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

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

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- targeted modification (selection, hybridization, operative manipulation);
- processing of meat raw materials;
- improvement of technologies for meat product manufacture;
- study of effects of meat and meat product consumption on human health;
- rational use of secondary resources of the meat industry and the ecological problems of the industry

The journal "Theory and practice of meat processing" publishes scientific and review articles, reports, communications, critical reviews, short research communications (letters to the editorial office), informative publications covering themes from the raw material base of the meat industry to innovative technologies of meat processing and food production, including the use of food biotechnology, from quality and safety management and risk analysis to information technologies, modeling in the meat industry as well as trophological chain management, marketing, economics, consumer demand analysis.

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MODERN FORMS OF IODINE-CONTAINING FOOD COMPONENTS

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Keywords: *classification, iodized products, iodized salt, source of iodine, organic iodine*

Abstract
The article presents the statistics of iodine deficiency disorders and the possible causes of their occurrence. The methods of iodine deficiency correction on the basis of state programs are reviewed. The recommendations from the World Health Organization on the amount of iodine added to iodized salt are given. A review of scientific databases on the topic of iodine-containing food components of various nature and their classification are given based on the form of the components (organic or inorganic). The analysis of iodine preservation in foods incorporating iodine-containing components under various conditions of technological processing and storage has been carried out.

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Introduction

Iodine is an essential micronutrient for the production of thyroid hormones. Iodine deficiency may lead to negative consequences for the body, especially for women and fetuses during pregnancy as well as for children in their first years of life [1, 2]. Sufficient iodine intake is vital to ensure the normal ability of the thyroid gland to synthesize triiodothyronine (T3) and thyroxine (T4) hormones [3]. Various forms of iodine found in natural and enriched foods are used to prevent iodine deficiency [4]. Salt iodization is by far the easiest way to achieve adequate iodine intake [5], but chemical stability and bioavailability of this form is a subject for further research.

The purpose of this review was to summarize and systematize modern scientific data on various forms of iodine-containing components, as well as to classify them and analyze the possibility of their use in food products.

Objects and methods

The sources of information were 4 scientific databases: elibrary, PubMed, Scopus, Google Scholar (accessed 01/27/2023). The search strategy included the following keywords: iodized foods, iodized salt, iodine source, organic iodine, iodine enrichment, iodine deficiency correction. The following acceptance criteria for research characterization were considered: foods, biofortification, micronutrients, original research. The parameters of publications were as follows: publication since 2013, language: English, Russian. Exclusion criteria: no access to the full-text articles. Statistics on iodine deficiency prevalence are given on the basis of data from the Iodine Global Network [6, 7]. Based on the review, the authors compiled a classification of iodine-containing components, identified possible ways to enrich foods with various forms of iodine, and also established factors affecting the preservation of iodine in food products and its absorption by the body.

Iodine deficiency statistics

According to World Health Organization statistics, about 2 billion people worldwide are at risk of insufficient iodine intake and approximately one third of the population lives in iodine-deficient areas. According to the Ministry of Health of the Russian Federation, in all regions of Russia from Sakhalin to the Central regions, there is a deficiency of iodine in the diet of local residents. A number of regions of the Russian Federation affected by the accident at the Chernobyl nuclear power plant are still endemic for goiter, the main cause of which is iodine deficiency[1]. The Iodine Global Network research indicates that the level of iodine consumption by the population of the Russian Federation is "insufficient". Consumption was estimated from the median urinary iodine concentration in school-age children (Figure 1). This technique is considered the most effective, since 90% of the consumed iodine is excreted from the body with urine [7].

[1] MU2.3.7.1064–01 "Control of the program for the prevention of iodine deficiency disorders through universal salt iodization" Guidelines, Moscow: Ministry of Health of the Russian Federation, 2001. Retrieved from https://docs.cntd.ru/document/1200026360 Accessed March 11, 2023

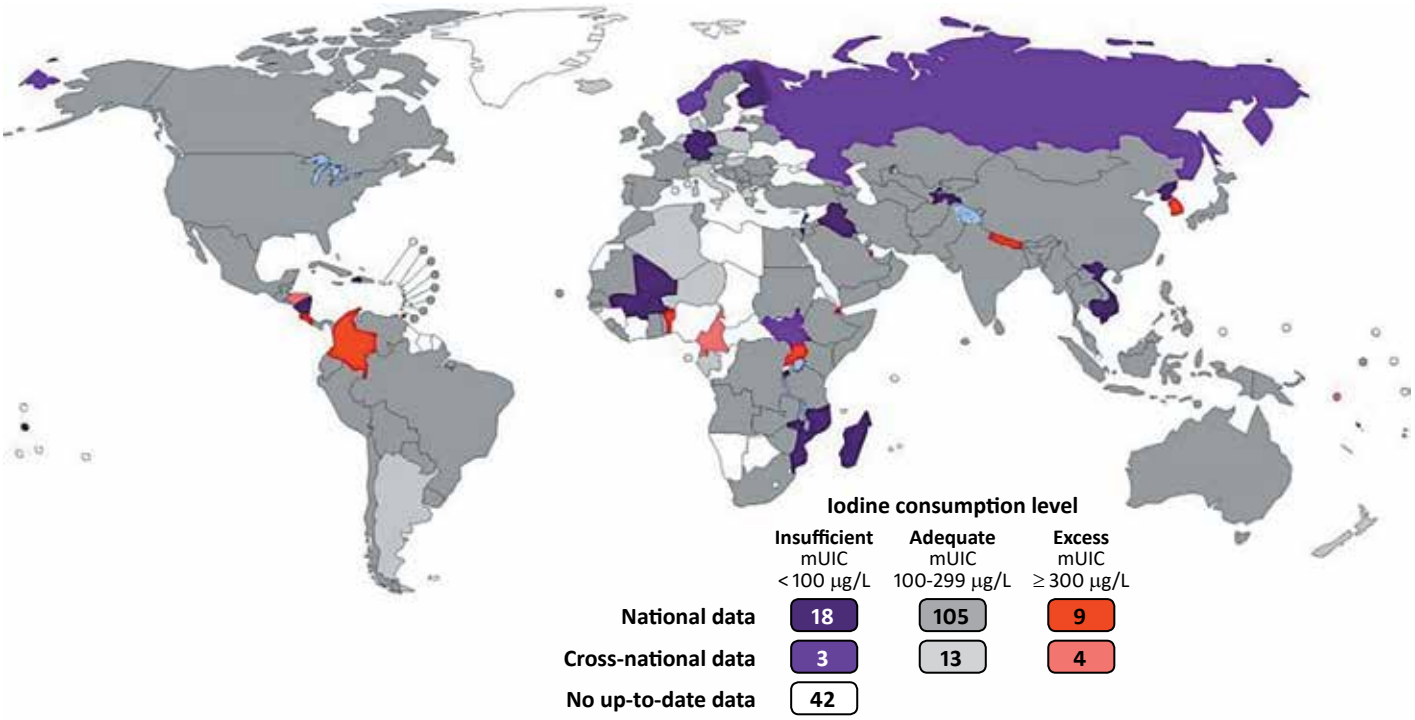

Figure 1. Global iodine consumption research 2005–2020 [8]

Iodine deficiency may interrupt biological functions and cause the development of endocrinological diseases such as goiter, hypothyroidism and cognitive impairment. A study by the National Research Center for Endocrinology of the Ministry of Health of the Russian Federation showed that annually, 1.5 million adult patients and 600 thousand children with endocrinological disorders receive medical care in the Russian Federation [9]. In monetary terms, the cost of treatment exceeds 250 billion rubles, which is 5 times more than the amount needed to organize measures to prevent all iodine deficiency disorders in the Russian Federation. The level of actual iodine consumption by people in Russia is 40–80 µg per day, which is 3 times lower than the established values of physiological need[2].

The growing interest in alternative products also reduces the level of iodine consumption by the population, since the raw materials for them do not contain the required amount of iodine, and only a small part of such products are additionally enriched [10]. Seaweed is often present in the diet of vegetarians, but this is often not enough to meet 100% of the need for iodine. According to the authors of [11], pescetarianism among people who do not eat meat implies the consumption of fish and seafood, which significantly increases the chances of such people to intake the necessary amount of iodine with food.

According to the strategy for improving the food quality in the Russian Federation until 2030[3], the development of methods for justification the shelf life of food products is a priority, as well as assessing the preservation of essential foods and biologically active substances. It should be noted that the preservation and absorption of iodine from foods depends on the form of iodine, technological modes of processing and storage conditions of finished products.

