layer spent hens. *Scientia Agricola*, 61(2), 234–239. https://doi.org/10.1590/S0103-90162004000200018
7. Mezenova, N. Yu., Agafonova, S.V., Mezenova, O. Ya., Baidalinova, L.S., Volkov, V.V., Shenderyuk, V.I. et al. (2020). The process of modifying cattle meat and bone raw materials by high-temperature hydrolysis. *Processes and Food Production Equipment*, 1(43), 18–26. https://doi.org/10.17586/2310-1164-2020-10-1-18-26 (In Russian)
8. Yessimbekov, Z., Kakimov, A., Caporaso, N., Suychinov, A., Kabdylzhar, B., Shariati, M.A. et al. (2021). Use of meat-bone paste to develop calcium-enriched liver pâté. *Foods*, 10(9), Article 2042. https://doi.org/10.3390/foods10092042
9. Wang, X., Shen, Q., Zhang, C., Jia, W., Han, L., Yu, Q. (2019). Chicken leg bone as a source of chondroitin sulfate. *Carbohydrate polymers*, 207, 191–199. https://doi.org/10.1016/j.carbpol.2018.11.086
10. Cansu, Ü., Boran, G. (2015). Optimization of a multi-step procedure for isolation of chicken bone collagen. *Korean Journal for Food Science of Animal Resources*, 35(4), 431–440. https://doi.org/10.5851/kosfa.2015.35.4.431
11. Kabdylzhar, B.K., Kakimov, A.K., Gurinovich, G.V., Suychinov, A.K. (2022). Research of compositions of amino acids, fatty acids and minerals in meat pate with addition of meat-and-bone paste. *Theory and Practice of Meat Processing*, 7(1), 66–72. https://doi.org/10.21323/2414-438X-2022-7-1-66-72
12. de Souza Cunha, R.C., de Carvalho, L.M., de Sousa Fontes, V.M., de Sousa Galvão, M., Olegário, L.S., de Medeiros, L.L. et al. (2023). Volatilomic evaluation of protein hydrolysates from free-range chicken bones treated with hot-pressure process. *LWT*, 174, Article 114368. https://doi.org/10.1016/j.lwt.2022.114368
13. Erge, A., Zorba, Ö. (2018). Optimization of gelatin extraction from chicken mechanically deboned meat residue using alkaline pre-treatment. *LWT*, 97, 205–212. https://doi.org/10.1016/j.lwt.2018.06.057
14. Dong, Z.Y., Li, M.Y., Tian, G., Zhang, T.H., Ren, H., Quek, S.Y. (2019). Effects of ultrasonic pretreatment on the structure and functionality of chicken bone protein prepared by enzymatic method. *Food Chemistry*, 299, Article 125103. https://doi.org/10.1016/j.foodchem.2019.125103
15. Cao, C., Wang, H., Zhang, J., Kan, H., Liu, Y., Guo, L. et al. (2023). Effects of extraction methods on the characteristics, physicochemical properties and sensory quality of collagen from spent-hens bones. *Foods*, 12(1), Article 202. https://doi.org/10.3390/foods12010202
16. Peshuk, L.V., Budnik, N.V., Halenko, O.O. (2011). Gerodietic meat products technology enriched with calcium and phosphorus *Food and Environment Safety*, 10(4), 18–23.
17. Fu, Y., Therkildsen, M., Aluko, R.E., Lametsch, R. (2019). Exploration of collagen recovered from animal by-products as a precursor of bioactive peptides: Successes and challenges. *Critical Reviews in Food Science and Nutrition*, 59(13), 2011–2027. https://doi.org/10.1080/10408398.2018.1436038
18. Losso, J.N., Ogawa, M. (2014). Thermal stability of chicken keel bone collagen. *Journal of Food Biochemistry*, 38(3), 345–351. https://doi.org/10.1111/jfbc.12059
19. Cortez-Vega, W.R., Fonseca, G.G., Feisther, V.A., Silva, T.F., Prentice, C. (2013). Evaluation of frankfurters obtained from croaker (Micropogonias furnieri) surimi and mechanically deboned chicken meat surimi-like material. *CyTA-Journal of Food*, 11(1), 27–36. https://doi.org/10.1080/19476337.2012.680199
20. Babji, A.S., Chin, S.Y., Seri Chempaka, M.Y., Alina, A.R. (1998). Quality of mechanically deboned chicken meat frankfurter incorporated with chicken skin. *International Journal of Food Sciences and Nutrition*, 49(5), 319–326. https://doi.org/10.3109/09637489809089405
21. Daros, F.G., Masson, M.L., Amico, S.C. (2005). The influence of the addition of mechanically deboned poultry meat on the rheological properties of sausage. *Journal of Food Engineering*, 68(2), 185–189. https://doi.org/10.1016/j.jfoodeng.2004.05.030
22. Oechsle, A.M., Akgün, D., Krause, F., Maier, C., Gibis, M., Kohlus, R. et al. 2016). Microstructure and physical–chemical properties of chicken collagen. *Food Structure*, 7, 29–37. https://doi.org/10.1016/j.foostr.2016.02.001
23. Munasinghe, K.A., Schwarz, J.G., Whittiker, M. (2015). Utilization of chicken by-products to form collagen films. *Journal of Food Processing*, 2015, Article 247013. https://doi.org/10.1155/2015/247013
24. Jin, S.K., Choi, J.S., Choi, Y.J., Lee, S.J., Lee, S.Y., Hur, S.J., 2015. Development of sausages containing mechanically deboned chicken meat hydrolysates. *Journal of Food Science*, 80(7), S1563-S1567. https://doi.org/10.1111/1750-3841.12920
25. Gezgin, T., Karaca, S., Atalay, M., Sinan, B., Erdem, T. (2022). Investigation of poultry meat products containing mechanically deboned meat by histological and chemical methods. *Gıda ve Yem Bilimi Teknolojisi Dergisi / Journal of Food and Feed Science — Technology*, 28, 57–64. (In Turkish)
26. Nagdalian, A.A., Rzhepakovsky, I.V., Siddiqui, S.A., Piskov, S.I., Oboturova, N.P., Timchenko, L.D. et al. (2021). Analysis of the content of mechanically separated poultry meat in sausage using computing microtomography. *Journal of Food Composition and Analysis*, 100, Article 103918. https://doi.org/10.1016/j.jfca.2021.103918
27. Botka-Petrak, K., Hraste, A., Lucić, H., Gottstein, Ž., Gomerčić, M. Đ., Jakšić, S. et al. (2011). Histological and chemical characteristics of mechanically deboned meat of broiler chickens. *Veterinarski Arhiv*, 81(2), 273–283.
28. Antipova, L.V., Polyanskikh, S.V., Orekhov, O.G., Sulina, Yu.A. (2013). Substantiation applied aspects of bird bone balance rational using. *Proceedings of the Voronezh State University of Engineering Technologies*, 1(55), 109–113. (In Russian)

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ANALYSIS OF AGRICULTURE SUSTAINABLE DEVELOPMENT IN RUSSIA

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Keywords: *analysis, sustainable development, sustainable development goals, agriculture, food security*

Abstract
The article is focused on determining the current situation in Russia on the way to achieving the goals of sustainable development in agriculture. When analyzing the literature, it was revealed that most of the goals and objectives of sustainable development are already, to a greater or lesser degree, incorporated into the main strategic and program documents in Russia. Achievements in the implementation of SDG 2 "End hunger, achieve food security and improved nutrition and promote sustainable agriculture" were considered. Statistical indicators of agriculture sustainable development in Russia are analyzed. The principles for development of the agrifood systems sustainability concept are formulated and presented. In the last century and a half, the main task of Russia in country's food security has been to feed its population. At the present day, it can be stated that this problem has been mainly solved.

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Introduction

On September 25, 2015, the UN General Assembly adopted the 2030 Agenda for Sustainable Development [1], which includes 17 goals aimed at eradicating poverty, conserving the planet's resources and ensuring prosperity for all (Figure 1) [2]. It is important to note that this agenda is universal and concerns all countries [3]. In the same year, the UN Statistical Commission established the Inter-Agency and Expert Group on Sustainable Development Goal Indicators [4]. The group included representatives of 28 national statistical agencies, including Russian one [5].

The 2030 Agenda for Sustainable Development [1] was designed to improve the lives and future of all people around the world [3]. Due to this, today the world community has not only the peacekeeping resolutions adopted by the General Assembly and the Security Council, but

Figure 1. The sustainable development goals [2]

also roadmaps. In fact, sustainable and inclusive development is not only an independent goal, but also the best tool available to the international community to prevent various problems [6–8].

Achieving the sustainable development goals requires the joint efforts of governments, private businesses [9,10], civil society and people of the Earth [11,12].

The sustainable development goals (SDGs) are increasingly integrated into the policies of modern states [13]. And the Russian Federation is no exception. This is expressed both in the inclusion of individual goals and objectives of sustainable development, as well as individual indicators reflecting the degree of their achievement, in the country's strategic and program documents, and in the formation of a comprehensive system for statistical accounting of the indicators. It is important to note that most of the goals and objectives of sustainable development are already, to a greater or lesser degree, incorporated into the main strategic and program documents adopted in Russia. The participation of civil society, business, non-governmental organizations, volunteers and scientific community is of great importance for achieving sustainable development goals [14].

The UN General Assembly recommended that countries should create their own national sets of indicators. Taking into account the national features and tasks defined in the strategic documents of the Government of the Russian Federation, in 2020 a list of national SDG indicators was approved, which initially included 160 indicators. At the same time, it was decided that the national list of SDG indicators should be a flexible tool for tracking progress in achieving the goals. In 2022, the national list of SDG indicators has been updated to include 175 indicators [15]. It is designed to monitor the achievement of sustainable development goals at the national level [15]. The list reflects national features and takes into account the tasks defined in the Decree of the President of the Russian Federation dated May 7, 2018, No. 204 "On the national goals and strategic objectives of the development of the Russian Federation for the period up to 2024"[1], strategic documents of the Government of the Russian Federation, as well as national and federal projects.

The Russian Federation is actively working to monitor the indicators of achieving the sustainable development goals. A review panel on information and statistical support for SDG monitoring has been created. A number of laws and doctrines have also been adopted at the legislative level [14]. Taking into account the importance of achieving the sustainable development goals, the article presents the analysis of statistical indicators for agriculture sustainable development in Russia.

[1] On the national goals and strategic objectives of the development of the Russian Federation for the period up to 2024 (Decree of the President of the Russian Federation dated May 7, 2018, No. 204). Retrieved from https://docs.cntd.ru/document/557309575. Accessed March 2, 2023. (In Russian).

Materials and methods

A review study method was used for analytical study of the databases: elibrary, Elsevier, Scopus, Russian Federal State Statistics Service, the Central Bank of the Russian Federation, the Ministry of Internal Affairs of the Russian Federation, the Ministry of Health of the Russian Federation, the Ministry of Natural Resources of the Russian Federation, the Ministry of Education of the Russian Federation, the Ministry of Agriculture of the Russian Federation, the Ministry of Construction of the Russian Federation, the Ministry of Transport of the Russian Federation, the Ministry of Finance of the Russian Federation, the Ministry of Digital Development of the Russian Federation, the Ministry of Economic Development of the Russian Federation, the Ministry of Emergency Situations of the Russian Federation, the Treasury of the Russian Federation, Russian Federal Road Transport Agency, Russian Federal Agency of Water Resources, Russian Federal Service for Hydrometeorology and Environmental Monitoring, Russian Federal Agency for Forestry, Russian Federal Service for Supervision in Education and Science, Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing, Russian Federal Service for Supervision of Natural Resources, Russian Federal Service for State Registration, Cadaster and Cartography, Russian Federal Agency for Fishery, Federal Tax Service of the Russian Federation, Federal Penitentiary Service of the Russian Federation, the Chamber of Commerce and Industry of the Russian Federation and the Russian Union of Industrialists and Entrepreneurs. The following inclusion criteria were considered: impact factor, combinations of keywords. Exclusion criteria were: irrelevant data. A strategic planning method was also applied, which consists in identifying factors of the internal and external environment for the sustainability of agrifood systems (SWOT analysis).

SDG 2 "End hunger, achieve food security and improved nutrition and promote sustainable agriculture"

One of the UN sustainable development goals up to 2030 is SDG 2 "End hunger, achieve food security and improved nutrition and promote sustainable agriculture" [2].

Hunger and malnutrition are common cause of diseases, a decrease in people's working capacity and, as a result, inability to increase earnings and improve living conditions [16]. Goal 2 aims to end all forms of malnutrition, build sustainable food production systems [17], and adopt agricultural practices [18–20] that increase production, save ecosystems [21], strengthen the ability to adapt to climate change, extreme weather events, droughts, floods and other disasters and gradually improve the quality of land and soil [22–25].

Without the elimination of hunger, it is impossible to achieve equality and effective functioning of the economics and the social sphere. Russia has made significant progress in achieving SDG 2 (Figures 2, 3, 4, 5) [26].

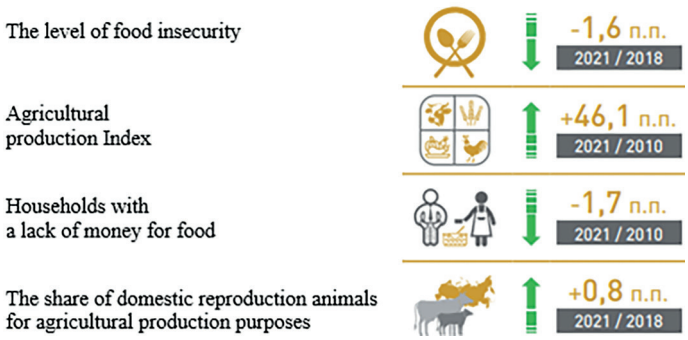

Figure 2. Achievements of Russia in the field of SDGs

It should be noted that the amount of lack of money for food is steadily decreasing. In 2021, only 0.1% of households reported this lack (Figure 3) [26] compared to previous years: 0.2% of households in 2020 [27], 0.5% of households in 2019 [28], 0.9% of households in 2018 [29], 0.9% of households in 2017, 1.0% of households in 2016, 1.2% of households in 2015, 1.8% of households in 2010.

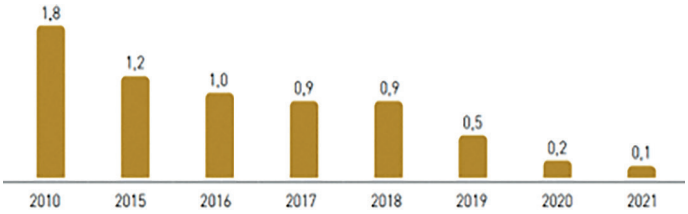

Figure 3. Households with a lack of money for food, %

The share of domestic reproduction animals for agricultural production purposes has increased, i. e. in 2021 it was 94.3% [26] compared to previous data: in 2020 it was 93.4% [27], in 2019 it was 93.4% [28], in 2018 it was 93.5% [29] (Figure 4) [26].

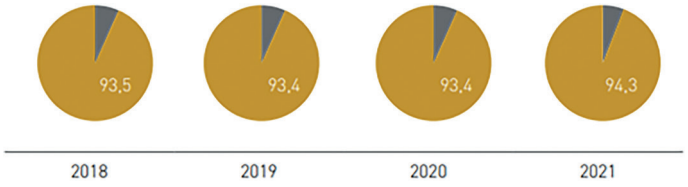

Figure 4. Share of domestic reproduction animals for agricultural production purposes, %

The priority areas in the development of animal husbandry in the Russian Federation in terms of social and food significance are dairy cattle breeding, specialized beef cattle breeding, and the development of crop fodder [30].

The Russian Federation has a unique gene pool of farm animals, which is represented by 42 species, 744 breeds, types and cross-breeds of worldwide and domestic selection. More than 2.3 thousand herds of breeding farm animals of 14 species are registered in the state breeding register [31].

In order to ensure food security in the country, the Doctrine of food security of the Russian Federation[2] is in force, the strategic goal of which is to provide the population of the country with safe, high-quality and affordable agricultural products, raw materials and food in volumes that ensure reasonable consumption rates [32,33]. Already in 2018, the prevalence of malnutrition was low, i. e. about 1.6% (among people over 18 years old). At the same time, in 2018, only 0.3% of the Russian population felt severe food insecurity, and 6.2% of the Russian population felt moderate or severe food insecurity [29]. In 2020, severe food insecurity was felt by 0.3% of the Russian population, and moderate or severe food insecurity was felt by 5.7% of the Russian population [27]. In 2021, severe food insecurity was experienced by 0.3% of the Russian population, while moderate or severe food insecurity was experienced by 4.6% of the Russian population (Figure 5) [26].

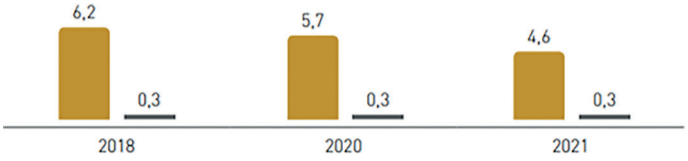

Figure 5. Level of food insecurity, %

In addition to this, there is a process of increasing the level of food security within the framework of interstate interaction of the EAEU member states on the basis of agreed directions and measures, which meets the main goal of the coordinated agro-industrial policy of the Union [34,35].

The basis for the formation of a national food quality management system in the Russian Federation is the Strategy on improvement of the quality of food products in the Russian Federation until 2030[3]. The strategy is focused on providing good nutrition, preventing diseases, increasing the duration and improving the quality of people's life, stimulating the development of production and circulation of good-quality food products on the market.

In 2019, the Long-term strategy for the development of the grain complex of the Russian Federation until 2035[4] was approved. The goal of the strategy is to form a highly efficient, scientifically and innovatively oriented, competitive and investment-attractive balanced system for the production, processing, storage and sale of basic grain and leguminous crops and their processed products, which guarantees Russia's food security, fully meets the country's domestic needs and creates a significant export potential.

From the point of view of ensuring the safety of products in the country, it should be noted that in the Russian Federation it is prohibited to grow and breed plants and animals whose genome has been changed using genetic engineering methods, with the exception of growing and breeding such plants and animals during examinations and research works [14].

[2] Doctrine of food security of the Russian Federation (Decree of the President of the Russian Federation dated January 30, 2010, No. 120). Retrieved from https://docs.cntd.ru/document/564161398. Accessed March 2, 2023. (In Russian).

[3] Strategy on improvement of the quality of food products in the Russian Federation until 2030 (Decree of the Government of the Russian Federation dated June 29, 2016, No. 1364-r). Retrieved from https://docs.cntd.ru/document/420363999. Accessed March 2, 2023. (In Russian).

[4] Long-term strategy for the development of the grain complex of the Russian Federation until 2035 (Decree of the Government of the Russian Federation dated August 10, 2019, No. 1796-r). Retrieved from https://docs.cntd.ru/document/560974985. Accessed March 2, 2023. (In Russian).

Russia is implementing the Federal scientific and technical program for the development of agriculture for 2017–2030[5], which sets the transition to highly productive and environmentally friendly agriculture and aquaculture, storage and efficient processing of agricultural products, and the creation of safe and high-quality food products as policy priorities in this direction [36,37].

Financial support and mechanisms for creating sustainable systems for the production of agricultural products are specified in the State program for the development of agriculture and the regulation of markets for agricultural products, raw materials and food[6] and the completed Federal target program "Sustainable development of rural areas for 2014–2017 and for the period up to 2020"[7]. In addition, the objectives of the ongoing Federal scientific and technical program for the development of agriculture are: the creation of scientific and technical results and products ("creation of knowledge"); transfer of scientific and technical results and products to practical use, implementation of training activities in order to ensure the development of agriculture ("technology transfer"); commercialization of scientific and technical results and products ("application of knowledge") [38,39].

Measures taken in the Russian Federation to solve the problem of excessive food price volatility include a mechanism for government procurement and commodity interventions. In addition, Russia is implementing a price regulation policy for socially significant food products[8]: some types of meat, dairy products, cereals, chicken eggs, sunflower oil, sugar, salt, wheat flour, tea, bread, some types of vegetables and fruits. Restrictions may be introduced for a period of no more than 90 days in case of price increase of more than 30% within three months [40–42].

This is how the Index of agricultural production was presented in comparable prices against the previous year (Figure 6) [26]. In 2021 it was 99.6%, in 2020 it was 101.3% [27], in 2019 it was 104.3% [28], in 2018 it was 99.8% [29], in 2017 it was 102.9%, in 2016 it was 104.8%, in 2015 it was 102.1%, in 2010 it was 87.9%.

Figure 6. Index of agricultural production in comparable prices against the previous year, %

The country is implementing a departmental project of the Ministry of Agriculture of the Russian Federation "Digital Agriculture" [43]. Within its framework, a set of measures is provided for the introduction of digital technologies and platform solutions in the agro-industrial complex, the achievement of productivity growth at "digital" agricultural enterprises by 2 times by 2024. Measures are also being implemented to stimulate the development and promotion of a healthy lifestyle and healthy nutrition of the population in order to change the eating habits of the population[9] to help eliminate the excess of the calorie content in the population's diet over the level of energy consumption, as well as the excess content of fat and sugar in consumed products.

It should be noted that the concept of agrifood systems sustainability [44] is constantly being worked on[10]. Thus, the following principles of development were adopted (Figure 7).

SWOT analysis for the agrifood systems sustainability

Taking in consideration the above, it is necessary to assess the internal and external factors that affect the sustainability of agrifood systems [45]. Thus, SWOT analysis [46] was applied, i. e. a strategic planning method for considering development opportunities by identifying strengths, weaknesses, opportunities and threats (Figure 8).

Based on the analysis, it can be concluded that the goal of policies for the development of agrifood systems sustainability should be to ensure food security, promote a balance between economic and environmental challenges and increase the resilience of the global agrifood system to shocks such as conflicts [47], pandemics and extreme weather events [48–50].

Conclusions

The Russian Federation is committed to achieving the goals set by the international community in the 2030 Agenda for Sustainable Development. Continuous efforts are being made to achieve the sustainable development goals (SDGs) at the national level.

[5] Federal scientific and technical program for the development of agriculture for 2017–2030 (Decree of the Government of the Russian Federation dated August 25, 2017, No. 996). Retrieved from https://docs.cntd.ru/document/436761964. Accessed March 2, 2023. (In Russian).

[6] State program for the development of agriculture and the regulation of markets for agricultural products, raw materials and food (Decree of the Government of the Russian Federation dated July 14, 2012, No. 717). Retrieved from https://docs.cntd.ru/document/902361843. Accessed March 2, 2023. (In Russian).

[7] Federal target program "Sustainable development of rural areas for 2014–2017 and for the period up to 2020" (Decree of the Government of the Russian Federation dated July 15, 2013, No. 598). Retrieved from https://docs.cntd.ru/document/499034090. Accessed March 2, 2023. (In Russian).

[8] On approval of the rules for establishing maximum permissible retail prices for certain types of socially significant essential food products, a list of certain types of socially significant essential food products, for which maximum permissible retail prices can be set, and a list of certain types of socially significant food products, for the purchase of a certain amount of which an economic entity engaged in trading activities is not allowed to pay remuneration (Decree of the Government of the Russian Federation dated July 15, 2010, No. 530). Retrieved from https://legalacts.ru/doc/postanovlenie-pravitelstva-rf-ot-15072010-n-530. Accessed March 2, 2023. (In Russian).

[9] Strategy for the formation of a healthy lifestyle of the population, prevention and control of non-communicable diseases for the period up to 2025 (Order of the Ministry of Health of the Russian Federation dated January 15, 2020 No. 8). Retrieved from https://rulaws.ru/acts/Prikaz-Minzdrava-Rossii-ot-15.01.2020-N-8. Accessed March 2, 2023. (In Russian).

[10] Strategy for the development of agro-industrial and fishery complexes of the Russian Federation for the period up to 2030 (Decree of the Government of the Russian Federation dated September 8, 2022, No. 2567-r). Retrieved from https://docs.cntd.ru/document/351735594. Accessed March 2, 2023. (In Russian).

- it is necessary to sharply increase the efficiency of the use of natural and labor resources for food production. This implies the use of fundamentally new technological approaches to production, but also the reduction of food losses all the way "from field to plate". It is necessary to obtain more end products per unit of resources.

- it is necessary to preserve and improve the state of natural resources used for food production. The agricultural sector is the main consumer of water resources, and with increasing aridity in key agricultural regions, the demand for water will only increase.

- the sustainability of agriculture requires the social development of rural areas, the inclusive development of the agricultural sector, in which all economically wealthy producers have access to natural and financial resources.

- the sustainability of agriculture involves increasing the resilience of human communities and ecosystems to external influences, in particular to climate change and market volatility. We need political and social mechanisms and new technological approaches and practices that would make it possible to cope with natural and man-made disasters, sharp fluctuations in world markets.

- we need a sustainable management system for agrifood complexes based on transparent and effective legislation observing a rational balance of private initiative and state regulation.

Figure 7. Principles of agrifood systems development

S - Strengths: - state support; - development of rural infrastructure; - implementation of complex sectoral strategic programs	**W - Weaknesses:** - the state of the material and technical base of agriculture; - weak renewal of funds; - backlog in technical and technological modernization of production; - degradation of the quality of agricultural land and a large amount of unused land
O - Opportunities: - increasing the efficiency of using natural and labor resources for food production; - introduction of a sustainable management system for agrifood complexes	**T - Threats:** - food threat; - social threat; - economic threat; - financial threat; - COVID-19 pandemic; - military security of the country

Figure 8. SWOT analysis of agrifood systems sustainability

Significant progress has been made in Russia towards achieving SDG 2 "End hunger, achieve food security and improved nutrition and promote sustainable agriculture", i. e. the level of food insecurity of the population is steadily decreasing, and only 0.1% of households report lack of money for food.

The development of Russia's potential in achieving SDG 2 is facilitated by such factors as the development of rural infrastructure and the implementation of comprehensive sectoral strategic programs.

Today, agriculture in Russia is the most dynamically developing sector, showing relatively high rates of development.

The situation with food safety has significantly improved over the past decade: the share of rejected products in the total volume of inspected goods is decreasing for all product groups, the share of products that do not meet hygienic requirements is decreasing sharply too. An equally important indicator of the positive situation with food security in the country is the presence of a clearly defined national policy in this area.

REFERENCES

1. United Nations. Department of Economic and Social Affairs. Sustainable Development. (2015). Transforming our world: the 2030 Agenda for Sustainable Development. Retrieved from https://sdgs.un.org/2030agenda Accessed January 15, 2023
2. United Nations. Department of Economic and Social Affairs. Sustainable Development. (2015). The 17 goals. Retrieved from https://sdgs.un.org/goals Accessed January 15, 2023
3. Arora-Jonsson, S. (2023). The sustainable development goals: A universalist promise for the future. *Futures,* 146, Article 103087. https://doi.org/10.1016/j.futures.2022.103087
4. United Nations. Department of Economic and Social Affairs. Sustainable Development. (2015). IAEG-SDGs Inter-agency and Expert Group on SDG Indicators. Retrieved from https://unstats.un.org/sdgs/iaeg-sdgs/ Accessed January 15, 2023
5. Bobylev, S.N., Grigoriev, L.M. (2016). Report on human development in the Russian Federation for 2016. Sustainable Development Goals. The UN and Russia. Moscow: Analytical Center under the Government of the Russian Federation, 2016. (In Russian)
6. Yin, C., Zhao, W., Fu, B., Meadows, M.E., Pereira, P. (2023). Key axes of global progress towards the Sustainable Development. Goals. *Journal of Cleaner Production*, 385, Article 135767. https://doi.org/10.1016/j.jclepro.2022.135767
7. Hirai, T., Comim, H. (2022). Measuring the sustainable development goals: A poset analysis. *Ecological Indicators*, 145, Article 109605. https://doi.org/10.1016/j.ecolind.2022.109605
8. Gyimah, P., Appiah, K. O., Appiagyei, K. (2023). Seven years of United Nations' sustainable development goals in Africa: A bibliometric and systematic methodological review. *Journal of Cleaner Production*, 395, Article 136422. https://doi.org/10.1016/j.jclepro.2023.136422
9. Schleifer, P., Brandi, C., Rupal Verma, R., Bissinger, K., Fiorini, M. (2023). Voluntary standards and the SDGs: Mapping public-private complementarities for sustainable development. *Earth System Governance,* 14, Article 100153. https://doi.org/10.1016/j.esg.2022.100153
10. Bogers, M., Biermann, F., Kalfagianni, A., Kim, R.E. (2023). Sustainable Development Goals fail to advance policy integration: A large-n text analysis of 159 international organizations. *Environmental Science and Policy,* 138, 134–145. https://doi.org/10.1016/j.envsci.2022.10.002
11. Encenzo, R.M., Asoque, R., Arceño, R., Aclao, J., Ramones, E., Orioque, J. et al. (2023). A comprehensive analytical framework for evaluating the similarity between organizations' strategic directions and the United Nations' sustainable development goals. *Decision Analytics Journal*, 6, Article 100176. https://doi.org/10.1016/j.dajour.2023.100176
12. Li, X., Wu, T., Zhang, H.-J., Yang, D.-Y. (2023). National innovation systems and the achievement of sustainable development goals: Effect of knowledge-based dynamic capability. *Journal of Innovation and Knowledge*, 8(1), Article 100310. https://doi.org/10.1016/j.jik.2023.100310
13. United Nations. Department of Economic and Social Affairs. Sustainable Development. (2022). The Sustainable Development Goals Report 2022. Retrieved from https://unstats.un.org/sdgs/report/2022/ Accessed January 23, 2023
14. Analytical Center under the Government of the Russian Federation. (2020). Voluntary national review of the implementation of the 2030 Agenda for Sustainable Development. https://www.economy.gov.ru/material/file/dcbc39abeafb0418d9d48c06c958e454/obzor.pdf Accessed January 23, 2023 (In Russian)
15. Rosstat Federal State Statistics Service. (2023). National set of SDG indicators. Retrieved from https://rosstat.gov.ru/sdg/national Accessed January 23, 2023 (In Russian)
16. Li, M., Liu, J., Chen, Y., Yang, Z. (2023). Can sustainable development strategy reduce income inequality in resource-based regions? A natural resource dependence perspective. *Resources Policy*, 81, Article 103330. https://doi.org/10.1016/j.resourpol.2023.103330
17. FAO. (2022). In Brief to The State of Agricultural Commodity Markets 2022. The geography of food and agricultural trade: Policy approaches for sustainable development. Rome, Italy, 2022. https://doi.org/10.4060/cc0475en
18. FAO. (2022). The State of Food and Agriculture 2022. Leveraging automation in agriculture for transforming agrifood systems. Rome, Italy, 2022. https://doi.org/10.4060/cb9479en
19. Gackstetter, D., von Bloh, M., Hannus, V., Meyer, S.T., Weisser, W., Luksch, C. et al. (2023). Autonomous field management — An enabler of sustainable future in agriculture. *Agricultural Systems,* 206, Article 103607. https://doi.org/10.1016/j.agsy.2023.103607
20. Shehata, N., Egirani, D., Olabi, A.G., Inayat, A., Abdelkareem, M.A., Kyu-Jung Chae, K.-J. et al. (2023). Membrane-based water and wastewater treatment technologies: Issues, current trends, challenges, and role in achieving sustainable development goals, and circular economy. *Chemosphere*, 320, Article 137993. https://doi.org/10.1016/j.chemosphere.2023.137993
21. Yin, C., Zhao, W., Ye, J., Muroki, M., Pereira, P. (2023). Ecosystem carbon sequestration service supports the Sustainable Development Goals progress. *Journal of Environmental Management,* 330, Article 117155. https://doi.org/10.1016/j.jenvman.2022.117155
22. Zhang, S., Anser, M.K., Peng, M.Y-P., Chen, C. (2023). Visualizing the sustainable development goals and natural resource utilization for green economic recovery after COVID-19 pandemic. *Resources Policy*, 80, Article 103182. https://doi.org/10.1016/j.resourpol.2022.103182
23. Bogers, M., Biermann, F., Kalfagianni, A., Kim, R.E., Treep, J., de Vos, M.G. (2023). The impact of the Sustainable Development Goals on a network of 276 international organizations. *Global Environmental Change,* 76, Article 102567. https://doi.org/10.1016/j.gloenvcha.2022.102567
24. Ahmad, N., Youjin, L., Žiković, S., Belyaeva, Z. (2022). The effects of technological innovation on sustainable development and environmental degradation: Evidence from China. *Technology in Society,* 72, Article 102184. https://doi.org/10.1016/j.techsoc.2022.102184
25. Hunjra, A.I., Hassan, M.K., Zaied, Y.B., Managi, S. (2023). Nexus between green finance, environmental degradation, and sustainable development: Evidence from developing countries. *Resources Policy*, 81, Article 103371. https://doi.org/10.1016/j.resourpol.2023.103371
26. Egorenko, S.N. (2022). Sustainable Development Goals in the Russian Federation. Moscow: Rosstat, 2022. Retrieved from https://rosstat.gov.ru/storage/mediabank/SGD_2022_RUS.pdf Accessed February 01, 2023 (In Russian)
27. Voluntary national review of Russia's achievement of sustainable development (2021). Retrieved from https://infogram.com/sdg-yearbook-2021–1hxr4zx9v8q7q6y?live Accessed February 01, 2023 (In Russian)
28. Egorenko, S.N. (2020). Sustainable Development Goals in the Russian Federation. Moscow: Rosstat, 2020. Retrieved from https://rosstat.gov.ru/storage/mediabank/ERqpLbXV/%D0%A6%D0%B5%D0%BB%D0%B8%20%D1%83%D1%81%D1%82%D0%BE%D0%B9%D1%87%D0%B8%D0%B2%D0%BE%D0%B3%D0%BE%20%D1%80%D0%B0%D0%B7%D0%B2%D0%B8%D1%82%D0%B8%D1%8F%20%D0%B2%20%D0%A0%D0%BE%

D1%81%D1%81%D0%B8%D0%B9%D1%81%D0%BA%D0%BE%D0%B9%20%D0%A4%D0%B5%D0%B4%D0%B5%D1%80%D0%B0%D1%86%D0%B8%D0%B8,%202020%20-%20%D1%81%D0%B1%D0%BE%D1%80%D0%BD%D0%B8%D0%BA.pdf Accessed February 01, 2023 (In Russian)
29. Egorenko, S.N. (2019). Sustainable Development Goals in the Russian Federation. Moscow: Rosstat, 2019. Retrieved from https://rosstat.gov.ru/storage/mediabank/SDG_in_Russia_2019_rus.pdf Accessed February 01, 2023 (In Russian)
30. Laykam, K.E. (2021). Agriculture in Russia. Moscow: Rosstat, 2021. Retrieved from https://rosstat.gov.ru/storage/mediabank/S-X_2021.pdf Accessed February 01, 2023 (In Russian)
31. Galkin, S.S. (2022). Russian Statistical Yearbook 2022. Retrieved from https://rosstat.gov.ru/storage/mediabank/Ejegodnik_2022.pdf Moscow: Rosstat, 2022. Accessed February 01, 2023 (In Russian)
32. Food safety, self-sufficiency of Russia according to the criteria of goods from the food consumer basket for the coming years: information edition. (2019). Moscow: Rosinformagrotekh, 2019. Retrieved from https://rosinformagrotech.ru/data/elektronnye-kopii-izdanij/normativnye-dokumenty-spravochniki-katalogi-i-dr/send/66-normativnye-dokumenty-spravochniki-katalogi/1362-prodovolstvennaya-bezopasnost-samoobespechennost-rossii-po-kriteriyam-tovarov-iz-prodovolstvennoj-potrebitelskoj-korziny-na-blizhajshie-gody-2019 Accessed February 01, 2023 (In Russian)
33. Altuhov, A.I. (2020). Food security in the context of implementation of the new edition of her doctrine. *Bulletin of the Kursk State Agricultural Academy*, 9, 82–89. (In Russian)
34. Kanamatova, D.A. (2021). Ensuring food security of the Russian Federation. *The Eurasian Scientific Journal,* 13(6), Article 70ECVN621. Retrieved from https://esj.today/PDF/70ECVN621.pdf Accessed February 01, 2023 (In Russian)
35. Vartanova, M.L. (2019). Problems and prospects of agricultural development of the EAEU. *Food Policy and Security*, 6(3), 159–172. https://doi.org/10.18334/ppib.6.2.41388 (In Russian)
36. Laykam, K.E. (2015). Agriculture in Russia. Moscow: Rosstat, 2015. Retrieved from https://rosstat.gov.ru/storage/mediabank/selhoz15.pdf Accessed February 01, 2023 (In Russian)
37. Laykam, K.E. (2019). Agriculture in Russia. Moscow: Rosstat, 2019. Retrieved from https://rosstat.gov.ru/storage/mediabank/sh_2019.pdf Accessed February 01, 2023 (In Russian)
38. Terkun, V. V. (2022). Problematic issues of agriculture in 2022. *The Applied Economic Researches Journal*, 4, 23–26. https://doi.org/10.47576/2313–2086_2022_4_23 (In Russian)
39. Patsala, S. V., Goroshko, N. V. (2021). Russian agriculture: Global positions, structural proportions, and development trends. *Bulletin of Kemerovo State University. Series: Political, Sociological and Economic Sciences,* 6(1), 96–108. https://doi.org/10.21603/2500–3372–2021–6–1–96–108 (In Russian)
40. Uzun, V. Ya., Fomin, A.A., Loginova, D.A. (2018). Position of Russia on the world agro-food map. *International Agricultural Journal*, 1(361), 68–76. https://doi.org/10.24411/2587–6740–2018–11016 (In Russian)
41. Barikaeva, A.F. (2019). Formation of the customer focus policy in the agro-industrial complex of Russia as a food security providing tool. *Vestnik of Economic Security*, 1,197–201. https://doi.org/10.24411/2414–3995–2019–10041 (In Russian)
42. Rudenko, M.N., Subbotina, Y.D. (2021). Food security of Russian Federation. *Izvestiâ Sankt-Peterburgskogo Gosudarstvennogo Ekonomičeskogo Universiteta*, 1(127), 84–90. (In Russian)
43. Ministry of Agriculture of the Russian Federation (2019). Departmental project "Digital Agriculture" (2019). Moscow: Rosinformagrotech, 2019. Retrieved from https://mcx.gov.ru/upload/iblock/900/900863fae06c026826a9ee43e124d058.pdf Accessed February 01, 2023 (In Russian)
44. Kadomtseva, M. E. (2022). Theoretical and methodological aspects of ensuring sustainable development of agro-food systems. *Izvestiya of Saratov University. Economics. Management. Law,* 22(3), 277–286. https://doi.org/10.18500/1994–2540–2022–22–3–277–286 (In Russian)
45. Cherednichenko, O.A., Dovgot'ko, N.A., Yashalova, N.N. (2018). Sustainable development of the agri-food sector: Russia's priorities and directions to adapt Agenda 2030 to Russian conditions. *Economic and Social Changes: Facts, Trends, Forecast*, 11(6), 89–108. https://doi.org/10.15838/esc.2018.6.60.6 (In Russian)
46. Nilashi, M., Abumalloh, R.A., Mohd, S., Azhar, S.N.F.S., Samad, S., Thi, H.H. et al. (2023). COVID-19 and sustainable development goals: A bibliometric analysis and SWOT analysis in Malaysian context. *Telematics and Informatics*, 76, Article 101923. https://doi.org/10.1016/j.tele.2022.101923
47. Blessley, M., Mudambi, S.M. (2022). A trade war and a pandemic: Disruption and resilience in the food bank supply chain. *Industrial Marketing Management,* 102, 58–73. https://doi.org/10.1016/j.indmarman.2022.01.002
48. Chen, L., Huang, H., Han, D., Wang, X., Xiao, Y., Yang, H. et al. (2023). Investigation on the spatial and temporal patterns of coupling sustainable development posture and economic development in World Natural Heritage Sites: A case study of Jiuzhaigou, China. *Ecological Indicators*, 146, Article 109920. https://doi.org/10.1016/j.ecolind.2023.109920
49. Kim, W., Song, C., Lee, S.K., Choi, C., Yang, R., Bak, I. et al. (2023). A way forward for climate technology transfer and sustainable development goals. *Environmental Science and Policy*, 142, 29–41. https://doi.org/10.1016/j.envsci.2023.01.009
50. Ren, X. (2022). Comprehensive evaluation model of rural financial ecological environment under the background of sustainable development. *Sustainable Energy Technologies and Assessments*, 9, Article 102899. https://doi.org/10.1016/j.seta.2022.102899

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COMPARISON OF THE BLOOD PARAMETERS WITH THE CHEMICAL COMPOSITION OF THE MUSCLE TISSUE OF MEAT-AND-EGG CHICKEN

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Keywords: *poultry, biochemical parameter, biomarker, muscle tissue, correlations, prediction*

Abstract
Basic blood and muscle tissue parameters have been analyzed in crossbred male Russian White and Cornish hens (♂, RW x CORN, n = 95, slaughtered at 63 days of age). According to BW at slaughter, males (n = 95) were divided into 3 groups (group 1–1,000–1,799 g, n = 31; group 2–1,800–2,099 g, n = 28; group 3–2,100–2,650 g, n = 36). It has been found that with an increase in the live weight at slaughter, the ratio of albumin to globulin (p = 0.038), aspartate aminotransferase (p = 0.003) increased in the serum of birds; the levels of globulins (p = 0.05), glucose (p = 0.02), Ca (p = 0.006), Mg (p = 0.05) decreased. With increasing BW, the crude protein content in thigh muscle decreased (p = 0.019) against a trend towards increasing moisture content in thigh meat (p = 0.058). Comparative assessment of biochemical blood parameters of nitrogen, carbohydrate-lipid, mineral metabolism, antioxidant protection parameters, some clinical blood parameters (hematocrit, erythrocytes and hemoglobin) and chemical composition of the breast and thigh muscle tissue has been carried out. The analysis (Pearson correlation coefficients) has revealed patterns between the concentration of some blood metabolites and the composition of muscle tissue in males. Thus, the accumulation and analysis of data on resource genetic populations is of interest for science and practice in order to establish relationships between blood parameters and the quality of chicken products, as well as to identify biomarkers for predicting poultry productivity in vivo.

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Introduction

Poultry is one of the actively developing branches of animal husbandry. It is quite capable of providing the population with high-quality meat associated with high growth energy and the bird's ability to reproduce quickly [1].

The study of the biochemical status of the bird's body is in great demand for assessing the state of health [2]. The authors have been studying the biochemical parameters of blood in birds of domestic breeds [3] and modern poultry crosses [4].

The high-quality food products are the basis for public health. The need of modern society poses the problem of deepening knowledge in the field of lifetime formation and improving the quality of poultry products. An urgent scientific problem is the fundamental study of the factors, contributed to the formation of the quality of poultry products by the integrated approach, including the complex of molecular genetic, biochemical, microbiological, hormonal mechanisms of homeostasis in the body of poultry [5].

The study of biochemical parameters of blood and their relationship with the antioxidant status and the composition of the poultry products is the most relevant with the advent of new bird genotypes. The modern market requirements determine the advantage of breeds and lines with good viability, high growth rate, good egg and meat qualities [6]. Crossbreeding of different chicken breeds can be a good strategy for the development of poultry farming and improvement of the poultry product quality. It can be useful for studying the biochemical and genetic aspects of product formation and obtaining new biomarkers of the health status and poultry product quality. The local poultry breeds of the meat productivity are promising for crossbreeding. Of particular interest is the assessment of the influence of the effect of heterosis on the biological characteristics of the offspring and the physiological and biochemical aspects of the formation of poultry health and poultry product quality.

An increase in the productive qualities of offspring and improvement in the intensity of live weight gain are among the main tasks of crossing different breeds of poultry. It is also important to obtain high-quality meat rich in biogenic nutrients for a high level of human nutrition. The study of the relationship between blood biochemical parameters and the meat chemical composition in accordance with the intensity of poultry growth is relevant. There are few data in the literature characterizing the correlation between biochemical indicators (including indicators of the antioxidant defense) and the poultry meat composition in accordance with the live weight and other growth indicators.

The influence of biochemical and molecular genetic factors on the poultry meat quality requires further study. The accumulation and analysis of correlations between blood biochemical and genetic parameters and the quality of animal products to identify biomarkers for predicting animals and poultry productivity of various genotypes is very interesting for science and practice.

The purpose of this study was to determine the biochemical and hematological parameters in roosters when crossing the Russian White and Cornish chicken breeds (RW x CORN) and to compare the parameters with the muscle tissue chemical composition.

Objects and methods

Animals

The experiment used meat-and-egg poultry (♂, RW × CORN) at the age of 63 days (n = 95). The birds were kept under the same conditions of feeding and keeping. Roosters (n = 95) were divided into groups according to BW at slaughter (age at slaughter was the same and was 9 weeks or 63 days): 1) 1,000–1,800 g, 2) 1,800–2,100 g, 3) 2,100–2,650 g.

The research was conducted in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No. 123, Strasbourg, 1986). The research was approved by the bioethical commission of the L. K. Ernst Federal Research Center for Animal Husbandry (protocol № 3, dated May 27, 2022).

The basis of the diet was industrial feed for young chickens, balanced in terms of nutrients and energy in accordance with modern requirements and the recommended feeding regimen [7]. The composition of feed was as follows: corn 48.0%, wheat 21%, soybean meal 13.0%, sunflower meal 12.0%, fish flour 1.0%, raw materials of animal origin, fish meal, vegetable oil, limestone meal, phosphates, salt, vitamins (including vitamin E analogue), minerals, amino acids, enzymes and other ingredients. The birds had constant access to water.

Analysis of biochemical and hematological variables

Blood collection was carried out when birds were slaughtered at 63 days of age. Two blood samples were transferred to Vacutainer tubes. The first blood sample was collected into 8 ml VACUETTE® serum tube with blood clotting activator (Greiner Bio-One, Austria) and centrifuged within 4 h of collection at 5,000 g for 5 min. The second blood sample was collected in a VACUETTE® tube (Greiner Bio-One, Austria) containing EDTA as the anticoagulant and used for hematological analysis.

Samples were sent to the laboratory (the Department of Physiology and Biochemistry of Farm Animals at the Federal Research Center for Animal Husbandry named after Academy Member L. K. Ernst) and analyzed on an automatic biochemical analyzer ChemWell (Awareness Technology, USA) using reagents from Analyticon Biotechnologies AG (Germany), Spinreact (Spain) and Deacon (Russia).

Methods used were as follows: protein total (TP) — by the biuret method (9104), albumin (ALB) — by the colorimetric method (9136), globulins (GLB) — by calculation, albumin to globulin ratio (ALB / GLB) — by calculation, creatinine (CREA)- by the Jaffe kinetic method (448), alanine aminotransferase (ALT) — by the UV kinetic (1187), aspartate aminotransferase (AST) — by the UV kinetic (1177), alkaline phosphatase (ALP) — by the UV kinetic (1625), glucose (GLU) — by the enzymatic-glucose oxidase (4341), triglycerides (TRIG) — by the enzymatic-colorimetric method (41031), total bilirubin (TBIL) — by the Walters and Gerarde method (804), cholesterol (CHOL) — by the enzymatic-colorimetric method (41021), chlorides (CL) — by the colorimetric method (1001360); calcium (Ca) — by the O-cresolphthalein complexone method (10100), phosphorus (P) — by the colorimetric method (1914), magnesium (Mg) — by the colorimetric method (1001280), iron (I) — by the colorimetric method (1001247). For hematology, hemoglobin (HGB) (spectrophotometric method), hematocrit (HCT), red blood cell (RBC) count were determined, using ABC VET (HORIBA ABX Diagnostics Inc) (France).

Lipid peroxidation assay

The lipid peroxidation level in serum samples was measured by the standard method (reaction with the thiobarbituric acid) by kits "Agat-Med" (Russia). The values of the thiobarbituric acid active products (TBA-AP) were expressed. The activity of ceruloplasmin (CP) was measured by the method of Revin [8].

The total amount of water-soluble antioxidants (TAWSA) was measured by the amperometric method using the device "TsvetYauza-01-AA" ("Khimavtomatika", Russia). The TAWSA values were determined by measuring the strength of the electric current arising during the oxidation of molecules on the surface of the working electrode at a potential of ~500 mV. TAWSA was measured in equivalent to gallic acid as in [9]. For this, the "working solutions" were prepared from a gallic acid solution (100 mg/dm^3) for calibration with a mass concentration of 0.2, 0.5, 1.0 and 4.0 mg/dm^3. An amount of 2.2 mmol/dm^3 of the phosphoric acid solution was used as an "eluent". The results of measuring the total antioxidant activity of the samples were statistically processed using the MS Excel program.

The TBK-AP/ CP ratio was calculated by the authors.