Methods for iodine deficiency correction

Currently, the problem of iodine deficiency is solved mainly by the introduction of iodized salt into the diet. In 2021, the Ministry of Health of the Russian Federation developed a draft federal law "On the prevention of iodine deficiency disorders"4, which established the measures for the prevention of iodine deficiency disorders, such as the use of iodized salt in food manufacturing and for salting, establishing requirements for the placement of iodized salt and products containing it at the point of sale, fortification of foods with iodine, as well as informing the population about iodine deficiency consequences and ways to prevent them. In addition, part 3 of article 6 of this draft federal law provides for the use of iodized salt in the manufacture of food products, except in cases where the technological process does not allow this. In this case, the products should be enriched with iodine by other possible methods.

Salt iodization programs have been adopted in 125 countries around the world (including Russia), but due to the salt reduction strategy, it is difficult to combine these two policies. Reducing the sodium content in food is implemented in 44 countries around the world [6]. The

[2] Draft federal law dated April 12, 2021 «On the prevention of iodine deficiency disorders», Ministry of Health of the Russian Federation. Retrieved from https://regulation.gov.ru/Regulation/Npa/PublicView?npaID=99202, Accessed March 11, 2023

[3] Strategy for improving the food quality in the Russian Federation until 2030 (approved by the order of the Government of the Russian Federation dated June 29, 2016 No. 1364-r) Retrieved from https://docs.cntd.ru/document/420363999, Accessed March 11, 2023

[4] Draft federal law dated April 12, 2021 «On the prevention of iodine deficiency disorders», Ministry of Health of the Russian Federation. Retrieved from https://regulation.gov.ru/Regulation/Npa/PublicView?npaID=99202, Accessed March 11, 2022

World Health Organization (WHO) promotes both the implementation of salt reduction programs as one of the cost-effective strategies for reducing the level of nutrition-related diseases, and universal salt iodization for the prevention and control of iodine deficiency disorders [13].

Salt iodization programs need to be constantly monitored to adjust the amount of iodine added to salt and to control the sodium intake in the population. Iodine loss depends on the iodization process, salt quality, packaging materials, and external factors. Given these conditions, WHO proposed to set the level of iodine added to salt as 65 mg of iodine per 1 kg of salt (Figure 2). Sodium carbonate/bicarbonate and sodium thiosulfate or dextrose are also added to the salt to stabilize the iodine.

The indicated concentration (Figure 2) was calculated based on the average recommended nutrient intake, i. e. 150 μg iodine/day + 30% process losses, considering iodine bioavailability of 92%. Iodine loss is variable depending on processing and storage conditions. The policy of the Food and Drug Administration (FDA), in close collaboration with WHO, supports the reasonable fortification of foods with additional nutrients, depending on the goal: 1) correction of recognized nutritional deficiencies; 2) restoration of the initial nutrient concentrations; 3) maintaining a balanced nutrient-versus-calorie profile; 4) improving the nutrition quality. In this regard, FDA has set a maximum amount of potassium iodide for use as a supplement in salt of 0.01 wt.% [14] to prevent the consequences of excess iodine in the diet.

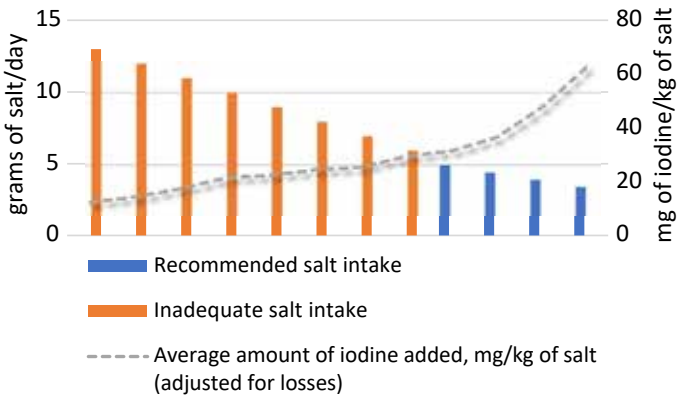

Figure 2. WHO recommended concentrations for salt fortification with iodine [12]

An alternative way to ensure sufficient iodine for the population is consuming foods rich in natural iodine or containing an organic form of iodine. Diversification of the diet with more seafood may be effective, but not always possible due to the high cost of such products. In the production of iodized products, it is necessary to ensure iodine level control throughout the entire shelf life due to the high volatility of iodine compounds. The effective use of iodine-enriched foods for iodine deficiency correction in the population is possible providing regulation by state, departmental and manufacturing control of the production and sales of enriched foods and iodized salt.

Components containing naturally occurring iodine

High concentrations of naturally occurring iodine are found in seafood [4] and seaweed [15] due to their ability to accumulate iodine from sea water. Eastern countries have long used seaweed as a source of iodine [16], and in the last 10 years this trend has begun to develop in Europe. At the same time, Europe allows products on its market that are safe for consumption and regulate their sales. For seaweed, France first established a regulation regarding their use as a food source in 1997, called "Novel Food", which includes a guideline for the evaluation of 25 algae and 3 microalgae suitable for human consumption [17].

A study by Norwegian scientists on brown and red algae of the North Atlantic showed that the consumption of 32 to 2150 mg of them in dried form completely meets the need for iodine in adults [18]. In addition, iodine content in these algae does not depend as much on season as in algae collected in the Pacific Ocean along the coast of China, as mentioned in the publication of Chinese researchers [19]. The effect of thermal processing on different types of algae is ambiguous, since in one case, boiling reduces iodine concentration, and in other case, it has no effect, which was proven in the study of four seaweed types [20]. Apparently, this is due to the impossibility of releasing iodine from its organic form in certain types of algae by thermal dehydration.

The study of numerous databases containing information on the amount of iodine in fish allows to conclude that it predominates in naturally grown cod, pollock, and hake [21]. A search for scientific publications on this topic showed that the average iodine content in fish fillets varies from 21 μg/100 g in raw Atlantic halibut to 790 μg/100 g in raw pollock [22] and depends on the geographic range. Iodine content in fish depends also on the season and spawning period. A study by Norwegian scientists [23] showed that fatty fish accumulate less iodine than non-fatty ones. Biofortification of aquaculture products with iodine is carried out by using feed supplements in the diet of fish and shellfish [24], as well as by integrating aquaculture products (seaweeds) into feed [25]. This method of animal products enrichment with iodine is called "antemortem formation of the functional properties in raw materials".

Eggs are also a source of iodine, as animal feed is enriched with calcium iodate, similar to fish feed. Initially, calcium iodide and calcium iodate were used to increase the productivity of farm animals, but later it was found that iodine is able to accumulate in animal tissues and remain in them until processed food is consumed. The authors of the article [26] studied the transfer of microelements and macronutrients into the egg from feed consumed by laying hens. Scientists claim that one egg of 60 g from laying hens who consumed iodine-containing feed supplements contains up to 122 μg of iodine. At the same time, bioavailability of iodine for the human body is higher when the feed supplements contain iodine in an organic form, i. e. in the form of iodized milk proteins. Since eggs, as well as

milk, are mandatory for inclusion in the diets of certain population groups for which iodine plays a crucial role in ensuring the growth and development of the body (pregnant, lactating women and children), the amount of iodine in them should be standardized. In addition, the amount of iodine in chicken eggs may vary depending on animal metabolism adaptation to enriched feed and the age of the animals [27].

In the organic method of milk production, animals are fattened on pastures without the use of feed supplements, which means that the animal does not receive a sufficient amount of many nutrients. There are studies proving that iodine concentrations in organic milk are much lower than in regular milk [28, 29], since the organic method of fattening does not involve the use of iodine-containing premixes. Further, iodine content in milk depends on season [30] and the method of milk thermal treatment at a dairy plant [31, 32]. In addition, for disinfection of the milking system and udder in the production of non-organic milk, a solution of iodofluorine is used, which is a chemical contaminant in dairy products [33] and, accordingly, increases the concentration of iodine in milk.

Higher plants, as a rule, are not a source of iodine. However, biofortification of agricultural crops is one of the promising ways of iodine deficiency correction due to the high level of consumption and large cultivated areas. Iodine is introduced into plants in various ways: through the soil with fertilizers, as well as with the help of hydroponic and irrigation systems [34]. To enrich plants, the method of root feeding with fertilizers containing inorganic forms of iodine (KI and KIO_3) is more often used. A comparative analysis of these two substances as fertilizers by Polish and Hungarian scientists showed that KI is more effective and does not have an inhibitory effect on crop growth [35, 36]. When iodine compounds are absorbed through the root system, its concentrations decrease from the root to stems, leaves, and fruits, which was proved in the study by Italian scientists [37], so it is advisable to use this method for enrichment of leafy vegetables. It is worth noting that the method of foliar fertilizing with solutions containing iodine is more suitable for mature plants of leguminous crops.

Iodized salt

Iodized salt is a mechanical mixture of salt (NaCl) and inorganic iodine compound, potassium iodide (KI) or potassium iodate (KIO_3). The disadvantage of this form is the low resistance to light and moisture, as well as the high volatility of iodine [38]. Studies by Russian scientists have shown that the content of inorganic iodine in foods containing iodized salt decreases by 50% after storage for 20 days from the date of production [39]. According to studies on the stability of iodine in the form of iodized salt under various processing conditions [40], a decrease in the iodine content in food products occurs due to moisture loss when heating.

The content of iodine in iodized salt depends on the specific manufacturer, conditions and term of storage. The World Health Organization has set a safe level of salt intake of up to 5 g/day for the adult population. The recommended intake of iodine is 150 μg /day for adults [41]. An analysis of sufficient iodine levels supply in different countries of the world, provided that all salt in the diet is iodized and adjusted for a 30% loss of iodine in the product during storage and cooking is presented in Table 1.

Table 1. Levels of iodine consumption with iodized salt in different countries [42]

Country	Iodine concentration in iodized salt, mg/kg	Iodine content in 5 g of iodized salt, taking into account losses (30%), μg	Level of iodine supply to the body when using 5 g of iodized salt,% of the daily requirement *	Actual level of iodine consumption with food for 2021,% of the daily requirement *	Actual level of iodine supply to the body for 2021, μg/L **
Canada	77	270	180	189	126
USA	76.5	267	178	190	127
Spain	60	210	140	173	115
South Africa	50	140	93	130	87
Russian Federation and CIS countries	40	140	93	<100	67
Italy	33	116	77	118	79
Brazil	30	105	70	276	184
Egypt	30	105	70	170	113
Greece	30	105	70	132	88
Thailand	30	105	70	179	119
UAE	27.5	97	65	162	108
China	26.5	93	62	200	133
India	25	88	58	178	119
Switzerland	25	88	53	137	91
Poland	23	81	54	112	75
Germany	20	70	47	89	59
Turkey	19.5	69	46	107	71
Indonesia	18	63	42	215	143
Australia	17.5	62	41	175	117
France	17.5	62	41	136	91
Serbia	15	53	35	195	130

* Recommended level of iodine supply to the body of an adult healthy person is 150 μg/day. This value was calculated by population survey.

** Actual intake was measured by median urinary iodine concentration. Data provided by the Iodine Global Network for the period of 2006 to 2021 [43].

The data presented in Table 1 indicate that the level of iodine supply to the body does not always depend on iodine concentration in salt. Obviously, this is directly related to the content of iodine in raw materials depending on the region of cultivation and indirectly related to the food preferences of different peoples of the world, as well as the level of consumption of iodine-enriched foods. For example, in Brazil, China, Indonesia, and Serbia, the actual level

of iodine supply to the body is several times higher than the theoretical level when using iodized salt. This means that other sources of iodine are present in the diet of the population, including natural and enriched ones.

Considering the relatively high consumption of bakery products by Russians in comparison with Western countries, i. e. USA, Australia, and New Zealand, the production of functional bakery products using iodized salt is promising in terms of iodine deficiency correction the Russian Federation [44]. In addition, the use of iodized salt improves sanitary and microbiological safety of bakery products, preventing the development of molds and *Bacillus mesentericus* [45]. However, the inorganic form of iodine is unstable and iodine loss during the technological processing and storage reaches 50% already on the ninth day of the finished product storage, which is reflected in the study of sausages and bakery products with the addition of iodized salt [46].

Considering the fact that iodized salt is now used for industrial purposes mainly in the production of bakery products, and its loss during storage reaches 50%, meeting the need of 150 μg/day with the use of adequate bread amounts is difficult. It is reasonable to develop recipes for other processed food products containing iodized salt or other ingredients rich in iodine. However, this requires the discussion and agreement of the technology with food manufacturers and the study of iodine preservation in its various forms under various technological regimes and during storage [47]. In addition, the loss of iodine during storage from iodized salt reaches 70%, which significantly reduces the level of iodine supply to the body when consuming 5 g of salt per day. Salt iodization programs are widely accepted all over the world, but legal regulation differs depending on the country [48] and therefore not all iodine-deficient regions take appropriate actions.

Iodine-containing components

In addition to potassium iodate and potassium iodide, the food industry uses iodine-containing supplements based on organic carriers: amino acids, fatty acids, and polysaccharides. Currently, the most common is iodine compound with milk protein, i. e. iodocasein, which is also used as Bioiodine dietary supplement. To obtain casein, cow's milk is defatted and the casein protein fraction is isolated by standard methods [49]. Elemental iodine is used for iodization. During the reaction, the temperature, pH of the medium, and the required degree of iodization are controlled. Iodine ions are linked to the amino acid tyrosine, which is a part of the casein structure, by the mechanism of electrophilic substitution. Iodine acquires an oxidation degree of +1 and forms a strong bond with carbon [50]. A solution of iodized protein is sterilized by short-term heating up to 90 °C, and then dried and ground to obtain a powder. The content of iodine in the Bioiodine dietary supplement is 7% to 9%, and the analysis of its preservation during technological processing and storage as a part of food products showed higher values in comparison with iodized salt [51].

The aforementioned milk protein-based supplement is successfully used in the production of fermented milk products with functional properties, such as cottage cheese [52] and yogurt [53]. In addition, Bioiodine may be used without reducing the effectiveness in relation to the normalization of the iodine index in the formulations of the products based on meat raw materials, which was proved in a study involving 20 students of the "Oryol State Institute of Economics and Trade" [54]. According to *in vivo* studies, its use is most effective in preventing destructive and degenerative changes in different organs [55]. Iodocasein is recommended by the Ministry of Health of the Russian Federation for preventing iodine deficiency disorders, particularly as a supplement when fortifying baby food. However, the production of this supplement is carried out according to technical specifications, and so far, no common standard that establishes general requirements and quality indicators is developed.

Iodized elastin is a hydrolyzate of elastin from connective tissue enriched with iodine by adding potassium iodide in the amount of 50 to 200 μg per 1 g of elastin. According to the data presented in the patent[5], raw elastic tissue is able to bind up to 70% of the of the iodine introduced, while iodine binding in the fine-cut boiled nuchal ligament reaches 100%. Iodized elastin hydrolyzate is incompatible with the technologies for whole milk, fermented milk products, and drinks production, but may be successfully used in meat products. The reliability of iodine delivery to the thyroid gland was confirmed by *in vivo* experiments on laboratory animals [38] using a blood test for the level of thyroid hormones as a result of artificial hypothyroidism simulation for 14 days.

Production of iodized soy protein involves the enrichment of the carrier (soy isolate or soy flour) with iodine by soaking in a potassium iodide solution under conditions of limited lighting for 30 minutes. Binding of iodine in this case reaches 480 μg of iodine per 1 g of soy protein, while high stability of iodine in the form of dietary supplements is observed during storage for 12 months [56]. The authors [57] also proved the effectiveness of this supplement against experimental hypothyroidism by the *in vivo* method. This technology has not yet been applied in practice, but is promising in the production of meat products. The properties of iodized soy protein were studied [38] in comparison with iodized wheat fibers and iodized salt. These studies also showed that the stability of iodine bound to soy proteins during storage under various temperature and humidity conditions is much higher than in iodized salt.

Iodine-chitosan is a dietary supplement containing iodine linked to an amino polysaccharide of animal origin,

[5] Bitueva E. B., Zhamsaranova S. D., Kapustina Yu. A. Biologically active food supplement. Patent RF no. 2266021, 2004. (In Russian)

i. e. chitosan. Iodine-chitosan is obtained using absorption of iodine from water-alcohol vapors of potassium iodide and potassium iodate by chitosan powder [58]. The result is a supplement containing inorganic iodine stabilized in an organic matrix. This supplement may be successfully introduced into the formulations of fermented milk products [59]. Studies of iodine preservation during the storage of products containing iodine-chitosan have not been carried out, but similarly to Bioiodine and iodocasein, it may be concluded that this form of iodine has a high resistance to external factors, and therefore is stable. The use of iodine-chitosan in the diet of laboratory animals with hypothyroidism activates the formation and maturation of blood cells, and *in vitro* studies prove its antioxidant properties [60].

Phytoiodine is a dietary supplement containing iodine in an organically bound form with a plant-based polysaccharide pectin. According to the data presented in the article [61], the production of Phytoiodine involves the introduction of pectin into iodine solution, thorough mixing and drying at room temperature. The author of the dissertation [62] proved the biological effectiveness of iodine-pectin compounds in terms of the impact on the main factors in the pathogenesis of endemic goiter and goiter transformations. This supplement is successfully used in the production of specialized enriched dairy products at canned milk plants in Bashkortostan [63]. High Phytoiodine stability during heat treatment and storage was proved by an experiment with adding it to bakery products and subsequent quantitative analysis of iodine [64].