Analysis of the chemical composition of meat

Meat samples were analyzed for dry matter (GOST 33319–2015[1]), crude fat (GOST 23042–2015[2]) and ash (ISO 936:1998[3]). Crude protein was calculated.

[1] GOST 33319–2015 "Meat and meat products. Method for determination of moisture content" Moscow: Standartinform, 2019. Retrieved from https://internet-law.ru/gosts/gost/60635/ Accessed December 15, 2022. (In Russian)

[2] GOST 23042–2015 "Meat and meat products. Methods of fat determination" Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200133107 Accessed December 15, 2022. (In Russian)

[3] ISO 936:1998 "Meat and meat products — Determination of total ash" Technical Committee: ISO/TC34/SC6 Meat, poultry, fish, eggs and their products, 1998. Retrieved from https://www.iso.org/standard/24783.html Accessed December 15, 2022.

Statistical analyses

Descriptive statistics (mean, median, SD, minimum and maximum values) were used with the software packages "Microsoft Office Excel 2003".

An ANOVA was carried out for indicators of blood and meat, taking into account the group of experimental poultry in terms of live weight (program Statistica 13RU, StatSoft, USA).

The Pearson correlation test to determine a relationship between the obtained biochemical parameters and chemical composition of meat was used. All the data were analyzed by using the software packages "Statistica" (Statistica 13RU, StatSoft, USA). The results of the statistical analysis were considered significant at $p < 0.05$.

The significance of the coefficient was determined by t-test, the closeness of connection on the Chaddock scale (0.3 or less — weak connection, 0.4–0.7 — medium, 0.7–0.9 — high connection, 0.9–1 — extremely high).

The calculation of the coefficient of variation (*CV*) was carried out according to the formula:

$$CV = (SD / \text{Median}) \times 100,$$

where *SD* is the standard deviation of the value; *M* — is the median value.

It was believed that when the value of the *CV* was less than 10%, then the spread of data values was insignificant; if from 10% to 20% — medium; greater than 20% and less than or equal to 33% — significant.

Results and discussion

Evaluation of an array of blood and meat indicators in roosters

Obtaining poultry with the highest performance indicators involves crossing poultry of different lines and breeds. This leads to the effect of heterosis with an increase in the scatter of genetic indicators and phenotypic manifestations. This affects blood parameters. It was noted that in CORN × RW poultry hybrids the studied biochemical blood parameters had a significant variation in values (Table 1).

Table 1. Metabolic indicators in roosters (CORN × RW)

Parameter	N	Mean	SEM	SD	Median	Min	Max
TP (g/L)	95	35.13	0.41	4.01	34.70	26.30	47.0
ALB (g/L)	95	13.07	0.36	1.09	13.00	10.30	16.40
GLB (g/L)	95	22.06	0.51	3.47	21.60	14.30	33.30
ALB / GLB	95	0.60	0.008	0.08	0.61	0.41	0.84
TBIL (μmol/L)	95	0.74	0.03	0.30	0.69	0.27	1.74
GLU (mmol/L)	95	14.86	0.16	1.59	14.90	11.27	19.74
CHOL (mmol/L)	95	3.45	0.04	0.76	3.40	2.17	9.98
Ca (mmol/L)	95	2.79	0.03	0.26	2.85	2.11	3.34
P (mmol/L)	95	2.02	0.03	0.35	2.04	0.07	2.89
Ca/P	95	1.75	0.37	3.59	1.38	0.90	36.28
Mg (mmol/L)	95	0.96	0.02	0.15	0.93	0.66	1.51
I (mmol/L)	95	20.41	0.39	3.79	19.90	13.25	32.23
CL (mmol/L)	95	112.95	0.42	4.16	112.50	102.67	122.70
ALT (IU /L)	95	7.35	0.20	2.04	7.10	2.70	13.80
AST (IU /L)	95	220.66	3.82	37.26	214.50	146.80	415.50
AST / ALT	95	31.97	0.97	9.50	30.10	13.27	77.85
ALP (IU /L)	95	1002.86	33.35	325.04	926.00	452.00	2359.00
CREA (mmol/L)	95	31.51	0.51	5.01	31.48	22.25	62.53
TRIG (mmol/L)	95	0.40	0.02	0.20	0.33	0.13	0.96

TRIG, TBIL, ALP, ALT, CHOL had the highest scatter (SD to mean ratio), I, P, AST, CREA, GLB, Mg, TP, GLU had the middle scatter, Ca, ALB, CL had the minimum scatter. The hematologic indices we studied (RBC, HCT, HGB) had average spread values (Table 2).

Table 2. Hematological parameters in roosters (CORN × RW)

Parameter	N	Mean	SEM	SD	Median	Min	Max
RBC (10^{12}/L)	95	3.28	0.06	0.66	3.49	1.50	4.45
HCT (%)	95	46.67	0.99	9.66	48.09	21.19	62.32
HGB (g/L)	95	108.54	1.54	15.01	110.00	9.82	136.00

Lipid peroxidation and antioxidant protection (Table 3) indices had a high scatter of values. With the high scatter of individual blood biochemical parameters (TRIG, TBIL, ALP, ALT, CHOL), it may indicate a high impact of crossbreeding on the stress parameters of hybrid roosters.

Table 3. Lipid peroxidation and antioxidant protection in roosters (CORN × RW)

Parameter	N	Mean	SEM	SD	Median	Min	Max
TBA-AP (μmol/L)	95	2.65	0.07	0.66	2.67	1.33	5.23
CP (mg/L)	95	40.72	1.05	10.31	39.00	23.00	78.00
TAWSA (mg/L)	95	39.80	0.87	9.54	39.34	22.80	69.14
TBA-AP/CP	95	0.07	0.002	0.02	0.07	0.02	0.12

The study of the parameters of the chemical composition showed little variability in dry matter, crude protein and total ash both in thigh meat and breast meat. There was a high scatter of ether extract values (Table 4). The meat chemical composition had less heterogeneity. It allows us to characterize a more stable fixation of these traits.

Table 4. Chemical composition of roosters' (CORN × RW) meat,%

Parameter	N	Mean	SEM	SD	Median	Min	Max
Thigh meat							
Dry matter	95	25.93	0.08	0.77	25.86	24.46	28.46
Crude protein	95	21.45	0.07	0.72	21.43	19.21	23.23
Crude fat	95	3.40	0.09	0.90	3.20	1.53	6.22
Total ash	95	1.10	0.005	0.05	1.10	0.91	1.23
Breast meat							
Dry matter	95	26.12	0.08	0.86	26.21	23.93	28.11
Crude fat	95	24.02	0.08	0.81	24.01	21.98	26.28
Ether extract	95	1.01	0.03	0.27	0.95	0.49	2.23
Total ash	95	1.18	0.008	0.08	1.17	1.01	1.51

Correlation of the blood parameters and chemical composition of meat

Pearson correlation coefficients (r) were calculated for the complex of studied blood and meat indicators. Table 5 shows correlations between the indicators in blood and meat of chickens (CORN × RW). High positive correlations between protein, carbohydrate, fat, mineral indicators of metabolism were established indicating a high degree of interconnection (Table 5) and were characterized by commonly known principles. A close positive correlation was established between TP and protein fractions (extremely

Table 5. Correlations of the studied blood and meat parameters (n = 95)

Parameter	TP	ALB	GLB	ALB / GLB	CREA	GIU	TBIL	TRIG	CHOL	ALT	AST	AST / ALT	ALP	Ca	P	Ca / P	Mg	I	CL	RBC	HGB	HCT	TAWSA	CP	TBA-AP	TBA/CP	Moisture of breast meat	Dry matter of breast meat	Crude protein of breast meat	Crude fat of breast meat	Ash of breast meat	Moisture of thigh meat	Dry matter of thigh meat	Crude protein of thigh meat	Crude fat of thigh meat	Ash of thigh meat
TP	1.00																																			
ALB	0.57	1.00																																		
GLB	0.96	0.32	1.00																																	
ALB / GLB	–0.62	0.27	–0.81	1.00																																
CREA	0.06	0.12	0.03	0.06	1.00																															
GLU	0.20	0.27	0.14	–0.04	0.06	1.00																														
TBIL	0.14	0.15	0.11	0.02	–0.05	0.08	1.00																													
TRIG	0.21	0.09	0.21	–0.18	–0.39	0.30	0.32	1.00																												
CHOL	0.33	0.34	0.26	–0.06	0.29	0.20	0.11	0.25	1.00																											
ALT	0.05	0.23	–0.02	0.17	0.18	–0.01	0.20	0.05	0.04	1.00																										
AST	0.00	0.14	–0.05	0.15	0.25	–0.14	0.03	–0.04	0.13	0.28	1.00																									
AST/ALT	–0.03	–0.09	–0.01	–0.07	–0.01	0.02	0.23	–0.09	0.06	–0.75	0.28	1.00																								
ALP	0.11	–0.32	0.24	–0.47	–0.27	0.23	0.06	0.31	–0.07	–0.19	–0.27	–0.03	1.00																							
Ca	0.31	0.18	0.30	–0.17	0.13	0.16	0.21	–0.01	0.05	–0.12	–0.26	–0.04	0.13	1.00																						
P	0.14	0.32	0.06	0.15	0.58	0.11	0.02	–0.25	0.22	0.29	0.14	–0.15	–0.29	0.15	1.00																					
Ca / P	0.07	–0.23	0.16	–0.31	–0.44	0.03	0.13	0.23	–0.19	–0.32	–0.32	0.07	0.40	0.48	–0.78	1.00																				
Mg	0.30	0.15	0.30	–0.21	0.25	0.29	0.16	0.43	–0.19	0.15	–0.13	–0.20	0.27	0.09	0.06	0.05	1.00																			
I	0.03	0.09	0.00	0.07	–0.20	–0.07	0.22	0.25	–0.25	0.16	–0.13	–0.23	0.05	0.00	0.03	0.01	0.14	1.00																		
CL	0.03	0.13	–0.01	0.09	–0.35	0.21	0.31	0.37	–0.31	0.12	–0.21	–0.24	0.16	0.25	–0.04	0.21	0.34	0.42	1.00																	
RBC	–0.07	–0.23	0.00	–0.10	0.41	–0.05	–0.11	–0.31	0.10	0.06	–0.08	–0.08	–0.08	–0.02	0.32	–0.26	–0.22	–0.14	–0.33	1.00																
HGB	0.26	0.13	0.26	–0.21	0.17	0.28	–0.07	0.09	0.11	0.03	–0.02	–0.01	0.07	0.02	0.15	–0.13	–0.02	–0.05	–0.11	0.27	1.00															
HCT	–0.07	–0.23	0.00	–0.10	0.40	–0.06	–0.08	–0.30	0.06	0.08	–0.09	–0.09	–0.07	0.01	0.30	–0.23	–0.19	–0.15	–0.34	0.99	0.28	1.00														
TAWSA	0.14	0.03	0.16	–0.17	0.03	0.23	0.13	0.14	0.00	0.16	0.07	–0.15	0.33	–0.24	–0.03	–0.08	0.22	0.12	–0.02	–0.26	0.09	–0.26	1.00													
CP	0.45	0.13	0.47	–0.40	0.01	0.25	0.16	0.45	–0.08	–0.07	–0.02	0.08	0.11	0.09	–0.01	–0.34	0.33	0.12	0.16	–0.15	0.15	–0.14	0.19	1.00												
TBA-AP	0.11	0.12	0.09	–0.01	0.32	0.14	–0.16	–0.34	0.31	0.12	0.04	–0.06	–0.07	0.09	0.43	0.08	0.13	–0.36	–0.23	0.37	0.23	0.38	–0.09	0.10	1.00											
TBA / CP	–0.23	0.02	–0.27	0.31	0.25	–0.10	–0.17	–0.57	0.32	0.15	0.08	–0.11	–0.20	0.06	0.34	–0.34	–0.19	–0.37	–0.29	0.35	0.07	0.36	–0.25	–0.69	0.60	1.00										
Moisture of breast meat	0.01	–0.23	0.09	–0.25	0.01	0.29	0.17	0.15	0.03	0.18	–0.01	–0.24	0.29	–0.03	0.04	–0.28	0.16	0.07	0.09	0.03	–0.12	–0.01	0.30	0.05	–0.11	–0.15	1.00									
Dry matter of breast meat	–0.01	0.23	–0.09	0.25	–0.01	–0.29	–0.17	–0.15	–0.03	–0.18	0.01	0.24	–0.29	0.03	–0.04	0.05	–0.16	–0.07	–0.09	–0.03	0.12	0.01	–0.30	–0.05	0.11	0.15	–1.0	1.00								
Crude protein of breast meat	0.01	0.25	–0.07	0.23	0.00	–0.26	–0.21	–0.15	0.00	–0.17	0.02	0.28	–0.29	–0.11	0.00	–0.16	–0.13	–0.01	–0.14	–0.04	0.15	–0.01	–0.22	–0.05	0.11	0.13	–0.94	0.94	1.00							
Crude fat of breast meat	–0.04	0.00	–0.05	0.09	–0.02	–0.08	0.07	–0.05	–0.08	–0.08	–0.06	–0.04	–0.03	0.38	–0.13	0.33	–0.11	–0.20	0.09	0.03	–0.04	0.05	–0.24	–0.02	0.05	0.07	–0.26	0.26	–0.09	1.00						
Ash of breast meat	–0.08	–0.01	–0.08	0.12	0.02	–0.35	0.07	0.02	–0.09	0.05	0.06	–0.07	–0.14	0.10	0.02	–0.01	–0.02	0.08	0.14	0.00	–0.04	0.05	–0.19	0.02	–0.01	0.03	–0.54	0.54	0.43	0.17	1.00					
Moisture of thigh meat	0.05	–0.03	0.06	–0.07	–0.50	–0.01	0.08	0.29	–0.07	0.00	0.08	0.01	0.17	–0.18	–0.37	0.25	0.12	0.06	0.07	–0.22	–0.11	–0.22	0.16	–0.01	–0.30	–0.23	0.41	–0.41	–0.35	–0.22	–0.22	1.00				
Dry matter of thigh meat	–0.05	0.03	–0.06	0.07	0.50	0.01	–0.08	–0.29	0.07	0.00	–0.08	–0.01	–0.17	0.18	0.37	–0.25	–0.12	–0.06	–0.07	0.22	0.11	0.22	–0.16	0.01	0.30	0.23	–0.41	0.41	0.35	0.22	0.22	–1	1.00			
Crude protein of thigh meat	–0.06	–0.14	–0.02	–0.06	0.10	–0.02	–0.13	–0.04	–0.06	0.05	–0.02	0.04	–0.07	–0.13	0.01	–0.08	–0.05	0.05	0.06	0.32	0.12	0.33	–0.16	0.24	0.12	–0.12	–0.18	0.18	0.22	–0.12	0.17	–0.26	0.26	1.00		
Crude fat of thigh meat	–0.02	0.12	–0.06	0.13	0.39	–0.02	0.01	–0.22	0.08	0.05	0.09	–0.05	–0.11	0.22	0.33	–0.19	–0.09	–0.09	–0.13	–0.04	0.00	–0.04	–0.01	–0.19	0.17	0.30	–0.23	0.23	0.14	0.29	0.07	–0.69	0.69	–0.47	1.00	
Ash of thigh meat	–0.03	–0.02	–0.02	–0.03	–0.20	0.15	–0.14	–0.03	0.11	–0.13	–0.12	0.06	0.16	0.03	–0.09	0.08	–0.07	–0.12	0.02	–0.10	0.13	–0.09	0.10	–0.08	–0.05	–0.01	–0.06	0.06	0.05	0.00	0.13	–0.07	0.07	0.07	–0.03	1.00

Red color indicates statistically significant values at $p < 0.05$; green color indicates positive (high and average) relations of the indicators, blue color indicates negative (high and average) relations of the indicators.

high between TP and GLB (r = 0.96), medium between TP and ALB (r = 0.57), weak correlation — between ALB and GLB (r = 0.32). The average correlation was established between the protein metabolism indicators with CHOL, the blood content of macro- and microelements (Ca, Ca/P, Mg, I) and between them. There was a negative average relationship between ALP and ALB (r = –0.42). The existing positive relationship (r = 0.99) between RBC and HCT was confirmed. Stress indicators had negative mean relationships with biochemical indices: CP and A/G (r = –0.40). TBA/CP and TRIG (r = –0.57). TBA-AP was positively correlated with blood CREA (r = 0.32). There were positive correlations between TBA-AP and TBA/CP with CHOL (r = 0.31 and r = 0.32, respectively). Stress and antioxidant protection indicators point to a negative effect on protein metabolism, accumulation of lipid peroxidation products during intensive growth of poultry. Correlations between the meat chemical composition and blood biochemical parameters were not as pronounced. The average correlation (r = 0.50) was established between dry matter of thigh meat and CREA. Crude protein (r = 0.94) and ash (r = 0.41) increased with increasing dry matter content of breast meat. Dry matter content of thigh meat had a high positive correlation with crude fat (r = 0.69).

Blood parameters and chemical composition of meat depending on the weight of poultry at slaughter

Crude protein of thigh muscle decreased with increasing slaughter weight (p = 0.019) against the backdrop of a trend towards increasing moisture content in thigh meat (p = 0.058) (Table 6, Figure 2).

Table 6. Metabolic and hematological indicators, meat chemical composition of roosters (CORN × RW)

Parameter	Group (by BW)						*p* -value
	1,000–1,799 g		1,800–2,099 g		2,100–2,650 g		
	n = 31		*n* = 28		*n* = 36		
	M	m	M	m	M	m	
TP (g/L)	36.28	0.85	34.29	0.63	34.79	0.65	0.136
ALB (g/L)	13.01	0.26	13.06	0.19	13.14	0.14	0.89
GLB (g/L)	23.27	0.74	21.23	0.48	21.66	0.57	0.052
ALB / GLB	0.57	0.02	0.62	0.01	0.62	0.01	0.038
CREA (μmol/ L)	30.95	0.65	32.95	1.43	30.87	0.57	0.196
GlU (mmol/L)	15.51	0.27	14.44	0.26	14.62	0.29	0.018
TBIL (μmol/ L)	0.78	0.06	0.70	0.05	0.74	0.05	0.574
TRIG (mmol/L)	0.45	0.05	0.34	0.03	0.38	0.03	0.092
CHOL (mmol/L)	3.45	0.09	3.47	0.07	3.44	0.06	0.958
ALT (IU /L)	6.96	0.41	7.49	0.37	7.58	0.33	0.435
AST (IU /L)	202.44	5.27	228.98	7.08	229.89	6.62	0.003
AST/ALT	31.93	2.16	32.33	1.79	31.73	1.26	0.969
ALP (IU /L)	1091.13	92.01	943.39	30.86	973.11	31.90	0.172
Ca (mmol/L)	2.90	0.04	2.77	0.05	2.70	0.04	0.006
P (mmol/L)	2.10	0.06	2.04	0.09	1.96	0.04	0.282
Ca / P	1.42	0.05	2.58	1.27	1.39	0.03	0.351
Mg (mmol/L)	1.01	0.03	0.93	0.03	0.94	0.02	0.051
I (mmol/L)	20.14	0.55	20.80	0.86	20.34	0.66	0.793
CL (mmol/L)	113.85	0.89	111.58	0.73	113.24	0.60	0.096
RBC (10^{12}/L)	3.43	0.12	3.29	0.11	3.16	0.12	0.262
HGB (g/L)	108.95	2.93	108.68	1.36	108.09	3.15	0.972
HCT (%)	48.05	1.72	45.40	1.61	43.83	1.79	0.202
TAWSA (mg/L)	38.58	1.87	38.99	1.98	41.48	1.50	0.421
CP (mg/L)	43.23	2.15	38.32	1.79	40.42	1.60	0.186
TBA-AP(μmol/L)	2.76	0.15	2.65	0.10	2.57	0.11	0.521
TBA / CP	0.07	0.00	0.07	0.00	0.07	0.00	0.445
Moisture of breast meat (%)	73.89	0.18	73.83	0.14	73.67	0.15	0.556
Dry matter of breast meat (%)	26.11	0.18	26.17	0.14	26.33	0.15	0.556
Crude protein of breast meat (%)	23.89	0.18	24.06	0.13	24.11	0.13	0.527
Crude fat of breast meat (%)	1.06	0.05	0.94	0.05	1.02	0.05	0.259
Ash of breast meat (%)	1.17	0.01	1.17	0.02	1.20	0.01	0.102
Moisture of thigh meat (%)	73.80	0.16	74.14	0.13	74.24	0.12	0.058
Dry matter of thigh meat (%)	26.20	0.16	25.87	0.13	25.76	0.12	0.058
Crude protein of thigh meat (%)	21.73	0.13	21.23	0.14	21.39	0.11	0.019
Crude fat of thigh meat (%)	3.36	0.20	3.54	0.16	3.32	0.13	0.608
Ash of thigh meat (%)	1.10	0.01	1.10	0.01	1.11	0.01	0.730

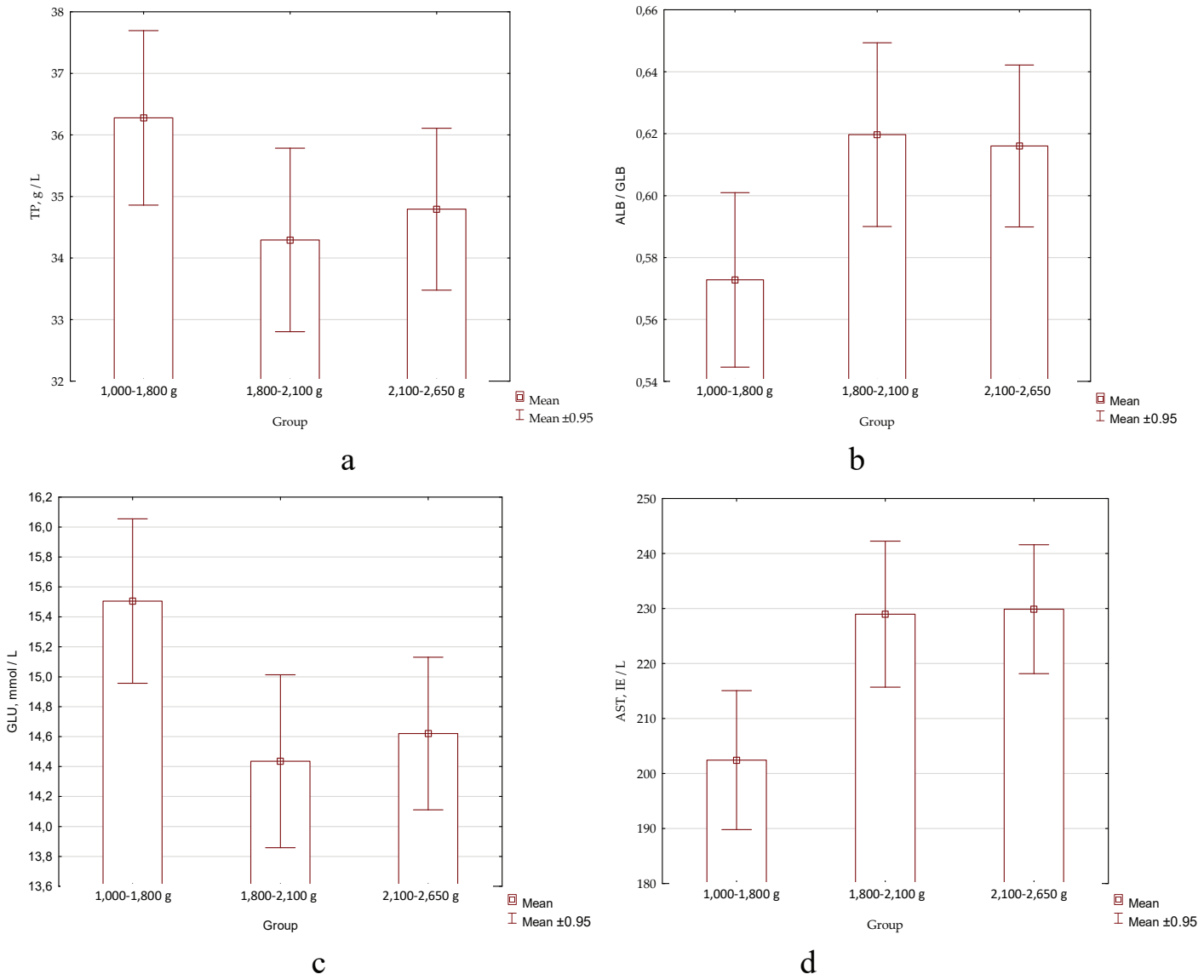

Figure 1. Relationship of indicators and their comparison among poultry groups with different weights (a — GLB, p = 0.052; b — A/G, p = 0.038; c — GLU, p = 0.017; d — AST, p = 0.003). Standard errors of the mean are calculated using the pooled ANOVA variance

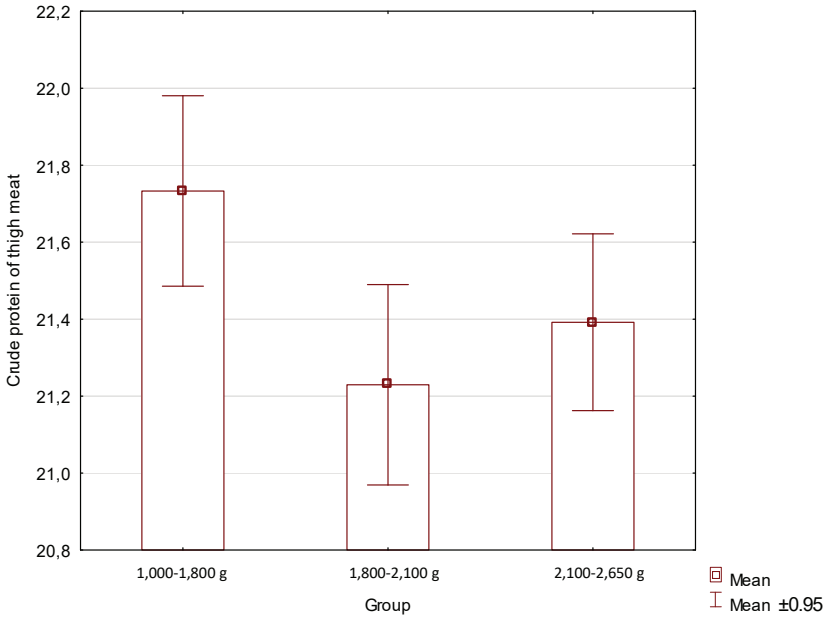

Figure 2. Crude protein of thigh meat in roosters as a function of weight. Standard errors of the mean are calculated using the pooled ANOVA variance

Comparative assessment of blood parameters and chemical composition of meat depending on the weight of poultry at slaughter

Table 7. Correlations of weight with blood parameters and meat chemical composition

Parameter	Group (by BW)		
	1,000–1,800 g	1,800–2,100 g	2,100–2,650 g
	n = 31	n = 28	n = 36
ADG	1.000	0.998	1.000
TP	–0.115	0.144	0.115
ALB	0.421	0.110	–0.146
GLB	–0.278	0.144	0.167
ALB / GLB	0.471	–0.113	–0.217
CREA	0.324	–0.124	–0.088
GlU	–0.037	–0.333	–0.077
TBIL	–0.212	0.048	–0.091
TRIG	–0.194	–0.080	0.174
CHOL	0.154	0.165	–0.241
ALT	–0.075	–0.178	0.020
AST	0.302	0.208	0.392
AST/ALT	0.225	0.179	0.258
ALP	–0.460	–0.166	0.052
Ca	–0.251	0.246	–0.208
P	0.498	–0.004	0.112
Ca / P	–0.662	–0.092	–0.300
Mg	–0.292	–0.238	–0.007
I	–0.205	–0.096	0.197
CL	–0.345	0.061	–0.032
RBC	–0.161	–0.308	0.128
HGB	0.121	–0.039	–0.095
HCT	–0.178	–0.288	0.134
TAWSA	–0.153	–0.205	–0.029
CP	–0.060	0.159	0.023
TBA-AP	0.246	0.026	–0.204
TBA / CP	0.337	–0.096	–0.139
Moisture of breast meat	–0.632	–0.069	0.191
Dry matter of breast meat	0.632	0.069	–0.191
Crude protein of breast meat	0.676	–0.060	–0.167
Crude fat of breast meat	–0.236	0.332	–0.165
Ash of breast meat	0.185	0.102	0.098
Moisture of thigh meat	–0.586	0.118	0.135
Dry matter of thigh meat	0.586	–0.118	–0.135
Crude protein of thigh meat	–0.160	–0.042	0.090
Crude fat of thigh meat	0.575	–0.078	–0.034
Ash of thigh meat	–0.086	0.300	–0.221

Red color indicates statistically significant values at $p < 0.05$

The main changes concerned the differences in protein metabolism (Figure 3–5) in low-weight (1,000–1,800 g) roosters. They were associated with different responses of birds to environmental conditions (feeding, stress, etc.).

Serum TP decreased with increasing ADG in the 1,000–1,799 g group (Figure 3), due to a GLB fraction decrease ($p = 0.052$, Table 6, Figure 5). It is shown by the ALB / GLB ratio too ($p = 0.038$, Table 6). The protein metabolism pattern is significantly different in the 2,100–2,650 g group (Figure 3–5). At almost the same serum TP level, there was a GLB fraction increase.

Cross-breeding is important for the development of new breeds and for the production of commercial poultry superior in performance and viability to purebred parental forms. The study of metabolic parameters in relation to meat quality carried out in this work is important to form approaches to obtaining poultry with improved/maintained quality parameters of the parent breeds and to understand the biochemical processes that determine the possible use of feed and production of a given quality.

Maintaining genetic diversity in farm animal and poultry populations has not lost its relevance in recent years [10]. To obtain the effect of heterosis in crossbreeding, birds with genetically determined traits of high productivity are used for the desirable combination and consolidation in the offspring. This is achieved if breeds, lines and individual animals tested for good compatibility with each other are used in crossbreeding. Our studies have allowed us to establish values of blood biochemical parameters in the body of hens when crossing birds of Russian white breed and Cornish. The Russian White breed belongs to the egg production direction; it was bred in the USSR by crossing White Leghorn cocks with local "outbred" hens [11]. The birds of this breed are characterized by high safety (91–96%), well developed and feathered wings, broad chest and back. The live weight of hens is about 1.8 kg, ales 2.3 kg [12]. The Cornish breed is a meat-producing bird based on the Malay and English fighting hens with a red Aseel hen. The bird is short in stature, with a strong and well-proportioned body in front, a large breast and a long back. The meat of the Cornish is tender and tasty, the weight of an adult hen reaches 2.75–3.25 kg, a rooster 3.75–4.5 kg.

The high coefficients of variability in biochemical indices established in our work indicate the cleavage of traits during crossing. Against the background of the heterosis effect, the distribution of phenotypic manifestations increases. There is a need for markers to trace and consolidate the desired effect in productivity, in particular metabolic indicators in the body.

Previously obtained data from biochemical studies have significant differences. This is due to different genetic, feed conditions and environmental factors. Thus, Kaiser J. C. et al. established reference values of biochemical parameters in domestic chickens of different breeds [2]. The results obtained in poultry at different breed combinations and at different age and physiological periods should be further studied, as direct comparison with available data is often incorrect.

Biochemical blood values reflect metabolic processes and depend on many factors, including housing conditions [13]. For example, in a study of biochemical processes in the body of yellow Wannan chickens, it was found that higher levels of CHOL and TRIG closely related to fat deposition were observed in the blood of non-pecked

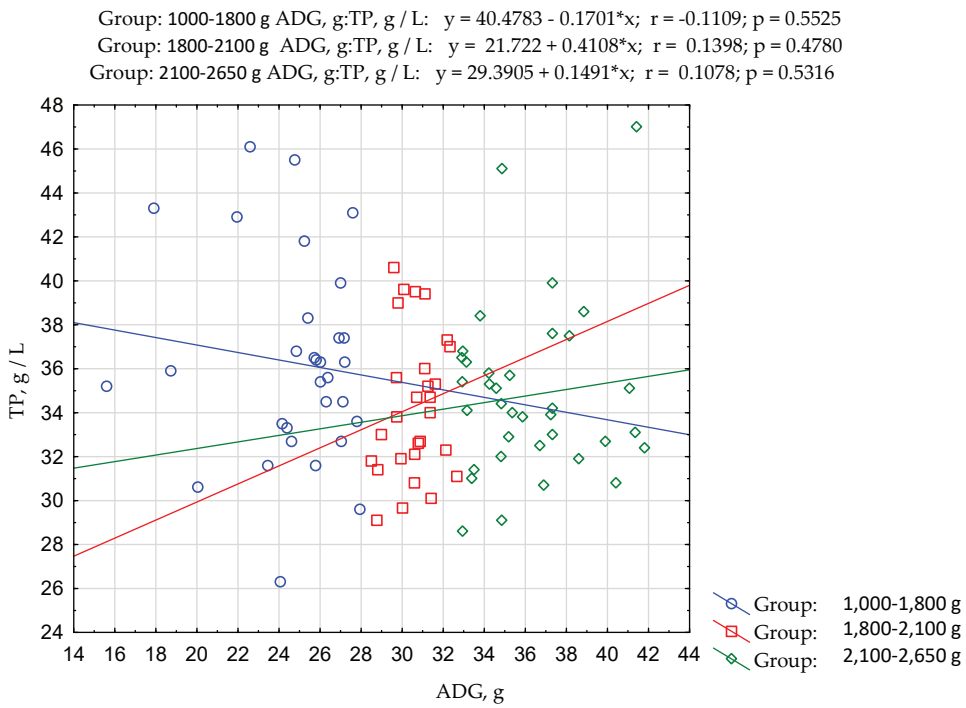

Figure 3. Categorical diagram of the relationship between serum TP and ADG in groups with different weights

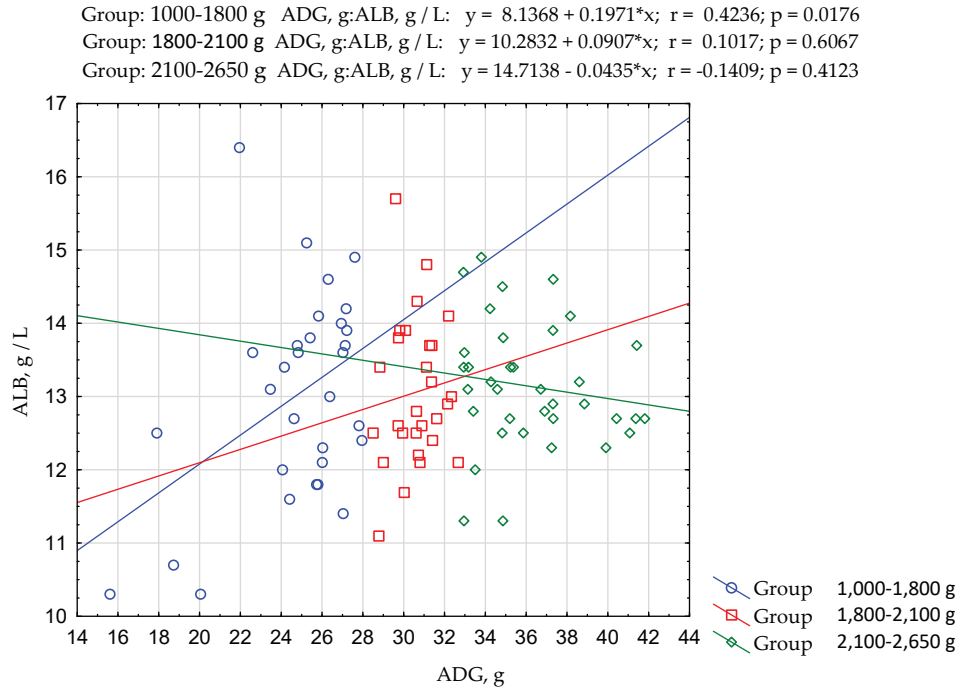

Figure 4. Categorical diagram of the relationship between serum ALB and ADG in groups with different weights

birds [14]. It was found that the activity of three enzymes (lactate dehydrogenase, aspartate aminotransferase and gamma-glutamyltransferase) was increased in the blood when the density increased above the standards (up to 25.3 birds/m^2). Further overpopulation of chickens up to 26.7 birds/m^2 is accompanied by increased serum glucose and creatinine levels, decreased calcium to phosphorus ratio, confirmed by increased alkaline phosphatase activity [15].

In our study of biochemical parameters, we found that TRIG and TBIL had the greatest variation (> 50%) (Table 1). It has been reported that TBIL increases after a long period of exercise due to accelerated erythrocyte destruction induced by exercise stress [16]. Lipolysis in muscle and adipose tissue and TRIG synthesis in the liver are increased due to reduced oxidative capacity of fat utilization during exercise. TRIGs also play an important role in replenishing intramuscular fat. Lipid metabolism is known to be one of the most important parts of adaptation, including the stress-releasing mechanism in birds. In stress-sensitive birds, compared to stress-resistant birds, there is a more pronounced increase in TRIG and CHOL concentrations due to the predominance of cholesterol included in very low density lipoproteins and a decrease in cholesterol included in low and high density lipoproteins [17]. ALP is involved in phosphoric acid metabolism, breaking it down

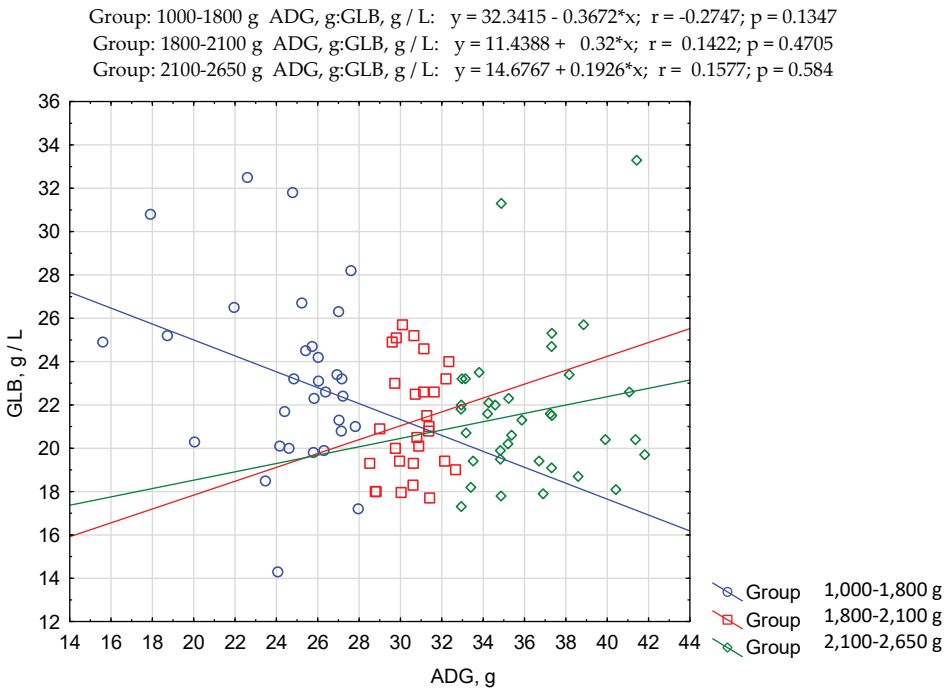

Figure 5. Categorical diagram of the relationship between serum GLB and ADG in groups with different weights

from organic compounds and contributes to phosphorus transport in the body, it affects bone growth, so its content is higher in intensively growing organisms. In turn, we have found that ALP, ALT and CHOL also had a high scatter of values in the studied livestock. In connection with the fact that these parameters (especially TRIG and TBIL) can be markers of the birds' condition, including their reaction to stress, we believe that the established differences should be considered in further work with poultry and in selecting them for further work based on the values of these biochemical parameters.

Carbohydrate metabolism is the key in energy metabolism in poultry [18]. During prolonged exercise, insulin sensitivity and glucose uptake increase, leading to a decrease in blood glucose levels, even if they remain at physiological levels [19]. According to our data, GLU had an average range of values, which generally corresponded to normal values, confirming that the birds were under standard rearing conditions, while the crossed birds, in addition to the effect of heterosis, had a high range of values for individual stress markers, indicating the display of susceptibility of individuals to environmental and nutritional conditions. This is also evidenced by the increased heterogeneity of antioxidant defence indicators (> 20%). The AOS data should also be taken into account when selecting birds for further work, as this may serve as an important factor in selecting birds with the best adaptogenic properties.

The TP level in the blood of the animals we studied was 35.13 g/l, GLB was 13.07 g/l. These indicators of protein metabolism differ greatly from the results of Fedorova et al. [20]. The authors studied adult Pushkin breed chickens (combined direction of productivity). According to the authors, these values were 52.59 and 34.64 g/l, respectively. The level of CREA, according to the authors, was 62.8 μmol/l, which is almost 2 times higher than in our study (31.51 μmol/l). This difference is due to both genetic differences and differences in the age of the poultry and once again confirms the need for separate studies for poultry of different breeds and combinations, as well as the direction of productivity and age.

Our biochemical results are close to those of the experiment on Ross × Ross 308 broiler chickens at 35 days of age, except for AST [21]. The AST activity in broiler chickens was 328 U/L. The mean value of the AST activity in our results was 220.66 IU/l.

Our work has established high positive correlations between indicators of protein, carbohydrate, fat and mineral metabolism, indicating a high degree of correlation between the studied parameters (Table 5). Of particular interest is the study of correlations between biochemical blood parameters and stress indicators. Stress markers had negative mean associations with biochemical parameters in our studies: CP and A/G (r = –0.40). TBA/CP and TRIG (r = –0.57). The TBA/CP ratio indicates a conjugation of lipid peroxidation and antioxidant defence. An increase in this index points to a decrease in the level of antioxidant protection and an increase in the synthesis of stress hormones.

TBA-AP was positively correlated with blood levels of CREA (r = 0.32). CP levels were negatively correlated with A/G. This may be due to the fact that decreased antioxidant protection leads to increased synthesis and secretion of corticoid hormones, as well as protein catabolism, and consequently to increased albumin levels, which determine A/G. An increase in TAS levels may lead to an increase in albumin and total serum protein. The weak positive correlations detected between TBA-AP and TBA/CP with CHOL (r = 0.31 and r = 0.32, respectively) are consistent with results obtained previously by researchers [22].

A significant correlation between serum biochemical indices and meat quality of farm animals has been reported previously. Serum biochemical indices determine the animal's resistance strength and oxygen transport and have a significant influence on growth intensity and metabolic specificity [23,24].

The study of the chemical composition of meat from the poultry stock we studied showed that these parameters had less heterogeneity, which allows us to characterize a more stable fixation of these traits in the production of offspring. Against this background, weak correlations were found between the chemical composition of meat and biochemical blood parameters. A medium correlation ($r = 0.50$) was found between the dry matter of thigh meat and serum CREA levels. The raw protein ($r = 0.94$) and ash content ($r = 0.41$) increased with increasing dry matter of thigh meat. Dry matter of thigh meat had a high positive correlation with the crude fat content ($r = 0.69$). CREA is an indicator of energy metabolism and is related to live weight of animals and poultry. This fact is probably the reason for the positive correlation between dry matter of thigh meat and serum CREA and in the future this parameter can be taken into account when predicting meat quality and when selecting poultry.

Studies by other authors have described the influence of factors on poultry meat quality, including the effect of the season of the year [5]. The influence of some biochemical indicators (stress markers) on poultry meat quality is shown in [25]. Different blood metabolites (stress biomarkers) and meat quality are evaluated in [26]. A correlation between serum biochemical indices and meat quality attributes based on pH, meat color and a number of other parameters has recently been reported [27]. The correlation between meat quality and serum biochemical indices has been studied in [28]. Albumin and serum water-holding capacity, serum somatotropin and pH1 (45–60 min after slaughter) were significantly and positively correlated with each other [29].

Thus, it is necessary to take into account correlations characterizing the interdependence of biochemical processes with quality parameters of meat, while expanding the range of studied parameters, including stress and AOS markers.

We have assessed blood biochemical parameters characterizing nitrogen, carbohydrate-lipid and mineral metabolism, antioxidant protection, hematological parameters (RBC, HCT, HGB), chemical composition of breast and thigh of 63-day-old cockerels ($n = 95$) depending on slaughter live weight. There were significant changes in the blood values (Table 6, Figure 1). A/G ($p = 0.038$) increased in animals with increasing slaughter weight. AST ($p = 0.003$); GLB ($p = 0.052$), GLU ($p = 0.018$), Ca ($p = 0.006$), Mg ($p = 0.051$) levels decreased. There was a downward trend in serum TRIG ($p = 0.092$), CL ($p = 0.096$). These figures indicate the important role of the study of stress tolerance in poultry and the peculiarities of the indication of the normal course of biochemical processes.

Analysis of the relationship between slaughter weight and blood parameters and the chemical composition of meat shows significant ($p < 0.05$) correlations mainly in the group of roosters with the low slaughter weight (1,000–1,800 g) (Table 7). Positive moderate correlations were observed between weight and protein metabolism, P, dry matter of breast and thigh meat, crude protein of breast meat, and crude fat of thigh meat. Negative correlations were observed between slaughter live weight and ALP, Ca/P. Against the background of low weight gain and increased protein content in meat, there was a decrease in blood ALB/GLB ratio and an increase in ALP (Tables 6, 7). Thus, these indicators can serve as markers for evaluating poultry growth.

The decrease in body weight was primarily characterised by differences in protein metabolism (Figures 3–5) in the group of roosters with the low body weight (1,000–1,799 g) related to the different responses of the birds to environmental conditions (feeding, housing, possible stress, etc.). In the group of animals with maximum slaughter weight, a significant positive correlation was observed between the live weight and serum AST activity. The increased activity of these enzymes may indicate activation of protein and amino acid metabolism, increased load on the liver and cardiovascular system [30]. The poultry live weight increases the load on these important functions and systems, causing an increase in the serum AST activity. Previously, ALT and ALB levels have been found to be of practical importance in predicting carcass quality in animals on the day of slaughter. ALB levels were moderately positively correlated with the live weight, hot carcass weight, cold carcass weight and dorsal fat thickness. Serum ALT levels were moderately positively correlated with the live weight, hot carcass weight and cold carcass weight [31].

Conclusion

Our study reaffirmed the importance of studying an extended range of biochemistry parameters (including AOS and stress markers) and in the relationship with meat quality parameters and growth intensity, which can serve as a basis for predicting growth parameters and as additional criteria for selecting poultry with given productivity parameters.

The metabolic status ($N = 95$), comparison of the biochemical blood indices characterizing the nitrogen, carbohydrate-lipid and mineral metabolism, antioxidant protection, some clinical blood indices (hemoglobin, erythrocytes, hematocrit), chemical composition of the breast and thigh meat of cockerels (♂ RW × CORN) at the age of 63 days have been analyzed. High positive correlations between the indices of protein, carbohydrate, fat and mineral metabolism have been established, indicating a high degree of interrelation and characterized in general by the commonly known principles. Correlations between biochemical parameters of protein, carbohydrate and lipid metabolism and stress markers have been established (first

of all, attention should be paid to protein metabolism parameters, but also to CHOL, TRIG and TBIL).

At the current stage of research, no highly significant links have been found between biochemical blood values and the chemical composition of meat. This indicates the importance of searching for additional markers for *in vivo* evaluation of the composition and quality of poultry products. Correlations have been established between cockerel body weight, blood parameters (TP, ALB/GLB, CREA, ALP, Ca/P and others) and the chemical composition of meat (primarily protein and fat content) in the poultry group with a slaughter weight of 1,000–1,799 g.

In the future, it is planned to expand the range of studying the relationships between biochemical, antioxidant, hormonal blood parameters, expression of antioxidant protection and immunity genes with regard to meat quality of modern chicken breeds to obtain new knowledge about the genetic determination of productivity traits. Development of express methods of predicting the biochemical composition of poultry products and health status of poultry based on extended analysis of blood biochemical composition is one of the priority tasks of practical approbation of our research in the future.

REFERENCES

1. Fisinin, V.I. (2019). World and Russian poultry farming: realities and challenges of the future. Moscow: Khlebprodinform, 2019. (In Russian)
2. Kaiser, J.C., Reider, H., Pabilonia, K.L., Moore, A.R. (2022). Establishment of biochemical reference values for backyard chickens in Colorado (*Gallus gallus domesticus*). *Veterinary Clinical Pathology*, 51(4), 577–584. https://doi.org/10.1111/vcp.13136
3. Board, M.M., Crespo, R., Shah, D.H., Faux, C.M. (2018). Biochemical reference intervals for backyard hens. *Journal of Avian Medicine and Surgery*, 32(4), 301–306. https://doi.org/10.1647/2017-310
4. Toghyani, M., Toghyani, M., Gheisari, A., Ghalamkari, G., Mohammadrezaei, M. (2010). Growth performance, serum biochemistry and blood hematology of broiler chicks fed different levels of black seed (*Nigella sativa*) and peppermint (*Mentha piperita*). *Livestock Science*, 129(1–3), 173–178. https://doi.org/10.1016/j.livsci.2010.01.021
5. Liang, F., Yan, L., Li, Y., Jin, Y., Zhang, J., Che, H. et al. (2022). Effect of season on slaughter performance, meat quality, muscle amino acid and fatty acid composition, and metabolism of pheasants (*Phasianus colchicus*). *Animal Science Journal*, 93(1), Article e13735. https://doi.org/10.1111/asj.13735
6. Cruz, F.L., Saraiva, L.K.V., Silva, G.E., Nogueira, T.M., Silva, A.P., Faria, P.B. (2018). Growth and carcass characteristics of different crosses of broiler chickens reared underan alternative system. *Ciencias Agrarias,* 39(1), 317–328. https://doi.org/10.5433/1679-0359.2018v39n1p317
7. Fisinin, V.I., Egorov, I.A., Draganov, I.F. (2011). Feeding poultry. Moscow: GEOTAR-Media, 2011. (in Russian)
8. Kondrakhin, N.P. (2004). Methods of veterinary clinical laboratory diagnostics. Moscow: Kolos, 2004. (In Russian)
9. Voronina, O.A., Savina, A.A., Bogolyubova, N.V., Zaitsev, S.Y. (2019). The total amount of water-soluble antioxidants in the blood serum of productive animals. *Veterinary, Animal Science and Biotechnology*, 12, 75–78. https://doi.org/10.26155/vet.zoo.bio.201912012 (In Russian)
10. Tyshchenko, V.I., Mitrofanova, O.V., Dementieva, N.V., Terletsky, V.P., Novikova, O.B. (2018). Molecular genetic assessment of diversity in populations of Cornish and Russian White chickens. *Modern Research and Development*, 9(26), 394–398. (In Russian)
11. Ernst, L.K., Dmitriev, N.G., Paronyan, I.A. (1994). Russian White. Genetic resources of agricultural animals in Russia and neighboring countries. All-Russian Research Institute of Genetics and Breeding of Farm Animals: St. Petersburg, 1994. (In Russian)
12. Vetokh, A.N., German, N. Yu. (2022). Chicken egg incubation results and growth rate of crossbreed chickens. *Agrarian Science*, 355(1), 53–57. https://doi.org/10.32634/0869-8155-2022-355-1-53-57. (In Russian)
13. Zhang, C., Ah Kan Razafindrabe, R.-H., Chen, K., Zhao, X., Yang, L., Wang, L. et al. (2018). Effects of different rearing systems on growth performance, carcass traits, meat quality and serum biochemical parameters of Chaohu ducks. *Animal Science Journal*, 89(4), 672–678. https://doi.org/ 10.1111/asj.12976
14. Jin, S., Fan, X., Yang L., He, T., Xu, Y., Chen, X. et al. (2019). Effects of rearing systems on growth performance, carcass yield, meat quality, lymphoid organ indices, and serum biochemistry of Wannan Yellow chickens. *Animal Science Journal*, 90(7), 887–893. https://doi.org/10.1111/asj.13220
15. Osadcha, Yu.V., Sakhatsky, M.I., Kulibaba, R.O. (2021). Serum clinical biochemical markers of Hy-Line W-36 laying hens under the influence of increased stocking densities in cages of multilevel batteries. *Regulatory Mechanisms in Biosystems*, 12(3), 425–429. https://doi.org/10.15421/022158
16. Allaam, M.A., Elseady, Y., Nayel, M.H., Elsify, A., Salama, A., Hassan, H.Y. et al. (2014). Physiological and hemato-chemical evaluation of thoroughbred race horse after exercise. *International Journal for Agro Veterinary and Medical Sciences*, 8, 81–93.
17. Lu, Z., He, X.F., Ma, B.B., Zhang, L., Li, J.L., Jiang, Y. et al. (2019). Increased fat synthesis and limited apolipoprotein B cause lipid accumulation in the liver of broiler chickens exposed to chronic heat stress. *Poultry Science,* 98, 3695–3704. https://doi.org/10.3382/ps/pez056
18. Sereda T. I., Derho M. A. (2011). Carbohydrates metabolism in laying hen of the "Lomann-Beliy" cross. *Izvestia Orenburg State Agrarian University*, 3(31), 334–337. (In Russian)
19. Miglio, A., Cappelli, K., Capomaccio, S., Mecocci, S., Silvestrelli, M., Antognoni, M.T. (2020). Metabolic and biomolecular changes induced by incremental long-term training in young thoroughbred racehorses during first workout season. *Animals (Basel)*, 10, Article 317. https://doi.org/10.3390/ani10020317
20. Fedorova, Z.L., Perinek, O. Yu. (2020). Biochemical indicators of blood of meat and egg chickens breeds in postnatal ontogenesis. *Proceedings of Lower Volga Agro-University Complex: Science and Higher Education,* 4(60), 253–262. https://doi.org/10.32786/2071-9485-2020-04-25 (In Russian)
21. Scott, A., Vadalasetty, K.P., Łukasiewicz. M., Jaworski, S., Wierzbicki, M., Chwalibog, A. et al. (2017). Effect of different levels of copper nanoparticles and copper sulphate on performance, metabolism and blood biochemical profiles in broiler chicken. *Journal of Animal Physiology and Animal Nutrition*, 102(1), e364-e373. https://doi.org/10.1111/jpn.12754
22. Keskinet, S., Berberoğlu, E., Sarıcaal, Ş. (2018). Examination of relationships between some biochemical and oxidative stress traits by canonical correlation analysis in broiler chickens. *Turkish Journal of Agriculture -Food Science and Technology*, 6(3), 255–259. https://doi.org/10.24925/turjaf.v6i3.255-259.1403
23. Anassori, E., Dalir-Naghadeh, B., Pirmohammadi, R., Hadian, M. (2015). Changes in blood profile in sheep receiving raw garlic, garlic oil or monensin. *Journal of Animal Physiology and Animal Nutrition*, 99(1), 114–122. https://doi.org/10.1111/jpn.12189
24. Lothong, M., Tachampa, K., Assavacheep, P., Angkanaporn, K. (2016). Effects of dietary betaine supplementation on back fat thickness and serum IGF-1 in late finishing pigs. *The Thai Journal of Veterinary Medicine*, 46(3), 427–434.
25. Xing, T., Gao, F., Tume, R. K., Zhou, G., Xu, X. (2018). Stress effects on meat quality: A mechanistic perspective. *Comprehensive Reviews in Food Science and Food Safety*, 18(2), 380–401. https://doi.org/10.1111/1541-4337.12417
26. Nelis, J.L.D., Bose, U., Broadbent, J.A., Hughes, J., Sikes, A., Anderson, A. et al. (2022). Biomarkers and biosensors for the diagnosis of noncompliant pH, dark cutting beef predisposition, and welfare in cattle. *Comprehensive Reviews in Food Science and Food Safety*, 21(3), 2391–2432. https://doi.org/10.1111/1541-4337.12935
27. Yu, J., Liu, G., Zhang, J., Zhang, C., Fan, N., Xu, Y. et al. (2021). Correlation among serum biochemical indices and slaughter traits, texture characteristics and water-holding capacity of Tan sheep. *Italian Journal of Animal Science*, 20(1), 1781–1790. https://doi.org/10.1080/1828051X.2021.1943014

28. Deng, L.J., Guo, Z.B., Bao, S.K., Han, L., Yu, Q.L. (2013). Correlation between meat quality and serum biochemical indices of yak. *Journal of Food Science*, 34(17), 57–60. https://doi.org/10.7506/spkx1002–6630–201317013 (In Chinese)
29. Yuan, J., Han, L., Wang, X.Y., Wang, Q. (2009). Study on correlations of meat quality with serum biochemical indexes of the silky fed by medlar. *Science and Technology of Food Industry*, 4, 116–121. (In Chinese)
30. Kudrin, A.G. (2006). Blood enzymes and forecasting the productivity of dairy cattle. Michurin: Publishing House of Michurinsk State Agrarian University, 2006. (In Russian)
31. Čobanović, N., Stanković, S.D., Dimitrijević, M., Suvajdžić, B., Grković, N., Vasilev, D. et al. (2020). Identifying physiological stress biomarkers for prediction of pork quality variation. *Animals*, 10(4), Article 614. https://doi.org/doi:10.3390/ani10040614

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SALMONELLA ENTERICA SPECIES ISOLATED FROM LOCAL FOODSTUFF AND PATIENTS SUFFERING FROM FOODBORNE ILLNESS: SURVEILLANCE, ANTIMICROBIAL RESISTANCE AND MOLECULAR DETECTION

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Keywords: *foodborne pathogen, frozen chicken meat, azithromycin resistance gene mphA.*

Abstract

The aim of this study was to determine the prevalence of Salmonella enterica in raw chicken meat, eggs, and ready-to-eat foods containing poultry products and among patients suffering from diarrhea as a result of ingestion of this foodborne pathogen in Baghdad, Iraq. It assesses the antibiotics susceptibility, virulence and pathogenicity of S. enterica isolates. Thirteen Salmonella spp. isolates from foodstuff and seven from clinical patients were recovered from 80 and 20 samples, respectively. Isolates from foodstuff samples displayed the highest resistance to nalidixic acid (69.23%), followed by chloramphenicol (53.84%). Salmonella spp. isolated from clinical samples showed resistance to both azithromycin and cefotaxime at the same percentage level (71.42%). The results of antibiotic resistance gene amplification (gyrA, mphA) were analyzed and showed that these genes were present in 100% and 50% of phenotypically resistant isolates, respectively. Virulence genes invA, avrA, and sipB were found on average in 86% of food isolates, accounting for 69.2%, 92.3%, and 95%, respectively. In addition, the detection of these virulence genes among clinical isolates showed their presence at the same level (85.7%). Our study revealed that unhygienic chicken slaughterhouses and lack of food safety management are strong indicators of a high probability of the Salmonella presence in our food products in the Iraqi markets.

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Introduction

The World Health Organization (WHO) has stated that foodborne diseases remain a significant problem and can show severity among children, the elderly, and people with immunosuppression [1]. More than 250 different foodborne illnesses are caused by various microbial pathogens and toxins [2], poisonous chemicals, or bio-toxins. *Salmonella* infections are a critical epidemiological and economic problem worldwide [4,5]. For example, the European Food Safety Authority (EFSA) reports that *Salmonella* spp. is recognized to be the second most common cause of human diseases and food poisoning associated with contaminated food [6]. *Salmonella* is a genus of gram-negative bacteria that belongs to the *Enterobacteriaceae* family and can infect various animal hosts [7]. Moreover, *Salmonella* can survive under harsh conditions for about a year in frozen meat [8]. *Salmonella* exists in different environments such as soil, water systems, sewage, and the gut flora of several animals [9]. The members of the genus *Salmonella* are categorized on the basis epidemiology, host range, biochemical reactions, and structures of the O, H, and Vi antigens [10]. *Salmonella*, according to their DNA relativeness, can be classified into two species; *S. bongori*, which populates cold-blooded animals, and *S. enterica*, which is able to inhabit both cold and warm-blooded hosts [11]. Strains belonging to *S. enterica* subsp. *enterica* are responsible for almost 99% of *Salmonella* infections in people and warm-blooded animals [12].

Antimicrobial drugs are utilized to prevent microbial infectious diseases in both humans and animals [7] as well as in animal feed for prophylaxis, therapeutics, and growth promotion [13]. The antimicrobial resistance of *Salmonella*, particularly multidrug resistance (MDR), has become a significant worldwide problem [14]. For example, more recently, *S. enterica* in food-producing animals has shown high MDR resulting in a global problem due to the widespread use of antibiotic drugs [13]. The excessive use of the same antibiotic drugs as a treatment in clinical and veterinary medicine may lead to emergence of resistant strains that can easily be transmitted to the human population from animal products, which is a serious public health problem/concern [15–17]. In Iraq, there is currently a widespread lack of food safety aspects including domestic production of poultry and its products, as well as restaurants and this is leading to the increased risk of exposure to *Salmonella* infection. In Baghdad and central

Iraq, non-typhoidal *Salmonella* was indicated as the second cause of gastroenteritis in children after enteric viruses [18]. Non-typhoidal *Salmonella* was recovered from 10.3% of diarrheal stool samples from children under the age of 5 years in a recent study in southern Iraq [18]. To our knowledge, there have been no published studies on the molecular epidemiology of non-typhoidal *Salmonella* isolated from local hens in Iraq. Given the importance of this pathogen to worldwide health, the current study presents an evaluation of the molecular detection of virulence and antibiotic resistance genes of *Salmonella enterica* from Iraqi foodstuff. We focused on three virulence genes of the SPI-1 region involved in *Salmonella* pathogenicity (*avr*A, *inv*A, and *sip*B), which play a critical role in the initial invasion of the host organism and cause salmonellosis infection as previously mentioned in [19–21]. Therefore, the aim of this study was to determine the prevalence of *Salmonella enterica* in raw chicken meat, eggs, and ready-to-eat foods containing poultry products and in patients suffering from diarrhea as a result of ingestion of this foodborne pathogen in Baghdad (Iraq). It assesses the antibiotic susceptibility as well as virulence and pathogenicity.

Materials and methods

Sample collection

From October 2020 to May 2021, one hundred samples were collected in Baghdad, including 80 samples of Iraqi raw and ready-to-eat food from eight processing points (frozen whole 9-piece chicken = 10; frozen chicken breasts = 10; frozen chicken thighs = 10; eggshell = 10; cooked chicken shawarma = 10; cooked chicken tika = 10; ready-to-eat cake cream = 10; food appetizers containing chicken derivatives = 10) from local supermarkets, and 20 samples from clinically suspected patients with foodborne diseases from private medical laboratories.

Salmonella isolation

Bacteria were isolated according to the ISO 6579–1:2017(E) procedure [22]. An analytical unit of 25 grams from each food sample chopped finely and fecal samples from patients taken with sterilized cotton swabs were inoculated into sterile flasks with 225mL of Buffered Peptone Water (BPW) broth (Himedia, India). The flasks were incubated at 37 °C for 18 hours. An amount of 0.1mL from pre-enriched culture was transferred to 10ml of Rappaport Vassiliadis (RV) broth medium (Himedia, India), thoroughly mixed and incubated at 41 ± 0.5 °C for 24 hours. A loopful from the incubated selective enrichment broth culture was streaked on Xylose Lysine Deoxycholate (XLD) agar plate and *Salmonella-Shigella* (SS) agar (Himedia, India). Both agar plates were incubated at 37 °C for 24 hours.

Salmonella identification

Suspected colonies with *Salmonella* morphology from each plate were identified biochemically using the VITEK-2 system (bioMérieux, France). In addition, isolates were investigated by the conventional method in the Iraqi National Centre for *Salmonella* at the Central Public Health Laboratories in Baghdad using the serological test (Anti-*Salmonella* H test) (Sifin/ Germany) kits that were designed for the use in examining the H-antigens of *Salmonella* strains via slide agglutination in Baghdad's Central Public Health Laboratory (CPHL). Bacteria from 16–20-hour-old subculture (nutrient) agar were applied to a clean microscope slide and mixed well with a drop of 25µl of anti-*Salmonella* H reagent (test serum), then slowly stirred with a sanitized stick. The slide was put on a dark surface and visible agglutination was observed in the case of the positive reaction, while a negative result was seen as a homogeneous milky turbid suspension. On the same slide, the positive and negative controls were tested in the same way. Typical *Salmonella* phenotypes were further confirmed by single-step PCR for the 16S rRNA gene of *S. enterica* [23].

Genomic DNA extraction

Extraction of DNA was carried out as recommended by the manufacturer of the HiPer® Bacterial Genomic DNA Extraction Teaching Kit (Solution Based), India.

16S rRNA gene direct sequencing

Conventional PCR was carried out to analyze all the genes of this study, the confirmatory 16S rRNA gene (621bp) was amplified by using GoTaq® G2 Green Master Mix (Promega, USA). PCR conditions in this study and primer design were used as shown in Tables 1 and 2. Purified PCR products (45µL) from the identified 16S rRNA gene target were forwarded to Macrogen comp. (Korea) for DNA sequencing. Then, using the BioEdit and Mega7 software, consensus sequences were created by aligning the forward and reverse DNA sequences for each sample. In addition, the final sequence from each sample was

Table 1. PCR conditions of this study

Gene name / Step conditions		16S rRNA	*inv*A	*avr*A	*sip*B	*mph*A	*gyr*A
1 CYCLE	Initial denaturation	95 °C, 5 min					94 °C, 5 min
30 CYCLE	Denaturation	94 °C, 40 sec					94 °C, 30 sec
	Annealing	60 °C	56 °C	58 °C	60 °C	58 °C	58 °C, 30 sec
		40 secs					
	Extension	72 °C, 40 sec					72 °C, 30 sec
1 CYCLE	Final extension	72 °C, 5 min					72 °C, 10 min
—	Holding	4 °C, 10 min					4 °C, 10 min

further analyzed by searching for similar matches in the NCBI gene bank database. This was achieved by employing the BLAST website tool, the BLAST search to assess the similarity.

Table 2. Primers used in PCR amplification

Gene name	Primer sequences (5′→3′)	Amplicon size (bp)	References
16S rRNA	F primer: GGAACTGAGACACGGTCCAG R primer: CCAGGTAAGGTTCTTCGCGT	671	[23]
*inv*A	F primer: TTGTTACGGCTATTTTGACCA R primer: CTGACTGCTACCTTGCTGATG	521	[24]
*avr*A	F primer: CCTGTATTGTTGAGCGTCTGG R primer: AGAAGAGCTTCGTTGAATGTCC	425	[25]
*sip*B	F primer: GGACGCCGCCCGGGAAAAACTCTC R primer: ACACTCCCGTCGCCGCCTTCACAA	875	[26]
*mph*A	F primer: GTGAGGAGGAGCTTCGCGAG R primer: TGCCGCAGGACTCGGAGGTC	403	[27]
*gyr*A	F primer: TGGGCAATGACTGGAACA R primer: GGTTGTGCGGCGGGATA	396	[28]

Antimicrobial susceptibility test

The disk diffusion method described by the Clinical and Laboratory Standards Institute (CLSI) [29] was chosen to test antibiotic resistance of *Salmonella* isolates. Ten antimicrobial agents (Bioanalysis, Turkey) were selected from several family groups according to [29], and tested against *Salmonella* isolates: phenicols (chloramphenicol, 30µg), aminoglycosides (gentamicin, 10µg), quinolones (nalidixic acid, 30µg), carbapenems (meropenem and imipenem, 10µg), folate pathway antagonists (trimethoprim-sulfamethoxazole, 30µg), macrolides (azithromycin, 15µg), cephalosporins (ceftazidime and cefotaxime, 30µg), and fosfomycins (fosfomycin, 200µg). *Escherichia coli* ATCC25922 was used as a quality control strain. The isolates were described as susceptible, intermediate, or resistant according to the CLSI [29] guidelines. An isolate was defined as multi-drug resistant (MDR) when showing resistance to three or more different classes of antimicrobials [30].

Detection of virulence and antimicrobial resistance genes

The virulence genes *inv*A (521bp), *avr*A (425bp), *sip*B (875bp), and the quinolone resistance gene *gyr*A (396bp), macrolide azithromycin resistance gene *mph*A (403bp) were analyzed in isolates that showed resistance to these two antimicrobials only. Bioneer's master mix (Bioneer's AccuPower PCR PreMix, Korea) was used for amplification. PCR conditions performed in this study and primer design were used as shown in Table 1 and Table 2.

Gel electrophoresis

The agarose powder (1 percent% w/v) was dissolved in 1X TBE buffer. The mixture was microwaved and allowed to cool at 50 °C before adding 8µl of RedSafe™ (iNtRON/Korea) (0.5 g/ml) to the agarose solution and pouring it onto the tray. After the gel hardened, the comb was removed.

Results

Occurrence of Salmonella spp.

Among 100 collected samples, 20 isolates (20%) were culture positive for *Salmonella* spp. Within the food sample groups, *Salmonella* spp. was isolated from 16.25% of samples (13 out of 80 food samples), all of which were collected from raw poultry meat and egg groups, whilst the other food groups were *Salmonella* free. Raw frozen chicken breasts had the highest level of contamination with *Salmonella* spp. (60%) followed by raw frozen chicken thighs (40%), raw frozen 9-piece chicken (20%), and eggshell (10%). As regards clinical diarrheal patients, *Salmonella* spp. was isolated from 7 (35%) out of 20 samples. The *S. enterica* isolates from food products identified in this study were *S. enterica* serovar Typhimurium (11 isolates, 84.6%) isolated from raw frozen 9-piece chicken (2 isolates, 15.3%), raw frozen chicken breasts (5 isolates, 38.4%), and raw frozen chicken thighs (4 isolates, 30.7%), *S. enterica* subsp. *enterica* (one isolate from eggs, 7.6%), and *S. enterica* subsp. *diarizonae* (one isolate from raw frozen chicken breasts, 7.6%). *S. enterica* serovar Typhi (4 isolates, 57.1%), and *S. enterica* serovar Typhimurium (3 isolates, 42.8%) were isolated from human diarrheal patients as shown in Table 3. The percentage of each serovar identified in this study is presented in diagrams (Figure 1A and Figure 1B).

Table 3. Identified isolates and their sources

Isolates No.	Source of isolates	Type of species
1	Whole raw frozen 9- piece chicken	*S. enterica* serovar Typhimurium
2		*S. enterica* serovar Typhimurium
3	Raw frozen chicken breasts	*S. enterica* subsp. *diarizonae*
4		*S. enterica* serovar Typhimurium
5		*S. enterica* serovar Typhimurium
6		*S. enterica* serovar Typhimurium
7		*S. enterica* serovar Typhimurium
8		*S. enterica* serovar Typhimurium
9	Raw frozen chicken thighs	*S. enterica* serovar Typhimurium
10		*S. enterica* serovar Typhimurium
11		*S. enterica* serovar Typhimurium
12		*S. enterica* serovar Typhimurium
13	Egg shell	*S. enterica* subsp. *enterica*
14	Diarrheal human patients	*S. enterica* serovar Typhi
15		*S. enterica* serovar Typhi
16		*S. enterica* serovar Typhi
17		*S. enterica* serovar Typhi
18		*S. enterica* serovar Typhimurium
19		*S. enterica* serovar Typhimurium
20		*S. enterica* serovar Typhimurium

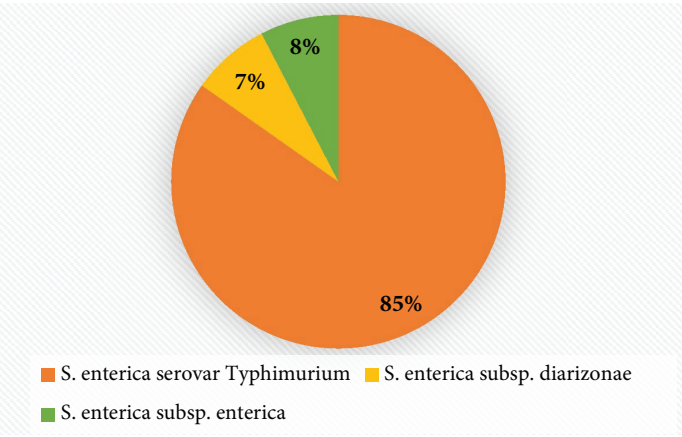

Figure 1A. Percentage of *S. enterica* serovars identified in raw food samples

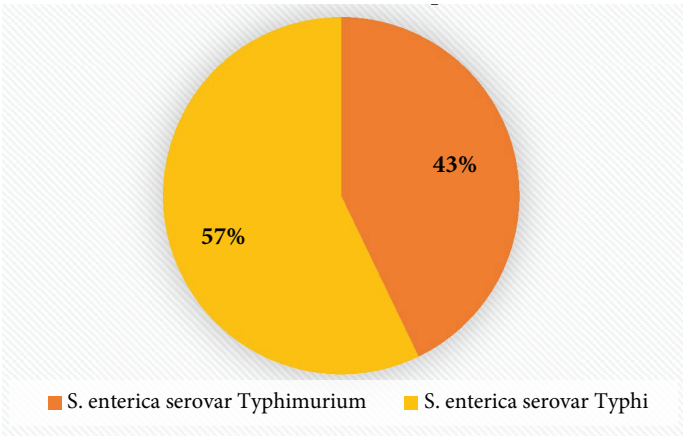

Figure 1B. Percentage of *S. enterica* serovars identified in clinical human diarrheal samples

As shown in Figures 1A and 1B, the most frequently identified serovars were *S. enterica* serovar Typhimurium (85%) and *S. enterica* serovar Typhi (57%) in raw food and clinical samples, respectively. The confirmatory molecular analysis indicated that all isolates (100%) had the 16S rRNA gene of *Salmonella*.

Sequencing analysis results

Several changes in nucleotide sequences were observed in isolates A4, A7, A13, A16, A17, and A19. There was approximately a maximum of 14 differences in base pairs as shown in Figure 2. With the presence of such polymorphisms, differences between isolates belonging to different subspecies and serovars were found.

During the further analysis for bacterial classification and detection of similarity in Figure 2, four clusters of bacterial strains were identified with reference strains. Two of them included AB680591.1 and MZ773245.1, and the other two included isolates A1 and A10. Each of them contained bacterial isolates with the highest similarity and the lowest genetic distance.

Differentiation of the reference strains from those of other serovars and subspecies was carried out as illustrated in Figure 3. It shows that isolates A11, AA2, A1, A8, A20, A12, A5, MZ773245.1, A4, A7, A17, A16, A13, A19, A6, A9, A10, A15, and A18 are more related to strain AB680591.1

Table 4. Alignment of partial 16S rRNA gene sequences of *Salmonella* spp. bacteria under consideration with the sequence of the NCBI database

Isolate no.	Result of strain sequencing	Similarity	e-value
A1	*S. enterica* **serovar Typhimurium**	100%	0.0
A2		100%	
A3		100%	
A4		99.53%	
A5		100%	
A6		100%	
A7	*S. enterica* **subsp.** *enterica*	100%	
A8	*S. enterica* **serovar Typhimurium**	99.50%	
A9	*S. enterica* **serovar Typhi**	100%	
A10	*S. enterica* **serovar Typhimurium**	99.84%	
A11	*S. enterica* **serovar Typhi**	100%	
A12		100%	
A13		99.05%	
A14	*S. enterica* **serovar Typhimurium**	100%	
A15		100%	
A16		98.73%	
A17		98.69%	
A18		100%	
A19		100%	
A20	*S. enterica* **subsp**. *enterica*	100%	

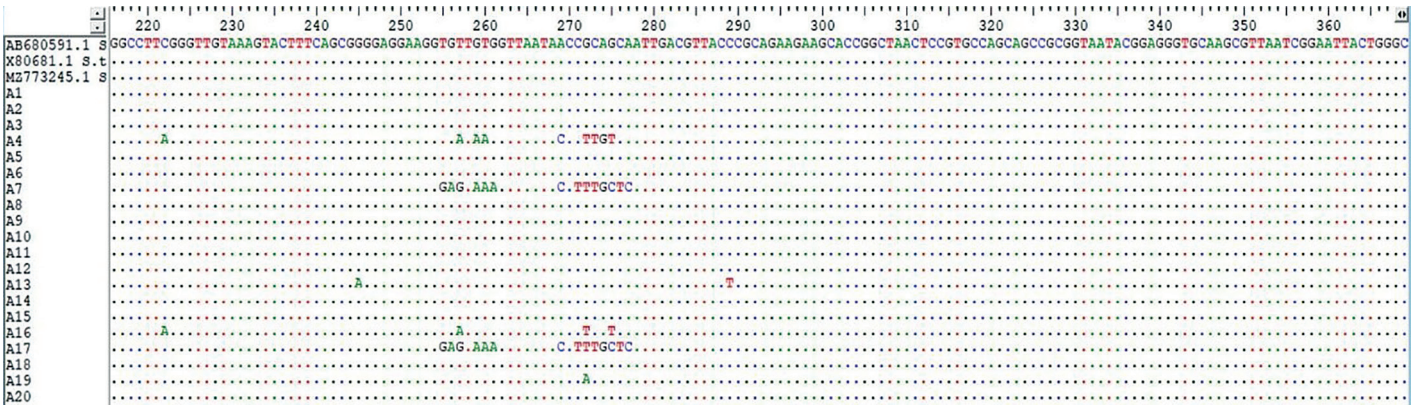

Figure 2. 16S rRNA gene sequence alignment of *S. enterica* isolates with the related reference sequence of the 16S rRNA gene by BioEdit software. Ref= Reference sequences of the 16S rRNA gene of *S. enterica* strains AB680591.1 S, X80681.1 S. t, and MZ773245.1 S (wild type). The black sign "A" denotes the names of isolates given in Table 4

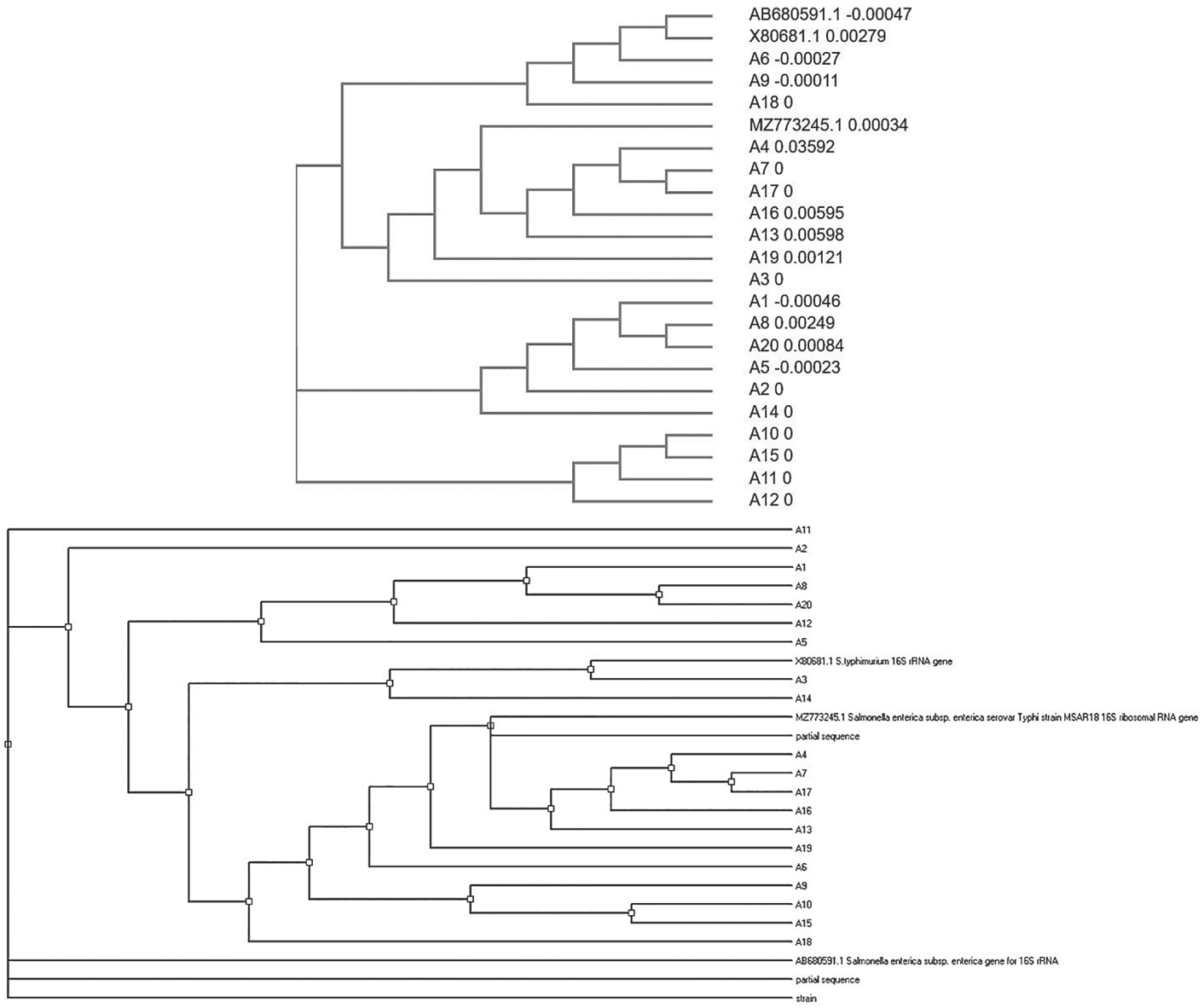

Figure 3. Phylogenetic tree of 16S rRNA gene sequences alignment of *S. enterica* spp. isolates with the related reference sequence of the 16S rRNA gene

and belong to different *S. enterica* serovars while isolates A14 and A13 belonging to *S. enterica* serovar Typhimurium have high similarity to X80681.1 as a common ancestor.

Additionally, the obtained data presented in Table 4 demonstrate the similarity (98–100%) of the isolated bacteria by the 16S rRNA gene, and show that the expected value (e-value) for all *Salmonella* spp. isolates was zero.

Antimicrobial susceptibility test

Antimicrobial susceptibility testing of *Salmonella enterica* in this study showed that all isolates tested except one were resistant to one antibiotic at least as shown in Table 5.

In general, the most effective antimicrobials against *Salmonella* isolated from food samples were imipenem (100%), cefotaxime, meropenem and ceftazidime (69.2% each). The highest resistance of all 13 isolates was recorded for nalidixic acid (69.23%), followed by chloramphenicol (53.84%), gentamicin (46.15%), trimethoprim-sulfamethoxazole (46.1%), fosfomycin (46.1%), and azithromycin (38.46%), whilst resistance to both cefotaxime and meropenem was 30.7% as shown in Table 6.

Different *Salmonella* serovars/subspecies tested in this study showed different levels of resistance. It was revealed that 63.63% (7/11) of isolates of *S. enterica* serovar Typhimurium recovered from food were resistant to nalidixic acid and chloramphenicol. Lower resistance was recorded for trimethoprim-sulfamethoxazole and gentamicin, which had the same percentage of 45.45% (6/11), followed by meropenem, cefotaxime, and azithromycin with the same proportion of 36.36% (4/11). Imipenem and ceftazidime were found to be effective antimicrobials against food isolates of *S. enterica* serovar Typhimurium (100% and 76.93%, respectively).

All food isolates (100%) of *S. enterica* subsp. *enterica* (n = 1) and *S. enterica* subsp. *diarizonae* (n = 1) were susceptible to meropenem, ceftazidime, cefotaxime, and chloramphenicol. Only *S. enterica* subsp. *diarizonae* showed high resistance (100%) to azithromycin, trimethoprim-sulfamethoxazole, gentamicin, nalidixic acid, and fosfo-

Table 5. Antibiotic resistance of each isolate to ten antibiotics chosen according to CLSI [29]

sample	Name of serovar/subspecies	NA	SXT	C	CTX	AZM	MEM	IPM	CN	CAZ	FOS	
1	*S. enterica* serovar Typhimurium	S	S	S	R	S	R	S	R	R	S	MDR
2	*S. enterica* serovar Typhimurium	S	S	S	R	S	S	S	S	S	S	—
3	*S. enterica* serovar Typhimurium	S	S	S	S	S	S	S	S	S	S	—
4	*S. enterica* serovar Typhimurium	S	S	R	S	S	S	S	S	S	R	—
5	*S. enterica* serovar Typhimurium	R	R	R	R	R	R	S	R	R	R	MDR
6	*S. enterica* serovar Typhimurium	R	R	S	S	S	R	S	S	S	S	MDR
7	*S. enterica* subsp. *diarizonae*	R	R	R	S	R	S	S	R	S	R	MDR
8	*S. enterica* serovar Typhimurium	R	S	S	S	S	S	S	S	S	S	—
9	*S. enterica* serovar Typhimurium	R	R	R	S	S	S	S	S	S	R	MDR
10	*S. enterica* serovar Typhimurium	R	R	R	R	R	R	S	R	R	S	MDR
11	*S. enterica* serovar Typhimurium	R	S	R	S	R	S	S	R	S	S	MDR
12	*S. enterica* serovar Typhimurium	R	R	R	S	R	S	S	R	S	R	MDR
13	*S. enterica* subsp. *enterica*	R	S	S	S	S	S	S	S	S	S	—
14	*S. enterica* serovar Typhi	S	R	S	R	S	S	S	R	R	R	MDR
15	*S. enterica* serovar Typhi	R	S	I	R	R	S	S	S	S	S	MDR
16	*S. enterica* serovar Typhi	R	S	R	R	S	S	S	S	S	S	MDR
17	*S. enterica* serovar Typhi	I	R	I	S	R	S	R	R	S	S	MDR
18	*S. enterica* serovar Typhimurium	R	S	S	R	R	S	S	I	S	R	MDR
19	*S. enterica* serovar Typhimurium	S	R	R	S	R	R	S	S	R	S	MDR
20	*S. enterica* serovar Typhimurium	R	S	S	R	R	S	S	I	R	S	MDR

NA: nalidixic acid; SXT: trimethoprim/sulfamethoxazole; C: chloramphenicol, CTX: cefotaxime; AZM: azithromycin; MEM: meropenem; IPM: imipenem; CN: gentamicin; CAZ: ceftazidime; FOS: fosfomycin; S: susceptible; I: intermediate; R: resistant; MDR: multidrug-resistant

mycin. *S. enterica* subsp. *enterica* showed high levels of resistance (100%) to nalidixic acid and high levels of susceptibility (100%) to gentamicin.

Regarding human isolates, the results obtained indicate that in general isolates (*S. enterica* serovar Typhi (n = 4) and *S. enterica* serovar Typhimurium (n = 3)) recovered from the diarrheal patient samples were resistant to azithromycin and cefotaxime showing the same percentage (71.42%; 5/7), while imipenem and meropenem were the ultimate effective antibiotics with the same proportion of susceptible isolates (85.7%; 6/7) as shown in Table7.

As for *Salmonella* serovars, *S. enterica* serovar Typhimurium (n= 3) isolates were completely susceptible (100%) to imipenem, followed by meropenem, fosfomycin, cefotaxime and chloramphenicol with the same proportion (66.67%, 2/3). However, they showed resistance to trimethoprim-sulfamethoxazole, nalidixic acid, and ceftazidime with the same proportion (66.67%, 2/3). The resistance to gentamicin was intermediate (66.67%, 2/3).

S. enterica serovar Typhi (n = 4) isolates exhibited intermediate resistance to chloramphenicol (50%; 2/4). Moreover, they showed resistance to trimethoprim-sulfonamide, nalidixic acid, azithromycin, and gentamicin, all of which had the same ratio (50%; n = 2/4). The highest susceptibility (100%) was observed for meropenem followed by ceftazidime, fosfomycin, and imipenem with the same proportion of 75% (3/4). *S. enterica* serovar Typhi was resistant (75%; 3/4) to cefotaxime.

All isolates except for five isolates from food samples (one from eggshell and four from frozen raw chicken breasts) exhibited multidrug resistance to ≥ 3 antibiotics as shown in Table 5.

This study illustrates a high proportion (77.7%) of food isolates of *S. enterica* serovar Typhimurium (n = 7) consid-

Table 6. Percentage of food isolates of *Salmonella* spp. resistant, intermediate and susceptible to antibiotics

Antibiotics	Food Isolates No.	Resistant (%)	Intermediate (%)	Susceptible (%)
Nalidixic acid (NA)	13	69.2%	0%	30.7%
Trimethoprim/ Sulfamethoxazole (SXT)	13	46.1%	0%	53.8%
Chloramphenicol (C)	13	53.8%	0%	46.1%
Cefotaxime (CTX)	13	30.7%	0%	69.2%
Azithromycin (AZM)	13	38.4%	0%	61.5%
Meropenem (MEM)	13	30.7%	0%	69.2%
Imipenem (IPM)	13	0%	0%	100%
Gentamicin (CN)	13	46.1%	0%	53.8%
Ceftazidime (CAZ)	13	30.7%	0%	69.2%
Fosfomycin (FOS)	13	46.1%	0%	53.8%

Table 7. Percentage of clinical *Salmonella* spp. isolates resistant, intermediate and susceptible to antibiotics

Antibiotics	Clinical Isolates No.	Resistant (%)	Intermediate (%)	Susceptible (%)
Nalidixic acid (NA)	7	57.1%	14.2%	28.5%
Trimethoprim/ Sulfamethoxazole (SXT)	7	42.8%	0%	57.1%
Chloramphenicol (CAM)	7	28.5%	28.5%	42.8%
Cefotaxime (CTX)	7	71.4%	0%	28.5%
Azithromycin (AZM)	7	71.4%	0%	28.5%
Meropenem (MEM)	7	14.2%	0%	85.7%
Imipenem (IPM)	7	14.2%	0%	85.7%
Gentamicin (GEN)	7	28.5%	28.5%	42.8%
Ceftazidime (CAZ)	7	42.8%	0%	57.1%
Fosfomycin (FOS)	7	28.5%	0%	71.4%

ered multidrug resistant (MDR). Two isolates were resistant to three antibiotic groups, two isolates were resistant to four antibiotic groups, one isolate was resistant to six antibiotic groups and two isolates were resistant to eight antibiotic groups as shown in Table 8. All (100%) clinical isolates of *S. enterica* serovar Typhimurium (n = 3) were MDR: one isolate was resistant to three antibiotic groups, one isolate was resistant to four antibiotic groups and one isolate was resistant to five antibiotic groups as shown in Table 8.

Table 8. MDR of food and clinical isolates of *S. enterica* serovar Typhimurium

No. of antimicrobial groups	Food isolates (n= 7)		Clinical diarrheal isolates (n= 3)	
	Antimicrobial resistance patterns	No. of isolates (%)	Antimicrobial resistance patterns	No. of isolates (%)
Three	Quinolones (NA), Sulfa drug)SXT(, Carbapenem (MEM). Cephems (CTX, CAZ), Carbapenem (MEM), Gentamycin (CN).	2 (28.56%)	Quinolones (NA), Cephems (CTX, CAZ), Macrolides (AZM).	1 (14.28%)
Four	Quinolones (NA), Sulfa drug)SXT(. Chloramphenicol (C), Fosfomycin (FOS). Quinolones (NA), Chloramphinical (C), Macrolides (AZM), Gentamycin (CN).	2 (28.56%)	Quinolones (NA), Chloramphenicol (C), Macrolides (AZM), Fosfomycin (FOS).	1 (14.28%)
Five	—	—	Cephems (CAZ), Carbapenem (MEM), Chloramphenicol (C), Macrolides (AZM), Sulfa drug (SXT(.	1 (14.28%)
Six	Quinolones (NA), Chloramphenicol (C), Fosfomycin (FOS) Carbapenem (IPM), Macrolides (AZM), Sulfa drug)SXT(.	1 (14.28%)	—	—
Eight	Cephems (CAZ, CTX), Carbapenem (MEM), Chloramphenicol (C), Macrolides (AZM), Sulfa drug (SXT(, Gentamycin (CN), Quinolones (NA), Fosfomycin (FOS). Cephems (CAZ, CTX), Carbapenem (MEM), Chloramphenicol (C), Macrolides (AZM), Sulfa drug (SXT), Gentamycin (CN), Quinolones (NA), Fosfomycin (FOS).	2 (28.56%)	—	—

Antibiotic resistance gene detection in S. enterica isolates

The fluoroquinolone resistance gene (*gyr*A) was associated with all fluoroquinolone (nalidixic acid) resistant isolates (16/16). All nine resistant isolates recovered from food (*S. enterica* serovar Typhimurium, n = 7; *S. enterica* subsp. *enterica,* n = 1; *S. enterica* subsp. *diarizonae,* n = 1) and all seven resistant clinical isolates (*S. enterica* serovar Typhi, n = 4; *S. enterica* serovar Typhimurium, n = 3) showed the presence of the *gyr*A gene. The azithromycin resistance gene (*mph*A*)* was associated with about half of the macrolide (azithromycin) resistant isolates (n = 6/10). The *mph*A gene was detected in four resistant isolates recovered from food (*S. enterica* serovar Typhimurium, 3/4; and *S. enterica* subsp. *diarizonae*; 1/1) with the proportion equaled 75% and 100%, respectively. Among resistant clinical isolates, 2 out of 5 isolates showed the presence of the *mph*A gene (*S. enterica* serovar Typhi,1/2; *S. enterica* serovar Typhimurium; 1/3), which accounted for 50% and 33.33%, respectively.

Virulence gene detection in S. enterica isolates

The *inv*A gene was revealed in 75% (15/20) of all *Salmonella* isolates. Among food isolates, *inv*A was found in 9 out of 13 (69.2%) *S. enterica* isolates. Detection of this gene gave negative results in four isolates, including three isolates from raw frozen chicken breasts and one isolate from raw whole 9-piece chicken. All these four negative isolates belonged to *S. enterica* serovar Typhimurium. So, the proportion of *inv*A gene detection was 63.6% (7/11) for *S. enterica* serovar Typhimurium isolates, whilst it was 100% for both *S. enterica* subsp. *enterica* (1/1) and *S. enterica* subsp. *diarizonae* (1/1). The *avr*A virulence gene was detected in 90% (18/20) of all *Salmonella* isolates. Among food isolates, *avr*A was detected in 12 out of 13 isolates (92.3%). Only one isolate of *S. enterica* subsp. *enterica* from eggshell showed a negative result of *avr*A gene detection. *S. enterica* serovar Typhimurium and *S. enterica* subsp. *diarizonae* showed a positive result of *avr*A gene detection in all isolates (100%) from local raw poultry meat. The *sip*B virulence gene was found in 95% (19/20) of all *Salmonella* isolates. Regarding local raw poultry meat and eggshell, the *sipB* gene was detected in all (100%) isolates of *S. enterica* serovar Typhimurium, *S. enterica* subsp. *diarizonae*, and *S. enterica* subsp. *enterica*.

All these virulence genes were detected in 85.7% (6/7) of clinical isolates. The proportion of clinical isolates of *S. enterica* serovar Typhi positive for these genes was 25% (1/4).

Discussion

To the best of our knowledge, this work is one of the first studies interested in the *Salmonella* prevalence in food and clinical samples in Iraq. The results of *Salmonella* isolates are approximate, with various studies done in many areas in Iraq between 2008 and 2017, with percentage of *Salmonella* isolation ranging from 1.07% to 16%. The great-

est proportion was recorded in Al-Hawijah, while the lowest percentage was recorded in Mosul [31–37].

Different studies on *Salmonella* prevalence were carried out in several countries. In [38] performed in China, *Salmonella* spp. were isolated from 249 out of 664 (37.5%) samples, including 190 (36.7%) chicken, 48 (40.7%) duck and 11 (39.2%) pigeon samples. *Salmonella* prevalence of 13.4% was documented by Rabby [39] in poultry meat retailed in wet- and super-markets in Dhaka city, Bangladesh. Salih *et al.* [40] examined the distribution of *Salmonella* spp. in 121 specimens from diarrheal patients in Duhok, Iraq, and showed that 72 isolates (59.5%) belonged to *Salmonella* spp. Sadeq *et al.* [41] analyzed 40 chicken samples, and found that 14 isolates (35%) belonged to *S. enterica* serovar Typhimurium with the presence of the *inv*A gene in 11 (78.5%) out of 14 isolates of *S. enterica* serovar Typhimurium. These results are almost identical to the results of our study since we recorded the presence of seven food isolates of *S. enterica* serovar Typhimurium positive for the *inv*A gene with a proportion of 53%. A recent study in Babil, Iraq, was carried out by Obayes et al. [42] who collected samples from 120 children with diarrhea and revealed that 58 samples were positive for different *Salmonella* spp. The most common serovar of *Salmonella enterica* in their study was *Salmonella enterica* serovar Typhi (29.3%) and this result agrees with the result of our study, which indicates the presence in the clinical human diarrheal samples of four isolates (57.1%) of *S. enterica* serovar Typhi as the most common serovar.

The most common serovars transferred from animals to humans are *Salmonella* Enteritidis and *Salmonella* Typhimurium. Typhoid fever, paratyphoid fever, food poisoning and gastroenteritis are all disorders caused by *Salmonella* [43]. The 16S rRNA gene sequence is considered an important approach toward identification of bacterial genus, species, and sub-species, and was used in this study as a confirmatory detection test. It is unique for each bacterial organism and can be considered a unique identification gene for bacterial species.