Preparation of iodized flour from wheat germs is described in [65] and involves the germination of grains in an aqueous solution of potassium iodide under conditions of limited lighting for 30 minutes, then drying at a temperature not exceeding 60 °C and grinding. In this case, high stability of iodine is observed during storage for 12 months. Sprouted wheat enriched with iodine may be used in the production of boiled-smoked sausages, which is reflected in the study of Russian scientists [66]. Western colleagues also conducted studies of this ingredient, which proved a high degree of iodine preservation in wheat dietary fibers compared with iodine preservation in the composition of iodized salt when stored at different temperature and humidity conditions for 12 months [38].

Substances of a carbohydrate nature may also act as a carrier for molecular iodine: sugars, dietary fibers and starches. Iodized arabinogalactan is obtained by introducing hydrated elemental iodine in the amount of 200 μg per 100 g into a solution of polysaccharide extracted from coniferous wood. This supplement has emulsifying and stabilizing properties, which makes it possible to use it in the production of iodized minced semi-finished products based on meat raw materials. However, iodine losses during storage of chilled semi-finished products containing it reach 48% within 24 hours after production [67].

Rebaudioside A iodization with the formation of iodine-glycoside described in the patent[6] involves mixing with molecular iodine in distilled water at high temperature and drying in a desiccator. The supplement contains 12% of iodine, and IR spectroscopy studies of the iodine-glycoside conjugate indicate the inclusion of iodine molecules in the structure of rebaudioside A molecules [68]. Studies of iodized rebaudioside in an experiment with laboratory animals showed that the use of the supplement compensates for the negative effect of mercazolil on parent rats [69], and therefore has a positive effect on cognitive functions in rat pups with artificial hypothyroidism. This supplement has not yet been applied in practice, but the physicochemical properties of low molecular weight carbohydrates open up broad prospects for their use in the food industry.

Iodized peanut butter is obtained using esterification of unsaturated fatty acids by linking iodine to double bonds [70]. The high efficiency of iodized peanut butter in the prevention of iodine deficiency disorders in comparison with iodized salt was proven 30 years ago by foreign scientists in experiments involving Algerian school-age children [71] and young people living in Zaire [65]. However, this supplement has not yet been applied in practice in food industry.

Foods containing added iodine

Iodization of food products may also be a part of the technology for processing raw materials into a finished product. Iodine is introduced in organic and inorganic form, most often as a component of salt or dietary supplement, since in this case it is easier to control the final iodine content in the product. This method of enrichment is applicable to products that undergo a technological mixing operation, for example, for bakery and confectionery products, pates, mousses and purees, minced meat products and sausages.

Since iodine is a highly volatile substance, its preservation may be affected by extreme technological regimes and storage conditions [72]. For the effective use of the above additives in food production, it is necessary to establish the levels of iodine preservation under various processing conditions and shelf life of products with supplement. The analysis of the literature presented in this review makes it possible to compare the degree of iodine preservation when it is added to products undergoing heat treatment. Storage period for various food products has a fairly wide range and comparison of iodine preservation in them should be made based on the shelf life for each individual product. Comparative analysis of iodine losses in the composition of finished food products containing iodized supplements is presented in Table 2.

[6] Kamilov F. Kh., Konkina I. G., Murinov Yu.I., Ivanov S. P., Baiburina G. A., Kozlov V. N. et al. Iodine-containing biologically active food supplement. Patent RF no. 2716971, 2019. (In Russian)

Table 2. Iodine losses during heat treatment and storage

Name of the iodine-containing component	Food product containing iodized component	Heat treatment conditions	Loss of iodine during heat treatment	Loss of iodine by the end of the shelf life of the finished product containing the dietary supplement*	Duration of product storage	Data source
Iodized salt	Sausages	85 °C	35.9%	More than 70%	20 days	[39, 51]
Bioiodine			12.2%	20.7%	21 days	
Iodocasein			3.2%	52.5%	21 days	
Iodized elastin	Dietary supplement	—	—	44%	6 months	[38]
Iodized soy protein	Minced meat products, steamed, frozen	100 °C	7%	54%	150 days	[38]
Iodized dietary fiber derived from wheat			3%	47%	150 days	[38, 66]
Iodine-chitosan	N/a					
Phytoiodine	Bakery products	220 °C	—	4%	90 hours	[64]
Iodized inulin			—	up to 80%	30 days	[73]
Iodine-glycoside	N/a					
Iodized arabinogalactan	Minced meat products, steamed, chilled	100 °C	0%	40%	24 hours	[67]
Iodized butter	N/a					

* data over the past 10 years for the studies published in scientific journals.

The data in Table 2 support the expediency of using iodine-containing supplements and biologically active additives in the production of mass consumption foods instead of iodized salt due to the high degree of iodine preservation during technological processing and storage of finished products. This will reduce the amount of added salt in the diet and increase the amount of iodine consumed with food. Apparently, resistance to high temperatures and external conditions during storage is inherent in iodine-containing supplements of an organic nature, i. e. linked to macronutrients: amino acids and polysaccharides, due to more stable iodine-carbon bonds.

Conclusion

Currently, salt iodization is the main method of iodine deficiency correction. However, salt intake should be limited to 5 g per day. This is especially true for those groups of the population for which iodine is a vital element that comes with water and food: pregnant and lactating women, young children. According to IGN (Iodine Global Network) statistics, the degree of iodine supply of the population does not always correlate with iodized salt consumption. The best way to fortify the diet with iodine is to eat natural sources of iodine such as milk, eggs, seafood, and seaweed, but often the amount of iodine from these foods is insufficient.

Foreign and domestic scientists have developed a number of iodine-containing foods and supplements that have the potential in the production of industrially processed iodized functional foods. The authors have classified the iodine-containing ingredients based on the form of iodine (organic/inorganic).

In the technology of processed food products, iodine is used mainly in the form of chemical compounds of various nature: salts of hydroiodic (iodide) and iodic (iodate) acids, amino acid derivatives, organically bound iodine with polysaccharides, as well as fatty acids esterified in the presence of iodine. As mentioned above, organic forms of iodine are more stable during storage, and also have high bioavailability and low toxicity compared to inorganic forms of iodine. These properties characterize the organic form of iodine as more suitable for the production of baby foods [54], as well as therapeutic, preventive and functional products in comparison with the inorganic one.

Iodine-chitosan is also included in the group of inorganic forms of iodine, since in this case, iodine is in the inorganic form of KI enclosed in a chitosan matrix. In addition, there are developments of iodized fatty acids, but since their use in food production has not been tested even in laboratory conditions, the authors considered it incorrect to include these substances in the classification of food components.

An analysis of scientific and technical literature allows to conclude that iodine-containing ingredients are logically divided into organic and inorganic ones (depending on the form of iodine present in the supplement). The form of iodine directly depends on the method of enrichment and, in this regard, the authors have compiled a hierarchical diagram that reflects the classification of iodine-containing ingredients (Figure 3).

Each mentioned iodine-containing ingredient is characterized by individual resistance to heat treatment and storage conditions. Based on the literature review, the authors concluded that organic forms of iodine are more resistant to technological conditions.