In general, the reasons for *Salmonella* spp. contamination detected in this study are the lack of HACCP control and due diligence. The Iraqi *Central Organization for Standardization and Quality Control* (COSQC, document No. 2270) indicates that the percentage of *Salmonella* growth should be zero in chicken cuts (thighs, breasts, wings), and for this reason the problem of the absence of quality control, HACCP and food handling instructions have to be dissolved and they should be applied in Iraqi slaughterhouses along the poultry processing chain until reaching consumers to prevent an increase in foodborne diseases as much as possible.

Concerning nalidixic acid, other studies also documented *Salmonella* resistance to this antimicrobial agent, and the resistance increased significantly (94.1%) in 2021 compared with the 2018 report (77.3%) indicating more applications of nalidixic acid in both veterinary medicine and human medicine fields [44, 45]. As for other antibiotics, a study conducted in Bangladesh reported that 95% of the isolates were resistant to azithromycin [46], another study reported low resistance (8%) to chloramphenicol [47]. In [48] 12% of *Salmonella* isolates were resistant to azithromycin and 1% to chloramphenicol, whereas the present study recorded much higher resistance to azithromycin and chloramphenicol compared to previous studies.

In comparison with [49], the partial similarity was noticed, particularly, the susceptibility of the isolates to imipenem. Resistance to SXT was higher in the present study than in [49], which recorded 31.3%, while the study by Velez [50] displayed that *Salmonella* isolates showed higher resistance (100%) to gentamicin than in our study. Moreover, in the current study, nine isolates were resistant to cefotaxime (approximately 30.7%), which is higher than the result reported in China by [14] where 2.44% of *Salmonella* isolates were found to be resistant to cefotaxime.

On other hand, resistance to ceftazidime, meropenem, and fostomycin was in the same line with an Iraqi study [51]. Antibiotic-resistant bacteria can cause life-threatening infections in people and constitute a serious danger to public health and wellbeing. Furthermore, the use of antimicrobials in veterinary medicine may increase the emergence of resistant bacteria harmful to humans and posing a possible threat to public health from zoonotic pathogens, such as *Salmonella*. As a result, the high resistance/MDR in human isolates of *Salmonella* spp. to antibiotics may be caused not by the misuse of drugs and their wrong consumption, but rather this resistance transfers through consumption of foods (e. g., poultry meat) from food-producing animals that received antibiotics on farm to enhance and promote their quality and prevent the growth and transmission of microbes.

Bacteria may acquire resistance genes via mobile genetic elements such as plasmids, which provide the flexibility to a host bacterium and aid in the dissemination and dispersion of these genes among various bacterial populations [52]. In our study, the *gyr*A and *mph*A genes conferring resistance to nalidixic acid and azithromycin, respectively, were detected among isolates that showed the phenotypic resistance. Similarly, there were high percentages of the *gyr*A genes found in nalidixic acid resistant *Salmonella* Albany (92%), *Salmonella* Corvallis (75%), and *Salmonella* Kentucky (85%) isolated in chicken food chains in Cambodia [53].

Quinolones with a broad spectrum of activity have a greater ability to inhibit gyrase in gram-negative bacteria. According to the researchers, antibiotic resistance in *Salmonella* spp. is mostly caused by mutations within the quinolone resistance-determining regions (QRDRs) of the target enzymes DNA gyrase (*gyr*A and *gyr*B) and topoisomerase IV (*par*C and *par*E) [54].

Wang et al. [28] identified the *mph*A gene in 15 out of 31 azithromycin-resistant *Salmonella* isolates. This is partially similar to the results of our study. Azithromycin is an

azalide antibacterial drug, which was shown to be equal to chloramphenicol, fluoroquinolones, and extended-spectrum cephalosporins for the treatment of uncomplicated typhoid fever [55]. The *mph*A gene is the key gene involved in *Salmonella* resistance to the macrolide azithromycin. It is typically found on plasmids and spreads rapidly, posing a significant threat to current *Salmonella* infection therapy [56].

Salmonella employs several virulence factors expressed at various phases of the disease process to develop a successful infection. A number of these parameters are linked to *Salmonella* Pathogenicity Islands (SPIs) on the *Salmonella* chromosomes [57]. The virulence *inv*A gene is involved in *Salmonella* pathogenicity. The *inv*A gene acts as a unique biomarker for *Salmonella* identification [58]. Previous studies [59–62] show that the *inv*A gene has been found in 100% of *Salmonella* strains, whilst, our study recorded a lower percentage (75%). Likewise, other authors, such as Mthembu *et al.* [21], revealed lower rates (54.4%; 106/195) and Somda *et al.* [63] showed the presence of the *inv*A gene in 91% (52/57) of non-typhoidal *Salmonella* isolates from human diarrhea, environment, and lettuce samples in Burkina Faso. Furthermore, Nikiema *et al.* [64] found that the *inv*A gene was present in 67% (61/91) of clinical isolates and 60% (9/15) of sandwich samples. In our study, 25% of *Salmonella* isolates did not harbor the *inv*A gene and hence would be unable to invade host cells. Salih and Yousif [65] conducted a study in Iraq to detect five virulence factors among four isolates of *S. enterica* serovar Typhimurium isolated previously from three puppies and one adult dog and reported that the *inv*A gene was detected in two isolates only. In the same line, another Iraqi study showed that the *inv*A gene was detected among eight *Salmonella* strains with a proportion of 50% [66]. Therefore, *Salmonella* might be virulent (*inv*A) or avirulent [67]. Furthermore, asymptomatic animals carrying either virulent or avirulent strains might be possible sources of transmission to humans through the food chain, as well as due to their close proximity to people and poor animal effluent management [21, 67]. All strains containing this *inv*A gene, which encodes a protein found in bacterial inner membrane and is crucial for invasion of host epithelium, are pathogenic [68,69]. Moreover, a key component of the pathogen's virulence phenotype is the virulence-associated effector protein AvrA of *Salmonella enterica*, which blocks the first line of defense of the host organism. AvrA expression increases the ability of the bacterium to invade the host [70,71]. Our study indicated that the *avr*A gene was revealed in 90% (18/20) of all *Salmonella* isolates. The other Iraqi researchers Jbar *et al.* [72] detected the *avr*A gene in 100% (30/30) of *Salmonella enterica* isolates. Similarly, an Egyptian study showed that all 6 (100%) *Salmonella* isolates carried the *avr*A gene [69]. This presence of the *avr*A gene in all isolates suggests a higher rate of gastroenteric illnesses in humans that may be transmitted from contaminated food. In addition, Hersh *et al.* [73] established the role of *sip*B in *Salmonella*-induced macrophage death, as well as the possible involvement of caspase-1 in this process. In our study, the *sip*B gene was found in 95% (19/20), while another Iraqi study [42] found that the *sip*B gene occurrence in *Salmonella* isolates was 18.9% (11/120).

Moreover, having regard to the serious roles of these three genes as illustrated briefly above and the outcome of our study, which shows the high occurrence of the *inv*A, *avr*A, and *sip*B genes (75%, 90% and 95%, respectively), it is safe to assume that the severity of infections that may occur in the Baghdad population will be increasing.

Differences in the presence of virulence genes in bacterial isolates from this investigation and prior studies might be attributed to geographic circumstances, dietary variables, and the migration of virulence genes through integrons and transposons. In addition to plasmids, [74] indicated that conjugation is a crucial method for the transmission of virulence genes in bacterial groups. These virulence genes, despite their high pathogenicity, are still in circulation, but the variables, as well as the mechanisms of the asymptomatic carriage, are poorly understood. Nonetheless, certain variables, such as a decrease in virulence gene expression or even the expression of bacterial components unique to a carrier, might be blamed. These pathogenicity island-encoded genes are critical in various phases of *Salmonella* pathogenesis [75,76].

Conclusions

This study shows that *Salmonella enterica* exists in local chicken meat and eggshell with high resistance to antibiotics, including multidrug resistance (75%). In addition, it demonstrates high proportions of *Salmonella enterica* isolates positive for virulence genes (*inv*A, *sip*B, *avr*A) responsible for initial invasion of the host cells.

REFERENCES

1. Cwiek, K., Korzekwa, K., Tabiś, A., Bania, J., Bugla-Płoskońska, G., Wieliczko, A. (2020). Antimicrobial resistance and biofilm formation capacity of Salmonella enterica serovar enteritidis strains isolated from poultry and humans in Poland. *Pathogens,* 9(8), Article 643. https://doi.org/10.3390/pathogens9080643
2. Pal, M., Ayele, Y. (2020). Emerging role of foodborne viruses in public health. *Biomedical Research International*, 5, 01–04.
3. Kawamoto, S., Bari, M. L. (2015). Emerging and re-emerging foodborne diseases: Threats to human health and global stability. Chapter in a book: Foodborne Pathogens and Food Safety. CRC Press, 2015.
4. EFSA (2020). The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018. *EFSA*, 18(3), Article 6007. https://doi.org/10.2903/j.efsa.2020.6007
5. Khalaf, Z. Z. (2018). Comparative study of antibiofilm activity of lime juice and lithium dioxide nanoparticles against Salmonella isolated from local cheese. *Journal of Biotechnology Research Center*, 12(2), 82–94. https://doi.org/10.24126/jobrc.2018.12.2.542
6. ECDC. (2020). Salmonella the Most Common Cause of Foodborne Outbreaks in the European Union. Retrieved from

https://www.ecdc.europa.eu/en/news-events/Salmonella-most-common-cause-foodborne-outbreaks-european-union Accessed December 15, 2022
7. Jajere, S. M. (2019). A review of Salmonella enterica with particular focus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance. *Veterinary World*, 12(4), 504–521. https://doi.org/10.14202/vetworld.2019.504–521
8. Muller, K., Aabo, S., Birk, T., Mordhorst, H., Bjarnadottir, B., Agersø, Y. (2012). Survival and growth of epidemically successful and nonsuccessful Salmonella enterica clones after freezing and dehydration. *Journal of Food Protection*, 75(3), 456–464. https://doi.org/10.4315/0362–028X.JFP-11–167
9. Rosenkrantz, J. T., Aarts, H., Abee, T., Rolfe, M. D., Knudsen, G. M., Nielsen, M. -B. et al. (2013). Non-essential genes form the hubs of genome scale protein function and environmental gene expression networks in Salmonella enterica serovar Typhimurium. *BMC Microbiology*, 13(1), Article 294. https://doi.org/10.1186/1471–2180–13–294
10. Brooks, G. F., Butel, J. S., Morse, S. A. (2004). Cultivation of Microorganisms. Chapter in a book: Medical Microbiology. New York, Cenveo Publisher Services, 2004.
11. Tindall, B. J., Grimont, P. A. D., Garrity, G. M., Euzeby, J. P. (2005). Nomenclature and taxonomy of the genus Salmonella. *International Journal of Systematic and Evolutionary Microbiology*, 55(1), 521–524. https://doi.org/10.1099/ijs.0.63580–0
12. Brenner, F. W., Villar, R. G., Angulo, F. J., Tauxe, R., and Swaminathan, B. (2000). Salmonella Nomenclature. *Journal of Clinical Microbiology*, 38(7), 2465–2467. https://doi.org/10.1128/JCM.38.7.2465–2467.2000
13. Harb, A., Habib, I., Mezal, E. H., Kareem, H. S., Laird, T., O'Dea, M. et al. (2018). Occurrence, antimicrobial resistance and whole-genome sequencing analysis of Salmonella isolates from chicken carcasses imported into Iraq from four different countries. *International Journal of Food Microbiology*, 284, 84–90. https://doi.org/10.1016/j.ijfoodmicro.2018.07.007
14. Ma, Y., Xu, X., Gao, Y., Zhan, Z., Xu, C., Qu, X. et al. (2020). Antimicrobial resistance and molecular characterization of Salmonella enterica serovar Corvallis isolated from human patients and animal source foods in China. *International Journal of Food Microbiology*, 335, Article 108859. https://doi.org/10.1016/j.ijfoodmicro.2020.108859
15. Lai, J., Wu, C., Wu, C., Qi, J., Wang, Y., Wang, H. et al. (2014). Serotype distribution and antibiotic resistance of Salmonella in food-producing animals in Shandong province of China, 2009 and 2012. *International Journal of Food Microbiology,* 180, 30–38. https://doi.org/10.1016/j.ijfoodmicro.2014.03.030
16. Yang, B., Qiao, L., Zhang, X., Cui, Y., Xia, X., Cui, S. et al. (2013). Serotyping, antimicrobial susceptibility, pulse field gel electrophoresis analysis of Salmonella isolates from retail foods in Henan Province, China. *Food Control*, 32(1), 228–235. https://doi.org/10.1016/j.foodcont.2012.11.022
17. Zhu, Y., Lai, H., Zou, L., Yin, S., Wang, C., Han, X. et al. (2017). Antimicrobial resistance and resistance genes in Salmonella strains isolated from broiler chickens along the slaughtering process in China. *International Journal of Food Microbiology*, 259, 43–51. https://doi.org/10.1016/j.ijfoodmicro.2017.07.023
18. Harb, A., O'Dea, M., Hanan, Z. K., Abraham, S., Habib, I. (2017). Prevalence, risk factors and antimicrobial resistance of Salmonella diarrhoeal infection among children in Thi-Qar Governorate, Iraq. *Epidemiology and Infection*, 145(16), 3486–3496. https://doi.org/10.1017/S0950268817002400
19. Fabrega, A., Vila, J. (2013). Salmonella enterica serovar typhimurium skills to succeed in the host: Virulence and regulation. *Clinical Microbiology Reviews*, 26(2), 308–341. https://doi.org/10.1128/cmr.00066–12
20. Lou, L., Zhang, P., Piao, R., Wang, Y. (2019). Salmonella Pathogenicity Island 1 (SPI-1) and its complex regulatory network. *Frontiers in Cellular and Infection Microbiology*, 9, Article 270. https://doi.org/10.3389/fcimb.2019.00270
21. Mthembu, T. P., Zishiri, O. T., El Zowalaty, M. E. (2019). Detection and molecular identification of Salmonella virulence genes in livestock production systems in South Africa. *Pathogens*, 8(3), Article 124. https://doi.org/10.3390/pathogens8030124
22. Mostafa, L., Aioub, K., Mousa, M., Ahmed, A. (2019). Effect of some essential oils on Salmonella Kentucky isolated from quail meat retailed in Alexandria markets. *Alexandria Journal of Veterinary Sciences,* 61(1), Article 179. https://doi.org/10.5455/ajvs.29683
23. Kaabi, H. K. J. A., AL-Yassari, A. K. S. (2019). 16SrRNA sequencing as tool for identification of Salmonella spp. isolated from human diarrhea cases. *Journal of Physics: Conference Series*, 1294(6), Article 062041. https://doi.org/10.1088/1742–6596/1294/6/062041
24. Van der Velden, A. W., Lindgren, S. W., Worley, M. J., Heffron, F. (2000). Salmonella pathogenicity island 1-independent induction of apoptosis in infected macrophages by Salmonella enterica serotype Typhimurium. *Infection and Immunity*, 68(10), 5702–5709. https://doi.org/10.1128/iai.68.10.5702–5709.2000
25. Huehn, S., La Ragione, R. M., Anjum, M., Saunders, M., Woodward, M. J., Bunge, C. et al. (2010). Virulotyping and antimicrobial resistance typing of Salmonella enterica serovars relevant to human health in Europe. *Foodborne Pathogens and Disease*, 7(5), 523–535. https://doi.org/10.1089/fpd.2009.0447
26. Skyberg, J. A., Logue, C. M., Nolan, L. K. (2009).Virulence genotyping of Salmonella spp. with multiplex PCR. *Avian Diseases*, 50(1), 77–81. https://doi.org/10.1637/7417.1
27. Gomes, C., Ruiz-Roldán, L., Mateu, J., Ochoa, T. J., Ruiz, J. (2019). Azithromycin resistance levels and mechanisms in Escherichia coli. *Scientific Reports*, 9(1), Article 6089. https://doi.org/10.1038/s41598–019–42423–3
28. Wang, J., Li, Y., Xu, X., Liang, B., Wu, F., Yang, X. et al. (2017). Antimicrobial resistance of Salmonella enterica serovar Typhimurium in Shanghai, China. *Frontiers in Microbiology*, 8, Article 510. https://doi.org/10.3389/fmicb.2017.00510
29. Clinical and Laboratory Standards Institute (CLSI). 2021. Performance standards for antimicrobial susceptibility testing. 31th (ed.). CLSI document M100. Wayne, USA.
30. Abraham, S., Groves, M. D., Trott, D. J., Chapman, T. A., Turner, B., Hornitzky, M. et al. (2014). Salmonella enterica isolated from infections in Australian livestock remain susceptible to critical antimicrobials. *International Journal of Antimicrobial Agents*, 43(2), 126–130. https://doi.org/10.1016/j.ijantimicag.2013.10.014
31. Alrifai, S. B., Alsaadi, A., Mahmood, Y. A., Ali, A. A. (2009). Prevalence and etiology of nosocomial diarrhoea in children < 5 years in Tikrit teaching hospital. *EMHJ — Eastern Mediterranean Health Journal,* 15(5), 1111–1118. https://doi.org/10.26719/2009.15.5.111
32. Rabatti, A.A., Rasheed, N.E. (2009). Etiology of bloody diarrhea among children admitted to maternity and children's Hospital-Erbil. *Al-Kindy College Medical Journal*, 4(2), 19–24.
33. Al-Mosawi, G.J., Al-Haris, F.M. (2008). The aetiology of bloody diarrhea in children of najaf governorate. *Kufa Medical Journal*, 11(1), 224–233.
34. Al-Taie, H. H. I. (2009). Prevalence rate of Salmonella in Babylon province. *The Iraqi Journal of Veterinary Medicine*, 33(2), 152–157. https://doi.org/10.30539/iraqijvm.v33i2.705
35. Al-Juboory, Y.H, Zenad, M.M, Hassen, R. H. (2014). Prevalence of Salmonella Serotypes in Diarrheic and Non-Diarrheic Patients in Mosul-Iraq. *Kerbala Journal of Medicine*, 7(2), 1937–1944.
36. Saleh, M.B., Hanan, Z.K., Mezal, E.H. (2016). Antimicrobial resistance, Virulence profiles of Salmonella enterica serovar Typhimurium isolated from diarrheal children in Thi-Qar province during 2015. *University of Thi-Qar Journal of Science*, 6(1), 3–8.
37. Abbas, K., Al-Wattar, W., Hasan, S., Kasim, H., Jasim, A. (2017). The incidence of Shigella and Salmonella in the stool of pediatric patients. *Iraqi Journal of Public Health*, 1(3), 2521–7267. https://doi.org/10.22317/ijph.12201705
38. Yang, X., Huang, J., Zhang, Y., Liu, S., Chen, L., Xiao, C. et al. (2020). Prevalence, abundance, serovars and antimicrobial resistance of Salmonella isolated from retail raw poultry meat in China. *Science of The Total Environment*, 713, Article 136385. https://doi.org/10.1016/j.scitotenv.2019.136385
39. Rabby, M. R. I., Shah, S. T., Miah, M. I., Islam, M. S., Khan, M. A. S., Rahman, M. S. et al. (2021). Comparative analysis of bacteriological hazards and prevalence of Salmonella in poultry-meat retailed in wet- and super-markets in Dhaka city, Bangladesh. *Journal of Agriculture and Food Research*, 6, Article 100224. https://doi.org/10.1016/j.jafr.2021.100224
40. Salih, D., Abdo, J., Saadi, A. (2019). Molecular identification of Salmonella enterica from patients with diarrhea in Duhok governorate Kurdistan region/Iraq. *Journal of Duhok University*, 22(1), 148–154. https://doi.org/10.26682/sjuod.2019.22.1.16
41. Sadeq, J. N., Esmaeel, J. R., Neama, A. A. (2017). Molecular detection of invA, ssaP in Salmonella typhimurium isolated from chicken in Al-Qadisiyah Province. *AL-Qadisiyah Journal of Veterinary Medicine Sciences*, 16(2), 8–13. https://doi.org/10.29079/vol16iss2art436
42. Obayes, M. S., Al-Bermani, O. K., Rahim, S. A. (2020). Genetic detection of invA, sipB, SopB and sseC genes in Salmonella spp. isolated from diarrheic children patients. *Eurasian Journal of Biosciences,* 14(2), 3085–3091.

43. Preethi, B., Shanthi, V., Ramanathan, K. (2015). Investigation of nalidixic acid resistance mechanism in Salmonella enterica using molecular simulation techniques. *Applied Biochemistry and Biotechnology*, 177, 528–540. https://doi.org/10.1007/s12010–015–1760–6
44. Fardsanei, F., Soltan Dallal, M. M., Douraghi, M., Memariani, H., Bakhshi, B., Zahraei Salehi, T. Z. et al. (2018). Antimicrobial resistance, virulence genes and genetic relatedness of Salmonella enterica serotype Enteritidis isolates recovered from human gastroenteritis in Tehran, Iran. *Journal of Global Antimicrobial Resistance*, 12, 220–226. https://doi.org/10.1016/j.jgar.2017.10.005
45. Wei, B., Shang, K., Cha, S.-Y., Zhang, J.-F., Jang, H.-K., Kang, M. (2021). Clonal dissemination of Salmonella enterica serovar albany with concurrent resistance to ampicillin, chloramphenicol, streptomycin, sulfisoxazole, tetracycline, and nalidixic acid in broiler chicken in Korea. *Poultry Science*, 100(7), Article 101141. https://doi.org/10.1016/j.psj.2021.101141
46. Ahsan, S., Rahman, S. (2019). Azithromycin resistance in clinical isolates of Salmonella enterica serovars typhi and paratyphi in Bangladesh. *Microbial Drug Resistance*, 25(1), 8–13. https://doi.org/10.1089/mdr.2018.0109
47. Mthembu, T. P., Zishiri, O. T., El Zowalaty, M. E. (2019). Molecular detection of multidrug-resistant Salmonella isolated from livestock production systems in South Africa. *Infection and Drug Resistance,* 12, 3537–3548. https://doi.org/10.2147/idr.s211618
48. Girish, R., Kumar, A., Khan, S., Dinesh, K. R., Karim, S. (2013). Revised ciprofloxacin breakpoints for Salmonella: Is it time to write an obituary? *Journal of Clinical and Diagnostic Research*, 7(11), 2467–2469. https://doi.org/10.7860/JCDR/2013/7312.3581
49. Yu, X., Zhu, H., Bo, Y., Li, Y., Zhang, Y., Liu, Y. (2021). Prevalence and antimicrobial resistance of Salmonella enterica subspecies enterica serovar Enteritidis isolated from broiler chickens in Shandong Province, China, 2013–2018. *Poultry Science*, 100(2), 1016–1023. https://doi.org/10.1016/j.psj.2020.09.079
50. Velez, D. C., Rodríguez, V., & García, N. V. (2017). Phenotypic and genotypic antibiotic resistance of Salmonella from chicken carcasses marketed at Ibague, Colombia. *Brazilian Journal of Poultry Science*, 19, 347–354. http://doi.org/10.1590/1806–9061–2016–0405
51. Hasan, T. O. (2021). Genomic diversity analysis of Salmonella spp. from broiler and layer flocks and their feed and water in Karbala, Iraq. Ph.D. thesis, University of Baghdad, 2021. https://doi.org/10.13140/RG.2.2.23584.71682
52. Blair, J. M. A., Webber, M. A., Baylay, A. J., Ogbolu, D. O., Piddock, L. J. V. (2014). Molecular mechanisms of antibiotic resistance. *Nature Reviews Microbiology*, 13(1), 42–51. https://doi.org/10.1038/nrmicro3380
53. Vuthy, Y., Lay, K. S., Seiha, H., Kerleguer, A., Aidara-Kane, A. (2017). Antibiotic susceptibility and molecular characterization of resistance genes among Escherichia coli and among Salmonella subsp. in chicken food chains. *Asian Pacific Journal of Tropical Biomedicine*, 7(7), 670–674. https://doi.org/10.1016/j.apjtb.2017.07.002
54. Preethi, B., Shanthi, V., Ramanathan, K. (2015). Investigation of nalidixic acid resistance mechanism in Salmonella enterica using molecular simulation techniques. *Applied Biochemistry and Biotechnology,* 177(2), 528–540. https://doi.org/10.1007/s12010–015–1760–6
55. Sharma, P., Kumari, B., Dahiya, S., Kulsum, U., Kumar, S., Manral, N. et al. (2019). Azithromycin resistance mechanisms in typhoidal Salmonellae in India: A 25 years analysis. *Indian Journal of Medical Research,* 149(3), 404–411. https://doi.org/10.4103/ijmr.IJMR_1302_17
56. Wang, H., Cheng, H., Huang, B., Hu, X., Chen, Y., Zheng, L., et al. (2023). Characterization of resistance genes and plasmids from sick children caused by Salmonella enterica resistance to azithromycin in Shenzhen, China. *Frontiers in Cellular and Infection Microbiology*, 13, 343. https://doi.org/10.3389/fcimb.2023.11161722
57. Golubeva, Y. (2010). Regulation of virulence in Salmonella enterica. Ph.D. thesis, University of Illinois at Urbana-Champaign 2010.
58. Li, Q., Cheng, W., Zhang, D., Yu, T., Yin, Y., Ju, H. et al. (2012). Rapid and sensitive strategy for Salmonella detection using an InvA gene-based electrochemical DNA sensor. *International Journal of Electrochemical Science,* 7(1), 844–856.
59. Chaudhary, J.H., Nayak, J.B., Brahmbhatt, M.N., Makwana, P.P. (2015).Virulence genes detection of Salmonella serovars isolated from pork and slaughterhouse environment in Ahmedabad, Gujarat. *Veterinary World,* 8(1), 121–124. https://doi.org/10.14202/vetworld.2015.121–124
60. Gassama-Sow, A., Wane, A.A., Canu, N.A., Uzzau, S., Aidara-Kane, A., Rubino, S. (2006). Characterization of virulence factors in the newly described Salmonella enterica serotype Keurmassar emerging in Senegal (sub-Saharan Africa). *Epidemiology and Infection*, 134(4), 741–743. https://doi.org/10.1017/S0950268805005807
61. Borges, K.A., Furian, T.Q., Borsoi, A., Moraes, H.L., Salle, C.T., Nascimento, V.P. (2013). Detection of virulence-associated genes in Salmonella Enteritidis isolates from chicken in South of Brazil. *Pesquisa Veterinária Brasileira*, 33, 1416–1422.
62. Deguenon, E., Dougnon, V., Lozes, E., Maman, N., Agbankpe, J., Abdel-Massih, R.M. et al. (2019). Resistance and virulence determinants of faecal Salmonella spp. isolated from slaughter animals in Benin. *BMC Research Notes*, 12(1), Article 317. https://doi.org/10.1186/s13104–019–4341-x
63. Somda, N.S., Bonkoungou, I.J.O., Sambe-Ba, B., Drabo, M.S., Wane, A.A., Sawadogo-Lingani, H. et al. (2021). Diversity and antimicrobial drug resistance of non-typhoid Salmonella serotypes isolated in lettuce, irrigation water and clinical samples in Burkina Faso. *Journal of Agriculture and Food Research*, 5, Article 100167. https://doi.org/10.1016/j.jafr.2021.100167
64. Nikiema, M.E.M., Kakou-Ngazoa, S., Ky/Ba, A., Sylla, A., Bako, E., Addablah, A.Y.A. et al. (2021). Characterization of virulence factors of Salmonella isolated from human stools and street food in urban areas of Burkina Faso. *BMC Microbiology*. 21(1), Article 338. https://doi.org/10.1186/s12866–021–02398–6
65. Salih, W., Yousif, A.A. (2018). Molecular detection of Salmonella typhimurium isolated from canine feces by PCR. *Advances in Animal and Veterinary Sciences*, 6(12), 542–547. https://doi.org/10.17582/journal.aavs/2018/6.12.542.547
66. Kadry, M., Nader, S.M., Dorgham, S.M., Kandil, M.M. (2019). Molecular diversity of the invA gene obtained from human and egg samples. *Veterinary World*, 12(7), Article 1033. https://doi.org/10.14202/vetworld.2019.1033–1038
67. Ahmer, B. M. M., Gunn, J. S. (2011). Interaction of Salmonella spp. with the intestinal microbiota. *Frontiers in Microbiology*, 2, Article 101. https://doi.org/10.3389/fmicb.2011.00101
68. Hassan, K. I., Saleh, S. H. (2016). A rapid method for PCR based on detection of Salmonella spp. and Staphylococcus aureus in spiked and naturally contaminated food. *Iraqi Journal of Agricultural Sciences*, 47(7 — special issue), 66–73.
69. Oludairo, O.O., Kwaga, J.K.P., Dzikwi, A. A., Junaid, K. (2013). Detection of invA virulence gene by polymerase chain reaction (PCR) in Salmonella spp. isolated from captive wildlife. *Bio-Genetics Journal*, 1(1), 12–14.
70. Lu, R., Wu, S., Liu, X., Xia, Y., Zhang, Y. -G., Sun, J. (2010). Chronic effects of a Salmonella type III secretion effector protein AvrA in vivo. *PLoS ONE*, 5(5), Article e10505. https://doi.org/10.1371/journal.pone.0010505
71. Saber, A. S., Abeer, H.A., (2019). Molecular characterization of Salmonella species isolated from chicken table egg content. *Assiut Veterinary Medical Journal*, 65(162), 83–92. https://doi.org/10.21608/avmj.2019.168950
72. Jbar, H.S., Al-Janabi, H. S. Kadhim, M.J. (2021). Molecular detection of some outer membrane proteins related to immune resistance in Salmonella Enterica. *Turkish Journal of Physiotherapy and Rehabilitation,* 32(3), 18739–18743.
73. Hersh, D., Monack, D. M., Smith, M. R., Ghori, N., Falkow, S., Zychlinsky, A. (1999). The Salmonella invasin SipB induces macrophage apoptosis by binding to caspase-1. *Proceedings of the National Academy of Sciences*, 96(5), 2396–2401.
74. Cabezon, E., Ripoll-Rozada, J., Pena, A., de la Cruz, F., Arechaga, I. (2015). Towards an integrated model of bacterial conjugation. Microbiol. Rev. *FEMS Microbiology Reviews*, 39(1), 81–95. https://doi.org/10.1111/1574–6976.12085
75. Ahmet, N. A., Bissoume, S. B., Abdoulaye, S., Amadou, D., Khota, F. N., Aziz, W. A. et al. (2020). Phenotypic and genotypic characterization of Salmonella isolated from Asymptomatic Carriers in the Suburb of Dakar. *Journal of Tropical Diseases and Public Health*, 8, Article 350. https://doi.org/10.35248/2329–891X.20.8.346
76. Allaith, S. A., Abdel-aziz, M. E., Thabit, Z. A., Altemimi, A. B., Abd El-Ghany, K., Giuffrè, A. M. et al. (2022). Screening and molecular identification of lactic acid bacteria producing β-glucan in Boza and Cider. *Fermentation*, 8(8), Article 350. https://doi.org/10.3390/fermentation8080350

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EFFECT OF BLUEBERRY (VACCÍNIUM MYRTÍLLUS) LEAVES EXTRACT OBTAINED BY MICROWAVE HEATING ON THE DYNAMICS OF ANIMAL FAT OXIDATION PROCESSES

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Abstract
The potential of antioxidant properties of blueberry leaves extract (vaccínium myrtíllus) that grows in the Trans-Baikal region has been studied in this work. In order to increase the efficiency of extraction of biologically active substances with antioxidant properties, this extract was obtained with the help of microwave field. The optimal parameters for the extraction of active substances from the blueberry leaves with the help of electromagnetic microwave field have been defined. The influence of the power of the electromagnetic field and the duration of the process of blueberry leaves extraction on the efficiency of extraction of biologically active polyphenolic substances has been studied. The following parameters of the technology for blueberry leaves extraction were obtained: extraction with a water-alcohol solution with a concentration of 40% in the ratio of raw materials to extractant as 1 to 5, with duration of stirring as long as 30 minutes, then the application of an electromagnetic field of microwave heating with a power of 850W for 8–10 minutes long. Blueberry leaves extract is a clear liquid with a high content of polyphenols, of rich brown color, tart taste, without bitterness. The antioxidant potential of the obtained extract has been studied. To do this, the extract has been added into the finely ground animal fat and left for storage in a closed dark container. During storage, the dynamics of the peroxide number has been measured, as this value characterizes the degree of lipid oxidation. It has been found that blueberry leaves extract inhibits the process of animal fat oxidation due to the action of biologically active substances that feature antioxidant properties.

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Introduction

Blueberry (*vaccínium myrtíllus*) is a low shrub of 20–30 cm high, which is widely spread in the taiga zone of the Trans-Baikal region. This plant is characterized by a creeping underground rootstock and numerous shoots. Its bluish-black berries are consumed. The berries are rich in tannins, anthocyanins, tannins, flavonols and other substances [1,2]. In addition to berries, the blueberry leaves and shoots are used as a remedy, which also contain various biologically active substances and are recommended against diseases of the eyes, gastrointestinal tract, diabetes, in gerontology, in the treatment of skin burns, stomatitis, etc. [3].

Blueberry leaves are a renewable raw material. These leaves are of high value due to the availability of biologically active substances, they can be dried and stored for a long time; therefore they are of interest for consuming as an antioxidant component [4,5].

The analysis of references and specialized literature showed that blueberry leaves contain a fairly large amount of antioxidants from bioflavonoids group, such as anthocyanins and polyphenols [6,7]. In addition, the flavonoids like rutin, hyperoside, isoquercetin, etc. were found there too. A number of authors in their studies underline that there are related substances like sugars and tannins up to 20%, organic acids (citric, oxalic, malic, succinic, quinic, lactic up to 7% [8], vitamin C up to 250 mg%, vitamin B, carotene, phenolic compounds — hydroquinone (1%), arbumin (1–2%), neomyrtillin (up to 2%), myrtillin (up to 1%) and many others [9].

The high antioxidant characteristics of blueberry leaves opens up an opportunity of its using in the composition of fat-containing foods to inhibit oxidative processes in lipids. Most meat products contain a large amount of saturated and unsaturated lipids, which undergo oxidation during their storage, which in its turn negatively affects the organoleptic characteristics and shelf life of the finished food [10].

To prevent oxidative spoilage, manufacturers use various antioxidant and antimicrobial food additives. The most prominent and rife antioxidants used in the meat industry are as follows: ascorbic acid (E300), sodium ascorbate (E301), butylhydroxytoluene (E321), sodium lactate (E325) and others [11,12]. So, E. V. Hardina et al. describe studies on the storage stability of chilled pork treated with rosemary extract and dihydroquercetin [13].

The authors of the research [14] have studied the effect of sage extract on the functional and technological properties, quality and shelf life of chopped semi-finished food products. It has been established that it is expedient to add the sage extract in the amount of 0.1% of raw materials weight into the recipe of minced meat semi-finished food products.

In their study, Lorenzo et al. [15] also note that oxidative reactions can reduce the quality of meat food. The authors considered the protective effects of active rosemary against oxidative degradation in meat food. Adding the rosemary essential oil or its extract can slow down the development of oxidative reactions and preserve redness pf food surface, reduce the accumulation of primary and secondary lipid and protein oxidation products, and slow down the increase of perceived rancidity in sensory analysis of foods. Consequently, more people want to use herbal extracts [16] because they are generally recognized as safe [17,18]. When cooking meat, natural extracts should be used to protect the food from external influences and extend its shelf life [19].

Reddy et al. evaluated the effectiveness of various natural antioxidants in improving the quality and shelf life of meat products [20]. Natural antioxidants keep food resistant to oxidation, and also effectively control microbial growth. However, further research is needed to explore how different duration of exposure and volumes of added extract, as well as interactions with other natural antioxidants and nutritional supplements, will affect the results.

The authors of the study [21] found that elderberry contain a significant amount of monosaccharides; citric acid dominates among organic acids. Berries also contain succinic acid, which is an antioxidant involved in the metabolic reactions in a human body. Elderberry features a hepatoprotective, anti-stress, adaptogenic effect. In addition, succinic acid reduces the formation of excess amounts of cholesterol, thus preventing the loss of calcium by cells. Antioxidants belong to the category of inhibitors — substances that slow down the oxidative processes [22]. Due to their interaction with reactogenic oxidizing agents and reactive forms of oxygen, as well as with other free radicals, they can lead to partial or complete inactivation of oxidizers. The substances of polyene group are particularly active. Some of the plants species are rich in these substances characterized by several unsaturated bonds and the mechanism of action which is associated with their ability to easily oxidize and thereby prevent oxidation of the molecules.

For more efficient use of the complex of biologically active substances in the recipe of food products several methods of their extraction are used, like herbal infusions, herbal decoctions and extracts. Various extractants are used to facilitate the extraction of useful components from the raw materials: water, acids, alkalis, alcohols, organic solvents, etc. With regard to floral and plant raw materials, a water-alcohol solution is most preferrable, since it is a food component and has a high extractive capacity in regards to the plant polyphenols [23].

As a result of the analysis of available methods of extraction, the method of extraction with a water-alcohol solution with a concentration of 40% in a ratio of 1:5 has been chosen [24]. This method is widely used in industrial food production. To intensify the extraction process, various methods are used: maceration, fractional extraction, exposure to thermal energy, microwave field, ultrasound, etc. For example, in the work [25], the authors present data on the study of the effect of microwave extraction modes on the yield of flavonoids from the leaves of the common gromwell (*Lithospermum officinale L.*). It was noted that the highest yield of the desired components is achieved at a microwaves generator power of 100W (2.5 min) at a field frequency of 2,450 MHz, which is comparable to the yield when using traditional convective heating for extraction by infusion at elevated temperature. Microwave extraction reduces the process time by 16 times and increases the yield of flavonoids by 23%. The authors state, that microwave energy destroy the plant tissues, which facilitates the efficient extraction of biologically active substances.

The authors in the work [26] present test data on the study of the effect of microwave radiation (100W, 20 min) on the degree of extraction of *Alceanudiflora*. It has been proven that the chosen conditions of the extraction, due to the ability of the substance to convert microwave energy into heat, made it possible to significantly reduce the duration of the process and obtain extracts enriched with new compounds. 13 acids were found, including 6 aromatic acids and 7 unbranched monobasic acids of unsaturated and saturated series, as well as 11 new neutral compounds, including 7 naphthalene derivatives.

The authors in the publication [27] described a method run on the sample of eucalyptus leaves. This method has proven the increase in the efficiency of extracting components from the plant materials by using microwave energy of 1 kW (microwave supply for about 5 minutes). The method allows obtaining a concentrated extract and high quality products through the use of microwave energy.

Thus, based on the analysis of the literature, it was found that herbal infusions, extracts and decoctions are used in the composition of food products to use biologically active substances with antioxidant properties from plant materials. To increase the efficiency, microwave heating is used for the preparation of herbal extracts.

It was noted that blueberry leaves and berries are rich in biologically active substances with antioxidant properties, including polyphenolic group. There are no studies on extraction of the active substances from blueberry leaves to find and confirm their antioxidant effect on animal fats. In connection with the above, the purpose of the work was to substantiate the modes of extraction from blueberry leaves growing in the Trans-Baikal region with the help of microwave field and to study its effect on the dynamics of animal fat oxidation.

Objects and methods

The objects of research were blueberry leaves and raw animal fat.

The blueberry leaves (*vaccínium myrtíllus*) were picked up in 2020 during the blossoming of the blueberry in Zaigraevsky area in the territory of Angirsky natural reservation of the Republic of Buryatia (latitude: 52.098721; longitude: 108.562931). The collected plant material — blueberry leaves — were sorted by their size and quality, yellow and damaged leaves were disposed. The usable leaves were washed with cold water, dried in room conditions at a temperature of 22 °C to 25 °C and packed in paper bags. The finished material was stored at a temperature from 4 °C to 5 °C.

The samples of animal fat were raw horse fat and raw pork fat obtained from the slaughter of healthy livestock. Raw fat was ground in a household meat grinder MFW4 (Bosch, Germany), with grate diameter of 2–3 mm. The obtained leaves extract was added to the test samples in amount of 5% of the fat weight. The amount was determined in accordance with the recommended amount of antioxidants added to minced meat — 0.05–0.075%. Based on the content of antioxidants in the leaves extract, usually they introduce blueberry leaves extract into animal fat in amount of 5%. After adding the extract, the ground fat was well mixed and the dynamics of the peroxide value was analyzed in comparison with control samples, without any extract. Control sample and test sample were sealed in jars with a tight lid and stored for 120 hours at a temperature of 2 °C to 6 °C. those jars were sampled after 24, 48, 72, 96 and 120 hours of storage, and the peroxide value was analyzed by a method based on the interaction of the oxidation products in raw fats with potassium iodide in a solution of acetic acid and chloroform, followed by a quantitative determination of the released iodine with sodium thiosulfate solution with the help of the titrimetric method (GOST R51487–991).

At the initial stage, control and test samples of the extract from dried blueberry leaves were made. To run the extraction process, dried blueberry leaves were ground in a coffee grinder (Bosch MKM6000 with a capacity of 75 grams, 180 W) (Bosch, Germany). After grinding the leaves powder was placed in a beaker flask of 100 ml with a tight lid, poured with a water-alcohol solution, which is traditionally used in as an extractant for vegetable raw materials. For extraction, the flasks with control and test samples were placed under the same conditions: they were stirred in a shaker (Elpan Bath Shaker type 357) (Elpan, Poland) for 30 min at room temperature (20–22 °C), 120 shakes/min. After extraction under the constant stirring in a shaker, the test sample was subjected to microwave waves in order to increase the efficiency of the process of extracting biologically active substances. To do this, a tightly closed flask with the liquid was placed in a Samsung GW712BR microwave oven (Samsung, Vietnam). During the experiment, the parameters of the extraction process were selected and defined: the duration and power of microwave exposure in regards to blueberry leaves. The obtained control and test samples of blueberry leaves extract were filtered through lavsan cloth and filter paper.

During the experiment, the total content of polyphenols was determined with the help of the Folin-Ciocalteu reagent. The samples were taken from the obtained extract, with a volume of 0.075 cm_3. The Folin-Ciocalteu reagent in volume of 0.075 cm^3 was diluted 5 times, added to the samples, stirred. In 3 minutes 0.15 cm^3 of 20% sodium carbonate solution and 1.2 cm^3 of distilled water were added. The mixture was covered with a lid, stirred and left at room temperature. After 1 hour of the optical density of the obtained tungsten blue (TB) was measured at a wavelength of 725 nm with the KFK-3–01 photoelectric photometer (Russia, OJSC ZOMZ), at the optical path length of 1 cm. The total content of TB is expressed in mg-equivalents of gallic acid per g of fresh weight of raw materials. The content of organic acids and water-soluble vitamins was determined by capillary electrophoresis with the Kapel-105M instrument (Russia, LLC Lumeks-Marketing) with indirect detection at a wavelength of 190 nm [28]. The acidity of the medium was determined by the potentiometric method. The obtained extract was organoleptically evaluated according to GOST 18078–722. The optical characteristics of the extracts were obtained with the help of KFK-3–01–30MZ photometer (Russia, OAO ZOMZ).

The experimental data were obtained in triplicate and statistically analyzed with MS Excel (Microsoft, USA) software. Results were presented as mean-root-square (S), mean-root-square (standard) deviation (±SD). Differences between control and test samples were recognized as significant at a probability level not higher than 0.05.

During the experiment, to find the optimal parameters of microwave heating during the extraction of blueberry leaves, the Mathcad 15 software (RTS, USA) was used. To

[1] GOST R51487–99 "Vegetable oils and animal fats. Method for determination of peroxide value". Moscow: Standartinform, 2003. Retrieved from https://docs.cntd.ru/document/1200028330 Accessed November 10, 2022 (In Russian)

[2] GOST 18078–72 "Fruit and berry extracts. Specifications" Moscow: Publishing House of Standards, 1998. Retrieved from https://docs.cntd.ru/document/1200022569 Accessed November 10, 2022 (In Russian)

begin with, two main variable factors affecting efficiency were determined — the duration of heating and the power of the microwave field, then the "desirability" function — the total content of antioxidants in the obtained extract — was established. The matrix of a two-factor experiment was composed. There was a condition that the level of the first factor crosses once with each level of the second factor. In result of solving the problem, the maximum values of the variables function were obtained.

Results and discussion

To extract the biologically active substances from the leaves of blueberry that grows in the Trans-Baikal region, the possibility of their extraction with a microwave field in order to increase the degree of extraction was studied. To extract useful components from the raw materials, including the antioxidant substances, various extractants were used. The most suitable among them was water-alcohol solution, as it is a food component and has a high extracting capacity.

To define the modes of technology for extracting, it is necessary to optimize the parameters of microwave extraction of plant components from the blueberry leaves. For this, a factorial experiment was used with involvement of mathematical methods.

The main extraction parameters to be optimized are the following:

- the duration of microwave extraction;
- the power of the microwave field.

Based on the planned parameters (Table 1), a matrix of a two-factorial experiment was compiled. This matrix served as the basis for a number of tests. The limitations of the studied parameters were chosen on the basis of the literature data of the authors, who recommend microwave processing for the plant substances extraction. The total value of antioxidants content was taken as the function of "desirability", which characterizes the efficiency of the extraction of biologically active antioxidant substances from the plant raw materials.

Table 1. Levels of factors studied

Factor	Level						
	1	2	3	4	5	6	7
X_1, Duration, minutes	0	2	4	6	8	10	—
X_2, Microwave power, W	0	450	550	650	750	850	950

The figure 1 below shows the contour of the "desirability" and its function of the microwave processing duration and microwave power on the content of polyphenols in the resulting extract.

In order to find the maximum value of the response function and the values of the corresponding factor, a standard procedure was performed to find the maximum of a function of two variables in a bounded domain of definition. When solving the extremum problem, the following solution was obtained (Table 2).

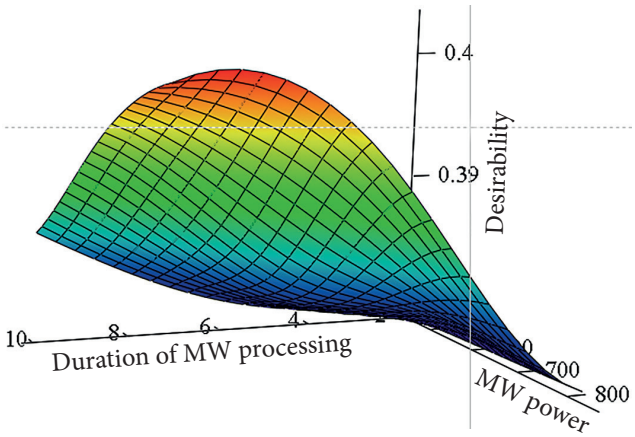

Figure 1. Effect of power and duration of microwave treatment on the content of antioxidants in the extract

Table 2. Results of solving the extremum problem

Duration at the peak value	Power at the peak value	Response function at the peak value
$X_1 = 9.2$ min	$X_2 = 850$ W	Y = 14.5%

The microwave field, due to its influence on the solvent dipoles orientation, converts microwave energy into thermal energy, thus heating up the entire bulk of the material. The heating induces destruction of plant tissue. This contributes to a more efficient extraction of biologically active substances from the plant materials into the solvent medium.

As a result of the experiments, the following parameters for obtaining an extract from blueberry leaves were chosen: extraction with a water-alcohol solution of 40% concentration, the ratio of raw materials to the extractant was 1:5 (the ratio recommended for plant raw materials ranges from 1:1 to 1:10), the microwave heating power was 850 W, duration of heating was 9–10 minutes in order to ensure the efficiency of the process.

Further, comparative studies of the characteristics of the control sample (without microwave heating) and test sample (exposed to the microwave heating) of blueberry leaves extracts were run (Table 3).

Table 3. Characteristics of blueberry leaves extract

Parameters	Blueberry leaves extract	
	Control	Test sample (with MW)
Sediment	Liquid without any sediment and foreign inclusions	Liquid without any sediment and foreign inclusions
Taste and smell	Weakly tart, without bitterness	Tart, slightly herbal, without bitterness
Color	Light brown	Rich brown
pH	4.22 ± 0.21*	4.17 ± 0.20
Sugar, %	14.50 ± 0.61*	15.40 ± 0.52
Organic acids, %	7.20 ± 0.42**	8.40 ± 0.53
Ascorbic acid, mg%	0.25 ± 0.03*	0.29 ± 0.02
Total content of antioxidants, %	9.23 ± 0.25**	14.42 ± 0.16
Total content of polyphenols, %	2.92 ± 0.22**	9.20 ± 0.34

* $p > 0.05$; ** $p < 0.05$

It was noted that blueberry leaves extracts obtained by maceration (control sample) and obtained by the microwave heating (test sample) were a liquid without sediment with a different shade of color.

The saturation of the extracts color, which depends on the amount of the extracted coloring pigments of anthocyanins of the polyphenolic group, was further determined by the spectrophotometric method.

When extracting the substances from the crushed plant raw materials, biologically active substances of blueberry leaves, including polyphenols, sugars, organic acids, ascorbic acid, etc., passed from the leaves into a water-alcohol solution. The test data presented in the Table 3 and their statistical processing showed that in terms of pH, sugar content and ascorbic acid content, no significant differences were found in the control sample and the test sample of the extract ($p > 0.05$).

Biologically active substances of the polyphenolic group showed the greatest antioxidant activity in plant raw materials. The data of the Table 3 indicate that the content of polyphenols significantly increased in the test sample of blueberry leaves extract — it increased three times ($p < 0.05$).

This fact proved the increase in the biologically active substances concentration, and hence it increased the efficiency of the extracting components from the blueberry leaves with the help of a microwave field.

Figure 2 shows the scheme for preparing an extract from the blueberry leaves.

To confirm the differences in saturated color of the extract obtained with the microwave heating, which proves a higher concentration of biologically active substances in the solution, the values of the optical density of the analyzed extract samples at the absorption maximum (340–400 nm) were studied (Table 4).

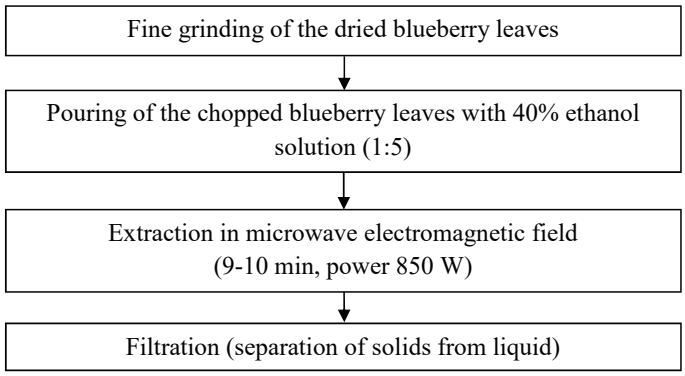

Figure 2. Scheme for preparing an extract from blueberry leaves

Table 4. The value of the optical density of the extract obtained from blueberry leaves

Sample	The value of optical density D, units	Total content of antioxidants (TCA), %
Control	1.75	9.23 ± 0.25
Test	2.72	14.42 ± 0.16

The obtained data confirmed the darker, more saturated color of the test sample. The value of the optical density of the test extract is higher by 55.4% compared to the control sample ($p < 0.05$). A higher value of the total content of antioxidants in the test sample of the extract obtained by microwave heating was noted in comparison with the control sample by 6.19%, which is 56.2 rel.% higher.

At the next stage an experiment was carried out to study the influence of the extract from blueberry leaves obtained with a microwave heating on the dynamics of oxidative process in animal fat. Samples without blueberry leaves extract were used as the control samples (Figures 3 and 4).

The data presented in the Figures 3 and 4 prove that blueberry leaves extract is able to inhibit oxidative processes in raw pork fat and raw horse fat. These processes are

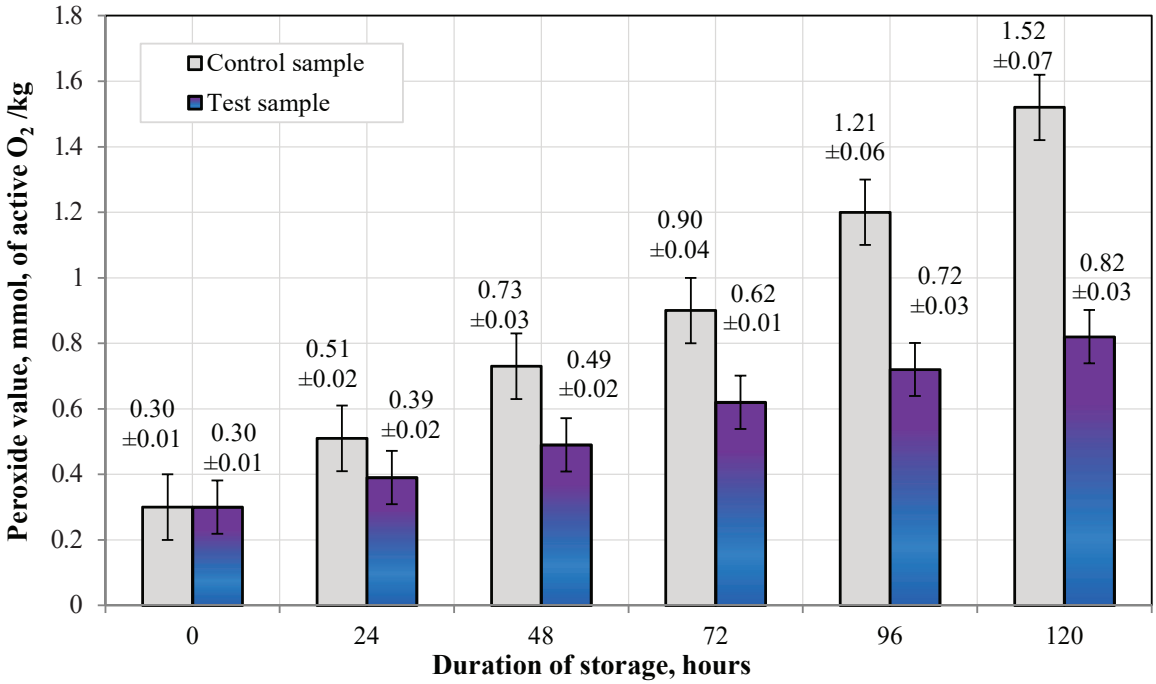

Figura 3. Dynamics of peroxide value change in the pork fat with blueberry leaves extract added

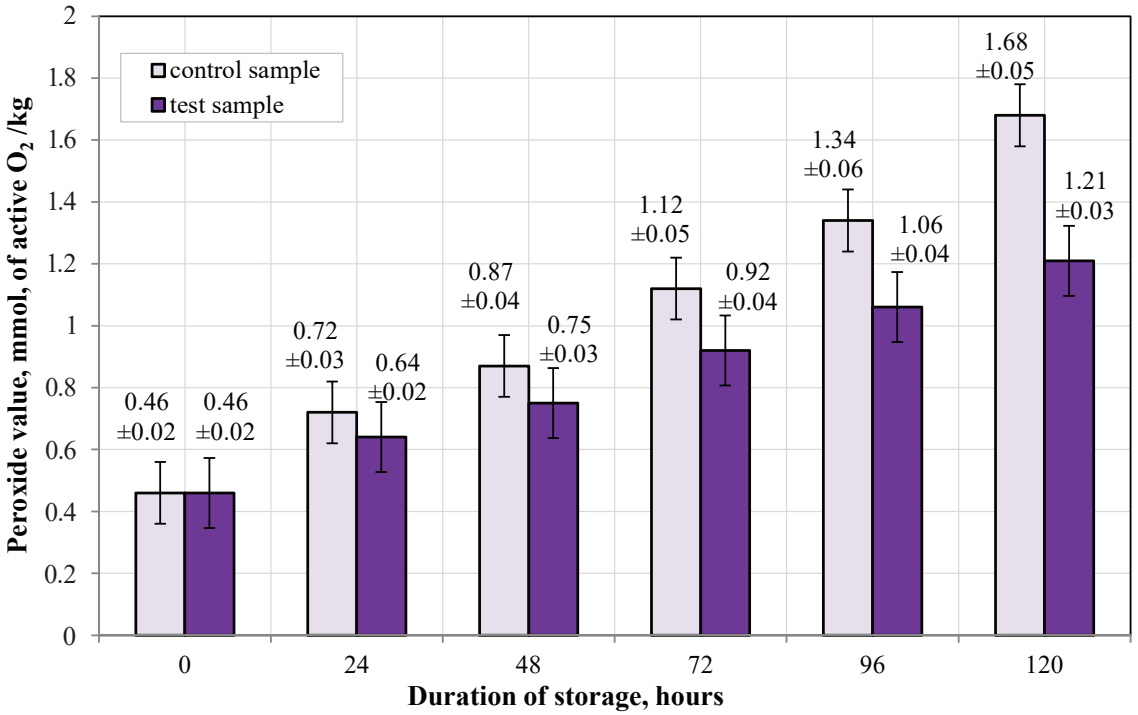

Figure 4. Dynamics of peroxide value of horse fat with blueberry leaves extract added

the continuous chain reaction, which can be inhibited by certain inhibitors with high antioxidant properties. The data of the Figure 3 showed that after 120 hours of storage of pork fat, inhibition of its peroxidation was observed, since the value of the peroxide value in the test sample is 46% lower in comparison with the same value in the control sample ($p<0.05$). In horse fat, the difference between the peroxide value in the control samples and test samples after 120 hours of storage reached 67% ($p<0.05$). In horse fat the oxidation process was more intense, since horse fat has more unsaturated fatty acids than pork fat. The influence of the biologically active substances in the extract is effective for both types of animal fat, therefore, the addition of the blueberry leaves extract as a renewable plant raw material into meat food can help inhibit oxidative processes, thereby extending the shelf life of finished food products.

The obtained test results are consistent with research data obtained by scientists in the food industry both in the Russian Federation and in foreign countries.

The researches of the Nanjing Agricultural University obtained data on the effect of fermented blueberry, added in amounts of 2, 4 and 6% to the meat food products, on the degree of oxidation of the fat fraction in the boiled sausage during its storage (4 °C) for 28 days. They found that adding of blueberry slows down the oxidative deterioration of fat, which was confirmed by a significant decrease in peroxide and thiobarbituric values [30].

The article [31] presents data on the effect of aromatic herbs (coriander, basil, parsley, rosemary) on preservation and food safety of sausages. It was found that the finished food product can be stored for up to five days without chemical additives due to the presence of substances with antioxidant properties among the ingredients.

Fat peroxidation value decreased during storage of the protein-fat composition with added thistle extract. That is explained by the interaction of flavonoids from the extract and free radicals, which interaction increases the storage duration of the fat-containing food [32].

The positive effect of aloe vera extract on slowing down the oxidative spoilage of fermented sausages has been established. After 30 days of storage in sausages containing only sodium nitrite and sausages containing only aloe vera extract, a decrease in the content of the thiobarbituric value was recorded in comparison with the control sample by 48% and 45%, respectively. The least TBV (decreased by 68%) was recorded in the sausages with added aloe vera extract and sodium nitrite ($p < 0.05$) [33].

The presented publications provide data on the positive effect of the plant raw materials on oxidative processes during the storage of animal fat.

Conclusions

The influence of electromagnetic field power and duration of extraction on the efficiency of extraction of substances of a polyphenolic group was studied. The following extraction parameters were obtained: extraction with a water-alcohol solution with a concentration of 40%; the ratio of raw materials to the extractant was 1:5; the solution was exposed to the microwave heating with a power of 850W for 9–10 minutes long. Experiments have shown that blueberry leaves extract is capable to inhibit oxidative processes in animal fat due to the action of polyphenols with antioxidant properties, thus extending the shelf life of fat-containing finished food products. The antioxidant properties of blueberry leaves extract are proven by the high total content of antioxidants (14.42%), which are able to neutralize the free radicals and prevent oxidation processes.

REFERENCES

1. Kurkin, V.A., Ryazanova, T.K., Petrukhina, I.K. (2014). Blueberry: modern approaches to the standardization of raw materials and the creation of medicines. Samara: Ofort, 2014. (In Russian)
2. Neuenfeldt, N.H., de Moraes, D.P., de Deus, C., Barcia, M.T., Menezes, C. (2022). Blueberry phenolic composition and improved stability by microencapsulation. *Food and Bioprocess Technology,* 15(3), 1–18. https://doi.org/10.1007/s11947–021–02749–1
3. Kowalczyk, E., Krzesiński, P., Kura, M., Szmigiel, B., Blaszczyk, J. (2003). Anthocyanins in medicine. *Polish Journal of Pharmacology*, 55, 699–702.
4. Tipsina, N.N., Yakovchik, N. Yu. (2013). Blueberry research. *Bulletin of KSAU*, 11(86), 283–285. (In Russian)
5. Belova, Y A., Tritek, V. S., Shulgau, Z. T., Gulyayev, A. Y., Krivykh, E. A., Kovalenko, L. V. et al. (2020). The study of phenolic compounds of the berries of three species of plants of the genus vaccinium, growing in the Khanty-Mansi autonomous area. *Khimija Rastitel'nogo Syr'ja*, 1, 107–116. https://doi.org/10.14258/jcprm.2020014534 (In Russian)
6. Ijaz, N., Bader, U., Ain, H., Bashir, S., Tufail, T., Ameer, K., Imran, S. et al. (2022). Health promoting properties and extraction of specific bioactive compounds in blueberry: Bioactive compounds in blueberry. *Pakistan BioMedical Journal*, 5(5), 18–20. https://doi.org/10.54393/pbmj.v5i5.492
7. Gumbrewicz, R., Calderwood, L. (2022). Fertility effects on blueberry gall midge (Diptera: Cecidomyiidae) in wild blueberry (*Vaccinium Angustifolium; Ericales; Ericaceae*). *Journal of Economic Entomology,* 115(3), 783–791. https://doi.org/10.1093/jee/toac043
8. Bazhenova, B.A., Leskova, S. Yu., Dobretsky, R.A., Burkhanova, A. G. (2022). *Blueberry are a source of natural antioxidants.* Food Innovations and Biotechnologies: Collection of Abstracts of the X International Scientific Conference of Students, Graduate Students and Young Scientists. Kemerovo. (In Russian)
9. Ryazanova, T.K. (2013). Pharmacognostic study of blueberry fruits and shoots. *Fundamental Research,* 8–5, 1136–1140. (In Russian)
10. Aslanova, M.A., Dydykin, A.S., Fedulova, L.V., Derevitskaya, O.K. (2017). Influence of electromagnetic treatment on oxidative stability and microbiological safety of meat intermediates. *Theory and Practice of Meat Processing,* 2(3), 9–48. https://doi.org/10.21323/2414–438X-2017–2–3–39–48. (In Russian)
11. Liu, F., Cheng, X., Liu, W., Miu, J., Wang, J., Cui, X. et al. (April 15, 2021). *Study on the extraction of polyphenols from blueberry leaves and their antioxidant properties.* International Conference on Tourism, Economy and Environmental Sustainability, 251, Article 02059. https://doi.org/10.1051/e3sconf/202125102059
12. Manessis, G., Kalogianni, A. I., Lazou, T., Moschovas, M., Bossis, I, Gelasakis, A.I. (2020). Plant-derived natural antioxidants in meat and meat products. *Antioxidants,* 9(12), Article 1215. https://doi.org/10.3390/antiox9121215
13. Hardina, E.V., Krasnova, O.A. (2019). Optimization of storage time of chilled pork through the use of natural antioxidants. *The Bulletin of Izhevsk State Agricultural Academy*, 2(58), 37–44. https://doi.org/10.21323/2071–2499–2022–2–10–13 (In Russian)
14. Kalinicheva, N.N. (2021). Investigation of the effect of sage extract on the functional and technological properties of chopped semi-finished products. *Biology in Agriculture*, 1(30), 30–34. (In Russian)
15. Lorenzo, J. M., Munekata, P.E., Pateiro, M., Domínguez, R., Alaghbari, M., Tomasevic I. (September 26–29, 2021). *Preservation of meat products with natural antioxidants from rosemary*. IOP Conference Series: Earth and Environmental Science, 854(1), Article 012053. https://doi.org/10.1088/1755–1315/854/1/012053
16. Ribeiro J. S., Santos, M.J.M.C., Silva, L.K.R., Pereira, L.C.L., Santos, I.A., da Silva Lannes, S.C. (2019). Natural antioxidants used in meat products: A brief review. *Meat Science*, 148, 181–188. https://doi.org/10.1016/j.meatsci.2018.10.016
17. Schegoleva, I. D., Molchanova, E. N. (2020). Decoction production waste as an additional resource of biologically active substances. *Health, Food & Biotechnology*, 2(1), 153–164. https://doi.org/10.36107/hfb.2020.i1.s297 (In Russian)
18. Kausar, T., Azad, Z. R. A. A., Anwar, S., Shahid, S.M.A., Kausar, M.A. (2021). Application of natural antioxidants for the formulation of functional meat products. *Neuro Pharmac Journal*, 6(3), 269–276. https://doi.org/10.37881/1.636
19. Tsurupa, M. A., Borovskaya, L.B. (2021). Methods of obtaining CO_2 exctracts of phytomaterials and their use in fish and meat products. *The Scientific Heritage*, 81(2(81)), 41–43. https://doi.org/10.24412/9215–0365–2021–81–2–41–43
20. Reddy, D. W., Reddy G. V. B., Mandal, P.K. (2018). Application of natural antioxidants in meat and meat products — A review. *Food and Nutrition Journal,* 3(3), Article 173. 1–12. https://doi.org/10.29011/2575–7091.100073
21. Burak, L. Ch. (2020). The content and significance of functional foods, using the juice of blueberry.*International Journal of Applied Sciences and Technology Integral*, 5, 41–49. https://doi.org/10.24411/2658–3569–2020–10089 (In Russian)
22. Reshetnik, E. I., Mandro, N. M., Sharipova, T. V., Maksimyuk, V. A. (2013). The possibility to use the flour of grape breed "Amursky" as an antioxidant additive when designing gerodietetical meat and vegetable prepared food. *Far East Agrarian Bulletin*, 4(28), 46–49. (In Russian)
23. Belokurov, S.S., Narkevich, I.A., Flisyuk, E.V., Kaukhova, I.E., Aroyan, M.V. (2019). Modern extraction methods for medicinal plant raw material (Review). *Pharmaceutical Chemistry Journal*, 53(6), 559–563. https://doi.org/10.1007/s11094–019–02037–5
24. Khishova, O.M. (2020). Technology for obtaining tincture of common blueberry leaves. *Bulletin of Pharmacy*, 4(90), 55–59. (In Russian)
25. Adamtsevich, N. Yu., Feskova, E. V., Boltovsky, V. S., Titok, V. V. (2021). Extraction of flavonoids from the leaves of the Little wale lithospermum officinale. I. (Boraginaceae) using microwave energy. *Khimija Rastitel'nogo Syr'ja*, 1, 85–92. doi.org/10.14258/jcprm.2021018244 (In Russian)
26. Pankrushina, N. A., Kukina, T. P. (2021). New components of Alceanudiflora extract after microwave extraction. *Khimija Rastitel'nogo Syr'ja,* 1, 79–84. https://doi.org/10.14258/jcprm.2021018361s (In Russian)
27. Rushchits, A. A., Shcherbakova, E.I. (2014). Application of microwave heating in the food industry and public catering. *Bulletin of the South Ural State University. Series: Food and Biotechnologies*, 2(1), 9–15. (In Russian)
28. Komarova, N.V. (2006). Practical guide to the use of capillary electrophoresis systems "Capel"». Saint Petersburg, Veda, 2006. (In Russian)
29. Yusupova, G.F. (2017). Using the desirability function in assessment of level of technospheric safety of the territory. *Socio-Economic and Technical Systems: Research, Design, Optimization*, 3(76), 67–81. (In Russian)
30. Zhou, H. Zhuang, X., Zhou, C., Ding, D., Li, C., Bai, Y. et al. (2019). Effect of fermented blueberry on the oxidative stability and volatile molecule profiles of emulsion-type sausage during refrigerated storage. *Asian-Australasian Journal of Animal Sciences,* 33(5), 812–824. https://doi.org/10.5713/ajas.19.0094
31. Martemyanova, L.E., Savelyeva, Yu.S. (2017). The application of antioxidants for the purposes of experation dates increase in meat industry. *Bulletin of Omsk State Agrarian University*, 4(28), 228–233. (In Russian)
32. Zhmurina, N.D., Parshina, E.A., Baranchikova, O.A. (2016). The effect of milk thistle extract on the oxidation processes of protein-fat composition. *Scientific Journal of OrelSIET,* 2(14), 160–164. (In Russian)
33. Uşan, E., Kılıç, G.B., Kılıç, B. (2022). Effects of Aloe vera utilization on physochemical and microbiological properties of Turkish dry fermented sausage. *Journal of Food Science and Technology,* 59(5), 1727–1738. https://doi.org/10.1007/s13197–021–05183–5

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MECHANICALLY DEBONED POULTRY MEAT AND ITS ROLE IN RATIONAL AND EFFICIENT USE OF RAW MATERIALS

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Abstract

The growth in poultry meat production is a worldwide trend. Industrial poultry meat processing is also developing with production of a wide range of products. The technology of mechanical deboning of poultry meat and carcass parts is widely used in complex non-waste production. Mechanically deboned poultry meat (MDPM) is believed to be of inferior quality and its use is restricted by certain rules in different countries of the world. At the same time, hand separated meat is accepted as conventional meat and is not subjected to any restrictions. Over the last decades, the technology and equipment have been created that allow approximating MDPM to the category "meat" in terms of quality characteristics and reducing risks in its use upon reduction of pressure in the process of its production. However, costs of new equipment that enables producing a product with higher quality do not provide the expected efficiency, and a positive effect will be achieved only in the case of clear legal solutions regarding separation of MDPM types and methods of their classification and identification. The volume of scientific publications concerning a solution to this problematic theme is significant and scientists from many countries search for approaches to its realization differently. The difficulty in finding a solution is caused by the multifaceted nature of the problem, the character of non-standardized raw materials, a type of equipment being used to obtain different MDPM types, and various methods of investigations. Nevertheless, the performed studies create conditions for improvement of the approach to classification of different MDPM types by the production method and maximum allowable threshold values of the main standardized parameters, assessment methods, detection of their characteristics and substantiation of terminology.

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Introduction

Poultry husbandry is a leading branch of production of animal husbandry products worldwide and is constantly increasing its outputs. According the forecasts presented at the 24th European Poultry Conference, poultry meat production should have reached 124.91 million tons in 2020 [1], but actually it accounted for 130 million tons already in 2019 [2]. Russia made a significant contribution to this quantity (5 million tons) and stably occupies the fourth place in the world by this indicator [3].

Radical changes have taken place in the world poultry husbandry over the last decades. As a result of the growth in production of poultry meat, it became a widely used raw material for further industrial processing. During last decades, the consumer demand shifted from whole carcasses to their parts and poultry meat products.

Equipment for deboning carcasses and their parts to obtain poultry pieces as well as to produce mechanically deboned poultry meat (MDPM) was designed with the aim of mechanization of labor-intensive processes and increase in efficiency of operations of meat separation from the bone fraction.

MDPM production volumes are growing in the world, including Russia. For example, about 15–20 thousand tons of mechanically deboned poultry meat out of 1,800 thousand tons of poultry meat in slaughter weight were produced in the country in 1990 [4]. In succeeding years, the MDPM use sharply grew due to its import from the USA and Europe. In 2002–2004, the import volume was 240–270 thousand tons annually. An increase in domestic poultry meat production, growth in its industrial processing into products, creation of the technical base of MDPM production using domestic and import equipment allowed producing and processing into products about 500–550 thousand tons of MDPM according to our estimates, which accounted for 14–15% of the total poultry meat production volume in agricultural enterprises. At the same time, resources (bone residue) for non-waste utilization of poultry raw materials have been created [5].

MDPM is widely used in industrial processing both in the poultry processing industry and meat industry. Due to its nutritional and functional characteristics, mechanically deboned poultry meat is suitable for production of a wide assortment of sausage products, frankfurters, nuggets and so on [6].

However, the process of MDPM production inevitably leads to changes in its chemical, physical, organoleptic and functional properties. The characteristics of mechanically deboned poultry meat are determined by a type and quality of the non-standardized raw material being processed that is sent to deboning (defect carcasses, different parts with skin or without skin) with different meat-bone index, as well as by parameters of its technological preparation, type of equipment for deboning, pressure in its working zone, condition of working bodies, content of calcium and bone inclusions, qualification of personnel, scheme of a technological process, the target parameter of quantity of produced products that ensures the balance between MDPM yield and quality in terms of functionality for further application [7,8,9,10].

According to the existing legislation of the majority of countries, the use of MDPM requires the mandatory indication of its presence on a product label as a separate component that is not included into the ingredient "meat". The development of new technologies based on the modern equipment for MDPM production under low pressure enabled making it closer to the characteristics of poultry meat, but does not allow obtaining the economic effect expected by producers.

Therefore, it is necessary to find the main criteria for its classification and determine under which conditions it can be assigned to the term "meat". In addition, the separate processing of its types depending on the content of wholesome components is expedient for rational use of raw materials intended for MDPM production.

Unfortunately, there are no clear boundaries for separation by quality of MDPM types compared to hand deboned meat, common terminology, methods for classification and identification. Different countries approach to this problem and search for ways of its solution differently.

The aim of this review is to analyze the state of regulatory normative rules for production of different types of mechanically deboned poultry meat in leading world countries, methods for their classification and identification, and determine ways for solving this problem in Russia based on the world scientific experience.

Mechanically deboned poultry meat in national normative documents

Previously, the term "mechanically deboned meat" was used in the USA and Europe to characterize MDM. Then, the term "mechanically separated meat" came to be regarded as more correct [11]. In Russia, the term "mechanically deboned meat" is officially used with indication of its type (chicken or turkey) and this term is analogous to the term mechanically separated meat[1]. Raw materials for MDPM are poultry carcasses with defects, carcass parts with previously removed meat in pieces (frames, back-shoulder part, wings, neck).

Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 [12] laying down specific hygiene rules for food of animal origin understands by the term "mechanically separated meat" (MSM) a product that was "obtained by removing meat from flesh-bearing bones after boning or from poultry carcasses, using mechanical means resulting in the loss or modification of the muscle fibre structure" and contains calcium insignificantly exceeding its presence in minced meat, for which Commission Regulation (EC) No. 2074/2005 [13] established a limit of no more than 0.1% (=100 mg/100 g or 1,000 ppm) of a fresh product.

According to the existing Code of Federal Regulations of the USA [14], when sending to mechanical deboning carcass parts, in which most of meat is retained as well as whole carcasses of non-standardized poultry, mechanically separated meat can be classified as "ground chicken meat". If the initial raw materials are frames, pieces or part of carcasses, from which most of meat was removed by hand, this meat should be defined as "mechanically separated meat" *with allowable limits for the calcium content and sizes of bone particles*.

According to FSIS Directive 7160.1 (1.09.96) (UDSA, USA) [15], two criteria were established to define the term "meat produced by advanced meat/bone separation machinery and meat recovery systems": the maximum calcium content should be no more than 0.15% and "the bones emerging from the advanced recovery systems must be essentially intact and recognizable to assure that the bones are not being crushed, ground, or pulverized". It is expected that the content of bones and bone constituents (for example, marrow) in a product obtained using these systems would not be higher than expected in a product obtained by hand deboning. Such meat should be produced under the control of inspectors of Food Safety and Inspection Service (FSIS).

GOST R 52313–2005 "Poultry-processing industry. Food products. Terms and definitions"[2] defines "mechanically deboned poultry meat" as a food product obtained as a result of deboning of an eviscerated poultry carcass or its parts by the method of separation and representing finely comminuted mass with the normed quantity and size of bone tissue.

EAEU TR 051/2021 "On the Safety of Poultry Meat and its Processed Products" [16] slightly changed the term introducing several amendments and defined "mechanically deboned poultry meat" as a product of poultry slaughter obtained as a result of deboning by the method of separation of an eviscerated poultry carcass or its parts including necks or bones with pieces of flesh no less than 30%, consisted of minced muscle, fatty and connective tissues with the normed size and mass fraction of bone inclusions. They are stated in the active GOST 31490–2012[3].

[1] GOST R 52313–2005. "Poultry-processing industry. Food products. Terms and definitions" Retrieved from https://docs.cntd.ru/document/1200039098 Accessed March 02, 2023. (In Russian)

[2] GOST R 52313–2005. "Poultry-processing industry. Food products. Terms and definitions" Retrieved from https://docs.cntd.ru/document/1200039098 Accessed March 02, 2023. (In Russian)

[3] GOST 31490–2012. "Poultry meat of mechanical separation. Specifications" Retrieved from https://docs.cntd.ru/document/1200095720 Accessed March 02, 2023. (In Russian)

The normative document approved by the Order of the Ministry of Health of Ukraine [17] introduces the following term: "Poultry meat separated with the use of mechanical means" (MSMM), which applies to all meat separated mechanically (its two types). The term MSM (mechanically separated poultry meat) defines the mass obtained by the mechanical separation of flesh from bones. With that, the calcium content in the indicated mass should not differ significantly from its content in minced meat obtained by hand deboning. If the content of calcium in the obtained mass is significantly higher than that in minced poultry meat, the mass is called MDM (mechanically deboned poultry meat).

In the guideline of the Canadian Food Inspection Agency "Meat Processing Controls and Procedures" [18], two terms are introduced for meat obtained using mechanical equipment to separate meat and bones: mechanically separated meat (MSM) and finely textured meat (FTM). Mechanically separated meat (MSM) should contain no more than 0.027% of calcium for every 1% of protein, no bone particles with a size of more than 2 mm, the minimum protein content of 10% (14% for retail sale). Finely textured meat (FTM) used as ground meat should contain no less than 14% of protein, no more than 0.15% of calcium, should not have bone particles with a size larger than 1.5 mm and the maximum of 20% of the bone particles with a size larger than 1 mm. Bones coming from the separation equipment should be basically intact and recognizable to guarantee that bones were not crushed, ground or pulverized.

Technical Regulations on Identity and Quality of Mechanically Separated Meat (CMS) from poultry, beef and pork (Brazil) [19] introduces the following definition: by mechanically separated meat (CMS) is meant meat obtained by mechanical comminution and separation of bones of meat-producing animals intended for production of *certain* meat products from poultry meat. It is characterized by the following indicators: protein (minimum) 12%, fat (maximum) — 30%, calcium content (maximum) — 1.5% (dry matter), bone diameter — 98% should have a size (maximum) of 0.5 mm, width (maximum.) 0.85 mm, (maximum) –1 meq KOH/1 kg fat [19].

The international organization for food quality Codex Alimentarius Commission establishes for MSM recommendations only for the calcium content — no more than 1.5% on dry matter basis [20].

Analysis of national normative-technical documents of several countries that are the main MDPM producers show significant differences in assessment criteria for "mechanically deboned poultry meat". For example, requirements of the content in MDPM of protein mass fraction are in a range from 10% to 15%, fat from 12% to 30%, calcium from 0.1% to 2.75%, amount of bone inclusions from 0,35% to 1%, their size from 400 μm to 2 mm. There are also other differences and requirements indicated in the above-mentioned documents [12,13,14,15,16,17,18,19,20]. The reason for such differences in various approaches to the problem includes non-standardized initial raw materials, poultry age, a ratio of meat and bone tissues in raw materials, initial temperature, type and design of the deboning equipment, its technical condition, used pressure and so on.

When using MDPM for meat product manufacture, the mandatory indication of its presence on a label as a separate component is necessary according to the international and national requirements. For example, Article XI (paragraph 110) of the TR CU "On the safety of meat and meat products" (TR CU 034/2013) [21] states that in the case of using mechanically deboned (finally deboned) meat in the manufacture of meat products, the information about its use shall be indicated in the composition of such products (for example, "mechanically deboned meat"). The similar requirement is in Article 12 (paragraph 106) of the EAEU TR 051/2021 "On the Safety of Poultry Meat and its Processed Products" [16].

In Europe, the sale of mechanically separated meat (MSM) as "meat" is also banned. If MSM is used as an ingredient of a product, it should be indicated in the list of ingredients as "mechanically separated meat". This rule also acts in other countries including the USA.

The foregoing analysis of the normative documents of several countries that dominate in the world by the share of mechanically deboned meat production shows that these documents reflect the search for the ways of increasing efficiency due to an improvement of MDPM characteristics.

Mechanically deboned meat (MDM) is usually regarded as low-quality and is used according to certain rules. It differs from hand separated meat by an increased risk of microbiological contamination, proportion of bone inclusions, their fractional composition, calcium and phosphorus content, chemical indicators (fat, protein, moisture) and by technological properties (water holding, water binding and emulsifying capacities).

At the same time, the biological value of MDPM protein is approximately the same as that of protein of hand deboned poultry meat and is predetermined by the amino acid composition. The deficiency of amino acids was not established in MDPM compared to chicken egg protein. It is necessary to note that part of connective tissue is separated from the muscle part of meat upon separation and enters the meat-and-bone residue. The relative biological value (RBV) determined using infusoria turned to be significantly higher ($P < 0.05$) compared to that of hand deboned meat in reference to casein [22].

The development of new modern technologies and equipment for mechanical deboning of meaty bones allows producing meat raw materials, which is difficult to distinguish from conventional minced meat; therefore, there are no objective reasons to classify all MDM as low-quality. This is stipulated in the normative documents of the USA [15], Ukraine [17] and Canada [18]. For example, according to the existing EU Regulations [13,23], MSM types are distinguished depending on whether low or high pressure was used in their production and are determined

according to the alterations in the bone structure and calcium content. The EU upper limit for the calcium content in low-pressure MSM is 100 mg/100 g (1,000 ppm), and MSM with the calcium content higher than this threshold is considered high-pressure MSM. Other terms are also used to define these types of meat: "soft and firm", "firm separation", and "soft separation", "Baader meat" [24,25].

The EU member states usually designate the pressure of up to 10^4 kPa (equal to 100 bar) for low-pressure MSM and the pressure higher than 10^4 kPa (up to 4×10^4 kPa or higher) for high-pressure MSM [26].

Nevertheless, classification of these values is not clearly determined and does not permit equating low-pressure MSM to the term "meat", although several European countries ignore this requirement. For example, the EU ban (Food Standards Agency — EFSA) to use low-pressure mechanically separated meat as a category "meat preparations" in the UK unlike existing EU norms led to significant economic losses, which amounted to £200 million according to the British Meat Processors Association. At the same time, the Food Standards Agency (FSA) declared that "there is no evidence of any increased food safety risks associated with DSM obtained by mechanical separation or the process by which it is produced" [27,28].

Modern approaches to distinguishing MDPM types

To straighten out approaches to distinguishing types of mechanically deboned poultry meat obtained on different types of equipment and on request from the European Commission, the Panel on Biological Hazards (BIOHAZ) of the European Food Safety Authority (EFSA) [26] carried out an expert examination of the published studies on the sanitary and hygienic risks associated with mechanically separated meat (MSM) from pork and poultry (high and low pressure) comparing them with non-MSM (fresh meat, minced meat and meat preparations) by criteria chosen as potential (chemical, histological, molecular, textural and rheological parameters). Mainly, the aim of the investigation was to determine whether it is possible to distinguish high-pressure MSM from low-pressure MSM and to establish whether low-pressure MSM is similar to hand deboned meat.

In several investigations, which compared meat of different types obtained by the method of low and high pressure, as well as hand deboning, the results were presented mainly on the basis of their histological assessment. It is quite difficult to distinguish low-pressure MSM and hand deboned meat using this method due to their similarity. Muscle structure of fibers is modified in hand deboned meat upon comminution or freezing and, therefore, it can be similar to meat obtained upon low pressure. With that, the presence of bones often can be higher in hand deboned meat depending on the experience of a boner and it also cannot be a reliable marker.

Indicators obtained by methods based on chemical and textural changes were contradictory as their levels in low-pressure MSM and hand deboned meat differed to such a degree by overlapping that they were not suitable for clear distinguishing.

Despite the large number of materials studied by EFSA, no individual parameter was chosen as an indicator of mechanical separation of minced meat types and it was concluded that there is no uniform method or approach that can be used to distinguish low-pressure MSM and hand deboned meat.

The EFSA recommended using the content of calcium and cholesterol in meat as well as a change in the muscle fiber structure as potential indicators of such difference [26].

In 2015, the English Food Standards Agency & DEFRA realized the project "An evidence based review of the state of knowledge on methods for distinguishing mechanically separated meat (MSM) from desinewed meat (DSM)" [27].

It was concluded based on the performed research and EFSA report that the study of differences can include a multivariate analytical approach with a decision tree as the best method. According to the authors' opinion, it should use calcium and fat levels, oxidation behavior, damage of nuclei, integrity of muscle fibers and a measure of texture. With that, it is necessary to determine categories, in which a sample corresponds to the high confidence limits and high certainty in the types of meat under study. It is also necessary to include overlap or "grey" areas and make a decision about their labeling for legislative purposes.

For future research, the project suggests taking into account the following:

- comparison of the residual material from hand deboning with that from machine deboning carried out depending on a type of meat remained on bones for correct assessment of a level of losses or modification of the muscle structure;
- use the histology method developed in the UK [30], which is similar to the method used in Germany [31], clearly distinguishes between low-pressure and high-pressure MSM and is suitable for measuring quality of a sample. It is necessary to develop this method for quantitative assessment supplying with high quality software for image analysis;
- investigation of microbial load in MSM production compared to hand deboned meat;
- formulation of clear requirements for types of comminuted meat that take into account not only losses or modification of fiber structure but also rheology as a measure of the property of the product itself;
- inclusion of inter-laboratory assessment for chosen methods.

The MACSYS project [32] on the "development of an objective method to perform quality classification of comminuted poultry meat" ended in 2016 was carried out within the framework of FP7-SME. It was financed by the EU and several companies. Three universities from Denmark (Kobenhavns Universitet, Aarhus Universitet) and

Germany (Max Rubner Institut), and seven private for-profit organizations (from Denmark, France, Spain, United Kingdom, Iceland) took part in the project.

The general goal of the MACSYS project was to overcome scientific and technical barriers linked with the development of efficient and objective solutions for quality classification of comminuted poultry meat. The main result of these investigations was an agreement on the common immune-histochemical method for quantitative assessment of muscle fiber degradation based on differentiation of their intact and non-intact membranes. This led to the other two main results of the MACSYS project: the cloud-based automated histochemical system of image analysis of intact and non-intact muscle fiber membranes and developed prototype based on near-infrared spectroscopy (NIRS) to measure muscle fiber destruction in comminuted poultry meat in real time, and this method should be calibrated against the immunohistochemical method [32].

The software for automated image analysis and fast acting device based on NIRS for objective quantitative assessment of the level of muscle structure degradation enables differentiating comminuted meat, classifying quality of MDM and also allows producers to obtain the economic benefit from this.

Another method was used to determine calcium — laser induced breakdown spectroscopy (LIBS). This method does not require sample preparation and is used for direct measurement of minerals in a sample, as well as for separation of samples with the very low level of calcium. With that, to obtain the representative sampling, it is necessary to determine the optimal number of measurements.

The project developers concluded that the positive effect will be achieved only if EU legislation is changed.

Raudsepp *et al.* [33] reported at the 61th International Congress of Meat Science and Technology about the results of investigations of histochemical methods based on staining of MDPM samples with Toluidine Blue, which is a well-proven method, and contemporary immunohistochemical labeling based on myosin and laminin, on which antibodies of comminuted chicken meat were applied to assess their potential in terms of objective detection of muscle tissue and its degradation. The researchers [33] concluded that the immunohistochemical method with myosin and laminin antibodies has a significant advantage as it uses fully automated equipment for visualization, ensures objective images with good representativeness for determination of the muscle tissue content and assessment of the degradation level in comminuted chicken meat. This method was used in the MACSYS project.

Since one of the main control parameters of MDPM is the calcium content as an indicator of residual bone, a method was proposed based on Raman spectroscopy to assess the calcium and ash content in bone and meat mixtures upon mechanical deboning of chicken meat and the partial least squares regression models were developed to predict their content [34].

Within the framework of the MPSQA project financed by the Ministry of Health of Italy, a study was carried out and a method was developed for identification of mechanically separated meat by irradiation of a sample coupled with electron spin resonance. Bone fragments were identified both in the samples of fresh meat with addition of different percentages of bones and in meat samples consisted of MDPM (chicken/turkey) obtained under low and high pressure [35].

Development of the technical base of MDPM production

To increase MDPM quality, measures are taken to improve equipment for its production upon reduced pressure with significant preservation of the meat structure.

In the middle of the last century, production of products from poultry meat increased along with the growth in its outputs. With that, a need emerged for the rational use of raw materials that are labor intensive for hand separation of meat from bones (frames, backs, necks, wings and so on) and not safe for working personnel. Creation of such equipment for these purposes allowed solving this task [6].

The initial use of equipment with high values of pressure in the working zone of separation (up to 200 bar and higher) for MDPM production allowed obtaining a product as finely comminuted paste-like mass with the presence of bone inclusions of different sizes, cartilages, increased calcium content, loss or modification of the muscle fiber structure of meat different from minced meat produced from raw materials in pieces [6,36].

By the principle of action, such units are classified into two types: batch-type (hydraulic) system and continuous (screw type and belt-drum). The equipment of the latter two types is mainly used to produce mechanically deboned poultry meat. MDPM production was mainly ground on the one-stage technology with the use of one unit of equipment. Upon using screw presses, the obtained mass usually has paste-like appearance with a high degree of comminution. This type of equipment is characterized by an impact of high pressure on raw materials with destruction of its structure and separation of soft fraction from it; with that, pressure of no less than 300×10^5 Pa is required for meat deboning [37].

At the same time, a belt-drum unit with the flexible elastic belt (Baader type) exerting soft impact on raw materials (up to 5 atm.) upon its corresponding adjustment allows obtaining a product with appearance of a granular minced meat (a degree of granularity depends on the diameter of drum holes) that is equal in quality to the requirements for the category "meat".

When studying "firm" and "soft" (Baader meat) separation of MDPM obtained on different equipment, the selected quality parameters (hydroxyproline, calcium, content of bone particles and their histological features) were compared. The average values of the hydroxyproline content, which characterizes an amount of collagen tissue in MDPM,

were more than two times higher (335.44 mg.100 g^{-1}) compared to those in Baader meat (140.73 mg.100 g^{-1}). More pronounced differences were revealed between the indicators in mechanically deboned products and those in poultry meat, mainly in breast muscles (32.62 mg.100 g^{-1} in pectoral muscle and 124.90 mg.100 g^{-1} in thigh muscle). Upon "firm" separation, the calcium content was 7.9 times higher, respectively. The average content of bone particles was 0.27% ("firm" separation) and 0.034% ("soft" separation). The results of the studies show that Baader meat was analogous to fresh poultry meat in terms of its properties [38]. Similar data were obtained when studying properties of Baader meat from chicken furcula (wishbone) [39].

In the process of MDPM production, the two-stage technology came into use. Under this technology, meat removal under low pressure of up to 20 atmospheres takes place at the first stage and under high pressure (more than 100 atm.) at the second stage. The work of the press — meat deboner enables obtaining part of a product that approximates the category "meat" (meat mass of large dispersity) under low pressure and producing part of MDPM with lower quality as a paste-like mass on the subsequent machine with high pressure. The one-stage and two-stage technologies are practically equal in terms of product yield. With that, the pressure can be regulated achieving different yields and quality indicators of a product.

German scientists carried out comparative studies on the mechanical deboning of parts of poultry meat using the two-phase system TWD8/Mado, modified separator POSS (drum sieve with a hole diameter of 3 mm) and original separator POSS (0.6 mm plates) [38]. The first two methods are characterized as methods of soft pressing; the third method gives meat of paste-like consistency (meat from a tough separator). As histological data show, the two-phase system TWD8/Mado gives the final product that is equivalent to minced meat in terms of quality provided that raw materials do not contain bones with a small amount of attached meat. With that such MDPM is recommended to use as fresh processed meat reviewing its legal classification as defined in Regulation (EC) No 853/2004 [12].

At present, different countries carry out work on producing MDPM of different grades on a single unit of equipment.

The All-Russian Scientific Research Institute of Poultry Processing Industry (ARSRIPPI) has received a patent on the method for separation and division of mechanically deboned meat by quality simultaneously on a single unit in the process of movement of raw materials through the multizone filter with holes of different diameters according to zones (from 4.0 to 0.5 mm) and creation of different pressure of pressing in the process of movement of raw materials along the filter (from 0 to 85 atm.). Additional processing of the secondary product on a separator is not required when using this method. The samples of the equipment were created, tested and showed positive results [40].

Testing of the screw press with the four-zone filter on keel bones of broiler chickens [41] enabled obtaining in the first two zones meat particles with a size of 3.5–2.5 mm, 85–75% of volumetric muscle tissue, including 70%-80% with the preserved structure, a size of bone inclusions of 150–200 μm; in the third and fourth zones, meat particles with a size of 1.5–0.1 mm and lower and a size of bone inclusions of 150 and less than 100 μm were obtained. A significant preservation of meat structure in the first and second zones, production of its two types (close to minced meat in terms of quality with a possibility to assign it to the category "meat" and MDPM) on a single unit allows expecting its further improvement.

Ukrainian scientists studied an effect of technological aspects of production using a screw-type press equipped with the perforated filter sleeve having a hole diameter of 3.0 mm on the quality characteristics of low-pressure MDPM [42]. Histochemical studies of this meat type showed in the micrographs the dominant presence of comminuted muscle tissue with the intact structure and less significant presence of fatty tissue (similar to minced meat from hand deboned poultry meat), as well as the presence of bone marrow fragments and bone inclusions in the structure. It was found by chemical methods that upon the same yield, the content of total protein and fat in low-pressure MDPM approximately corresponded to minced meat from hand deboned poultry meat. The content of calcium was not higher than the norm (0.07%) established by regulatory documents. Macrostructural analysis demonstrated that the linear dimensions of the bone inclusions basically did not exceed 1.0 mm, and the sizes of incidental inclusions were less than 2 mm. These data served as an evidence base for identification of low-pressure MDPM obtained in the experiment.

The performed studies showed that it was possible to obtain low-pressure MDPM close to hand deboned meat in terms of quality using several technical means. With that, it is necessary to recognize such MDPM at the official level as the category "meat" with agreed deviations.

Methods for detection of falsification of raw materials and products with MDM

The presence of MDM in meat and sausage products is subjected to declaration. Due to the economic benefits, however, unfair producers more and more often replace expensive raw materials with cheaper and allow inclusion of MDM into meat product recipes without indication on a label. Nowadays, the existing methods have been improving and new methods have been developing to detect falsification.

Detection of bone inclusions in the multi-component meat products (sausages and other products) produced with MDM using the method of their gravimetric determination by chemical treatment of samples broadens possibilities of revealing falsification of these products as the level of qualitative and quantitative expertise [43].

To detect meat product authenticity, protect consumers from falsification of products due to the presence of non-declared MDM, histological methods are actively used with staining sections of samples with hematoxylin and eosin, and trichrome blue along with the investigation of technological properties, content of ash, bones, cartilages and calcium [44].

The investigations performed based on the histological methods for detection of unauthorized inclusions in meat sausage randomly collected on Iranian markets by staining sections with hematoxylin and eosin, Masson's trichrome, periodic acid- Schiff/Alcian blue and Verhoeffe/Van Gieson allowed revealing a wide spectrum of unauthorized tissues including dense connective tissue, cartilages, bones, skin, smooth muscles and blood vessels. The researchers believe that histological methods, especially Masson's trichrome staining are practical methods for routine assessment of possible falsification [45].

To detect MDM in meat products, invasive destructive methods are mainly used. At the same time, the Czech researchers developed a new non-invasive method for detection of bone fragments as accompanying structures of MDM based on X-ray micro computed tomography (μCT). Bone tissue detected on the basis of higher density using μCT was confirmed by the image analysis and histochemical method with alizarin red staining. The method allows analyzing bone fragments in meat products with a possibility of determining parameters of their shape [46].

A study based on the application of computed tomography using a computed tomography analyzer (CTAn) was carried out to detect the presence of bone inclusions in sausage products with MDPM. On the basis of its results, characteristics of bone and cartilage inclusions in the experimental samples were determined. It was concluded that it is possible to use this method for microstructural analysis of food products to ensure quality of production or reveal food falsifications [47].

On the request of the European Food Safety Authority (EFSA), a study was performed to identify meat products with MSM using a liquid scintillation counter of ultra-low levels of the 90Sr activity concentrations in combination with other parameters: 88Sr, Ca and ash percentage via the multivariate approach. The accuracy of this method ensured the correctness of identification (87%) higher than in the reference method (Ca level; 76%). According to the authors' opinion, this is a new approach to identification of products with MSM [48].

To reveal non-declared MDPM presence in sausage products, an analysis was developed and approved based on pseudo-MRM–LC–MS/MS, which uses peptides specific to intervertebral discs and cartilages assigned to collagen II alpha 1. This method allowed detecting MDPM in real samples of the unknown composition upon its content of up to 10% in meat [49].