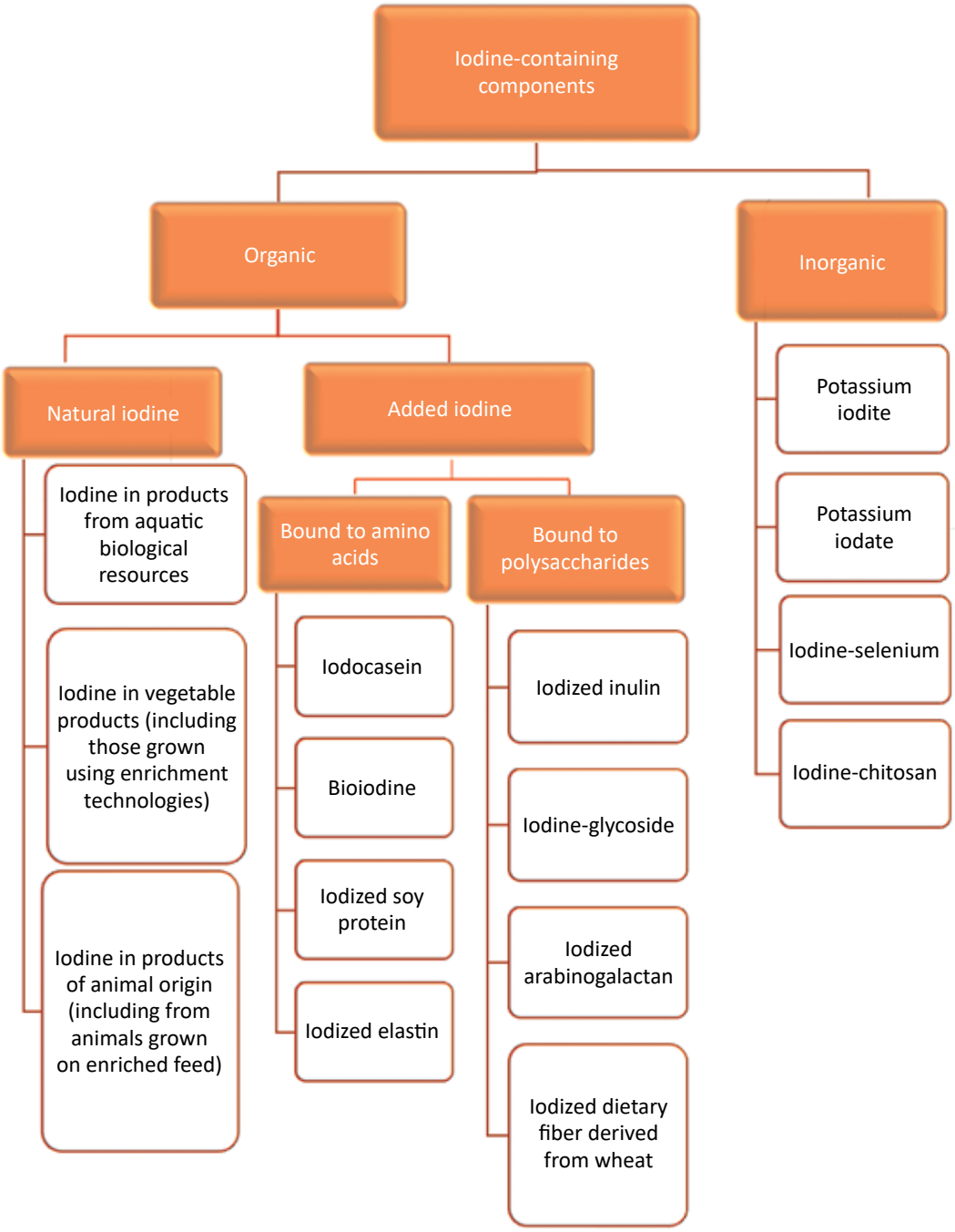

Figure 3. Classification of iodine-containing components proposed by the authors

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INVESTIGATION OF THE CHEMICAL COMPOSITION, PHYSICOCHEMICAL PROPERTIES, AND MICROSTRUCTURE OF MEAT PATTIES WITH AMARANTH FLOUR

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Keywords: *composite mixture, beef, organoleptic, physico-chemical parameters, water-holding capacity, microstructure*

Abstract

This study aimed to investigate the effect of adding amaranth flour to meat patties on their chemical composition. Four different variations of meat patties were prepared, with amaranth flour added at concentrations of 5%, 10%, and 15% in place of beef. The control sample was prepared without any addition. The results of the study showed that the addition of amaranth flour led to a significant decrease in the moisture content of the meat patties, while the proportions of carbohydrates, fat, and ash increased. Specifically, the patties with the highest concentration of amaranth flour (15%) had the highest proportions of carbohydrates and fat with the lowest proportion of moisture. The control sample had the highest moisture content and the lowest proportion of carbohydrates, fat, and ash. The addition of amaranth flour increased the water-holding capacity of the meat patties, with the highest increase observed in the sample with 15% amaranth flour (82.21%). The overall score of sensory evaluation of the meat patties did not significantly decrease with the addition of up to 10% amaranth flour, according to the sensory evaluation. The study provides evidence that up to 10% amaranth flour can be used as a substitute for beef in meat patties, which can lead to an increase in the fat and carbohydrate content and mineral composition and improvement of the water-holding capacity of the final product.

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Introduction

In recent decades, there has been a growing trend in the meat product market toward the consumption of combined meat-plant products [1,2]. Meat products with plant ingredients are food products that contain both animal-based protein and plant-based ingredients. Adding plant ingredients to meat products can enhance their nutritional value by increasing the protein, fiber, vitamin, and mineral content [3–5]. One approach to enhance the textural characteristics of meat products is to incorporate plant-based ingredients. Plant-based ingredients can provide a range of functional benefits, such as enhancing water-binding capacity, emulsification, and gelling properties, which can improve the texture and mouthfeel of meat products [6,7].

Using plant ingredients in meat products can help to reduce the amount of meat that is consumed, which is beneficial for both health and environmental reasons [8–10]. Plant-based proteins are often less expensive and have a smaller environmental impact compared to animal-based proteins, so incorporating them into meat products can help to reduce the cost and environmental footprint of meat production [11,12].

Amaranth is a group of plants that belong to the genus Amaranthus [13]. Amaranth is grown and consumed in many parts of the world, but the largest producers of amaranth are mainly concentrated in Latin America (Peru, Mexico) and Asia (China, India, Nepal). Amaranth has been cultivated for thousands of years for its edible leaves, seeds, and stems, and is an important source of nutrition and income for many communities [14,15]. The consumption of amaranth is recommended for patients with ischemic heart disease and atherosclerosis, diabetes and obesity, cancer, and a weakened immune system. Amaranth flour and products made from it have a preventive effect on many body systems: they lower cholesterol levels, improve the condition of arteries, reduce the risk of cardiovascular and oncological diseases, promote the elimination of toxins [16,17].

The seeds of the amaranth plant are also highly nutritious and are often used as a gluten-free grain. Amaranth seeds are a good source of protein, fiber, iron, magnesium, and other essential nutrients. According to the chemical composition, amaranth contains 18.0–19.6% protein, 8.0–8.6% fat, 3.5–5.5% fiber, and 65.0–70.0% carbohydrates [18,19]. Amaranth is particularly high in essential amino acids, which are amino acids that the body cannot produce and must obtain from food. The amino acid profile of amaranth is notable for its high content of glutamic acid, aspartic acid, and arginine. These three amino acids alone make up over 45% of the total amino acid content of amaranth, with glutamic acid being the most abundant at 23.82 g/100g [20]. Other amino acids that are present in high amounts in amaranth include glycine, leucine, lysine, serine, phenylalanine, and valine. These amino acids are all important for various functions in the body, including protein synthesis, energy production, and the maintenance of healthy tissues [21,22].

In addition, amaranth seeds are rich in a range of B-group vitamins, tocopherol, provitamin A, ascorbic acid, and vitamin D. Amaranth flour is also a good source of various minerals, including iron, calcium, potassium, phosphorus, magnesium, copper, and others. Moreover, amaranth flour contains dietary fibers, which have an important advantage in reducing the calorie content of the diet and promoting a persistent sense of satiety [23]. Amaranth flour can be used in sausage production as a gluten-free and nutrient-dense ingredient that can improve the texture, flavor, and nutritional value of the final product [24,25].

The aim of this study is to investigate the influence of amaranth flour on the physicochemical properties, sensory characteristics, and nutritional value of meat patties.

Materials and Methods

Production of meat patties

Chicken meat and beef were deboned and trimmed of tendons, and connective tissues. The weighed meat was cut into pieces and ground using a meat grinder with a 3 mm plate. To prepare the forcemeat, the ground meat and non-meat ingredients, water, and spices were weighed and loaded into a meat mixer and mixed for 3–5 minutes.

Next, oval-shaped patties with a thickness of 2–2.5 cm were formed from the prepared forcemeat. The forming process was done using the IPCS-123M patty-making machine (Elf 4M Company, Russia). After forming, the semi-finished products were placed in a single row and sent to a freezing unit (at a temperature of –35 °C). The frozen semi-finished products were then packaged in a polymer, cardboard, or other packaging material and stored at a temperature of –10 °C to –30 °C.

According to the recipe (Table 1), four variations of meat patties were prepared, with the addition of 5%, 10%, and 15% amaranth flour instead of beef, along with a control sample without any addition.

Table 1. Recipe variants of meat patties, %

Ingredient	% of amaranth flour			
	1	2	3	4
Chicken meat	28	28	28	28
Beef	37	32	27	22
Amaranth flour	0	5	10	15
White bread	10	10	10	10
Milk	11	11	11	11
Egg	4.5	4.5	4.5	4.5
Creamy butter	4.5	4.5	4.5	4.5
Onion	4.5	4.5	4.5	4.5
Salt	0.4	0.4	0.4	0.4
Black pepper	0.1	0.1	0.1	0.1

Determination of chemical composition

The determination of the chemical composition (moisture, fat, ash, and protein) was based on the methods previously described in [26].

Determination of the mineral composition

A high-pressure Teflon container was used to hold between one and two grams of the sample. The sample was then burned in a muffle furnace for 4 hours at 400 °C, followed by an additional 2 hours at 600 °C. One gram of the resulting ash (measured by dry weight) was then digested by adding 3 mL of HNO_3 and 2 mL of HF. The mixture was then heated in a Berghof Speed Wave microwave system at 200 °C for 20 minutes. After this process, the samples were placed in a 10 mL container and diluted with 1% HNO_3. The content of elements in muscle samples was determined with an inductively coupled plasma mass spectrometric method (ICP-MS, Varian-820 MS, Varian Company, Canberra, Australia) [27].

pH determination

To determine the active acidity (pH), the potentiometric method was used. The sample was ground twice and mixed with distilled water in a ratio of 1:10. This mixture was then stirred on a magnetic stirrer for 30 minutes. The pH value was determined by HI 99163 instrument (Hanna Instruments Inc., USA) [28].

Determination of water-binding capacity

Water-binding capacity (WBC) was identified by the method proposed by Grau and Hamm using filter paper and a weighted press. The weight of the test sample was 0.3 g [29].

Statistical analysis

The experiments were carried out in triplicate. Standard deviation values are given for all measurements. Differences between the experimental and control groups were calculated using a one-way ANOVA with Tukey test. $p < 0.05$ was considered significant.

Results and discussion

Chemical composition of meat patties

The addition of amaranth flour significantly affected the moisture, fat, ash, and carbohydrate content of meat patties. For instance, replacing beef mince with amaranth flour at a concentration of 5% to 15% resulted in a decrease in moisture from 70.25% to 66.49%-58.91%, respectively (Table 2). This can be primarily attributed to the high content of dry matter in the flour, which, in turn, substantially increased the carbohydrate content from 5.86% to 16.36%. A slight increase in ash and fat was observed in the experimental samples of patties with amaranth flour. The protein content underwent little significant change, which characterizes the equivalent substitution of animal protein with plant protein.

The observed changes in the composition of meat patties resulting from the addition of amaranth flour have important implications for the nutritional and sensory quality of the final product. The reduction in the moisture content may contribute to a firmer texture and longer shelf life, as lower moisture levels limit microbial growth and spoilage. However, excessively low moisture levels can also result in a dry, unappetizing product [30]. The significant increase in the carbohydrate content resulting from the addition of amaranth flour is noteworthy, as it can provide an additional source of dietary fiber and nutrients for consumers [31,32].

Table 2. Chemical composition of meat patties, %

Indicator	% of amaranth flour			
	0	5	10	15
Moisture	70.25 ± 0.93^{d}	66.49 ± 0.91^{c}	62.69 ± 0.68^{b}	58.91 ± 0.76^{a}
Protein	14.83 ± 0.34^{a}	14.74 ± 0.30^{a}	14.66 ± 0.29^{a}	14.56 ± 0.22^{a}
Fat	7.88 ± 0.17^{a}	8.16 ± 0.14^{a}	8.47 ± 0.16^{b}	8.78 ± 0.13^{b}
Ash	1.16 ± 0.02^{a}	1.26 ± 0.02^{a}	1.30 ± 0.02^{b}	1.37 ± 0.03^{b}
Carbohydrate	5.86 ± 0.13^{a}	9.33 ± 0.18^{b}	12.86 ± 0.12^{c}	16.36 ± 0.20^{d}

$^{a, b, c, d}$ means within the same row, with different letters meaning there is a significant difference among different samples of sausages ($p < 0.05$)

Verma *et al.* [33] reported comparable findings, indicating that goat meat nuggets containing 3% amaranth flour and 1.5% quinoa had substantially lower moisture levels compared to the control group. Similar to our findings, Bağdatli [34] determined that the protein content of meatballs with quinoa flour did not change significantly, but had a significant effect on the fat and moisture contents of beef meatballs. The study showed that substituting wheat rolls with a combination of hemp seeds, amaranth, and golden flaxseed resulted in a positive impact on the protein content of poultry pates [35].

Mineral composition of meat patties

The mineral composition of meat patties underwent changes upon the addition of amaranth flour (Table 3). The calcium content increased to 48.91 mg/100g in variant 2, 55.46 mg/100g in variant 3, and up to 62.01 mg/100g in variant 4 in comparison to variant 1 without amaranth flour. A similar trend was observed for potassium, magnesium, phosphorus, iron, manganese, and copper (Table 3). Meanwhile, the sodium, sulfur, and zinc content were slightly reduced. The changes in the quantitative composition of the mineral substances in different variants of the meat patties can be explained by the content of these mineral substances in amaranth flour. Amaranth is rich in calcium (up to 150 mg/100g), magnesium (200–240 mg/100g), potassium (430–490 mg/100g), iron (up to 7.6 mg/100g), and other elements.

Table 3. Mineral composition of meat patties, mg/100g

Element	% of amaranth flour			
	0	5	10	15
Potassium	318.13 ± 4.83^{a}	326.53 ± 4.59^{a}	334.93 ± 3.31^{b}	343.33 ± 3.60^{b}
Calcium	42.36 ± 0.68^{a}	48.91 ± 0.91^{b}	55.46 ± 0.58^{c}	62.01 ± 0.89^{d}
Magnesium	21.07 ± 0.16^{a}	32.32 ± 0.42^{b}	43.57 ± 0.59^{c}	54.82 ± 0.96^{d}
Sodium	100.13 ± 2.16^{b}	97.48 ± 0.72^{b}	94.83 ± 0.57^{a}	92.18 ± 0.57^{a}
Sulfur	138.78 ± 2.01^{d}	127.70 ± 1.62^{c}	116.61 ± 1.07^{b}	105.53 ± 1.36^{a}
Phosphorus	160.49 ± 2.51^{a}	178.14 ± 2.39^{b}	195.79 ± 2.66^{c}	213.44 ± 2.99^{d}
Iron	1.21 ± 0.02^{a}	1.61 ± 0.03^{b}	1.93 ± 0.04^{c}	2.17 ± 0.04^{d}
Manganese	0.13 ± 0.002^{a}	0.30 ± 0.003^{b}	0.46 ± 0.004^{c}	0.61 ± 0.01^{d}
Copper	0.07 ± 0.001^{a}	0.09 ± 0.002^{b}	0.12 ± 0.002^{c}	0.14 ± 0.002^{d}
Zinc	1.75 ± 0.03^{b}	1.69 ± 0.02^{b}	1.62 ± 0.03^{ab}	1.56 ± 0.02^{a}

$^{a, b, c, d}$ means within the same row, with different letters meaning there is a significant difference among different samples of sausages ($p < 0.05$)

The findings revealed that the addition of amaranth flour to meatballs significantly increased their iron content. The control sample without the addition of amaranth flour had a mean iron content of 1.21 mg/100g. In contrast, the experimental samples with 5%, 10%, and 15% amaranth flour had mean iron contents of 1.61 mg/100g, 1.93 mg/100g, and 2.17 mg/100g, respectively. The highest iron content was observed in the experimental sample with 15% amaranth flour, indicating a dose-dependent response. The observed increase in the iron content may be attributed to the high iron concentration of amaranth flour. Amaranth is a good source of bioavailable iron, which is essential for human health [36].

The control sample without adding amaranth flour had a magnesium content of 21.07 mg/100g. This value served as a reference point for the other experimental samples. The results show that as the percentage of amaranth flour increased in the experimental samples, the magnesium content also increased. Specifically, the sample with 5% amaranth flour had a magnesium content of 32.32 mg/100g, while the sample with 10% of amaranth flour had a magnesium content of 43.57 mg/100g. The highest magnesium content was observed in the experimental sample with 15% of amaranth flour, which had a magnesium content of 54.82 mg/100g.

Magnesium is an essential mineral that plays a crucial role in many bodily functions, such as muscle and nerve function, blood pressure regulation, and bone health [37,38]. These findings suggest that the addition of ama-

ranth flour to meat cutlets can increase their magnesium content. Amaranth flour is a good source of magnesium, and its incorporation into meat cutlets can be an effective way to increase the magnesium content of meat-based dishes. The observed increase in the magnesium content with increasing amounts of amaranth flour could be due to the higher magnesium content of amaranth flour, as well as the fact that the amaranth flour may have facilitated the absorption of magnesium in the meat patties.

The control sample without amaranth flour had the highest zinc content at 1.75 mg/100g. In contrast, the experimental samples with 5%, 10%, and 15% amaranth flour had progressively lower zinc content of 1.69 mg/100g, 1.62 mg/100g, and 1.56 mg/100g, respectively. The results show that as the proportion of amaranth flour increased, the zinc content decreased, indicating an inverse relationship between the two variables. The difference in the zinc content between the control and experimental samples was minimal, with a maximum reduction of 0.19 mg/100g.

Zinc is an essential nutrient that plays a critical role in various physiological functions, including immunity, wound healing, and growth and development. However, zinc overdose can cause gastrointestinal issues, copper deficiency, reduced immune function, anemia, headaches, fatigue, and reduced HDL cholesterol [39,40]. The decrease in zinc the content with the addition of amaranth flour could be due to various factors. Amaranth flour is rich in phytates, which are compounds that can bind to minerals like zinc, making them less available for absorption in the body. Additionally, the heat processing during cooking could have also contributed to the decrease in the zinc content [41].

The study conducted by Kobzhasarova *et al.* [42] found that the use of amaranth flour in cooked sausage increased the content of macroelements such as calcium by 69.7%, potassium by 6.4%, phosphorus by 39.96%, and magnesium by 59.5% compared to cooked sausage without amaranth flour. According to Behailu's research conducted in 2020, the amount of calcium present in beef sausage demonstrated a significant rise (at a significance level of 0.05) when the level of soybean and millet flours inclusion increased [43].