Wubshet *et al.* [34] reported about the first use of Raman spectroscopy as a fast tool for assessment of the calcium and ash content in bone and meat mixtures with mechanically deboned poultry meat. This analysis allows detecting much lower quantities of MDPM (10%) in commercially available meat samples compared to all currently established standard methods, such as microscopy, calcium detection and liquid scintillation counting (20%) or total reflection X-ray fluorescence (TXRF) method (40%). In addition, the method has another advantage as it enables abandoning thorough biochemical and chemical characterization of a sample material (lipids, proteins, ash, calcium, carbohydrates and so on) because high specificity of pMRM-transitions allows selective detection of MDPM specific marker peptides.

Electron spin resonance (ESR) spectroscopy is widely used for identification of irradiated meat and fish that contain bones [35]. This is associated with the characteristic signals obtained upon bone irradiation. When executing the MPSQA project (Italy), an innovative analytical method for MDPM identification after irradiation was developed as this type of meat products contains bone fragments. Ashing of samples allowed achieving the full removal of interfering signals. Bone fragments were identified both in the samples of fresh meat with addition of different percentages of bones and in the meat samples consisted of low-pressure and high-pressure MDPM (chicken/turkey).

Sarakatsianos *et al.* [50] studied the use of inductively coupled plasma/mass spectrometry to detect and differentiate the content of high-pressure mechanically deboned meat (MDM) in meat products. Of all tested elements, barium had a clear tendency of dependence of its concentration on the content of bone particles in MDM, which enabled detecting the presence of MDM in processed meat products by its correlation with the barium concentration.

With that, large variations among batches of mechanically deboned chicken meat that depended more on its processing rather than on initial raw materials will require consideration for this factor when improving methods for detecting falsification [51].

State of production regulation and quality and safety control of MDPM in Russia

GOST 31490–2012[4] applies to mechanically deboned poultry meat (chicken and turkey) intended for industrial processing. According to this document, mechanically deboned meat should correspond by the organoleptic and physico-chemical parameters to the following main requirements: it should be viscous finely ground paste-like mass in terms of appearance with the moisture mass fraction no more than 70%, protein no less than 12%, fat no more than 18%, calcium no more than 0.26%, bone inclusions in reference to mass of mechanically deboned meat no more than 0.6% with their specified differentiated sizes.

[4] GOST 31490–2012. "Poultry meat of mechanical separation. Specifications" Retrieved from https://docs.cntd.ru/document/1200095720 Accessed March 02, 2023. (In Russian)

Quality indicators also include norms of the presence in MDPM of the quantity of volatile fatty acids, fat peroxide value (% of iodine), acid value (mg KOH/g fat), mass fraction of total phosphorus (%).

It is not permitted to use raw materials with the mass fraction of fleshy tissues of less than 30% for production of mechanically deboned meat.

According to the GOST, the following restriction is introduced: raw materials in a form of poultry carcasses and/or their parts should be obtained directly in an enterprise that performs cutting and deboning of chilled poultry carcasses and/or their parts. At the same time, several production facilities were created in Russia, including in large holdings, where raw material production for MDPM and MDPM production are located at different sites, which contradicts to this GOST, although it is possible upon correspondence to the time of delivery, necessary temperature regimes and sanitary rules.

The GOST does not take into account new technical possibilities of producing MDPM of different types, and new scientifically based criteria for dividing such products for MDPM of different quality are required. In addition, its narrower specifications by parameters are necessary due to possible significant variations of MDPM by the protein and fat content depending on a type of initial raw materials.

Several interstate standards specify methods for controlling various indicators of MDPM. For example, according to GOST 31466–2012[5] approved based on the investigations carried out by ARSRIPPI [52], determination in MDPM of the calcium mass fraction is carried out by flame atomic absorption spectrometry, sizes of bone inclusions by the microscopic method, mass fraction of bone inclusions and mass fraction of bone inclusions, which size is higher than the specified (normed) value, by the gravimetric method.

To assess quality of meat raw materials and meat products and their correspondence to the normative document, including MDPM, the method[6] is used, which is based on identification in histological preparations of animal and plant components in different types of canned meats and meat products according to their microstructural features as well as on the determination of the ratio of muscle and connective tissues in meat raw materials. General staining of sections is performed with hematoxylin and eosin, staining for detection of fat with Sudan III and Sudan IV, staining for detection of starch with Lugol's solution. Semi-quantitative assessment of one or another component can be also carried out using either ocular-micrometer or ocular inserts attached to light microscopes.

The existing normative document used to reveal product falsification is based on the fast histological method for identification of animal and plant structural components of the compositions in different types of meat and meat products[7]. It enables revealing the presence of unenvisaged components and the correspondence of the real composition of a sample to the existing documentation or to the composition indicated on a product package.

At the same time, it is necessary to search for a method to detect not only semi-quantitative but also quantitative parameters of differences between MDPM types upon its using for objective assessment of the presence in products.

Improvement of the organizational forms of MDPM production and processing

Today, processing of poultry carcasses and their parts sent to mechanical deboning is performed on practice using several schemes. The main scheme among them includes the following: poultry slaughter, MDM production and its processing into products are carried out in the same enterprise — poultry processing plant. The next scheme is production of MDM from purchased raw materials in a specialized enterprise with the following product shipping to a customer. Several processing enterprises purchase raw materials for MDPM from poultry processing plants and produce it for their own needs.

The most effective production with lower allowable risks is MDPM production according to the first scheme. With that, it is possible to control each factor that determines its quality at each previous stage of processing. Production or purchase of MDPM in a specialized enterprise leaves producers relatively few possibilities to influence their own part of the process as an effect of other factors was applied at earlier stages (breeding, slaughter or preliminary deboning of meat from poultry carcasses). MDPM production operations that are disrupted in time and space negatively affect quality characteristics of products and their microbiological safety [53]. Furthermore, certain producers violate recommended time for raw material processing and overstate the yield of the final product. To avoid this, producers and consumers of MDPM need to apply maximum integration of production chain links, strictly adhere to production instructions and temperature regimes, ensure robust logistics and reciprocal control of production.

The next important specific feature of MDPM production is linked today with its production volumes, first of all in large enterprises. Appearance of enterprises with daily production volumes from 60 to 400 tons of poultry meat in Russia and assignment of a significant part of poultry meat for semi-prepared products and finished products create a possibility of separate processing of poultry carcass parts into MDPM after preliminary mechanical or hand deboning with different initial quality characteristics of raw

[5] GOST 31466–2012. "Products of processed poultry meat. Methods of determination of mass fractions of calcium and dimensions and mass fraction of bond particles" Retrieved from https://docs.cntd.ru/document/1200096477 Accessed March 02, 2023. (In Russian)

[6] GOST 31479–2012. "Meat and meat products. Method of histological identification of composition" Retrieved from https://docs.cntd.ru/document/1200097485 Accessed March 02, 2023. (In Russian)

[7] GOST 31796–2012. "Meat and meat products. Fast histological method of identification of composition structural components" Retrieved from https://docs.cntd.ru/document/1200100067 Accessed March 02, 2023. (In Russian)

materials. The calculations of the author show that upon cutting into parts broiler chicken carcasses in an amount of 50 tons and sending to mechanical deboning 4–5 tons of breast parts after preliminary separation of fillet from them, it is possible to obtain 2.8–3.5 tons of MDPM close to the initial raw materials in terms of quality. A producer obtains a product with higher quality and value when processing these raw materials separately from others.

Previous research [54] notices an effect of quality of produced MDPM from separately deboned carcasses of broiler chickens, layer hens and their parts on quality of the final products. Chemical and histological analyses (sections were stained with hematoxylin and eosin according to Mayer) showed their significant differences in terms of the content of total protein, lipids, moisture, cartilages, bones, connective, lipid and lymphoid tissues. For example, the average content of lipids was the lowest in the neck samples (4.87%). It was higher in back samples (7.74%) and whole carcasses (9.51%), while the highest content was found in wings (11.56%). Such complex investigations give a reliable insight into the raw material composition, its effect on the final product quality and prospects for the rational use.

In [55], which assessed quality of the poultry raw material sent to mechanical deboning, the main indicator for classification was its protein content and key factor was its quality. The protein mass fraction in the raw material with account for meat pieces on bones serves as a basis for detecting its quantity, while the nutritional and biological value serves for detecting quality indices: meat/bone, fat/protein, tryptophan/oxyprolin, proportion of complete protein in%, a ratio of complete protein to incomplete, energy value of raw materials, kkal/100g. The calculated values of the above-mentioned indices of raw material groups taken from large production batches of different suppliers showed their significant differences. For example, a ratio of the complete protein proportion to the incomplete protein proportion was the highest (3.26) for keel bone, which indicates the highest quality of raw materials among all compared types (2.43 times higher than in necks, 3.1 times higher than in frames and 65.2 times higher than in wings). With that, the study revealed significant differences in quality of raw materials represented by different parts of carcasses and less significant differences between batches of raw materials of the same type supplied by different producers.

Based on the data obtained, the authors propose using such raw material types as keel bones, backs and necks to produce MDPM of differentiated quality and use it for new products.

Abaldova *et al.* [56] proved the difference in chicken meat quality by the amino acid composition and the biological value depending on the carcass part, deboning method (hand or mechanical) and separation pressure (low or high) compared to hand deboned meat. For example, the total protein content in MDPM from keel bone was 4.7% lower than in hand deboned fillet, but 20.1% higher than in whole carcass which indicates its higher quality. When using low pressure, the content of pure protein (without connective tissue protein) in MDPM from keel bone was significantly higher than in the control (by 12.7%) and in fillet (by 3.3%), but it was lower by 9.7% when using high-pressure separation.

Similar results were obtained upon separate processing of turkey raw materials into MDPM using screw press with the six-zone filter with different diameters of holes in zones [57].

It is possible to increase the yield of high quality MDPM by improving criteria of its assessment by types [58]. With that, it is important to coordinate a type of the initial raw materials with the desired quality of the obtained MDPM as well as the produced final product. The process of meat removal with consideration for categorization of incoming meat-and-bone raw materials (pork) at the input stage is typical for foreign processors [59]. Therefore, there has been a long-standing need for classification of raw materials by quality characteristics with consideration for their morphological composition (meat-bone index, content of protein and fat) already at the input stage for the efficient use of MDPM.

Conclusion

Mechanically deboned poultry meat is widely used in significant volumes worldwide and in the national practice to produce sausages and other products. Quality characteristics of MDPM have been actively studied upon its production using the equipment with high pressure in the working zone. With that, the product had an appearance of paste-like mass with the destroyed structure, the presence of bone inclusions of different sizes and cartilages, increased content of calcium and several other inclusions that distinguished it from hand deboned poultry meat. The term “mechanically deboned poultry meat” is used for its definition.

Over the last decades, the technology and technical means for MDPM production have been improving worldwide. The equipment with low pressure has been designed and is used, which allows obtaining a product with quality approximating that of hand deboned meat. Boundaries for division by quality of all MDPM types compared to hand deboned poultry meat, conventional terminology, methods for their classification and identification are not clearly defined. Scientists from different countries search ways for solving these tasks.

At present, scientific studies are carried out widely in the world on the whole spectrum of indicators of new MDPM types produced under different pressures compared to hand deboned meat (content of calcium, barium and cholesterol, damage of muscle tissue and so on) and products of its using. Conditions are being created for improving classification of different MDPM types by method of production and maximum allowable threshold values, standardized parameters, determination of their characteristics, methods for assessment and substantiation of terminology.

Similar problems are also relevant to our country. With the appearance of domestic equipment and use of import equipment for MDPM production that allow production of low-pressure MDPM, the existing normative base requires correction and addition.

Based on the international and domestic scientific research, it is necessary to:

- substantiate the most objective parameters of quality characteristics of mechanically deboned meat based on poultry type (meat and egg chickens, turkey), their parts and pressure upon its production (destruction of the meat structure, presence of calcium, bone inclusions, their sizes as well as bone marrow, iron, cholesterol and so on), determine limits of maximum allowable values of destruction of the muscle tissue structure, the content of calcium and bone inclusions in low-pressure MDPM that is close in quality to hand deboned poultry meat, as well as to perform their comparative microbiological assessment;
- develop new and improve existing methods for detecting objective criteria of quality characteristics of MDPM based on physico-chemical, histological investigations and others, both arbitration and express analyses;
- classify MDPM depending on pressure upon its production, take into account changes in the sphere of production organization, actualize the national normative base;
- develop methods for controlling falsification when using MDPM for product manufacture (above norms specified in a recipe).

Creation of such a normative base will allow the rational use of poultry meat raw materials, increase in production efficiency and creation of conditions for active introduction of new technique for these purposes.

There has also been a long-standing need for classification of raw materials coming for processing by their quality characteristics with regard to the morphological composition, meat-bone index, protein and fat content already at the input stage to increase MDPM quality.

It is necessary to pay attention to improvement of the equipment design both for the one-stage and two-stage technology for MDPM production toward formation of feedback between the finished product, initial raw materials and pressure in the process, which will enable increasing its quality characteristics.

Producers and consumers — processors of MDPM should pay attention to the logistics schemes of movement along the life cycle linking its parameters with quality of the final product.

REFERENCES

1. Desouzart, O. (June 23–27, 2014). *Future trends in feed ingredients availability*. European Poultry Conference 2014. Stavanger, Norway, 2014.
2. Yıldız, D. (2021). Global Poultry Industry and Trends. Retrieved from https://www.feedandadditive.com/global-poultry-industry-and-trends/ Accessed March 15, 2023
3. Fisinin, V. (2021). Expanding scientific horizons and increasing intellectual potential. *Animal Husbandry of Russia,* S3, 2–7. (In Russian)
4. Gustchin, V.V. (2020). The reserves of effectiveness of poultry meat processing: the new potentials for improving the quality of poultry meat of mechanical boning. *Topical Issues of the Dairy Industry, Intersectoral Technologies and Quality management Systems*, 1, 149–154. https://doi.org/10.37442/978–5–6043854–1–8–2020–1–149–154 (In Russian)
5. Leenen, G. (2016). Waste-free production: Technical solutions in meat deboning. *Poultry and Poultry Products,* 6, 15–17. (In Russian)
6. Froning, G.W., McKee, S.R. (2010). Mechanical separation of poultry meat and its use in products. Chapter in a book: Poultry Meat Processing. CRC Press, 2010.
7. Froning, G.W. (1981). Mechanically deboning of poultry and fish. *Advances in Food Research*, 27, 109–147. https://doi.org/10.1016/S0065–2628(08)60298–0
8. Field, R.A. (1981). Mechanically deboned red meat. *Advances in Food Research*, 27, 23–107. https://doi.org/10.1016/S0065–2628(08)60297–9
9. Gonotskii, V.A., Fedina, L.P., Dubrovskaya, V.I., Gonotskaya, V.A. (2000). Poultry meat of mechanical deboning. *Poultry and Poultry Products,* 1, 22–26. (In Russian)
10. Gonotskii, V.A., Fedina, L.P., Dubrovskaya, V.I. (2000). Poultry meat of mechanical deboning. *Poultry and Poultry Products,* 2, 21–23. (In Russian)
11. Froning, C.W., McKee, S.R. (2001). Mechanical separation of poultry meat and its use in products. Chapter in a book: Poultry Meat Processing. CRC Press, 2001.
12. Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. Retrieved from https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ: L:2004:139:0055:0205:en: PDF Accessed March 15, 2023
13. Commission Regulation (EC) No 2074/2005 of 5 December 2005. Retrieved from https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32005R2074 Accessed March 15, 2023
14. Code of Federal Regulations. (1996). Mechanically Separated (Kind of Poultry): Title 9 / Chapter III / Subchapter A / Part 381 / Subpart P / § 381.173. Retrieved from https://www.ecfr.gov/current/title-9/chapter-III/subchapter-A/part-381/subpart-P/section-381.173 Accessed March 15, 2023
15. USDA. (1996). Meat produced by advanced meat/bone separation machinery and meat recovery systems. Retrieved from https://www.fsis.usda.gov/policy/fsis-directives/7160.1 Accessed March 15, 2023
16. Technical Regulations of the Eurasian Economic Union "On the Safety of Poultry meat and its processed Products" (EAEU TR 051/2021). Adopted by the decision of the Council of the Eurasian Economic Commission dated October 29, 2021 N110. Retrieved from https://docs.cntd.ru/document/726913772 Accessed March 15, 2023 (In Russian)
17. Ministry of Health of Ukraine (2013). On approval of hygienic requirements for poultry meat and individual indicators of its quality. Retrieved from https://ips.ligazakon.net/document/RE23911?an=2 Accessed March 15, 2023 (In Ukrainian)
18. Government of Canada. (2018). Meat processing controls and procedures: Archived — Chapter 4. Retrieved from https://inspection.canada.ca/food-safety-for-industry/archived-food-guidance/meat-and-poultry-products/manual-of-procedures/chapter-4/eng/1367622697439/1367622787568 Accessed March 15, 2023
19. Brasilia normative instruction SDA (2000). Normative instruction SDA No. 4 of 31 March 2000 — technical regulations on the identity and quality of mechanically separated meat (CMS), mortadella, sausage and sausage. Retrieved from http://www.agais.com/normas/carne/carnes_linguica.htm Accessed March 15, 2023 (In Portuguese)
20. European Commission. Food Safety. Codex Alimentarius. Retrieved from https://food.ec.europa.eu/horizontal-topics/international-affairs/international-standards/codex-alimentarius_en Accessed March 15, 2023
21. Technical Regulations of the Customs Union "On the safety of meat and meat products" (TR CU 034/2013)". Decision of the Council of the Eurasian economic Commission of Oktober 9, 2013 № 68. Moscow, 2018. Retrieved from https://docs.cntd.ru/document/499050564 Accessed March 15, 2023 (In Russian)

22. Gonotskii, V.A. (2008). Scientific substantiation, development and implementation of poultry meat products technology. Author's abstract of the dissertation for the scientific degree of Doctor of Technical Sciences. Moscow: 2008. (In Russian)
23. Commission Regulation (EC) No 2074/2005 of 5 December 2005 laying down implementing measures for certain products under Regulation (EC) No 853/2004 of the European Parliament and of the Council and for the organisation of official controls under Regulation (EC) No 854/2004 of the European Parliament and of the Council and Regulation (EC) No 882/2004 of the European Parliament and of the Council, derogating from Regulation (EC) No 852/2004 of the European Parliament and of the Council and amending Regulations (EC) No 853/2004 and (EC) No 854/2004 (Text with EEA relevance). Retrieved from https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32005R2074 Accessed March 15, 2023
24. Nagy, J., Lenhardt, L., Korimová, L., Dičáková, Z., Popelka, P., Pipová, M. et al. (2007). Comparison of the quality of mechanically deboned poultry meat after different method of separation. *Meso,* 9(2), 92–95.
25. Hać-Szymańczuk, E., Cegiełka, A., Karkos, M., Gniewosz, M., Piwowarek, K. (2019). Evaluation of antioxidant and antimicrobial activity of oregano (*Origanum vulgare* L.) preparations during storage of low-pressure mechanically separated meat (BAADER meat) from chickens. *Food Science and Biotechnology,* 28(2), 449–457. https://doi.org/10.1007/s10068–018–0491
26. EFSA. (2013). Scientific opinion on the public health risks related to mechanically separated meat (MSM) derived from poultry and swine. *EFSA Journal,* 11(3), Article 3137. https://doi.org/10.2903/j.efsa.2013.3137
27. Groves, K., Knight, A. (2015). An evidence based review of the state of knowledge on methods for distinguishing mechanically separated meat (MSM) from desinewed meat (DSM). Maryland, USA: Food Standards Agency & DEFRA, 2015.
28. Patrick, R. (2012). Guidance on the moratorium on the production and use of desinewed meat from non ruminant bones or poultry carcases in the United Kingdom: a letter by Rachel Patrick, Enforcement and Local Authority Delivery Division of Food Standards Agency, from 18 Sept. 2012. Retrieved from https://webarchive.nationalarchives.gov.uk/ukgwa/20171207203200/https://www.food.gov.uk/sites/default/files/multimedia/pdfs/enforcement/enfe-12–027.pdf. Accessed March 15, 2023.
29. Food Standards Agency. (2013). Processes used in the UK to manufacture MSM and former DSM meat products from poultry and pork and an initial assessment of microbiological risk: research project. Retrieved from https://www.food.gov.uk/research/foodborne-disease/processes-used-in-the-uk-to-manufacture-msm-and-former-dsm-meat-products-from-poultry-and-pork-and-an-initial-assessment-of-risk Accessed February 15, 2023.
30. Groves, K. (2011). Q01101 — Evaluation of a Simple Microscopy Protocol for Identifying Mechanically Separated Meat (MSM) in Pork, Chicken and Turkey. Retrieved from https://sciencesearch.defra.gov.uk/ProjectDetails? ProjectId=18019 Accessed March 15, 2023.
31. Branscheid, W., Bauer, A., Troeger, K. (August 7–12, 2011). *Modification of muscle structure in poultry meat caused by different meat recovery systems.* 57th International Congress of Meat Science and Technology. Ghent, Belgium, 2011.
32. European Commission. CORDIS. EU research results. (2014). Development of an objective method to perform quality classification of comminuted poultry meat. Retrieved from https://cordis.europa.eu/project/id/605621. Accessed February 20, 2023.
33. Raudsepp, P., Henckel, P., Groves, K., Therkildsen, M., Brüggemann, D. (August 23–28, 2015). *Reliability of different histological methods for estimation of muscle fiber structure in MSM.* 61st International Congress of Meat Science and Technology. Clermont-Ferrand, France, 2015.
34. Wubshet, S.G., Wold, J.P., Böcker, U., Sanden, K.W., Afseth, N.K. (2018). Raman spectroscopy for quantification of residual calcium and total ash in mechanically deboned chicken meat. *Food Control,* 95, 267–273. https://doi.org/10.1016/j.foodcont.2018.08.017
35. Tomaiuolo, M., Chiaravalle, A.E., Mangiacotti, M, Petrella, A., Taranto, A. D., Iammarino, M. (2019). Innovative techniques for identifying a mechanically separated meat: sample irradiation coupled to electronic spin resonance. *European Food Research and Technology*, 245(10), 2331–2341. https://doi.org/10.1007/s00217–019–03340-x
36. Abaldova, B.A. (2021). MDM: structure and quality depending on the equipment used. *Meat Branch,* 5(221), 14–20. https://doi.org/10.33465/2308–2941–2021–05–14–20 (In Russian)
37. Ostroukh, A.S., Abaldova, V.A. (2016). Calculation of performance for mechanical deboning screw presses considering counterpressure. *Theory and Practice of Meat Processing,* 1(3), 66–80. https://doi.org/10.21323/2414–438X-2016–1–3–66–80
38. Branscheid, W., Troeger, K. (2012). Mechanical recovery of meat and residual meat in poultry. *Fleischwirtschaft Frankfurt*, 92(1), 98–105.
39. Branscheid, W., Judas, M., Wagner, H., Troeger, K. (2008). Investigations on the characterisation of mechanically deboned broiler meat. *Fleischwirtschaft Frankfurt*, 88(11), 106–111.
40. Mazur, V.M., Abaldova, V.N. A method of producing meat of mechanical deboning of different quality and a device for its implementation. Patent RF, no. 2541406C, 2015. (In Russian)
41. Khvilya, S.I., Abaldova, V.A. (2015). Mechanical deboning of poultry meat using a multi-zone filter. Characteristics of the microstructure of the MDCM of the thoracic bones. *Poultry and Poultry Products,* 3, 57–60. (In Russian)
42. Usatenko, N.F., Kalashnik, M.G., Verbytskyi, S.B., Oxrimenko, Y.I. (2021). Non-standardized raw material for the meat industry. *Food Industry: Science and Technologies*, 14(4(54)), 34–40. https://doi.org/10.47612/2073–4794–2021–14–4(54)-34–40 (In Russian)
43. Usatenko, N., Verbytskyi, S. (2022). Determination of the content of bone inclusions in multicomponent meat products. *Veterinary Sciences and Practices*, 17(1), 20–25. https://doi.org/10.54614/VetSciPract.2022.983393
44. Mohamed, M.A., Zahran, D.A., Kassem, G.M.A., Emara, M.M.T., Mansour, N.M. (2011). Detection of mechanically recovered poultry meat (MRPM) of mechanically recovered poultry meat (MRPM) in traditional Egyptian luncheon (Emulsion Type Sausage). *Polish Journal of Food and Nutrition Sciences,* 66(1), 17–23. https://doi.org/10.1515/pjfns-2015–0013
45. Moghtaderi, A., Raji, A., Khanzad, S., Nabipour, A. (2019). Application of histological method for detection of unauthorized tissues in meat sausage. *Veterinary Research Forum*, 10(4), 357–360. https://doi.org/10.30466/vrf.2018.89154.2160
46. Pospiech, M., Zikmund, T., Javůrková, Z., Kaiser, J., Tremlová, B. (2019). An innovative detection of mechanically separated meat in meat products. *Food Analytical Methods*, 12, 652–657. https://doi.org/10.1007/s12161–018–1394–8
47. Nagdalian, A.A., Rzhepakovsky, I.V., Siddiqui, S.A., Piskov, S.I., Oboturova, N.P., Timchenko, L.D. et al. (2021). Analysis of the content of mechanically separated poultry meat in sausage using computing microtomography. *Journal of Food Composition and Analysis,* 100(4), Article 103918. https://doi.org/10.1016/j.jfca.2021.103918
48. Iammarino, M., Miedico, O., Petrella, A., Mangiacotti, M., Chiaravalle, A.E. (2020). Innovative approaches for identifying a mechanically separated meat: evaluation of radiostrontium levels and development of a new tool of investigation. *Journal of Food Science and Technology,* 57(2), 484–494. https://doi.org/10.1007/s13197–019–04076-y
49. Wilhelm, C., Hofsommer, M., Wittke, S. (2022). Detection of mechanically separated meat from chicken in sausages and cold meat by targeted LC–MS/MS analysis. *Food Analytical Methods*, 15(2), 1899–1908. https://doi.org/10.1007/s12161–022–02231–4
50. Sarakatsianos, I., Manousi, N., Georgantelis, D., Goula, A., Adamopoulos, K., Samanidou, V. (2018). Detection of mechanically deboned meat in cold cuts by inductively coupled plasma/mass spectrometry. *Pakistan Journal of Analytical and Environmental Chemistry,* 19(2), 115–121. http://doi.org/10.21743/pjaec/2018.06.01
51. Mello, M.R.P.A., Neto, J.M.M., Torres, E.A.F.S. (2017). Application of multivariate analysis to the study of mechanically deboned chicken meat (MDCM). *International Food Research Journal*, 24(3), 1102–1109.
52. Krasyukov, Yu.N., Gromov, I. Yu., Savinkova, I.P., Pavlenko, N.M., Danshova, L.L. (2006). Determination of bone inclusions and calcium in poultry meat of mechanical deboning. Collection of proceedings. Rzhavki township: All-Russian Scientific Research Institute of Poultry Processing Industry, 2006. (In Russian)
53. Van der Steen, F. (2016). Relevant: Optimal use of poultry carcasses. *Poultry and Poultry Products,* 6, 17–19. (In Russian)
54. Botka-Petrak, K., Hrast, A., Lucić, H., Gottstein, Z., Gomerčić, M.G., Jakšić, S. (2011). Histological and chemical characteristics

of mechanically deboned meat of broiler chickens. *Veterinarski Arhiv*, 81(2), 273–283.
55. Abaldova, V.A., Filippova, G.V., Sorokina, I.M. (2021). On the issue of assessing the quality of raw materials for mechanical deboning. *Poultry and Poultry Products*, 6, 14–16. https://doi.org/10.30975/2073–4999–2021–23–6–14–16 (In Russian)
56. Abaldova, V.A., Ovcharenko, V.I. (2022). Investigation of the quality of raw materials for mechanical deboning in the poultry processing industry. *Poultry and Poultry Products*, 3, 65–68. https://doi.org/10.30975/2073–4999–2022–24–3–65–68 (In Russian)
57. Abaldova, V.A., Ovcharenko, V.I. (2022). Factors affecting the yield and safety of mechanically deboned turkey meat of differentiated grade. *Poultry and Poultry Products*, 4, 21–24. https://doi.org/10.30975/2073–4999–2022–24–4–21–24 (In Russian)
58. Lisitsyn, A.B., Tatulov, Yu.V., Chernukha, I.M. (2001). The world practice of forming the quality of meat raw materials and the requirements for it of the processing industry. *Meat Industry*, 9, 6–9. (In Russian)
59. Meerdink, J. (2016) Modern approach to the process of mechanical deboning of meat. *Poultry and Poultry Products*, 6, 19–21. (In Russian)

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EVALUATION OF PHYSICAL CHARACTERISTICS OF CHEVON AS AFFECTED BY POST-MORTEM CARCASS DRESSING AND FREEZING PRESERVATION

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Keywords: *Red-Sokoto, male goats, scalding, skinning, singeing, freezing preservation, analysis of variance*

Abstract
This study was conducted to investigate the effects of post-mortem dressing methods and freezing preservation on the physical characteristics of chevon. Twenty-seven Red-Sokoto male goats between 10 and 12 months of age weighing 18–20 kg were purchased, stabilized and slaughtered. The carcasses were randomly allotted to three post-mortem dressing procedures (scalding, skinning and singeing) and meat from thigh cuts was frozen for 0, 7, 14 and 21 days. Meat samples were excised each day after thawing for physical analysis and data collected were subjected to analysis of variance (ANOVA) in a completely randomized design experiment with 3x4 factorial arrangement. The significant means were separated with the Duncan multiple range test at $p < 0.05$. The results showed that the post-mortem dressing methods and freezing affected Red-Sokoto chevon significantly with the singeing method exerting the highest detrimental effects on physical attributes of meat with the exception of color, yield and pH, while the skinning method exerted the least detrimental effects. Also, cold, cooking and drip losses as well as thermal shortening, cold shortening and pH values increased between the 14th and 21st day, while color, yield, water holding capacity, texture and shear force values decreased across the three treatments during freezing periods. The effects were more significant in singed and scalded meat than in skinned chevon. It was recommended, therefore, that skinning method be encouraged if meat from Red-sokoto male goats is to be frozen and the period of freezing be limited to 14 days for wholesome meat.

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Introduction

There is a high demand for animal protein in developing countries. However, the existing conditions and facilities for slaughtering and handling meat in most of these countries without proper and efficient utilization of facilities result in meat deterioration and heavy post-slaughter losses, and therefore, pose a threat to assuaging do not allow producing sufficient amounts of animal protein in these countries [1]. Moreover, civilization and urbanization have made it difficult for most consumers to allot time to purchase meat on a daily basis, hence they purchase meat in bulk and preserve it in a home refrigerator or freezer in order to meet their daily and immediate future needs [2]. Freezing preservation is a post-slaughter handling of meat that emphasizes and facilitates meat reserve stocking, regulates periodical fluctuations in meat supply and reduces storage losses as well as limits physical, bio-chemical and microbiological changes that reduce meat shelf-life and quality [3]. It also lowers the inner temperature of meat, meat products and food items below the cryogenic point thereby increasing meat and meat product quality [4]. Reports showed that freezing preservation of meat caused some physical alterations in meat due to thawing and refreezing of meat, which resulted in the destruction of the tissue structure and meat spoilage [5]. Post-mortem dressing of carcasses has also been reported to be one of the sources of meat quality variability [6]. However, Monin et al. [7] reported that dressing of animal carcasses using the singeing method imparted better meat eating qualities. It was anticipated, therefore, that the combined effects of post-mortem dressing (namely, scalding, skinning and singeing) and preservation with freezing on meat quality attributes can be enormous. Thus, this study was carried out to evaluate the effects of post-mortem dressing methods and freezing preservation on physical characteristics of Red-Sokoto chevon.

Materials and methods

Experimental Animals

Twenty seven matured Red-Sokoto male goats between 10 and 12 months of age weighing 18–20 kg were used for this study carried out at the Department of Animal Science, University of Ibadan. The animals were quarantined and stabilized for two weeks on cowpea chaff and a standard diet. They were fasted for 16 hours after two weeks,

but had access to clean water ad libitum. Goats were slaughtered and their carcasses were weighed and allotted randomly (n = 9) to one of the post-mortem dressing methods, namely, scalding, skinning and singeing.

Dressing of carcasses

Scalding: Carcasses were dressed by modifying the scalding method described by Monin et al. [7]. Hot water (75 °C) was poured on each carcass instead of dipping to soften the hairs before scrapping with a metal scrapper. **Skinning:** Skins on the carcasses were removed completely using a sharp knife following the procedures of Omojola and Adesehinwa [6]. A ring was made round one of the hind legs just above the hock. The knife was inserted into the skin of the leg and opened down to the root of the tail, the same operation was done to the second leg and another incision was made from the pelvic region to the neck. The skin was pulled gradually until it was removed. **Singeing:** Carcasses in this group were placed on fire (about 250 °C) made with hard wood, teakwood (*Tectona grandis*) until all the hairs were carefully burnt off with minimal damage to the skin according to the procedures of Okubanjo [8].

Evisceration and Fabrication of Carcasses

Carcasses were decapitated, shanked and eviscerated by removing the gastrointestinal tract and other interval organs. The carcasses were washed and split into two mirror halves using a hand meat saw and fabricated into primal cuts: leg, loin, rack, shoulder, breast, shank and flank (BSF) and neck following the procedures of Field et al. [9]. The carcasses primal cuts were chilled immediately after evisceration in a refrigerator at 4 ^{0}C for 24 hours before freezing .

Freezing Preservation of Meat

Leg cuts from scalded, skinned and singed carcasses were frozen at –18 °C in a freezer for 0, 7, 14 and 21 days [2]. The leg cuts were thawed and meat samples were excised from them for the determination of physical variables of meat and were refrozen on the same day.

Measurement of physical variables

The physical properties of frozen chevon measured included the followings:

Frozen meat visual color

The visual color of frozen meat was determined using the subjective visual method as described by [10]. Meat samples from leg cuts of each treatment were placed on a tray in the laboratory and a 10-member panel was used to evaluate the meat color based on the color intensity (redness) and homogeneity using a scale ranging from 1 to 8 with higher scores representing more attractive and homogenous red color after 0, 7, 14 and 21 days of freezing.

Cooking loss and thermal shortening

The cooking loss was determined following the method of Malgorzata et al. [11]. A meat piece with a weight of about 10 g and a length of 6 cm from the leg cut was wrapped in a polythene bag and boiled in a pressure cooking pot at 80 °C for 20 minutes after thawing on 0,7, 14 and 21 days. The meat samples were boiled on an adjustable PIFCO Japan Electric hot plate model No (ECP 202) until reaching a temperature of 72 °C in the geometric center of the meat samples. The meat samples were removed and cooled to room temperature (27 °C) for 10 minutes and were reweighed. The difference in weight was expressed as loss. Thus:

$$\text{Cooking loss} = \frac{Wt_1 - Wt_2}{Wt_1} \times 100 \quad (1)$$

where:

Wt_1 = initial weight of meat (g)

Wt_2 = final weight of meat (g)

Thermal shortening

The thermal shortening of meat was determined with the same meat samples used to measure the cooking loss according to Apata [12]. The length of meat samples was remeasured after cooking and cooling. The difference in length of meat samples was expressed as thermal shortening percentage:

$$\text{Thermal shortening} = \frac{L_{h1} - L_{h2}}{L_{h1}} \times 100 \quad (2)$$

where:

L_{h1} = initial length of meat (cm)

L_{h2} = final length of meat (cm)

Cold loss

The cold loss of meat samples from frozen leg cuts was determined after thawing meat on 0, 7, 14 and 21 days. A piece of deboned meat with a weight of 10 g and a length of 6 cm was excised from the leg cuts, wrapped in a polythene bag and refrozen for 48 hours following the procedures of Lawrie and Ledward [13]. The meat samples were removed and reweighed. The difference in weight was expressed as percentage of cold loss, thus:

$$\text{Cold loss} = \frac{Wt_{c1} - Wt_{c2}}{Wt_{c1}} \times 100 \quad (3)$$

where:

Wt_{c1} = initial cold weight of meat (g)

Wt_{c2} = final cold weight of meat (g)

Cold shortening

The cold shortening was determined using the same meat samples used to measure the cold loss according to Lawrie and Ledward [13]. The length of the meat samples was remeasured after refreezing for 48 hours and the reduction in the initial length was expressed as the cold shortening percentage, thus:

$$\text{Cold shortening} = \frac{L_{c1} - L_{c2}}{L_{c2}} \times 100 \quad (4)$$

where:

L_{c1} = initial length of meat samples (cm)

L_{c2} = final length of meat samples (cm)

Cooking yield

The cooking yield was determined after thawing meat on 0, 7, 14 and 21 days. A piece of deboned meat (25 g) was removed from leg cuts, wrapped in a polythene bag and boiled for 20 minutes. Then, meat was removed, cooled to room temperature (27 °C) and reweighed. The weight of the boiled meat was used to calculate the cooking yield according to Apata [12].

$$\text{Cooking yield} = \frac{Wt_{m1}}{Wt_{m2}} \times 100 \quad (5)$$

where:

Wt_{m1} = initial weight of meat samples (g)
Wt_{m2} = final weight of meat samples (g)

Drip loss

The drip loss was determined following the procedures of Insausti [14]. Slices of meat samples (10 g) from leg cuts were suspended in polythene bags sealed under atmospheric pressure. The meat samples were then hanged in a refrigerator at 4 °C for 48 hours so that juice could drain. After that, the meat samples were reweighed. The drip loss was calculated as follows:

$$\text{Drip loss} = \frac{(W_p + j) - W_p}{(W_p + m) - W_p} \times 100 \quad (6)$$

where:

$W_p + j$ = weight of pack + juice
W_p = weight of pack
$W_p + m$ = weight of pack + meat

Water Holding Capacity (WHC)

The water holding capacity was determined as expressible juice following the procedures of Malikajuna and Mittal [15]. An approximately 1 g of a meat sample from the leg cut was placed between two pre-weighed 9 cm Whatman No 1 filter papers (Model C, Caver Inc. Wabash USA). The meat sample and the filter papers were pressed between two 10.2 × 10.2 cm^2 plexiglass plates at about 32.7 kg/cm^3 absolute pressure for 1 minute with a vice. The wetted filter papers were removed and reweighed. The WHC was calculated as follows:

$$\text{WHC} = \frac{W_{wp} - W_{dp}}{W_{dp}} \times 100 \quad (7)$$

where:

W_{wp} = weight of wetted papers (g)
W_{dp} = weight of dry papers (g)

Meat Texture

This variable was determined following the procedures of [16] using the subjective visual method. A 10-member panel was used to score the texture of the meat samples from the leg cut. The scores were based on a scale ranging from 1 to 8, on which 1 = extremely coarse and 8 = extremely fine texture.

Shear force value

The Warner Bratzler shear values of meat samples were determined following the procedures of Malgorzata et al. [11]. Meat samples (10 g) from the leg cut were wrapped in polythene bags and boiled for 20 minutes in a pressure pot on a PIFCO Japan Electric hot plate (Model No ECP 202) to an internal temperature of 73 °C. The meat samples were then cooled to room temperature (27.8 °C) and were reweighed, wrapped in polythene bags and chilled at 4 °C for 18 hours. The meat samples were removed and were held to equilibrate to room temperature. Then, 1.25 cm diameter cores parallel to muscle fiber orientation were made and were sheared at three locations with a Warner Bratzler V-Notch blade shearing instrument. The average shear values were recorded for each treatment.

Meat pH

The pH values of meat samples were determined following the method described by Marchiori and de Felicio [17]. A meat sample (10 g) from the leg cut was homogenized with 90 ml of distilled water for 5 minutes using a laboratory blender (plate 5mm, Model 242, Nakai Japan). The pH values of meat samples were measured with a pH meter (model H-18424 micro-computer, Hanna instruments, Romania).

Experimental design n and statistical analysis

The completely randomized design was used for this study. There were three treatments (scalding, skinning, singering) and four different periods of freezing preservation (0, 7, 14 and 21 days). All data collected from this study were subjected to analysis of variance (ANOVA) using [18] and the significant means were separated with the Duncan multiple range test of the same statistical system.

Results and discussion

The results for the chevon visual color as affected by carcass dressing and freezing preservation are presented in Table 1. The scores for the chevon color were higher ($p < 0.05$) in meat samples from the carcasses dressed with the singeing method and were lower ($p < 0.05$) in those from the carcasses dressed with the skinning method. The scores for the color of frozen chevon decreased as the period of freezing preservation increased from 0 to 21days ($p < 0.05$).

Color or appearance of meat or any food substance is an attribute most valued by consumers, and it is used in categorizing meat quality [6]. Meat samples from the singed and scalded carcasses had higher scores for color compared to meat samples from the skinned carcasses probably because of heat treatment of the carcasses. The heat might have stabilized the content of oxygen, which could not be attacked by oxygen consuming enzymes [19]. The scores for color of meat decreased steadily as the time of freezing increased probably because of non-steady flow of oxygen in the freezer due to the epileptic nature of electricity supply, therefore the meat samples could not be oxygenated as reported by Apata [12]. The meat color values were high on 0 and 7th day of freezing probably due to blooming

that took place in the freezer as a result of availability of oxygen both in the open air where meat was processed and in a freezer when electricity was at maximum [13]. Other researchers [20,21,22] also reported that when meat was frozen color changes progressed over a long period, which supports the results obtained from this study.

Table 1. Visual color scores of chevon as influenced by carcass dressing methods and frozen storage

	Treatments		
Time/Day	Scalding	Skinning	Singeing
0	7.00 ± 0.01^{a}	7.00 ± 0.01^{a}	7.00 ± 0.01^{a}
7	7.00 ± 0.01^{a}	6 .00 ± 0.02^{a}	7.00 ± 0.01^{a}
14	5.00 ± 0.03^{b}	4.00 ± 0.05^{c}	6.00 ± 0.02^{a}
21	5.00 ± 0.03^{b}	4.00 ± 0.05^{c}	6.00 ± 0.02^{a}

abc: Means in the same row with different superscripts are statistically significant ($p < 0.05$)

Table 2 shows the results of determination of the chevon cooking loss as influenced by carcass dressing and freezing preservation. Meat samples from the carcasses dressed with the singeing method had higher ($p < 0.05$) percentage of cooking loss followed by meat samples from the carcasses dressed with scalding, while meat from the carcasses dressed with the skinning method had the lowest ($p < 0.05$) cooking loss percentage. The percentage of cooking loss of chevon increased ($p < 0.05$) as the period of freezing increased and was highest ($p < 0.05$) on the 21st day of preservation.

The highest cooking loss observed in meat from the singed carcasses (Table 2) could be connected with the action of heat resulting in weakening and puncturing the myofibrils as well as the connective tissue of meat, which facilitated the loss of juices from heated carcasses and subsequently meat [16]. In this study, the cooking loss followed the intensity of heat applied. The heat intensity was higher in singeing than in scalding and no heat was applied to the skinned carcasses, hence it was expected that the cooking loss in meat from the skinned carcasses would be minimal [6]. The loss of juices in meat from the singed carcasses could have been aggravated by thawing that accompanies freezing. Kondratowicz and Mamsevicius [3] reported that freezing and thawing destroy the structure of meat, and since the meat samples were removed from partially heated carcasses by singeing and scalding, these procedures might have added to the damage unlike meat samples from the skinned carcasses [16].

Table 2. Percentage of cooking loss in meat as influenced by carcass dressing methods and frozen storage

	Treatments		
Time/Day	Scalding	Skinning	Singeing
0	13.12 ± 0.04^{b}	10.22 ± 0.06^{c}	14.20 ± 0.03^{a}
7	13.17 ± 0.04^{b}	11.25 ± 0.05^{c}	15.42 ± 0.02^{a}
14	13.29 ± 0.04^{b}	11.60 ± 0.05^{c}	15.50 ± 0.02^{a}
21	13.31 ± 0.04^{b}	11.68 ± 0.05^{c}	16.64 ± 0.01^{a}

abc: Means in the same row with different superscripts are statistically significant ($p < 0.05$)

The results of cooking yield percentage of chevon as influenced by carcass dressing and freezing preservation are shown in Table 3. Meat from the skinned carcasses had the highest ($p < 0.05$) cooking yield compared with other two treatments, while meat from the skinned carcasses had the lowest ($p < 0.05$) cooking yield. The results showed further that cooking yield of frozen chevon decreased as the period of freezing preservation increased and was lowest ($p < 0.05$) on the 21st day.

Meat yield is an important aspect of carcass processing as it indicates the economic value of meat for the processors [12]. In this study, the yield was highest in meat from the skinned carcasses (Table 3). The high yield observed in meat from scalded carcasses could be due to the skin cover and lower juice loss from meat during processing. Although there was skin cover in meat from singed carcasses, but there could be loss of juices during singeing, which could have decreased the yield in meat samples from the singed carcasses. The yield was very low in singed meat due to heat applied to the carcasses which also reflected in the preserved meat (Table 3). Other researchers also reported that singed meat has a higher tendency to loose juices than scalded and skinned meat because of the heat applied [6,8,19]. The yield decreased as the time of freezing preservation increased due to higher draining as a result of softness and tenderness of the meat which would have warranted more drains from the meat.

Table 3. Cooking yield of chevon as affected by carcass dressing methods and frozen storage

	Treatments		
Time/Day	Scalding	Skinning	Singeing
0	86.88 ± 0.01^{b}	89.78 ± 0.01^{a}	85.80 ± 0.03^{c}
7	86.83 ± 0.01^{b}	88.75 ± 0.02^{a}	84.58 ± 0.04^{c}
14	86.71 ± 0.01^{b}	88.40 ± 0.02^{a}	84.50 ± 0.04^{c}
21	86.69 ± 0.001^{b}	88.32 ± 0.02^{a}	83.36 ± 0.05^{c}

abc: Means in the same row with different superscripts are statistically significant ($p < 0.05$)

The results of cold loss values of chevon as affected by carcass dressing and freezing preservation are presented in Table 4. The results indicated that meat from the skinned carcasses had the highest ($p < 0.05$) cold loss percentage, while meat samples from the scalded carcasses had the lowest ($p < 0.05$) cold loss values. The percentage of the cold loss of meat samples increased across the treatments as the time of freezing preservation increased and was highest ($p < 0.05$) on the 21st day of freezing.

The cold loss was highest in meat samples from the singed carcasses and least in meats from scalded carcasses (Table 4). Heating of singed carcasses during processing (dressing) could have weakened the muscle structure of meat and led to higher draining from meat unlike skinned meat, which did not pass through heat at all.

Table 4. Cold loss of chevon as influenced by carcass dressing methods and frozen storage

Treatments			
Time/Day	Scalding	Skinning	Singeing
0	10.09 ± 0.12^{c}	11.20 ± 0.10^{b}	12.88 ± 0.09^{a}
7	11.14 ± 0.10^{c}	12.25 ± 0.09^{b}	14 .08 ± 0.08^{a}
14	11.31 ± 0.10^{c}	12.59 ± 0.09^{b}	14.23 ± 0.08^{a}
21	11.90 ± 0.09^{c}	13.68 ± 0.08^{b}	15.33 ± 0.07^{a}

abc: Means in the same row with different superscripts are statistically significant (p < 0.05)

Table 5 presents the results of drip loss percentage in chevon as influenced by carcass dressing and freezing preservation. There were significant (p < 0.05) differences in the drip loss values of chevon due to carcass dressing and freezing preservation. Meat samples from the singed carcasses had the highest (p < 0.05) drip loss values, while meat from the skinned carcasses had the lowest (p < 0.05) drip loss percentage. The values of drip losses in chevon increased from 0 day to 21day due to freezing with the highest (p < 0 .05) drip loss observed on the 21st day.

Table 5. Drip loss of chevon as affected by carcass dressing methods and frozen storage

Treatments			
Time/Day	Scalding	Skinning	Singeing
0	9.25 ± 0.02^{b}	8.20 ± 0.03^{c}	10.28 ± 0.01^{a}
7	10.28 ± 0.03^{b}	9.24 ± 0.04^{c}	11.32 ± 0.02^{a}
14	11.30 ± 0.04^{b}	10.27 ± 0.05^{c}	12.49 ± 0.03^{a}
21	12.42 ± 0.05^{b}	11.33 ± 0.06^{c}	13.53 ± 0.04^{a}

abc: Means in the same row with different superscripts are statistically significant (p < 0.05)

The same pattern as found for the drip loss was observed in the values of thermal shortening of chevon as affected by carcass dressing and freezing (Table 6). Thermal shortening values were higher (p < 0.05) in meat samples from the carcasses dressed with singeing and lower in those from the carcasses that were skinned. Meat from the scalded carcasses had thermal loss values close to the values obtained for meat from the singed carcasses. The highest (p < 0.05) values for thermal shortening of chevon were recorded on the 21st day of freezing preservation.

The results on thermal shortening of chevon stored for 21days had similar pattern as obtained for the drip loss in meat (Table 6). Lawrie and Ledwards [13] reported that any muscle that underwent thermal processing (heating) would have a tendency to shorten more when kept in a freezer over time. The same results were observed in this study, which confirmed the report of Dalatowski et al. [4]. Meat samples from the singed carcasses showed the highest shortening followed by meat from the scalded carcasses, while the lowest values were in skinned meat because skinned meat was not subjected to heating during processing.

Table 6. Thermal shortening of chevon as influenced by carcass dressing methods and frozen storage

Treatments			
Time/Day	Scalding	Skinning	Singeing
0	18.78± 0.08^{b}	15.27± 0.09^{c}	20.24± 0.06^{a}
7	20.27± 0.06^{b}	17.31± 0.08^{c}	21.37± 0.05^{a}
14	21.52± 0.05^{b}	18.40± 0.07^{c}	23.67± 0.04^{a}
21	21.70 ± 0.05^{b}	18.47± 0.07^{c}	23.71± 0.04^{a}

abc: Means in the same row with different superscripts are statistically significant (p < 0.05)

Table 7 presents the results of cold shortening of chevon as affected by carcass dressing and freezing preservation. The singed carcasses gave meat with the highest (p < 0.05) cold shortening values followed by meat samples from the scalded carcasses. Meat from the skinned carcasses had the lowest (p < 0.05) cold shortening values. The results also revealed that the values of meat cold shortening increased progressively as the time of freezing increased to 21days.

The effect of carcass processing (dressing) was very evident in the cold shortening results of the meat samples (Table 7). It was obvious that as the heat intensity was highest in singed carcass, cold shortening was also very high in the singed meat, whereas meat from the skinned carcasses shortened very poor because no heat was applied and no muscle weakening took place. These results confirm the reports of Lawrie and Ledwards [13] as well as Apata [12].

Table 7. Cold shortening of chevon as influenced by carcass dressing methods and frozen storage

Treatments			
Time/Day	Scalding	Skinning	Singeing
0	11.27 ± 0.10^{b}	10.20 ± 0.12^{c}	12.45 ± 0.10^{a}
7	11.42 ± 0.10^{b}	10.27 ± 0.12^{c}	12.60 ± 0.10^{a}
14	12.60 ± 0.10^{b}	11.35 ± 0.12^{c}	13.65 ± 0.09^{a}
21	12.67 ± 0.10^{b}	11.46 ± 0.12^{c}	13.82 ± 0.09^{a}

abc: Means in the same row with different superscripts are statistically significant (p < 0.05)

The results of the water holding capacity (WHC) of chevon as influenced by carcass dressing and freezing showed significant (p < 0.05) differences in the value of WHC across the three dressing treatments with meat samples from the skinned carcasses having the highest (p < 0.05) WHC followed by meat from the scalded carcasses, while the lowest (p < 0.05) WHC values were recorded in meat samples from the singed carcasses. The water holding capacity values decreased as the number of days of freezing preservation were prolonged to 21days.

The water holding capacity (WHC) is very important in determining a degree of other eating attributes of meat because when it is relatively high it influences the tenderness, juiciness, texture and overall acceptability of meat or meat products (Table 8). This could be because skinned meat did not pass through heat like singed meat. Therefore,

the muscle structure in skinned meat was intact unlike that in singed meat and scalded meat, whose muscle architecture could have been destroyed by heat and this might have paved the way for more water in form of drains to exudate from meat. This result correlates with the studies by Omojola and Adesehinwa [6], Monin et al. [7], [8] and aApata [12] who reported that skinned meat has higher WHC than either scalded or singed meat. The WHC decreased as the time of freezing increased in this study, which agrees with the results of Apata [12].

Table 8. Water holding capacity of chevon as affected by carcass dressing methods and frozen storage

Treatments			
Time/Day	Scalding	Skinning	Singeing
0	66.70 ± 5.39^{b}	72.18 ± 2.31^{a}	55.41 ± 6.22^{c}
7	61.57 ± 8.82^{b}	66.70 ± 5.39^{a}	55.27 ± 6.88^{c}
14	59.72 ± 9.46^{b}	63.87 ± 7.16^{a}	53.32 ± 8.28^{c}
21	57.15 ± 10.29^{b}	60.64 ± 9.94^{a}	49.61 ± 8.13^{c}

abc: Means in the same row with different superscripts are statistically significant ($p < 0.05$)

The meat texture is influenced by a level of the water holding capacity and heat treatment. In this study the texture scores for meat from the skinned carcasses were highest (Table 9), followed by meat from the scalded carcasses, while meat from the singed carcasses received the lowest scores for texture profile. The reason for these results could be the fact that meat from the skinned carcasses retained a high amount of water, which resulted in meat with very fine texture. In addition, heat did not affect skinned meat, while meat from both singed and scalded carcasses was exposed to heat. Moreover, the temperature applied to singed meat was higher than that applied to scalded meat. Therefore, although scalded meat also underwent heat treatment, it was not as severe as in the case of singed meat. As a result, the lowest scores for the texture profile were recorded for singed meat, while finer texture of meat was observed in case of scalding. These results agree with the reports of Omojola and Adesehinwa [6] and Apata [12].

Table 9.Texture scores of chevon as affected by carcass dressing methods and frozen storage

Treatments			
Time/Day	Scalding	Skinning	Singeing
0	6.47 ± 0.02^{b}	7.63 ± 0.01^{a}	5.37 ± 0.06^{c}
7	6.35 ± 0.02^{b}	7.60 ± 0.01^{a}	4.35 ± 0.07^{c}
14	5.32 ± 0.03^{b}	6.52 ± 0.02^{a}	4.30 ± 0.07^{c}
21	4.30 ± 0.04^{b}	6.45 ± 0.02^{a}	3.28 ± 0.10^{c}

abc: Means in the same row with different superscripts are statistically significant ($p < 0.05$)

The results of the shear force values of chevon as affected by carcass dressing and freezing preservation are shown in Table 10. Meat samples from the singed carcasses had the highest ($p < 0.05$) shear force values followed by meats from the scalded carcasses, while the lowest ($p < 0.05$) shear force values were recorded in meat from the skinned carcasses. The meat shear force values decreased ($p < 0.05$) as the time of freezing preservation increased up to the last freezing day.

The criteria that affected the texture of meat would also apply to the shear force value of meat or meat products. The shear force represents a degree of tenderness or toughness of a meat sample and is predicated on an amount of water a meat sample can hold or bind. The shear force in singed and scalded meat was significantly higher than that in skinned meat because heat applied to the two meat samples reduced their moisture content drastically compared with skinned meat, which was not subjected to heat treatment (Table 10). The previous studies by Okubanjo [8], Omojola and Adesehinwa [6], and Apata [12] demonstrated that singed and scalded meat was tougher due to the skin cover and heat treatment when compared to skinned meat without the skin cover and heat treatment. The shear force values decreased as the time of freezing increased, which suggests that freezing contributed to the value of shear force reducing the muscle tone and rendering the muscles more tender due to ageing as reported by Apata [12].

Table 10. Shear force values of chevon as affected by carcass dressing methods and frozen storage (kg/cm3)

Treatments			
Time/Day	Scalding	Skinning	Singeing
0	5.20 ± 0.02^{b}	4.12 ± 0.03^{c}	6.28 ± 0.01^{a}
7	5.10 ± 0.02^{b}	4.08 ± 0.03^{c}	6.20 ± 0.01^{a}
14	4.10 ± 0.03^{b}	3.05 ± 0.07^{c}	5.18 ± 0.10^{a}
21	4.07 ± 0.03^{b}	3.01 ± 0.07^{c}	5.12 ± 0.10^{a}

abc: Means in the same row with different superscripts are statistically significant ($p < 0.05$)

Table 11 presents the results of pH values of chevon as affected by carcass dressing and freezing preservation. The results indicated that there were no significant ($p < 0.05$) differences in the pH values of meat samples irrespective of the treatment between 0 and 7th day of freezing preservation, but significant ($p < 0.05$) differences were observed on the 14th and 21st days of preservation.

The pH of meat determines whether meat is PSE, DFD or normal and this in effect determines other indicators of eating quality such as color, tenderness and juiciness, as well as WHC, which in combination dictate the overall acceptability of a meat sample. A change in pH of meat from all three treatments was not significant in scalded and singed meat on 0 and 7th day of freezing, but it was significant in skinned meat within the same period of time. The pH increased from day 14 in all treatments and was highest on the 21st day of freezing. These results indicated that both singed and scalded frozen meat was still within the range of pH that allows longer shelf life of meat, whereas, pH of skinned meat was moving to the range that could encour-

age the proliferation of microorganisms, thereby shortening the shelf life of skinned meat. Apata [12] reported about similar observations.

Table 11. pH values of chevon as influenced by carcass dressing methods and frozen storage

Treatments			
Time/Day	Scalding	Skinning	Singeing
0	5.40 ± 0.02	5.40 ± 0.01	5.52 ± 0.01
7	5.44 ± 0.10	5.45 ± 0.10	5.54 ± 0.10
14	5.46 ± 0.01^{b}	6.57 ± 0.01^{a}	5.56 ± 0.10^{b}
21	5.52 ± 0.01^{b}	6.90 ± 0.00^{a}	5.58 ± 0.01^{b}

ab: Means in the same row with different superscripts are statistically significant ($p < 0.05$)

Conclusion

It can be concluded from the results of this study that carcass dressing methods (scalding, skinning and singeing) and freezing have a significant effect on physical characteristics of chevon, with singeing method exerting a higher effect than scalding and skinning. Also, freezing chevon for 21days impacted negatively on the chevon physical attributes. It is therefore, recommended that skinning method be adopted by butchers since most of the physical attributes of chevon were very low in skinned meat, and that chevчon should not be frozen beyond 14 days to avoid an increase in the physical attributes of chevon, which could make meat unacceptable.

REFERENCES

1. Chambers, P.G., Grandin, T., Heinz, G., Srisuvan, T. (2001). Guidelines for humane handling, transport and slaughter of livestock. FAO, Bangkok, Thailand, 2001.
2. Kandeepan, G., Biswas, S. (2005). Effect of low temperature preservation on microbial and sensory quality of buffalo meat. *Livestock Research for Rural Development,* 17(11), Article 124.
3. Kondratowicz, J., Matusevicius, P. (2002). Use of low temperature for food preservation. *Veterinarija ir Zootechnika*, 17(39), 88–92.
4. Dolatowski, Z., Stasiak, D.M., Latoch, A. (2000). Effect of ultra sound processing of meat before freezing on texture after thawing. *Electronic Journal of Polish Agricultural Universities. Series Agricultural Engineering,* 3(2), 1–13.
5. Kondratowicz, J., Bak, T., Melter, Z. (1999). Effect of enrichment and different methods of freezing on the weight losses and taste qualities of horse meat during cold storage. *Polish Journal of Food and Nutrition Sciences*, 49(2), 185–193.
6. Omojola, A.B., Adesehinwa, A.O.K. (2006). Meat characteristics of scalded, skinned and conventionally dressed rabbit carcasses. *World Journal of Zoology*, 1(1), 24–29.
7. Monin, G., Tahimant, A., Aillery, P., Collas, G. (1995). Effects of carcass weight and meat quality of pigs dehaired by scalding or singeing post-mortem. *Meat Science*, 39(2), 247-254. https://doi.org/10.1016/0309–1740(94) P1825-G
8. Okubanjo, A.O. (1997). Meat characteristics of singed and conventionally dressed chevon carcasses. *Journal of Food Science and Technology,* 34(6), 494–497.
9. Okubanjo, A.O., Omojola, A.B., Ogunsola, O.O., Adewunmi, M.K., Ajiboro, O.G., Alabi, G.F. et al. (2003). Meat characteristics of Bunaji, Gudali and Keteku cattle. *Tropical Animal Production Investigation*, 6, 185–193.
10. AMSA. (2012). Meat color measurement guidelines Retrieved from https://meatscience.org/docs/default-source/publications-resources/hot-topics/2012_12_meat_clr_guide.pdf Accessed April 15, 2023
11. Sobczak M., Lachowicz K., Kamieniecki H., Wojcik J., Gajowiecki L., Zochowska J. et al. (2005). The effect of cattle genotype on texture of selected muscles during post-mortem ageing. *Electronic Journal of Polish Agricultural Universities. Series Food Science and Technology,* 8(3), 1–9.
12. Apata, E.S. (2011). Quality attributes of Red-sokoto buck meat as influenced by post-slaughter processing methods. Ph.D Thesis in the Department of Animal Science, University of Ibadan, Ibadan, Oyo state, Nigeria.
13. Lawrie, R.A., Ledwards, D. (2006). Lawrie's meat science. Cambridge: Woodhead Publishing Limited, England, 2006.
14. Insausti, K., Berian, M.J., Purroy, A., Alberti, P., Lizaso, L., Hernandez, B. (1999). Colour of beef from local Spanish native cattle breeds stored under vacuum and modified atmosphere. *Meat Science,* 53(4), 241–249. https://doi.org/10.1016/S0309–1740(99)00063–7
15. Mallikarjunan, P., Mittal, G.S. (1994). Meat quality kinetics during beef carcass chilling. *Journal of Food Science*, 59(2), 291–294. https://doi.org/10.1111/j.1365–2621.1994.tb06950.x
16. AMSA. (2015). Research guidelines for cookery, sensory evaluation and instrumental tenderness measurement of meat. Retrieved from https://meatscience.org/docs/default-source/publications-resources/amsa-sensory-and-tenderness-evaluation-guidelines/research-guide/2015-amsa-sensory-guidelines-1–0.pdf?sfvrsn=6 Accessed April 15, 2023
17. Marchiori, A.F., de Felicio, P.E. (2003). Quality of wild boar meat and commercial pork. *Scientia Agricola*, 60(1), 1–5. https://doi.org/10.1590/S0103–90162003000100001
18. SAS (2002). Statistical Analysis System. SAS stat, version 9 SAS Institute Inc. Garry, NC, USA.
19. Veiseth, E., Shackelford, S.D., Wheeler, T.L., Koohmaraie, M. (2001). Effect of post-mortem storage on μ-calpain and m-calpain in ovine skeletal muscle. *Journal of Animal Science,* 79(6), 1502–1508. https://doi.org/10.2527/2001.7961502x
20. Lee, K.-T., Yoon, C.-S. (2000). Quality changes and shelf-life of imported vacuum packaged beef chuck during storage at 0 °C. *Meat Science*, 59(1), 71–72. https://doi.org/10.1016/S0309–1740(01)00054–7
21. Gonzatez, C.B., Salitto, V.A., Carduza, F.J., Pazos, A.A., Lasta, J.A. (2001). Effect of calcium chloride marination on bovine cutaneous trunci muscle. *Meat Science*, 57(3), 251–256. https://doi.org/10.1016/S0309–1740(00)00099–1
22. Banani, R.C., Rabi, S.M., Runu, C. Utpal, R.C. (2006). Effect of combination pre-treatment on physicochemical, sensory and microbial characteristics of fresh aerobically stored minced goat (Black Bengal) meat organs. *African Journal of Biotechnology*, 5(12), 1274–1283.
23. Aduku, A.O., Olukosi, J.O. (2001). Animal products handling and processing in the tropics. GU publications, Abuja, Nigeria, 2001.

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OPTICAL-SPECTROSCOPIC ANALYSIS OF COLORIMETRIC CHANGES IN MEAT DURING ITS STORAGE

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Abstract
The colorimetric and spectral characteristics of meat and their changes during the period of storage were researched. It was shown that spectral methods of analysis can potentially be used to assess the properties of meat during its storage in order to define the degree of autolytic changes which occur in the meat along with its histological-structural and proteomic changes. The work studies the quality characteristics of meat on the base of an array of its parameters and a correlation between them. Considerable attention is paid to the determination of colorimetric and spectral characteristics of autolytic and other changes in meat during its storage. The possibility of using the method of optical spectrometry for assessing the quality of meat is considered. The data obtained by processing the absorption spectra of aqueous extracts from muscle tissue confirm the promising prospects of using this method in a comprehensive study of the raw meat materials properties. The work proves possibility of classifying raw meat according to the degree of its autolysis for further assessment of its colorimetric characteristics, the value of extinction coefficients and the relative area of peaks at the wavelength λ_{415}, λ_{525}, λ_{542}, λ_{555}, *u* λ_{582}.

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Introduction

Color evaluation is an integral part of meat quality research, development of a high-quality and safe food product and elimination of errors during its processing. Tomashevich I. B. has noted that the improvement in color stability of meat and meat products is closely related to their shelf life. It is desirable to increase the period during which the meat is still visually acceptable to the consumers in retail trade. Measuring the color of meat with a colorimeter or computer vision system allows evaluating its suitability for the processing, the quality of the finished food product, the correctness of the technological processes, etc. [1,2]

It is known that the optical properties of meat play an important role in color formation. The color of meat dramatically depends on its pH [3], the chemical condition and amino acid sequence of myoglobin [4,5], redox processes and interactions between muscle pigments [6,7]. However, studies [8,9,10] showed that, in addition to myoglobin, other compounds also influence the final formation of meat color, the most significant of which are the endogenous pigments (chromoproteids). Among meat chromoproteids, hemoglobinogenic pigments (ferritin, hemosiderin, bilirubin), proteinogenic pigments (melanin, adrenochrome) and lipopigments (lipofuscin, ceroid, lipochromes) are denoted.

The content of chromoproteids in meat and its optical characteristics depend on many factors, for example, the type of animal, its genetic characteristics, its diet, post-slaughter changes in muscles, mode of refrigeration, storage time, way of packaging, etc. [11].

As shown in the work [12], the optical properties and color of meat can be assessed by two main methods: chemical and physical. For an objective measurement of the color of food products by chemical methods, the pigments from the samples are extracted and their concentration is measured by a spectrophotometric method. Physical methods are based on the interaction of light with the object of study — its reflective, absorbing or transmissive capacity.

The optical properties of meat and meat products are also determined by the complexity of their microstructure and physicochemical properties. Absorption and scattering of radiation are determined by four main processes: resonant absorption of radiation by dry matter molecules, as well as molecules of structural and bound moisture; scattering of radiation due to fluctuations in the density of a substance, as well as scattering on molecules of pro-

teins, polysaccharides, ions, etc.; scattering of radiation on suspended colloidal particles, cells, pigment particles, etc., as well as scattering of radiation on optical inhomogeneities — capillaries, pores [13].

Thus, the correct visual and instrumental study of the optical properties and color of meat can be powerful and beneficial for the meat processing industry. However, this study must be run with the help of thoroughly designed techniques to avoid artifacts or incorrect data.

Currently, there are many options for instrumental analysis of the optical properties and color of meat. The most common color measuring instruments are colorimeters and spectrophotometers [14].

Colorimeters determine the proportions of primary additive light sources that match the color reflected from the sample or transmitted by the sample.

Spectrophotometers measure the amount of light of various wavelengths reflected from the sample or transmitted through the sample, resulting in a reflectance, absorption, or transmission spectrum. The transmission or absorption spectrum of the sample can be used together with the standard CIE observer function and the relative spectral energy distribution of the light source to calculate tricolor CIE XYZ values for the given sample with the selected light source.

As shown by Chernousova O. V., Rudakov O. B. [15], spectrophotometric methods for studying the optical properties and color of meat are very promising, as they provide accuracy and high speed in obtaining results and are often characterized by ease of their application. Currently, there is an active introduction of instruments and devices based on the spectroscopy principles.

The principle of operation of the devices described above serves as basis in various portable analyzers of the raw meat materials quality. Thus, the authors of the work [16] described a device for the integrated measurement of physical-chemical parameters and food color, which provides fast measurements in the standard CIELAB format.

The paper [17] presents the results of the development of a portable color analyzer for assessing the qualitative characteristics of poultry meat. The optical methods allowed establishing a relationship between the change in the dominant wavelength and duration of the carcasses storage.

The papers [18,19] proved the prospects of using optical spectroscopy for colorimetry of cognac products to detect the fact of product adulteration.

Pochitskaya I. M. proposed to evaluate the color characteristics of food products using the Adobe Photoshop program in the RGB (red-green-blue) coordinate system, which makes it possible to predict the color of the resulting finished product in order to create new food products with pre-defined color and taste characteristics [20].

The influence of broiler age on color characteristics (lightness L*, redness a*, yellowness b*) was shown by Janisch et al [21], who found a significant difference between the values of electrical conductivity, volume of meat juice loss, shear force and color in meat of 28 and 41-days old broilers.

The authors [9,22] proved that the study of meat by optical spectroscopy methods makes it possible to differentiate the muscle tissue of wild and domestic animals depending on the processing conditions: freezing, water extraction, lyophilization, and fermentation. This method makes it possible to reveal significant differences in changes in the spectral characteristics of the main components and components of the muscle tissue and muscle fiber of pork and beef, depending on the degree of oxidation, degree of mechanical destruction, strength of myofibrils and bond of myoglobin pigment protein with them.

Holman et al. [23] used a linear model to establish the relationship between color values, volume of meat juice losses, pH values, and shear forces during storage.

The high sensitivity, resolution and analytical features of optical spectroscopy methods in the study of biological tissues of animal origin in solid and liquid state of aggregation make it possible to obtain factual information about changes in the spectral characteristics of their main components.

Optical spectroscopy makes it possible to judge the depth of destructive chemical processes at the level of all components of biological material. Thus, as a result of storing chilled meat for a week, the myoglobin doublet disappears and absorption decreases in all areas of the electromagnetic spectrum, while the spectrum of thawed muscle tissue, on the contrary, lies above the spectrum of the original sample [9, 22].

Over the past five years a lot of researches into the optical properties and color of meat have been run, but several fundamental concepts still remain unsolved. In particular, insufficient attention has been paid to the role of autolytic changes in the formation of meat color during its storage. Additional study of the fundamental relationships between protein fractionation, histological-structural changes, and optical properties of meat can help solve numerous practical color stabilization issues.

Thus, for deeper understanding of autolytic processes and changes in the spectral characteristics of meat during its storage, it is advisable to study the characteristics of meat quality as the complex of its parameters array and establish a correlation between them in order to improve the methodology for meat color measuring.

Materials and methods

When constructing the experimental plan, the factors that provide the strongest effect on the meat quality were considered. These factors include ante-mortem and post-mortem conditions and storage duration.

Meat raw materials. Chilled samples of the porcine rib eye (*Sus scrofa M. longissimus dorsi*) were used as raw materials. Meat samples were put into storage at a temperature from 0 °C to plus 4 °C. Sampling and preparation of

Table 1. Change in the color parameters of meat during its storage

Day	L*	a*	b*	λ_{dom}	Color purity	Saturation	Color shade
1	98.29 ± 0.2	0.97 ± 0.04	5.15 ± 0.30	581.17 ± 0.04	6.84 ± 0.35	5.24 ± 0.28	0.57 ± 0.03
2	96.43 ± 0.8	2.43 ± 0.04	9.74 ± 0.31	582.36 ± 0.43	13.11 ± 0.42	10.04 ± 0.29	1.44 ± 0.02
3	97.10 ± 0.4	1.88 ± 0.05	8.36 ± 0.15	581.90 ± 0.09	11.17 ± 0.23	8.57 ± 0.16	1.11 ± 0.03
4	98.08 ± 0.3	0.98 ± 0.04	5.26 ± 0.28	581.14 ± 0.04	7.00 ± 0.35	5.35 ± 0.28	0.57 ± 0.03

Note: The values in the columns are statistically significantly different ($p < 0.05$)

samples for the analysis complied with GOST 7269–2015[1] and GOST R 51447–99 (ISO 3100–1–91)[2].

Determination of color and spectral parameters of meat. The color of meat was determined in an aqueous extract of muscle tissue with the help of a spectrophotometer PE-5400VI (LLC "Ecokhim", Russia) in the visible wavelength range within 340–830 nm with an increment of 5 nm. To obtain an aqueous extract, a lump of meat samples (10.00 ± 0.02 g) was ground in a manual meat grinder of the brand "Motor Sich 1 MA-s" (manufactured by JSC "MOTOR SICH", Ukraine), placed in a 100 ml flask and extracted with distilled water in a ratio of 1:5 on a SHR-1D laboratory shaker (Daihan Scientific, South Korea) for 30 min at 20 °C and stabilized with 1% gelatin solution. The resulting extract was filtered through a folded paper filter with a pore size of 8–12 μm and exposed to spectrophotometry to determine the curves of light absorption and color. Light absorption curves were obtained in the coordinates $A = f(\lambda)$, where λ is the wavelength, nm; A is the optical density.

The absorption bands were resolved by calculating the second derivative. Noise was filtered out during the second derivative analysis by approximating the absorption spectra by non-uniform rational Bezier splines of various orders (Non-Uniform Rational Bezier Spline, NURBS,) with oversampling. In case of ambiguity in the interpretation of weak signals, Savitzky-Golay Smoothing Filters (SGSF) were used as an alternative. For a relative quantitative assessment of the certain component content in an aqueous extract, the relative peak area was used.

The color coordinates in the CIE system L* a* b*, color purity, degree of saturation, color shade and dominant wavelength λ_{dom} were calculated as described in the paper [24]. Color coordinates were represented in the CIE L* a* b* system, which were calculated using standard light source A.

Analysis of the fractional composition of proteins of the longissimus porcine muscle was carried out by one-dimensional electrophoresis in 12.5% polyacrylamide gel in the presence of sodium dodecyl sulfate in a VE-10 chamber (Helicon, USA). As a standard for electrophoresis, a marker from the company "Thermo", USA, was used, which marker is a mixture of 11 recombinant proteins (250, 150, 100, 70, 50, 40, 30, 20, 15, 10 kDa). Staining was performed by Coomassie G-250 followed by densitometric quantification.

Study of the histological structure of meat. Histological examination of the sample was carried out in accordance with GOST R 31479–2012[3] and GOST 19496–2013[4]. Slices were evaluated using a microscope "Micromed-1 var.2–20" (Micromed, Russia).

The pH value was determined by the potentiometric method according to GOST R 51478–99[5] using a pH meter "HI98163" from the company "Hanna Instruments" (USA). Its measurement range of the active acidity of the medium lies within from — 2.00 till 20.00 units with an error of ±0.01 units.

Statistical analysis of the results was performed using Excel 2019 software (Microsoft, USA). The results obtained were considered significant at $p < 0.05$. Pearson's correlation coefficients were calculated to evaluate any relationships between various factors.

Results and discussion

Table 1 shows the changes in the main colorimetric characteristics of chilled meat during its storage. On the first day of storage, the lightness value (L*) was 98.29 units. On the second day of storage the L* value of aqueous extracts from muscle tissue decreased by 1.86 units, which was clearly visible to the human eye (color difference in reference to the original sample $\Delta E_{2000} = 3.92$ units.).

The values a* and b*, that characterize the color transitions of meat, increased by 1.46 and 4.59 units on the second day of storage, respectively. The color of the extract increased by 6.3%, thereby approaching the spectral (100%), as can be seen from the changes in color purity on the second day of storage. The saturation and hue of meat extracts also changed: from 5.24 to 10.04 and from 0.57 to 1.44, respectively.

Further storage of chilled pork samples was accompanied by a monotonous increase in lightness L* almost to the initial value. Thus, the value of L* on the third day of storage was 97.10 units, and on the fourth day it was 98.08. It should be noted that as the chilled meat was stored, the

[1] GOST 7269–2015 "Meat. Methods of sampling and organoleptic methods of freshness test". Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200133105 Accessed April 10, 2023 (In Russian)

[2] GOST R 51447–99 "Meat and meat products. Methods of primary sampling". Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/1200028183 Accessed April 10, 2023 (In Russian)

[3] GOST R 31479–2012 "Meat and meat products. Method of histological identification of composition". Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200097485 Accessed April 11, 2023 (In Russian)

[4] GOST 19496–2013 "Meat and meat products. The method of histological investigation". Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200107317 Accessed April 11, 2023 (In Russian)

[5] GOST R 51478–99 "Meat and meat products. Reference method for measurement of pH". Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/1200028185 Accessed April 11, 2023 (In Russian)

color difference in aqueous extracts of muscle tissue decreased, and on the 4th day was equal to 0.15 units.

The values of a* and b* on the 4th day approached the initial values and amounted to 0.98 and 5.26 units, respectively. The purity of color, saturation and color shade of meat extracts also reached their values recorded on the first day of storage.

Such changes in colorimetric characteristics can be explained by the influence of post-slaughter changes in meat. On the first day of storage of chilled meat, *rigor mortis* occurs, accompanied by peculiar changes in the histological structure of tissues and the fractional composition of proteins [25]. With the resolution of rigor mortis and subsequent storage of meat, its colorimetric characteristics changed similarly to the ongoing biochemical processes.

In addition to chromoproteids there are three key mechanisms that affect the color of meat [11]:

1) variations in the distance between myofilament lattices, which size changes due to osmotic swelling or contraction, or due to a change of the muscle sarcomere length [26], as a result, changing the diameter of myofibrils and muscle fibers. The lightness value (L* value) of the muscles increases together with a change in the diameter of muscle fibers;
2) variations in the length of sarcomeres, if this is associated with changes in the diameter of myofilaments and myofibrils;
3) variations in the sarcoplasmic proteins distribution.

Thus, the change in the color of meat occurs as a result of the influence of post-mortem biochemical mechanisms that occur in its tissues during storage, which is confirmed by the results of studies of the histological structure of tissues and the fractional composition of proteins.

When analyzing the absorption spectra of aqueous extracts from the muscle tissue of chilled meat during storage (Figure 1), several absorption bands were found that are peculiar for the biomolecules involved in color formation. It is known from the specialized literature that 320–380 nm is the area of absorption of unsaturated fatty acids of lipid components; an intense broad band with a maximum in the region of 400–430 nm is caused by the absorption of mucopolysaccharides (glucosaminoglycans), and the muscle pigment myoglobin, which provides red color to the tissue. This band appears as a doublet within the visible area at 540–580 nm [9].

The absorption spectra of the four redox species overlap and intersect (isobestic point) at 525 nm, and spectrophotometric absorption at 525 nm was used to estimate the total concentration of Mb in aqueous meat extracts.

Among the detected absorption peaks (Table 2), the peaks characteristic of cytochromes and various redox forms of myoglobin were identified.

Throughout the entire period of storage of chilled meat, a hypsochromic shift of the λ_{415} absorption band by 5 nm was observed and a slight hyperchromic effect on the second day was observed too (Figure 1). This effect can be caused by the fact that, as a result of biochemical processes, the viscosity of the aqueous extract drops down and an increase in its optical density is also observed. Thus, the shielding level of organic compounds decreases, and they absorb more light [27].

The relative content of cytochromes (λ_{415}) remained practically unchanged during the storage of meat, however, on the third day there was an increase in their content by 1.13 times in comparison with the initial content on the first day of storage. That was confirmed by an increase in the relative area of the λ_{415} peak from 63.93% up to 72.29% (Table 2). This effect could be associated with loosening of muscle fibers, which was confirmed by the results of histological tests (Figure 3a).

The relative content of various forms of myoglobin changed insignificantly, which could be observed within the area of the $\lambda_{540-580}$ peaks. The extinction coefficient of the solution at λ_{525} on the second day increased by 2.18 times in comparison with this level on the first day, which can be

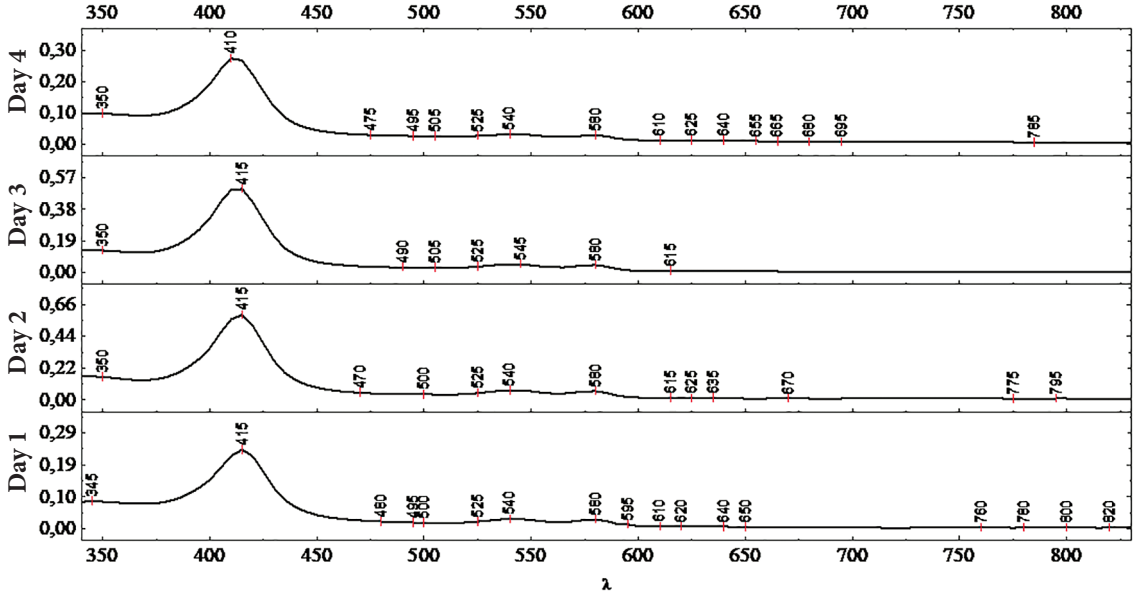


Figure 1. Absorption spectra of muscle tissue extracts

seen from the changes in the peak height from 0.022 to 0.048 (Table 2).

Table 2. Characteristics of bands of some muscle tissue extraction

1st day			2nd day		
λ_{of} the peak, nm	Relative area of the peak, %	Height of the peak	λ_{of} the peak, nm	Relative area of the peak, %	Height of the peak
345	13.78	0.084	350	7.67	0.159
415	63.93	0.238	415	65.06	0.588
480	1.80	0.023	470	2.86	0.051
495	0.56	0.020	500	1.05	0.041
500	1.64	0.019	525	2.14	0.048
525	1.16	0.022	540	5.41	0.067
540	5.37	0.030	580	4.63	0.061
580	4.52	0.029			
595	0.80	0.014			
3rd day			4th day		
λ_{of} the peak, nm	Relative area of the peak,%	Height of the peak	λ_{of} the peak, nm	Relative area of the peak,%	Height of the peak
350	6.14	0.133	350	6.97	0.097
415	72.29	0.503	410	64.15	0.273
490	0.99	0.032	475	2.67	0.029
505	0.98	0.031	495	1.23	0.025
525	1.54	0.036	505	0.61	0.025
545	5.44	0.051	525	1.83	0.026
580	3.36	0.045	540	4.89	0.032
			580	4.91	0.028

Note: The values in the columns are statistically significantly different ($p < 0.05$)

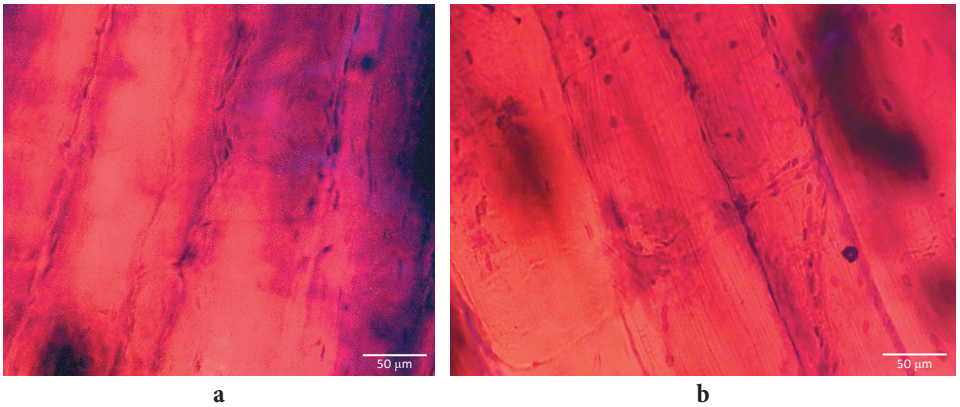
Sharp loom of the extinction coefficient on the second day at the isobestic point of various redox forms of myoglobin caused a hyperchromic effect, which was later replaced by a hypochromic effect, starting from the 3rd day. These effects characterize an increase or decrease in the intensity of absorption, which were caused by biochemical transformations in meat during its autolysis, which was confirmed by the results of the analysis of the protein composition in meat. The results obtained do not contradict to this assumption [28].

It was found that the change in the colorimetric characteristics of aqueous extracts of muscle tissue occurred synchronously with the change in the microstructure of the meat, as evidenced by the data obtained from the histological analysis of chilled pork samples.

Thus, on the first day of meat storage, an asynchronous contraction of muscle fibers was detected with a weakening of the transverse striation and intensification of the longitudinal striation, along with the formation of oval contraction nodes (refer Figure 2a). Muscle fibers had an irregular shape due to deformation changes during *rigor mortis*. In muscle fibers, due to the uneven development of postmortem rigidity, both rod-shaped and round-shaped nuclei were observed. The different shape of the nuclei was caused by the fact that the fibers either were not completely contracted or were relaxed, or they were in a contracted state. The color of the cross-section was uniform. The nuclei were clearly colored. The muscle fiber diameter was 80±20 μm. The fibers were arranged in a wavy pattern, adjoining to each other pretty tightly.

On the second day of storage of meat histological sections, some signs of resolution of rigor mortis were observed. The rigidity resolution signs were accompanied by a hyperchromic effect at λ_{415} and λ_{525} wavelength, a significant lightening and an increase in the color saturation of muscle tissue extracts were observed too. During this period, relaxation of the muscle fibers was observed, although some fibers were found in a contracted state (Figure 2b). Most of the nuclei were elongated and well colored. Restoration of transverse striation was found. Longitudinal striation was clearly visible. In some places, in the nodes of contraction, ruptures of the sarcolemma of muscle fibers were found with the preservation of the fiber contents and its internal structure. The diameter of muscle fibers decreased by 18.7% and amounted to 65±15 μm. The fibers were arranged evenly, adjoining to each other pretty tightly.

On the third day of meat storage, signs of resolution of rigor mortis were clearly expressed, which indicated the beginning of the stage of meat maturation (refer to the Figure 3a). Histological tissue changes were characterized by development of destructive processes in the meat. The onset of fiber fragmentation and loosening of connective tissue fibrous elements with their detachment from



a b

Figure 2. Microstructure of meat on the first (a) and second (b) days of storage (vol. 40×)

muscle fibers was observed. No signs of the muscle fiber contracture were found. The fiber diameter was 40 ± 10 μm. In cross-sections an enlargement of interfiber space and increasing of microcracks number were noticeable. The transverse and longitudinal striations were clearly visible. In some cases, the disintegration of the sarcolemma and its granular disintegration were found. In some places, microflora foci in the form of separate diffuse overlays were detected. Condensation and margination of chromatin were observed in the nuclei; chromatin was built up under the nuclear membrane in the shape of small lumps.

On the fourth day of storage the destructive changes in muscle tissue were more pronounced than histological changes on the third day of pork storage. Muscle fibers were arranged loosely and unevenly colored. Localized lysis was observed in some places. The number of transverse slit-like ruptures of the muscle fibers integrity increased along with partial preservation of the structure of the nuclei, of transverse and longitudinal striation in fragments. Homogeneous nuclei with signs of karyolysis were found in places (Figure 3b). Due to the disintegration of chromatin, the nuclei acquired a shadowy color of varying intensity. The transverse striation was less pronounced. The fibers slightly increased in diameter up to 55 ± 15 μm. In the structure of the fibers, granular and granular inclusions were locally detected. The sections revealed the presence of coccal and rod-shaped microflora in the shape of foci and diffuse overlays. The staining of most sections was basophilic.

Morphological changes in muscle tissue during its storage were accompanied by a change in colorimetric characteristics. With the resolution of rigor mortis, a slight hypsochromic shift and hypochromic effect of some peaks, a decrease in the number of absorption bands, and discoloration of aqueous extracts, which was expressed as a decrease in redness a* and yellowness b*, were observed (Table 1).

The data obtained indirectly pointed to the proteolytic destruction of protein structures, as a result of which muscle fibers got loosened and fragmented, and muscle proteins extracted into the solution shifted the color coordinates. It is known [29] that during autolysis meat proteins undergo a line of changes, like aggregation and partial decomposition, which was confirmed by the results of studies of the proteins fractional composition.

Identification of the protein composition of raw materials is a very important aspect, since it allows you to directly determine the qualitative composition of the finished product. The use of the electrophoretic method at the initial stage of research makes it possible to evaluate the quantitative and qualitative distribution of structural and tissue-specific protein molecules; evaluate the influence of autolytic processes, etc.

As a result of the analysis of 1D electropherograms of meat proteins (Figure 4), differences in the fractional composition of proteins and their concentration, determined by the color intensity of the protein zones, were found.

On the 1D electrophoregram (Figure 4), the areas were marked in yellow where changes in the protein structures and their relative content during storage were assessed by changing the color staining intensity of the protein zones.

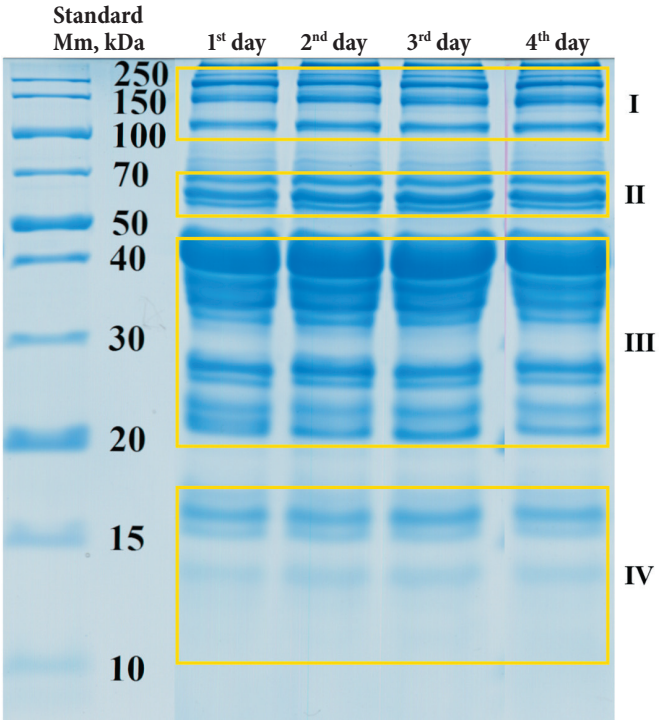
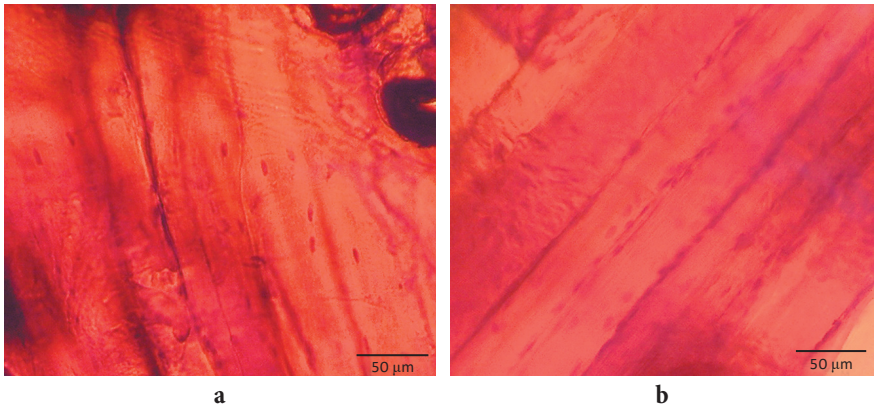


Figure 4. Electrophoregram of *Sus scrofa M. longissimus dorsi* proteins during their storage



a **b**

Figure 3. Microstructure of meat on the third (a) and fourth (b) days of storage (vol. 40×)

The proteins of the identified zones had a different origin according to the database [30], and were identified as connective tissue, myofibrillar proteins and metabolic proteins.

As can be seen from the Figure 5 above, the relative content of protein fractions with a molecular weight in the area of 110–291 kDa (area I on the electropherogram, Figure 4), 51–67 kDa (area II on the electropherogram, Figure 4), 20–42 kDa (area III on electrophoregram, Figure 4) and 10–18 kDa (area IV on the electrophoregram, Figure 4) changed in different degree, depending on the duration of meat storage.

It was revealed that in the process of rigor mortis, evidenced by the results of histological studies (Figure 2), on the first day of storage the relative content of high-molecular fractions I and II of proteins increased along with the decrease in the average molecular fraction III (Figure 5).

During the further storage, the amount of macromolecular structures of fraction I increased from 13.26% to 15.27%. In fraction III, an inverse dependence was observed. It was noted that during the storage of meat, the amount of low molecular weight proteins increased, as it was confirmed by changes in the intensity of the protein zones of the fraction with a molecular weight within the area of 10–18 kDa (region IV on the electropherogram, Figure 4).

These changes indicated the processes of autolysis that occurred in the meat, as a result of which changes in various protein structures occurred in parallel, such as aggregation and partial decay. Autolytic changes in the meat were confirmed by the results of histological studies.

Based on changes in the color intensity of protein bands with a molecular mass of 239–248 kDa and 205–213 kDa, it is possible to come to assumption about changes in the myosin fractions, which does not contradict to the researches [31, 32]. Thus, as a result of autolytic processes, a decrease in the relative amount of the myosin fraction by 13.9% was observed on the second day of storage. This could be caused by the development of rigor mortis and the formation of complexes between F-actin and myosin that characterizes it. The subsequent increase in the relative content of protein fractions II and IV up to the fourth day of storage clearly evidenced the destruction of actomyosin molecules and myosin aggregates down to their heavy chains (200–223 kDa) and light chains (16–20 kDa).

It was noted that the change in the relative content of various protein fractions was accompanied by an increase in redness a*, yellowness b*, in purity, color saturation and extinction coefficient at the isosbestic point of various redox forms of myoglobin by almost 2 times.

As the relative content of fraction II increased on the third day of storage (10.92%) at the same moment the number of absorption bands in aqueous extracts of muscle tissue dropped down from 17 to 8.

An increase in the color intensity of the protein zones with a molecular weight of 52–67 kDa and concomitant changes in the color intensity of the protein zones of the

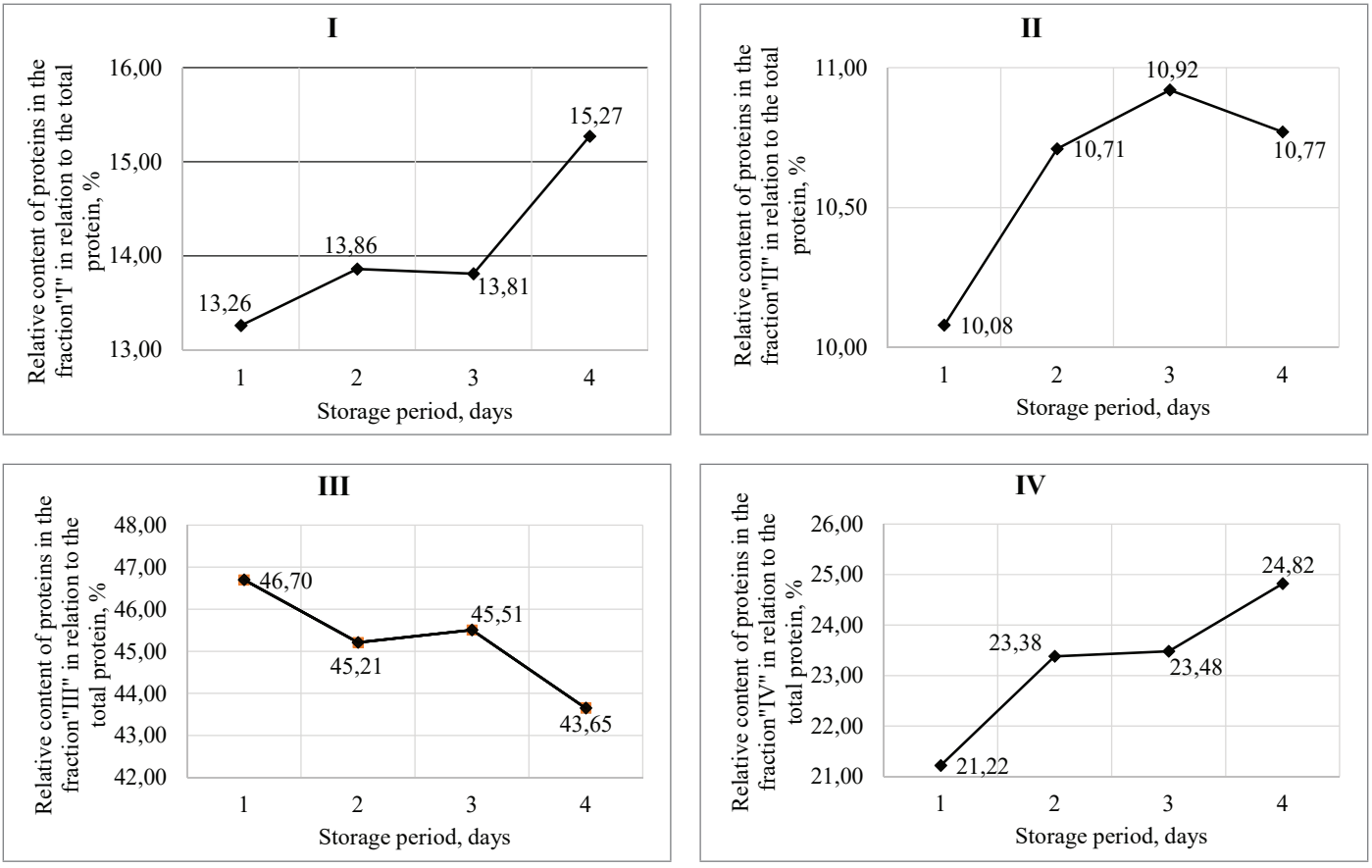


Figure 5. Change in the relative content of proteins in the various fractions:
I — 110–291 kDa, II — 51–67 kDa, III — 20–42 kDa, IV — 10–18 kDa

fraction with a molecular weight in the area of 27–33 kDa could indicate changes with desmin (53 kDa), vimentin (54 kDa), glucose-6-phosphate isomerase (63 kDa) and tropomyosin (33–28 kDa). The change in color intensity of zones with a molecular weight within 30–33 kDa and 17–19 kDa evidence the troponin changes.