Microstructure of meat patties

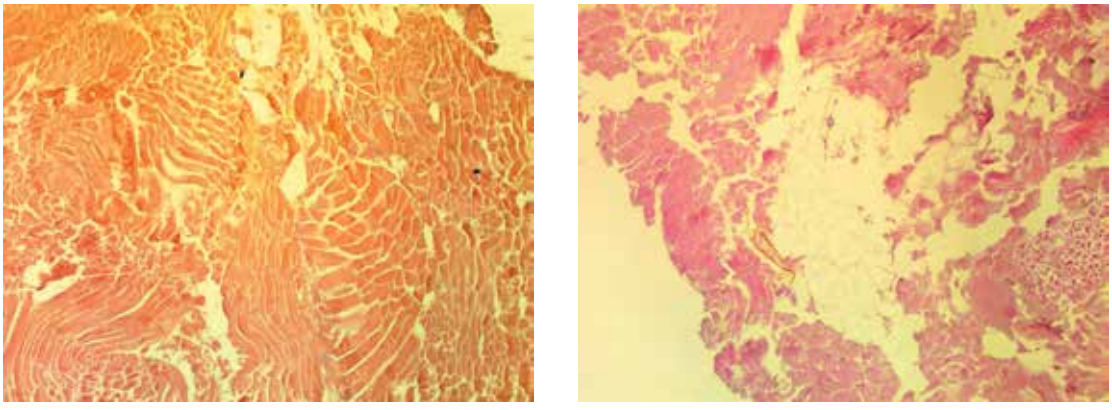
While examining the microstructure of meat patties, it was discovered that there were sections in the ground meat composition that contained plant ingredients. In the microstructure of the meat mixture that contained plant additives, muscle fibers that were cut both longitudinally and transversely were arranged with small gaps in between them (Figure 1). Furthermore, there was a finely structured pink-colored mass. The fatty tissue had a distinctive cellular structure with visible cell membranes and unpigmented content. Additionally, the presence of amaranth flour may increase the number of small spaces and gaps between muscle fibers, as the flour particles may interfere with the arrangement of the fibers during cooking. Microscopic analysis may reveal the presence of amaranth flour particles within the meat matrix, as well as changes in the organization and spacing of the muscle fibers. Furthermore, the addition of flour may alter the color and appearance of the meat, as the flour particles may absorb or reflect light differently than the meat tissue [44,45].

Overall, the microstructural changes that occur when mixing ground meat with amaranth flour may have implications for the texture, nutritional content, and sensory properties of the final product. Further research is needed to fully understand the impact of different types and levels of amaranth flour on the microstructure and quality of meat products.

In the histological preparation, fragments of connective tissue and large and small particles of amaranth flour were observed among loosely arranged muscle fibers. The nuclei of the plant additive cells were round and significantly larger than the nuclei of muscle fibers and connective tissue cells. The nuclei of muscle fibers and connective tissue cells were poorly visible compared to the nuclei of plant tissue cells when viewed under a x4 objective lens.

Sensory evaluation

The evaluation results of the sensory characteristics by the taste panel showed the highest score for variant 3 with the addition of 10% amaranth flour. In this variant, consistency, aroma, taste, and appearance of the meat patties were rated the highest compared to the other variants (Figure 2).



Control | Meat patties with 10% of amaranth flour

Figure 1. Microstructure of meat patties

Overall, variants 1, 2, and 3 were positively evaluated by the tasters, with minor differences. The lowest score was recorded for variant 4 (15% amaranth flour in the composition). This variant showed crumbliness, deterioration in taste, the appearance of a pronounced odor not typical for meat patties, and a disruption in consistency. The results of the sensory analysis indicate that adding up to 10% amaranth flour to the composition of meat patties does not lead to significant losses in quality and appearance.

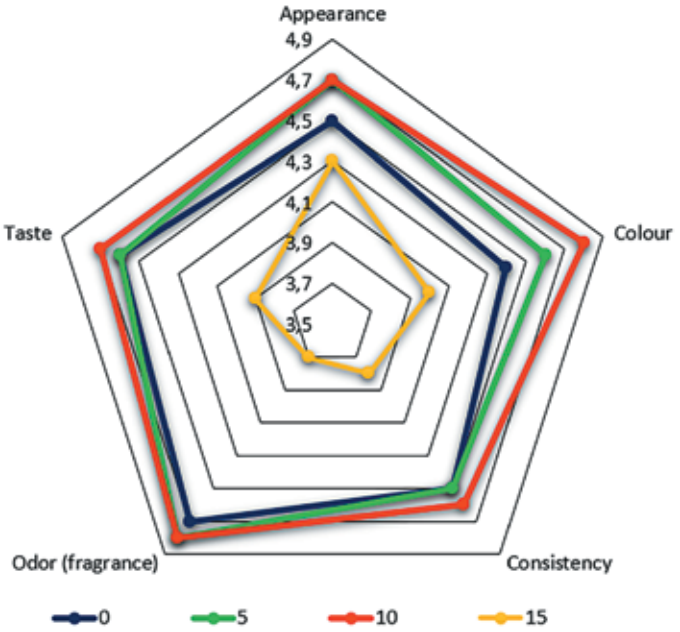


Figure 2. Sensory score diagram of meat patties

Behailu *et al.* [43] reported that the addition of the addition of soybean and finger millet flours to the beef sausage product enhanced the sensory quality of the sausage. The sausage products were generally accepted, and 20% soy and millet flour inclusion was "liked very much" [43]. In the study conducted by Muchekeza *et al.* [46] indicated no significant differences in the color of the sausages made with amaranth, quinoa, and corn-starch binder flours.

Determination of pH and water-holding capacity

The pH of meat is an important characteristic that influences product quality. In this study, pH values of meat patties were investigated after adding different concentrations of amaranth flour. The control sample without amaranth flour had a pH value of 6.14, which is in line with the normal pH range for meat products (Figure 3). The experimental samples with amaranth flour showed a slight decrease in pH as the concentration of amaranth flour increased. The sample with 5% amaranth flour had a pH of 6.10, while the samples with 10% amaranth flour had a pH of 6.10%.

The lowest pH value was observed in the sample with the highest concentration of amaranth flour (15%), which had a pH value of 6.0. This result suggests that the addition of amaranth flour to meat patties can lead to a slight decrease in pH, which may be attributed to the acidity of amaranth flour. It is important to note that the observed differences in pH values were relatively small and may not have a significant impact on the overall quality of the product.

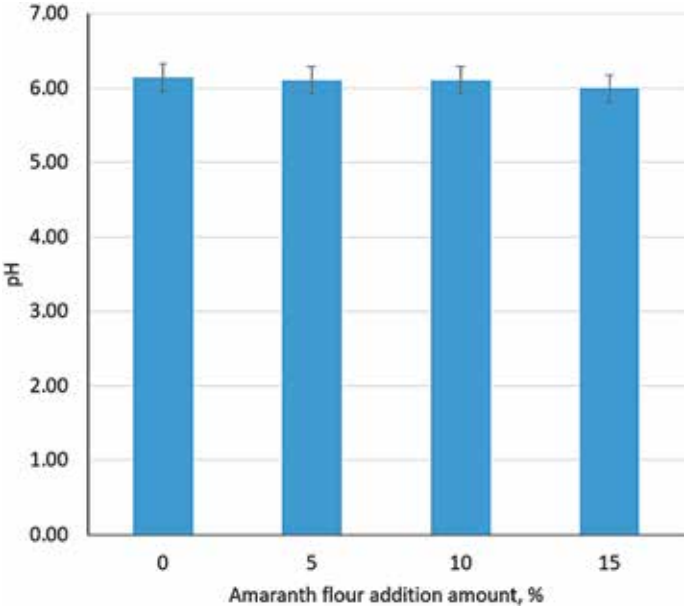


Figure 3. pH of meat patties

Water-holding capacity is a key characteristic of meat products, as it affects juiciness, texture, and overall quality. The results indicate that the addition of amaranth flour led to an increase in the water-holding capacity compared to the control sample. The water-binding capacity of the control sample and experimental samples with 5% and 10% of amaranth flour was 68.54%, 71.24%, and 74.58%, respectively (Figure 4). The maximum water-binding capacity was observed in the sample with 15% amaranth flour, which was 81.21%. These findings suggest that amaranth flour has the potential to improve the water-binding capacity of meat products. Ostoja *et al.* [47] found that the use of crude amaranth seed grit improved the ability of meat-fat batter to hold water, resulting in decreased cooking losses of canned meat after pasteurization or sterilization.