The dynamics of changes in fractions III and IV (Figure 5), which is represented by troponins and cytochromes, had a strong correlation with the change in the extinction coefficient of the muscle tissue extract at λ410–415 (R = 0.94).

Softening and loosening of meat during the beginning of the ripening stage on the third day is caused by a weakening of the structure of myofibrils due to the degradation of desmin (50–53 kDa), and intramuscular connective tissue under the action of calcium ions [25].

Upon reaching the maximum value (10.92%) on the third day, the mass content of proteins with a molecular weight of 51–67 kDa (Figure 5) started dropping down till 10.77% on the fourth day, which coincides with the onset of the resolution of rigor mortis according to data of histological analysis (Figure 3).

As can be seen from the data presented above, during storage the high-molecular protein substances decomposed partially or completely and formed the medium-molecular and low-molecular fractions.

The longer the meat was stored, the more protein substances went into solution during water extraction. During intermolecular interaction in an aqueous solution, fractions of various proteins formed aggregated particles, which were subsequently removed during filtration, which became the main reason for the clarification of aqueous extracts of muscle tissue.

As a result of the analysis of the histological structure of meat and the fractional composition of proteins, an average and strong negative and positive correlation (R = minus 0.98 … 0.86) was found between the color intensity of protein zones with a molecular weight of 159–167 kDa, 94–97 kDa, kDa, 52–65 kDa, low molecular weight fractions of the area III and IV (Figure 5) and muscle fiber diameter. This dependence indicates that changes of muscle tissue structure are strongly influenced by changes in myofibrillar proteins, primarily myosin, troponin, desmin, calpastatin, as well as pyruvate kinase and cathepsins [31,33].

The dynamics of changes in color purity, degree of saturation, color shade, as well as the values of a* and b* possess a strong correlation with changes in the relative content of protein fractions with a molecular weight within the area of 205–213 kDa, 82–85 kDa, 56–58 kDa, 40–42 kDa and below 27 kDa, which could evidence the involvement of fractions of endoenzymes, cytochromes, myoglobin, and troponin in color transitions, which is consistent with the results of studies [34]. Thus, a change in the colorimetric characteristics of a* and b* could indicate the transformation of chromoproteids and some other proteins, which is confirmed by the results of densitometric analysis of proteins.

When storing the chilled meat, a correlation was found between lightness (L*) on the one hand, and histostructural changes and changes in fractional composition of proteins on the other hand (R = –0.81 and 0.98, respectively). It was found that a change in the relative content of proteins with a molecular weight of 205–213 kDa, 82–85 kDa, 56–58 kDa, 40–42 kDa and below 27 kDa led to a change of lightness (L*) (Table 1). This dependence evidenced to participation of other proteins in color formation, except for myoglobin. Thus, the colorimetric characteristics of aqueous extracts from muscle tissue were affected by a row of factors.

When running the experiment, it was assumed that the myoglobin protein can act as a kind of "indicator", as its color intensity can depend on pH value. In order to confirm this assumption, aqueous extracts of meat and myoglobin solutions were prepared. pH of these solutions was changed and the color difference was observed. The pH ranged from 3 to 9 units.

As a result of the experiment, it was found that when the pH value of aqueous extracts of meat and myoglobin solutions changes from 5 units up to 7 units the value of ΔE_{2000} did not exceed 0.2 units. The color difference increased only when the pH was decreased down to less than 3 and when it was increased up to more than 9 units, which, possibly, occurred as a result of conformational changes in protein molecules. The obtained results prove that changes in the optical properties of myoglobin solutions and aqueous meat extracts are affected by conformational changes in proteins rather than by the pH value.

The obtained data do not contradict to [11,32]. So the variation of the pH value is accompanied by a change in the scattering of light within the structure of the muscle fiber. The increase in light dispersion explains why the surface of the meat looks paler. Thus, the meat surface looks pale in muscle with a low pH value (pH 5.4–5.7) and looks dark in a muscle with a high pH (more than 5.8 units).

Thus, the pH value provides only an indirect effect on the meat color and extracts obtained from it. The obtained results confirm that not only chromoproteids, but also myofibrillar proteins are involved into meat color formation.

Changes in the native structure of proteins during storage affect the shielding of biomolecules, which, in its turn, affects the color of meat. Thus, the analysis of the color of meat extracts allows full evaluation of its quality characteristics and better understanding the biochemical processes.

Conclusion

In addition to the classical methods of meat quality analysis, the spectrophotometric analysis of aqueous extracts of muscle tissue is quite promising and reliable method, since the changes of optical characteristics in raw meat evidence the biochemical processes in meat. Analysis

of the absorption spectra showed that the extinction coefficients and the relative area of the peaks at λ_{415}, λ_{525}, λ_{542}, λ_{555}, and λ_{582} significantly correlated ($R > 0.8$) with histological and structural changes and the relative content of proteins like troponins, cytochromes, endoenzymes, and myoglobin.

It was determined that during the storage of raw meat, changes in optical characteristics take place along with the typical changes in the composition of proteins components and histological structure of tissues. The effect of autolytic changes on the colorimetric characteristics of meat was confirmed.

In accordance with the recommendations of the International Commission on Lighting CIE, the values of true color for chilled meat were determined in dependence to its shelf life.

The possibility of applying the method of optical spectrometry of meat to determine the quality of meat is evaluated. The data obtained by processing the absorption spectra of aqueous extracts from muscle tissue prove the promising prospects of using this method in a comprehensive research of properties of raw meat materials. It is shown that, depending on the stage of meat autolysis, characteristic changes in the absorption spectra and color of meat take place.

REFERENCES

1. Tomasevic, I.B. (2018). Computer vision system for color measurements of meat and meat products: A review. *Theory and Practice of Meat Processing*, 4(4), 4–15. https://doi.org/10.21323/2414–438X 2018–3–4–4–15 (In Russian)
2. Milovanovic, B.R., Djekic, I.V., Tomović, V.M., Vujadinović, D., Tomasevic, I.B. (2021). Color measurement of animal source foods. *Theory and Practice of Meat Processing*, 6(4), 311–319. https://doi.org/10.21323/2414–438X-2021–6–4–311–319
3. Jankowiak, H., Cebulska, A., Bocian, M. (2021). The relationship between acidification (pH) and meat quality traits of polish white breed pigs. *European Food Research and Technology*, 247, 2813–2820. https://doi.org/10.1007/s00217–021–03837–4
4. Pujol, A., Ospina-E, J. C., Alvarez, H., Muñoz, D. A. (2023). Myoglobin content and oxidative status to understand meat products' color: Phenomenological based model. *Journal of Food Engineering*, 348, Article 11439. https://doi.org/10.1016/j.jfoodeng.2023.111439
5. Suman, S.P., Poulson, P. (2014). Chemical and physical characteristics of meat color and pigment. Chapter in a book: Encyclopedia of Meat Sciences. Academic Press, 2014. https://doi.org/10.1016/B978–0–12–384731–7.00084–2
6. Suman, S. P., Joseph, P. (2013). Myoglobin chemistry and meat color. *Annual Review of Food Science and Technology*, 4, 79–99. https://doi.org/10.1146/annurev-food-030212–182623
7. Murashev, S.V., Bolshakova, O.S. (2014). Effect of the metal-ligand interaction in the heme group on the colour of myoglobin forms. *Processes and Food Production Equipment,* 3, 152–163. (In Russian)
8. Hunt, M. C. King, David. (2012). AMSA meat color measurement guidelines. American Meat Science Association, Champaign, Illinois, USA, 2012.
9. Nechiporenko, A.P., Orehova, S.M., Plotnikova, L.V., Plotnikov, P.P. (2019). Diffuse-reflection electron spectroscopy in the study of muscle tissue of wild and domestic animals. *Proceedings of Universities. Applied Chemistry and Biotechnology*, 9(3), 489–499. https://doi.org/10.21285/2227–2925–2019–9–3–489–499 (In Russian)
10. Murashov, S.V., Vorobyov, S.A., Zhemchuzhnikov, M.E. (2010). Physical and chemical reasons of meat red color appearance. *Processes and Food Production Equipment,* 1, 61–68. (In Russian)
11. Purslow, P. P., Warner, R. D., Clarke, F. M., Hughes, J. M. (2020). Variations in meat colour due to factors other than myoglobin chemistry; a synthesis of recent findings (invited review). *Meat Science,* 159, Article 107941. https://doi.org/10.1016/j.meatsci.2019.107941
12. Elmasry, G., Barbin, D.F., Sun, D.-W., Allen, P. (2012). Meat quality evaluation by hyperspectral imaging technique: An overview. *Critical Reviews in Food Science and Nutrition,* 52(8), 689–711, https://doi.org/10.1080/10408398.2010.507908.
13. Swatland, H. J. (June 17–20, 2012). *Optical properties of meat.* Proceedings of the 65th Reciprocal Meat Conference of the American Meat Science Association., Fargo, North Dakota, USA.
14. Bekhit, A. E.-D., Morton, J.D., Bhat, Z. F., Zequan, X. (2019). Meat colour: Chemistry and measurement systems. Chapter in a book: Reference Module in Food Science. Academic press, 2019. https://doi.org/10.1016/B978–0–08–100596–5.22419–0
15. Chernousova, O.V., Rudakov, O.B. (2019). Digital images in analytical chemistry for quantitative and qualitative analysis. *Chemistry, Physics and Mechanics of Materials,* 2(21), 55–125. (In Russian)
16. Tobijaszewska, B., Mills, R., Jøns, J. (2018). Using spectrometry for simultaneous measurement of colour and composition in food samples. Retrieved from https://www.fossanalytics.com/en/landingpages/global/dairy/foodscan-2-dairy Accessed March 25, 2023
17. Palchikova, I.G., Alejnikov, A.F., Smirnov, E.S., Chugui, Yu.V., Shvydkov, A.N., Nitsievskaya, K.N. et al. (2019). Portable color analyzer of qualitative changes of poultry meat. *Achievements of Science and Technology in Agro-Industrial Complex,* 29(9), 80–83. (In Russian)
18. Tsyupko, T.G., Gunkin, I.N., Temerdashev, Z.A. (2010). Spectroscopic and electrophoretic research of changes of qualitative-composition cognac production in the process of ageing. *Izvestiya VUZOV. Food Technology,* 5–6, 25–28. (In Russian)
19. Tolokontseva, E.O., Polovetsraya, O.S., Nikishina, M.B., Ivanova, E.V. (2018). Determination of the color parameters of cognac products. *Theory. Practice. Innovation*, 12(36), 247–251. (In Russian)
20. Pochitskaya, I.M. (2022). Scientific and practical basis for the development of a system of integrated assessment of food quality. Author's abstract of the dissertation for the scientific degree of Doctor of Technical Sciences. Krasnodar: Kuban State Technological University, 2022. (In Russian)
21. Janisch, S., Krischek, C., Wicke, M. (2011). Color values and other meat quality characteristics of breast muscles collected from 3 broiler genetic lines slaughtered at 2 ages. *Poultry Science*, 90(8), 1774–1781. https://doi.org/10.3382/ps.2010–01073
22. 22. Kolodyaznaya, V.S., Broyko, Y.V. (2015). Effect of cold treatment on structural changes of muscle tissue veal. *Processes and Food Production Equipment*, 3, 51–57. (In Russian)
23. Holman, B. W. B., Kerr, M. J., Morris, S., Hopkins, D. L. (2019). The identification of dark cutting beef carcasses in Australia, using Nix Pro Color Sensor™ colour measures, and their relationship to bolar blade, striploin and topside quality traits. *Meat Science*, 148, 50–54. https://doi.org/10.1016/j.meatsci.2018.10.002
24. CIE2018. CIE015:2018. Colorimetry. The International Commission on Illumination, Vienna, Austria, 2019.
25. Ding, Z., Wei, Q., Liu, C., Zhang, H., Huang, F. (2022). The quality changes and proteomic analysis of cattle muscle postmortem during rigor mortis. *Foods*, 11(2), Article 217. https://doi.org/10.3390/foods11020217
26. Millman, B. M. (1998). The filament lattice of striated muscle. *Physiological Reviews*, 78(2), 359–391. https://doi.org/10.1152/physrev.1998.78.2.359
27. Denaturation of nucleic acids, molecular hybridization Retrieved from https://www.wikilectures.eu. Accessed March 28, 2023
28. Bağdatli, A., Kayaardi, S. (2015). Influence of storage period and packaging methods on quality attributes of fresh beef steaks. *CyTA-Journal of Food,* 13(1), 124–133. https://doi.org/10.1080/19476337.2014.919029
29. Chernukha, I.M., Akhremko, A.G. (2018). Application of proteomic tools: the autolytic changes of pork muscular tissue. *Theory and Practice of Meat Processing,* 3(4), 32–37. https://doi.org/10.21323/2414–438X-2018–3–4–32–37 (In Russian)
30. Universal Protein Resource (UniProt) Retrieved from https://www.uniprot.org. Accessed March 20, 2023

31. Lisitsyn, A.B., Ivankin, A.N., Vostrikova, N.L., Stanovova, I.A. (2014). Study of the fractional composition of meat proteins during prolonged cold storage. *Vsyo o Myase*, 2, 236–40. (In Russian)
32. Warner R. (2016). Meat: Conversion of muscle into meat. Chapter in a book: Encyclopedia of Food and Health. Academic Press, 2016. https://doi.org/10.1016/B978–0–12–384947–2.00452–9
33. Lametsch, R., Roepstorff, P., Bendixen, E. (2002). Identification of protein degradation during post-mortem storage of pig meat. *Journal of Agricultural and Food Chemistry*, 50(20), 5508–5512. https://doi.org/10.1021/jf025555n

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EVALUATING THE EFFECT OF VARIOUS TYPES OF DISINFECTANTS ON BACTERIAL BIOFILMS

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Keywords: *biofilms, pathogens, disinfectants*

Abstract
Biofilm formation on equipment surfaces is a potential food safety hazard, providing increased resistance and persistence of pathogens and spoilage microorganisms in food production environments. The issue of preventing the biofilm formation is extremely important, since a wide range of disinfectants does not always provide the proper effect. The article discusses the antimicrobial effectiveness of disinfectants with various active ingredients (based on active chlorine, peracetic acid and quaternary ammonium compounds (QAC) with enzymatic substances) on binary biofilms. The objects of the study were the strains of pathogenic and opportunistic microorganisms isolated from abiotic surfaces of food production environments and food products. Different effects of disinfectants on biofilms formed by bacteria have been established. Disinfectant based on peracetic acid and chlorine had the greatest effect on binary biofilms of Brochothrix thermosphacta/Salmonella spp. and Staphylococcus equorum/Salmonella spp. The greatest antimicrobial effect on biofilm of Listeria monocytogenes 12/Pseudomonas azotoformans 6 was shown by a chlorine-based disinfectant. Disinfectants based on chlorine and QAC with enzymatic substances were most effective against the binary biofilm of L. monocytogenes 12/Salmonella spp. 14. However, none of the disinfectants had absolute antimicrobial effectiveness against the studied binary biofilms. Biofilm-forming microorganisms have shown resistance to the recommended concentrations of disinfectants. Therefore, currently, it is extremely important to revise approaches to hygiene at enterprises by finding working concentrations of new antimicrobial agents and new procedure that are effective for destroying biofilms.

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Introduction

The most dangerous phenomenon in the food industry is the ability of microorganisms to form biofilms on abiotic surfaces. The main and auxiliary equipment at food enterprises have an abiotic surface characterized by roughness, porosity, presence of joints, seams and other hardly accessible areas [1–3]. Such structural features of the equipment are a favorable environment for the development and attachment of biofilms.

The phenomenon of biofilm formation was discovered in the mid-1980s [4,5]. Over the next years, biofilm research proved that biofilm formation is inherent in a large number of pathogenic and opportunistic microorganisms [6, 7]. Some researchers consider biofilm formation as a pathogenicity factor [8].

Biofilm is a population of surface-associated microbial cells enclosed in polymeric extracellular matrix. According to literature, many pathogenic bacteria are associated with biofilms and in some cases actually grow in them, including *Legionella pneumophila* [9], *Staphylococcus aureus* [10], *Listeria monocytogenes* [11], *Campylobacter* spp. [12], *Escherichia coli* O157: H7 [13], *Salmonella typhimurium* [14], *Vibrio cholera* [15] и *Helicobacter pylori* [16].

Compared to planktonic cells, biofilm-associated cells are much more resistant to antimicrobials, including disinfectants. This increased resistance has a significant impact on the quality of hygienic measures at food enterprises.

Effective disinfection is necessary at food enterprises, since wet surfaces of objects in the production environment create favorable conditions for the growth of microorganisms [17,18,19]. Modern disinfectants used in the food industry include oxidizing agents such as hypochlorite, hydrogen peroxide, and peracetic acid; denaturing agents, for example alcohol-based products; non-oxidizing agents and agents that reduce interfacial tension; and enzyme-based compounds [20,21]. Disinfectants must be effective, safe, rinseable, and easy-to-use [20].

The resistance of biofilm-associated cells to disinfectants is explained by many factors, often acting simultaneously, which include the presence of extracellular polymers that interfere with diffusion/reaction and differences in physiological status depending on the biofilm layer [22,23].

There is also growing evidence that interspecies interactions within the biofilm matrix further enhance resistance to disinfectants compared to single-strain biofilms [24–27].

The permeability of the matrix may be reduced by various factors such as changes in the microenvironment, cell density and biofilm age. The last two factors are highly correlated and difficult to separate as the biofilm matrix becomes thicker and denser with age and the number of colony-forming units (CFU) increases. Despite this, biofilm age has been shown to play a more important role than cell density [28] in relation to increased tolerance to disinfectants.

The limited time for penetration of disinfectants into biofilm during hygienic procedures at food enterprises may result in low levels of antimicrobial agent exposure in the deeper layers of the biofilm. Consequently, microorganisms in the biofilm will develop adaptive responses to sublethal concentrations of disinfectants. Surface-associated bacteria are more difficult to kill than planktonic cells, so biofilm contamination of production environments increases microbial load and potentially reduces food safety and quality.

The complex nature of biofilm structure and the ability of biofilm-associated cells to be firmly attached to hardly accessible surfaces make the antimicrobial activity of currently used disinfectants less effective [29,30]. For all these reasons, it is necessary to consider whether the modern cleaning and disinfection procedures currently used in the food industry are effective, or whether new methodologies and strategies are needed to solve the problem.

The aim of this study was to determine the effectiveness of disinfectants used in the food industry for destructing the binary biofilms of pathogenic and opportunistic microorganisms.

Objects and methods

To determine the antimicrobial effectiveness of various disinfectants against formed biofilms, the following microorganisms were selected: *Pseudomonas azotoformans 6, Salmonella* spp. *14, Listeria monocytogenes 12* isolated from the environment of food enterprise, *Brochothrix thermosphacta 2726* and *Staphylococcus equorum 2736* isolated from pig carcass wipe samples, *Salmonella* spp. *38* isolated from a food product. Binary biofilms were formed from microorganisms: *Brochothrix thermosphacta 2726/Salmonella 38, Staphylococcus equorum 2736/Salmonella* spp**.** *38, L. monocytogenes 12/P. azotoformans 6, L. monocytogenes 12/Salmonella* spp. *14*.

The following substances were used as disinfectants:

— Disinfectant No. 1 for decontamination of equipment and premises at meat industry enterprises. Ingredients: tertiary amines (N, N-bis(3-aminopropyl) dodecyl amine 3 ± 0.5%), enzymes (carbohydrase 4 ± 1%, enzyme complex 4 ± 1%), quaternary ammonium compounds (QAC) (benzalkonium chloride 8 ± 0.6%, dodecyl dimethylammonium chloride 6 ± 0.4%). For the study, a working solution of the agent with a concentration of 0.085% was prepared.
— Disinfectant No. 2 for decontamination of process equipment and production facilities at meat industry enterprises. Ingredients: sodium salt of dichloroisocyanuric acid, as well as functional components that contribute to better dissolution of the agent. When dissolved in water, 2.7 grams of the product releases 1.5 grams of active chlorine. For the study, a working solution of the agent with an active chlorine concentration of 0.015% was prepared.
— Disinfectant No. 3 for decontamination of process equipment and production facilities at meat industry enterprises. Ingredients: peracetic acid (15.5–17.0%), hydrogen peroxide (15.8–18.0%), acetic acid, functional additives. For the study, a working solution of the agent with peracetic acid concentration of 0.05% was prepared.

Biofilm formation

Biofilms were formed at the solid surface/air interface. Biofilms of this type were obtained using glass fiber filters as substrates, which are an easily dispersed material, according to the method described earlier (Plakunov et al., 2016) [31]. Glass fiber filters (Whatman GF/F, UK) were cut into 15x15 mm squares and sterilized by autoclaving (20 min, 120 °C), then laid out on the surface of LB agar medium (Becton Dickinson, USA) in plates.

Bacterial cultures were separately grown in LB broth until stationary phase. Turbidity was prepared in pure 0.5 LB broth according to McFarland using DEN-1B McFarland Densitometer (Biosan, Latvia). Next, 40 µL of the obtained binary bacterial culture were applied in triplicate onto prepared sterile glass fiber filters in sterile plates with PCA agar medium. Cultures were grown in a thermostat for 48 h at 30 °C.

Effect of disinfectants on biofilms

After 48 hours of biofilm growth, they were treated with disinfectants. Solutions of disinfectants in sterile water were prepared immediately before being applied to the filters. Biofilms were removed from the surface of the growth medium, transferred to sterile plates, each was treated with disinfectant solutions in the amount of 100 µL, until the filter was completely wetted. The exposure time of disinfectants was 10 minutes. As a positive control, instead of disinfectant, sterile water in the amount of 100 µL was added to the surface of the medium with a formed biofilm.

The glass fiber filter was then placed in a flask with sterile saline. A sterile glass mortar and beads were used to homogenize the glass fiber filter. The resulting content of the flask was considered a first dilution. Aliquots of the obtained homogenates (100 µL) were diluted in 900 µL of sterile saline and a series of decimal dilutions was prepared. Then, homogenates were incubated in a thermostat at 30 °C for 24 h, followed by counting the colonies on the

plates. In each dilution, a number of viable cells (CFU/cm³) was determined by the microscopy method, after which the CFU titer in the primary filter homogenate was calculated. The experiments were performed in three independent biological replicates.

Statistical analysis

Statistical data processing was carried out using the Statactical software ver. 10.0.1011 (StatSoft). The results were calculated as "mean ± standard error". Differences with p-values of ≤ 0.05 were considered statistically significant.

Results and discussion

In their natural environment, biofilms are complex populations of different types of microorganisms, rather than single-species biostructures. Multispecies biofilms in their structure are more resistant to environmental conditions, including the action of disinfectants. A quantitative assessment of the antimicrobial effect of modern disinfectants on binary (multispecies) biofilms was carried out.

Based on information about the frequent detection of *Brochothrix thermosphacta* in various food products and objects at the production environment [32,33] and its genomic heterogeneity in terms of the presence of a gene potentially involved in the formation of a biofilm matrix [34], the effect of various disinfectants on binary biofilm formed by *Brochothrix thermosphacta* and pathogenic *Salmonella* spp. was studied. The antimicrobial effectiveness of disinfectant working solutions on biofilm is shown in Figure 1. Analysis of the obtained data shows the different effects of disinfectants on the biofilm formed by two types of microorganisms.

At the concentrations studied, disinfectant No. 2 showed an antimicrobial effect in contrast with the other two disinfectants. Disinfectant No. 1 based on QAC with enzymatic substances was the most ineffective against biofilm. Absolute antimicrobial effectiveness against biofilm was not observed with any of the disinfectants.

A different pattern regarding the effectiveness of disinfectants was observed with biofilm of *Staphylococcus equorum/Salmonella* spp. (Figure 2). Disinfectant No. 3 showed

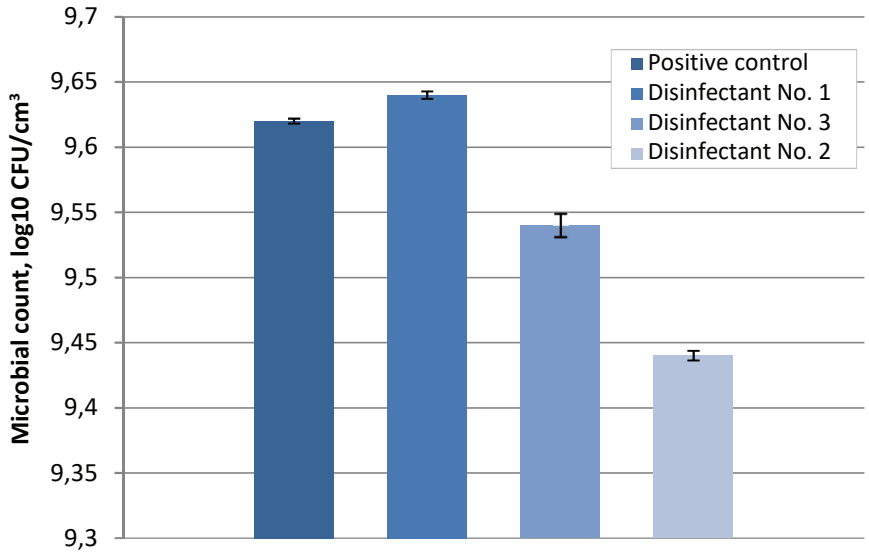

Figure 1. Results of disinfectants' effect on the binary biofilm of *Brochothrix thermosphacta/Salmonella* spp.

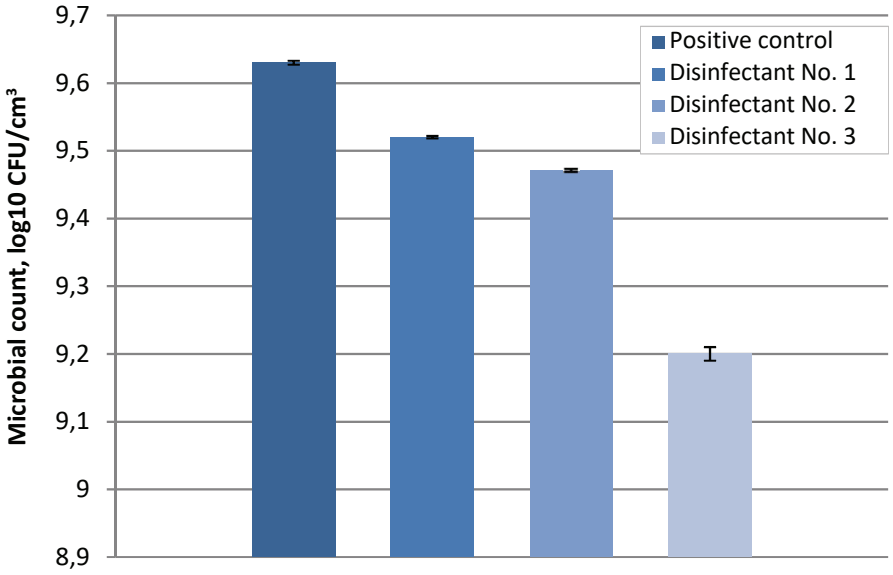

Figure 2. Results of disinfectants' effect on the binary biofilm of *Staphylococcus equorum/Salmonella* spp.

the highest antimicrobial activity against it. No significant differences in the effects of disinfectants No. 1 and No. 2 on the binary biofilm of *Staphylococcus equorum/Salmonella* spp. were established.

At the concentrations studied, disinfectants have shown to be ineffective against binary biofilms. According to the literature, biofilms composed of many types of bacteria may be more resistant to antibacterial agents, including antibiotics and disinfectants [35,36].

The persistence of *Salmonella* spp. in biofilm raises food safety concerns. *Salmonella* spp. are one of the main causes of foodborne infectious diseases [37]. Bacteria may enter food enterprises and spread through raw ingredients, dirty packaging, equipment, workers' hands and clothes. The ability to form biofilms only increases the rate and area of pathogen spread. The control and prevention of *Salmonella* spread in food production facilities depends on the correct implementation of comprehensive hygienic measures. However, the results obtained may indicate the need to revise the approaches to decontamination at enterprises.

Multispecies biofilms may include in their consortium not only pathogens, but also spoilage microorganisms such as *Brochothrix thermosphacta*. *Brochothrix thermosphacta* is one of the main spoilage microorganisms in meat products. *B. thermosphacta* has recently been identified in 80% of biofilms sampled at a meat processing facility, including both food contacting and non-contacting surfaces [32].

An equally significant pathogen is *L. monocytogenes*. The control of this pathogen has become one of the main goals in the food industry [38]. Biofilms of *L. monocytogenes* on food contacting surfaces have been identified as an important pathway for pathogen persistence and subsequent product contamination [39–41]. The highest antimicrobial effect on the biofilm of *L. monocytogenes 12/P. azotoformans 6* was showed by disinfectants No. 1 and No. 2, where microbial count after exposure decreased by 3.45 log and 3.15 log, respectively (Table 1).

Disinfectants No. 1 and No. 2 showed the best antimicrobial effect on cell combination in the biofilm of *L. monocytogenes 12*/*Salmonella* spp. *14*, where microbial count after exposure decreased by 2.14 log and 2.33 log, respectively. Disinfectant No. 3 based on peracetic acid showed the worst antimicrobial properties against the studied binary biofilms of *L. monocytogenes 12/P. azotoformans 6* and *L. monocytogenes 12*/*Salmonella* spp. *14*.

The results obtained showed that microbial counts in multispecies biofilms reduced when exposed to disinfectants, but these values were not significant, and absolute antimicrobial effectiveness was not observed. The results show that the use of disinfectants studied in this work is not always effective in elimination of the bacterial biofilms from the surfaces of food production environments. Definitely, after treatment with disinfectants in the studied concentrations, microorganisms remain on the surface.

The resistance of the formed biofilms to disinfectants based on active chlorine and peracetic acid was directly reflected in this study. This finding is of concern because the concentrations used in the experiment are commonly used to disinfect food equipment, especially in meat industry.

The ability to form biofilms is inherent not only in opportunistic microorganisms, but also in pathogenic ones. Recent data obtained by other scientists have shown that the formed biofilm of foodborne pathogens is highly resistant to sodium hypochlorite and peracetic acid [42]. In this study, binary biofilms of pathogenic *Salmonella* spp. and *L. monocytogenes*, as well as opportunistic bacteria, showed resistance to the working concentrations of solutions recommended for decontamination and used for disinfection at food enterprises. In the work by Byun et al. [43], chlorine-based disinfectants (NaOCl and ClO_2) were used to reduce the counts of planktonic cells and biofilms of *S. enteritidis*. As a result, it was shown that both preparations are effective as disinfectants when applied against planktonic cells at a dose of more than 100 μg/mL for 1 min, while biofilms were destroyed only when ClO_2 was applied at the same concentration for 5 minutes. In general, ClO_2 effectively reduced the counts of planktonic cells and biofilms of *S. enteritidis* compared to NaOCl under the same conditions. However, the presence of organic substances significantly reduced the effectiveness of the studied disinfectants [43]. The results obtained indicate that various disinfectants based on active chlorine, but with different active substances, may have different antimicrobial effects.

Disinfectant No. 1 based on QAC and containing additional enzymes for the destruction of biofilm matrix was effective against biofilms of *L. monocytogenes 12/P. azotoformans 6* and *L. monocytogenes 12/Salmonella* spp. *14*. As an environmentally friendly alternative for industrial surface cleaning, disinfectants with the addition of enzymatic agents have proven to be an effective tool against biofilms in the food industry [44]. It becomes apparent that biofilms must be destroyed by one of the accepted methods before decontamination.

The need to revise the approaches to decontamination at food enterprises arises. The development and evaluation of approaches to biofilm destruction on various objects at food enterprises are carried out all over the world.

Table 1. Results of disinfectants' effect on binary biofilms

Microbial composition of the biofilm	Disinfectant No. 1	Disinfectant No. 2	Disinfectant No. 3	Control
	Microbial count, log10 CFU/cm^3			
L. monocytogenes 12/P. azotoformans 6	**6.32 ± 0.08**	**6.62 ± 0.12**	**8.88 ± 0.08**	**9.77 ± 0.09**
L. monocytogenes 12/Salmonella* spp. *14	**6.48 ± 0.08**	**6.29 ± 0.11**	**7.71 ± 0.07**	**8.62 ± 0.08**

Conclusion

The present study examined the antimicrobial effectiveness of three disinfectants with different active ingredients used for decontamination at food enterprises against binary biofilms of pathogenic bacteria and spoilage microorganisms. The results of the study showed that the biofilm-associated microorganisms were resistant to the recommended concentrations of disinfectants used at food enterprises. It is evident that the objects in the production environment may act as containers for disinfectant-resistant bacteria. The search for fundamentally new methods of resistant bacteria elimination, including their biofilms, and the revision of approaches to decontamination at food enterprises are becoming increasingly important. The results of this study confirm the need to change approaches to ensuring microbiological safety at food industry enterprises.

REFERENCES

1. Tomaras, A. P., Dorsey, C.W., Edelmann, R.E., Actis, L.A. (2003). Attachment to and biofilm formation on abiotic surfaces by Acinetobacter baumannii: Involvement of a novel chaperone-usher pili assembly system. *Microbiology,* 149(12), 3473–3484. https://doi.org/10.1099/mic.0.26541–0
2. Huhu, W., Xinxiao, Z., Qiuqin, Z., Keping, Y.X., Zhou, X.G. (2015). Comparison of microbial transfer rates from Salmonella spp. biofilm growth on stainless steel to selected processed and raw meat. *Food Control,* 50, 574–580. https://doi.org/10.1016/j.foodcont.2014.09.049
3. Kravchenyuk, Kh. Yu., Kukhtin, M.D., Lazaryuk, V.V. (2016). E. coli biofilm formation on the stainless steel aisi 321 surface in terms of surface roughness. *Visnyk of Kherson National Technical University,* 1(56), 95–100. (In Russian)
4. Characklis, W.G., Cooksey, K.E. (1983). Biofilms and microbial fouling. *Advances in Applied Microbiology,* 29, 93–137. https://doi.org/10.1016/S0065–2164(08)70355–1
5. Marshall, P.A., Loeb, G.I., Cowan, M.M., Fletcher, M. (1989). Response of microbial adhesives and biofilm matrix polymers to chemical treatments as determined by interference reflection microscopy and light section microscopy. *Applied and Environmental Microbiology,* 55(11), 2827–2831. https://doi.org/10.1128/aem.55.11.2827–2831.1989
6. Somers, E.B., Schoeni, J.S., Wong, A.C.L. (1994). Effect of trisodium phosphate on biofilm and planktonic cells of Campylobacter jejuni, Escherichia coli O157: H7, Listeria monocytogenes and Salmonella typhimurium. *International Journal of Food Microbiology*, 22(4), 269–276. https://doi.org/10.1016/0168–1605(94)90178–3
7. Costerton, J.W., Stewart, P.S., Greenberg, E.P. (1999). Bacterial biofilms: a common cause of persistent infections. *Science,* 284(5418), 1318–1322. https://doi.org/10.1126/science.284.5418.1318
8. Lin, S., Yang, L., Chen, G., Li, B., Chen, D., Li, L. et al. (2017). Pathogenic features and characteristics of food borne pathogens biofilm: Biomass, viability and matrix. *Microbial Pathogenesis*, 111, 285–291. https://doi.org/10.1016/j.micpath.2017.08.005
9. Khweek, A.A., Amer, A.O. (2018). Factors mediating environmental biofilm formation by *Legionella pneumophila*. *Frontiers in Cellular and Infection Microbiology*, 8, Article 38. https://doi.org/10.3389/fcimb.2018.00038
10. Idrees, M., Sawant, S., Karodia, N., Rahman, A. (2021). Staphylococcus aureus biofilm: Morphology, genetics, pathogenesis and treatment strategies. *International Journal of Environmental Research and Public Health,* 18(14), Article 7602. https://doi.org/10.3390/ijerph18147602
11. Lee, B.-H., Cole, S., Badel-Berchoux, S., Guillier, L., Felix, B., Krezdorn, N. et al. (2019). Biofilm formation of *Listeria monocytogenes* strains under food processing environments and pan-genome-wide association study. *Frontiers in Microbiology,* 10, Article 2698. https://doi.org/10.3389/fmicb.2019.02698
12. Araújo, P.M., Batista, E., Fernandes, M.H., Fernandes, M.J., Gama, L.T., Fraqueza, M.J. (2022). Assessment of biofilm formation by Campylobacter spp. isolates mimicking poultry slaughterhouse conditions. *Poultry Science*, 101(2), Article 101586. https://doi.org/10.1016/j.psj.2021.101586
13. Sheng, H., Xue, Y., Zhao, W., Hovde, C.J., Minnich, S.A. (2020). *Escherichia coli* O157: H7 curli fimbriae promotes biofilm formation, epithelial cell invasion, and persistence in cattle. *Microorganisms,* 8, Article 580. https://doi.org/10.3390/microorganisms8040580
14. Shatila, F., Yaşa, İ., Yalçın, H.T. (2021). Biofilm Formation by *Salmonella enterica* Strains. *Current Microbiology,* 78, 1150–1158. https://doi.org/10.1007/s00284–021–02373–4
15. Silva, A.J., Benitez, J.A. (2016). Vibrio cholerae biofilms and Cholera Pathogenesis. *PLOS Neglected Tropical Diseases*, 10(2), Article e0004330. https://doi.org/10.1371/journal.pntd.0004330
16. Yonezawa. H., Osaki, T., Kamiya, S. (2015). Biofilm formation by Helicobacter pylori and its involvement for antibiotic resistance. *BioMed Research International,* 2015, Article 914791. https://doi.org/10.1155/2015/914791
17. Dzieciolowski, T., Boqvist, S., Rydén, J., Hansson, I. (2022). Cleaning and disinfection of transport crates for poultry—comparison of four treatments at slaughter plant. *Poultry Science*, 101(1), Article 101521. https://doi.org/10.1016/j.psj.2021.101521
18. Medina-Rodríguez, A.C., Ávila-Sierra, A., Ariza, J. J., Guillamón, E., Baños-Arjona, A., Vicaria, J.M. et al. (2020). Clean-in-place disinfection of dual-species biofilm (Listeria and Pseudomonas) by a green antibacterial product made from citrus extract. *Food Control*, 118, Article 107422. https://doi.org/10.1016/j.foodcont.2020.107422
19. Khamisse, E. Firmesse, O. Christieans, S. Chassaing, D. Carpentier, B. (2012). Impact of cleaning and disinfection on the non-culturable and culturable bacterial loads of food-contact surfaces at a beef processing plant. *International Journal of Food Microbiology*, 158(2), 163–168. https://doi.org/10.1016/j.ijfoodmicro.2012.07.014
20. Li, Q., Liu, L., Guo, A., Zhang, X., Liu, W., Ruan, Y. (2021). Formation of multispecies biofilms and their resistance to disinfectants in food processing environments: A review. *Journal of Food Protection*, 84(12), 2071–2083. https://doi.org/10.4315/JFP-21–071
21. Møretrø, T., Schirmer, B. C.T., Heir, E., Fagerlund, A., Hjemli, P., Langsrud, S. (2017). Tolerance to quaternary ammonium compound disinfectants may enhance growth of Listeria monocytogenes in the food industry. *International Journal of Food Microbiology*, 241, 215–224. https://doi.org/10.1016/j.ijfoodmicro.2016.10.025
22. Stewart, P. S., Franklin, M. J. (2008). Physiological heterogeneity in biofilms. *Nature Reviews Microbiology,* 6199–210. https://doi.org/10.1038/nrmicro1838
23. Bridier, A., Briandet, R., Thomas, V., Dubois-Brissonnet, F. (2011). Resistance of bacterial biofilms to disinfectants: a review. *Biofouling,* 27, 1017–1032. https://doi.org/10.1080/08927014.2011.626899
24. Burmølle, M., Webb, J. S., Rao, D., Hansen, L. H., Sørensen, S. J., Kjelleberg, S. (2006). Enhanced biofilm formation and increased resistance to antimicrobial agents and bacterial invasion are caused by synergistic interactions in multispecies biofilms. *Applied and Environmental Microbiology,* 72(6), 3916–3923. https://doi.org/10.1128/AEM.03022–05
25. Bridier, A., del Pilar Sanchez-Vizuete, M., Le Coq, D., Aymerich, S., Meylheuc, T., Maillard, J.-Y., et al. (2012). Biofilms of a *Bacillus subtilis* hospital isolate protect *Staphylococcus aureus* from biocide action. *PLoS ONE,* 7, Article e44506. https://doi.org/ 10.1371/journal.pone.0044506
26. Schwering, M., Song, J., Louie, M., Turner, R. J., Ceri, H. (2013). Multi-species biofilms defined from drinking water microorganisms provide increased protection against chlorine disinfection. *Biofouling,* 29(8), 917–928. https://doi.org/10.1080/08927014.2013.816298
27. Wang, R., Kalchayanand, N., Schmidt, J. W., Harhay, D. M. (2013). Mixed biofilm formation by Shiga toxin-producing Escherichia coli and *Salmonella enterica* serovar Typhimurium enhanced bacterial resistance to sanitization due to extracellular polymeric substances. *Journal of Food Protection,* 76(9), 1513–1522. https://doi.org/ 10.4315/0362–028X.JFP-13–077

28. Stewart, P.S. (2015). Antimicrobial tolerance in biofilms. *Microbiology Spectrum,* 3(3). https://doi.org/ 10.1128/microbiolspec.MB-0010–2014
29. González-Rivas, F., Ripolles-Avila, C., Fontecha-Umaña, F., Ríos-Castillo, A.G., Rodríguez-Jerez, J.J. (2018). Biofilms in the spotlight: Detection, quantification, and removal methods. *Comprehensive Reviews in Food Science and Food Safety,* 17, 1261–1276. https://doi.org/ 10.1111/1541–4337.12378
30. Martínez-Suárez, J.V., Ortiz, S., López-Alonso, V. (2016). Potential impact of the resistance to quaternary ammonium disinfectants on the persistence of *Listeria monocytogenes* in food processing environments. *Frontiers in Microbiology,* 7, Article 638. https://doi.org/10.3389/fmicb.2016.00638
31. Plakunov, V.K., Mart'yanov, S.V., Teteneva, N.A., Zhurina, M.V. (2016). A universal method for quantitative characterization of growth and metabolic activity of microbial biofilms in static models. Microbiology, 85(4), 509–513. https://doi.org/10.1134/S0026261716040147
32. Wagner, E.M., Pracser, N., Thalguter, S., Fischel, K., Rammer, N., Pospíšilová, L. et al. (2020). Identification of biofilm hotspots in a meat processing environment: Detection of spoilage bacteria in multi-species biofilms. *International Journal of Food Microbiology*, 328, Article 108668. https://doi.org/10.1016/j.ijfoodmicro.2020.108668
33. Wagner, E.M., Fischel, K., Rammer, N., Beer, C., Palmetzhofer, A.L., Conrady, B. et al. (2021). Bacteria of eleven different species isolated from biofilms in a meat processing environment have diverse biofilm forming abilities. *International Journal of Food Microbiology*, 349, Article 109232. https://doi.org/10.1016/j.ijfoodmicro.2021.109232
34. Illikoud, N., Klopp, C., Roulet, A., Bouchez, O., Marsaud, N., Jaffrès, E. et al. (2018). One complete and three draft genome sequences of four Brochothrix thermosphacta strains, CD337, TAP 175, BSAS1 3 and EBP 3070. *Standards in Genomic Sciences,* 13, Article 22. https://doi.org/10.1186/s40793–018–0333-z
35. Burmølle, M., Ren, D., Bjarnsholt, T., Sørensen, S. J. (2014). Interactions in multispecies biofilms: do they actually matter? *Trends in Microbiology*, 22(2), 84–91. https://doi.org/10.1016/j.tim.2013.12.004
36. Lee, K. W.K., Periasamy, S., Mukherjee, M., Xie, C., Kjelleberg, S., Rice, S. A. (2014). Biofilm development and enhanced stress resistance of a model, mixed-species community biofilm. *The ISME Journal,* 8(4), 894–907. https://doi.org/10.1038/ismej.2013.194
37. Alenazy, R. (2022). Antibiotic resistance in Salmonella: Targeting multidrug resistance by understanding efflux pumps, regulators and the inhibitors. *Journal of King Saud University — Science*, 34(7), Article 102275. https://doi.org/10.1016/j.jksus.2022.102275
38. Pagadala, S., Parveen, S., Rippen, T., Luchansky, J.B., Call, J.E., Tamplin, M.L. et al. (2012). Prevalence, characterization and sources of Listeria monocytogenes in blue crab (Callinectus sapidus) meat and blue crab processing plants. *Food Microbiology*, 31, 263–270. https://doi.org/10.1016/j.fm.2012.03.015
39. Nowak, J., Cruz, C.D., Tempelaars, M., Abee, T., van Vliet, A.H.M, Fletcher, G.C. et al. (2017). Persistent Listeria monocytogenes strains isolated from mussel production facilities form more biofilm but are not linked to specific genetic markers. *International Journal of Food Microbiology*, 256, 45–53. https://doi.org/ 10.1016/j.ijfoodmicro.2017.05.024
40. Pažin, V., Jankuloski, D., Kozačinski, L., Dobranić, V., Njari, B., Cvrtila, Ž. et al. (2018). Tracing of Listeria monocytogenes contamination routes in fermented sausage production chain by pulsed-field gel electrophoresis typing *Foods,* 7(12), Article 198. https://doi.org/10.3390/foods7120198
41. Rodríguez-Campos, D., Rodríguez-Melcón, C., Alonso-Calleja, C., Capita, R. (2019). Persistent Listeria monocytogenes isolates from a poultry-processing facility form more biofilm but do not have a greater resistance to disinfectants than sporadic strains. *Pathogens,* 8(4), Article 250. https://doi.org/ 10.3390/pathogens8040250
42. Iñiguez-Moreno, M., Gutiérrez-Lomelí, M., Javier Guerrero-Medina, P.J., Avila-Novoa, M.G. (2018). Biofilm formation by Staphylococcus aureus and Salmonella spp. under mono and dual-species conditions and their sensitivity to cetrimonium bromide, peracetic acid and sodium hypochlorite. *Brazilian Journal of Microbiology*, 49(2), 310–319. https://doi.org/10.1016/j.bjm.2017.08.002
43. Byun, K.-H., Han, S.H., Yoon, J.-W., Park, S.H., Ha, S.-D. (2021). Efficacy of chlorine-based disinfectants (sodium hypochlorite and chlorine dioxide) on Salmonella Enteritidis planktonic cells, biofilms on food contact surfaces and chicken skin. *Food Control,* 123, Article 107838. https://doi.org/10.1016/j.foodcont.2020.107838
44. Delhalle, L., Taminiau, B., Fastrez, S., Fall, A., Ballesteros, M., Burteau, S., Daube, G. (2020). Evaluation of enzymatic cleaning on food processing installations and food products bacterial microflora. *Frontiers in Microbiology,* 11, Article 1827. https://doi.org/10.3389/fmicb.2020.01827

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