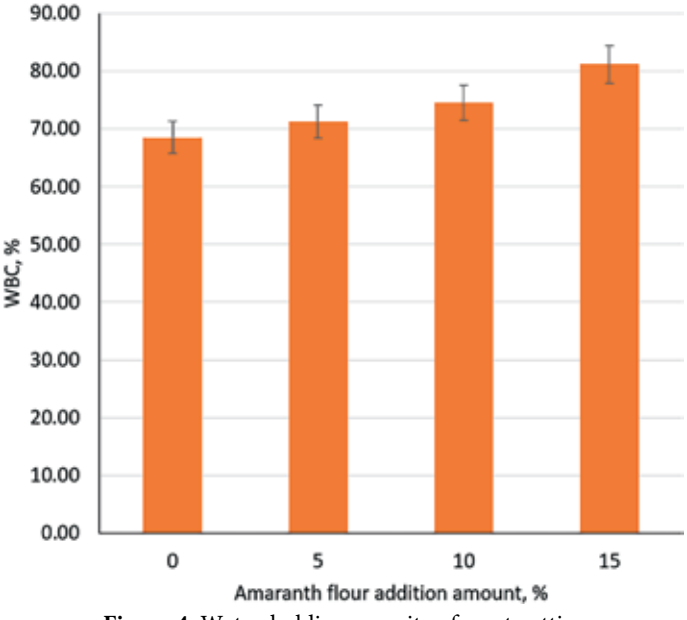


Figure 4. Water-holding capacity of meat patties

The mechanism behind this effect is likely due to the unique properties of amaranth flour, which contains high levels of soluble fiber and protein. Soluble fiber has been shown to increase water-binding capacity by forming gels that trap water molecules, while protein can contribute to the formation of a stable emulsion that helps retain water [48,49].

Conclusion

Based on the research findings, it can be concluded that the addition of amaranth flour to meat patties can lead to significant improvements in their nutritional and sensory properties. The chemical composition of the patties was affected, with a decrease in moisture and slight increases in ash and fat observed in the experimental samples. The addition of amaranth flour also led to a significant increase in the content of important minerals such as calcium, potassium, magnesium, phosphorus, iron, manganese, and copper. The sensory evaluation of the patties showed that the addition of 10% amaranth flour resulted in the highest score for overall sensory characteristics. Furthermore, the incorporation of amaranth flour resulted in an enhanced water-binding capacity of the meat patties compared to the control sample. Overall, these findings suggest that using amaranth flour in meat patties formulation could be a promising strategy for improving their nutritional value and sensory quality.

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HISTOLOGICAL CHARACTERISTICS AND FUNCTIONAL PROPERTIES OF RED AND WHITE PARTS OF M. *SEMITENDINOSUS* OF SLAUGHTER PIGS

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Keywords: *meat quality, meat defects, PSE, hypercontraction, m. semitendinosus, type of muscle tissues, histology*

Abstract

A unique muscle of pigs (Sus scrofa domesticus) is m. semitendinosus, which contains the "red" (dark) part located mainly in the depth of the leg cut and the "white" (light) part located in the close proximity to the subcutaneous fat layer. Differences in the characteristics of its "red" and "white" parts can exert a significant effect on quality and economic indicators of meat products. The aim of this research was to study histological features of the microstructure and technological properties of muscle tissue from different parts of m. semitendinosus, obtained from slaughter pigs of Russian production. M. semitendinosus was excised from chilled porcine carcasses (N=20) 24 hours after slaughter in the process of deboning. Histological examination showed that the dark part of the muscle was characterized by a higher package density of fibers, higher number of capillaries and higher sarcomere length. On the contrary, the light part was characterized by a higher diameter of muscle fibers. Analysis of muscle fiber types showed that the proportion of type I, intermediate and type IIb fibers was higher by 9.3, 5.2 and 4.1%, respectively, in the dark part. Significant differences between the dark and light parts of m. semitendinosus were revealed in terms of the number and size of giant fibers: the light part was characterized by a larger number (by more than 5 times) of giant fibers with the fibers of a larger size (almost by 11%). The samples of minced meat from the dark and light parts showed significant ($p<0.05$) differences in the mean values of lightness, redness and yellowness (L^*, a^* and b^*) by 6.00, 4.68 and 3.01 units, respectively, in raw samples, and by 6.53, 2.99 and 1.81, respectively, after curing with the nitrite mixture and cooking ($p<0.05$). The dark part of m. semitendinosus had higher pH values ($p<0.05$) both for raw and cooked samples. The consistency of the samples from the light part was less elastic, looser and more crumbly than that in the samples produced from the dark part of m. semitendinosus, which was confirmed by the structural-mechanical investigations. Therefore, this study showed significant differences between the dark and light parts of m. semitendinosus by microstructural and functional-technological characteristics. Significant variability by muscle fiber diameter, which was observed in the light part of this muscle, apparently should be taken into account in breeding work and quality assessment of pork from slaughter animals.

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Introduction

Two quality defects of meat are widely known in the meat industry: pale, soft and exudative (PSE) and dark, firm and dry (DFD) meat. DFD meat has the dark red or red color and pH values higher than 6.0. On the contrary, PSE meat has the pale pink color and pH values lower than 5.6. It is traditionally believed that the PSE defect is typical of "white" muscles with the high content of white glycolytic fibers (IIb type). At the same time, "white" muscles are more prone to the development in the process of autolysis of the so-called "giant" fibers emerging mainly as a result of hypercontraction of IIb type muscle fibers [1]. "Red" muscles, which have a significantly lower content of such fibers, are distinguished, as a rule, by a high pH value after rigor mortis and high uniformity of muscle fibers by diameter. Muscles that are different in color usually have different metabolism: "red" muscles are characterized by aerobic metabolism and "white" muscles by anaerobic metabolism. Prominent examples of "white" and "red" muscles in pigs are *m. longissimus dorsi* and *m. masseter*, respectively [2].

Skeletal muscles of mammals, birds and fish demonstrate a great variety of shapes, sizes, anatomic location and physiological functions. Certain muscles of slaughter animals can simultaneously have different types of metabolism, various physiological functions and can show different ultimate result of meat quality formation during ageing after slaughter [3]. Such a unique muscle in pigs (*Sus scrofa domesticus*) is *m. semitendinosus*, which contains the "red"

(dark) part located mainly in the depth of the leg cut (ham) and the "white" (light) part located in the close proximity to the subcutaneous fat layer. However, both light and dark parts constitute a single muscle having the same origin and location [3,4].

Characteristics of muscle tissue are of fundamental importance for meat and meat product quality. Producers, distributors and consumers impose various and specific requirements for quality that depend on the use of products [3]. The leg cut of pork carcass is a valuable raw material for production of expensive delicacy products such as cooked, cooked-smoked, raw smoked and raw air dried hams and other meat products from whole muscles (group of muscles), for which color uniformity is an important characteristic of production quality [5]. The presence of PSE meat (PSE zones) in ham also negatively affects sensory characteristics such as consistency, juiciness, aroma and taste [3]. For processors, the best technological qualities of pork are always associated with low losses in production of the final product, including upon cold storage, cutting, cooking and other operations. Due to the low water holding capacity of PSE pork, its processing leads to a significant decrease in the final product yield and an increase in the risks of manufacturing products with low quality or defects [3,6].

The structure and composition of skeletal muscles and, consequently, the technological properties of pork are influenced by many factors *in vivo* and *post mortem*, such as species and genotypes of animals, nutritional and environmental factors, conditions of slaughter and primary processing of carcasses. As there are a large number of such factors, their interaction with meat quality is not always clear due to their mutual influence [3]. Studying this interrelation is expedient when undesirable reduction of certain characteristics of raw materials and their effect on meat technological properties were established.

In the Russian Federation, fast growing hybrid animals from three-breed crossing (LWxDxL) are mainly used for growing pigs for slaughter [7]. Earlier, we studied manifestation of myopathic changes, which are typical of PSE meat and emerge under an effect of lifetime and slaughter factors, in the microstructure of *m. longissimus dorsi* taken from slaughtered hybrid animals [8,9]. This muscle is the most frequently chosen object of investigations and its characteristics are studied quite well. Several foreign studies aimed at investigation of *m. semitendinosus* from regional phenotypes of animals showed significant differences in characteristics of "red" and "white" parts and their impact on quality and economic indicators of meat products. The number of studies dedicated to pork quality carried out on the samples of *m. semitendinosus* has been increasing over the last years [10–14]. There are only few studies aimed to investigate the microstructure of "red" and "white" parts of *m. semitendinosus*, as well as a possible effect of special features of growing, transportation and slaughter of pigs in Russia on quality of muscles from the leg cut. Therefore, the aim of this study was to investigate the histological characteristics and technological properties of muscle tissue of the dark and light parts of *m. semitendinosus* obtained from slaughter pigs of Russian production.

Objects and methods

Objects of research

Objects of research were *m. semitendinosus* excised from chilled porcine carcasses 24 hours after slaughter in the process of deboning of leg cuts.

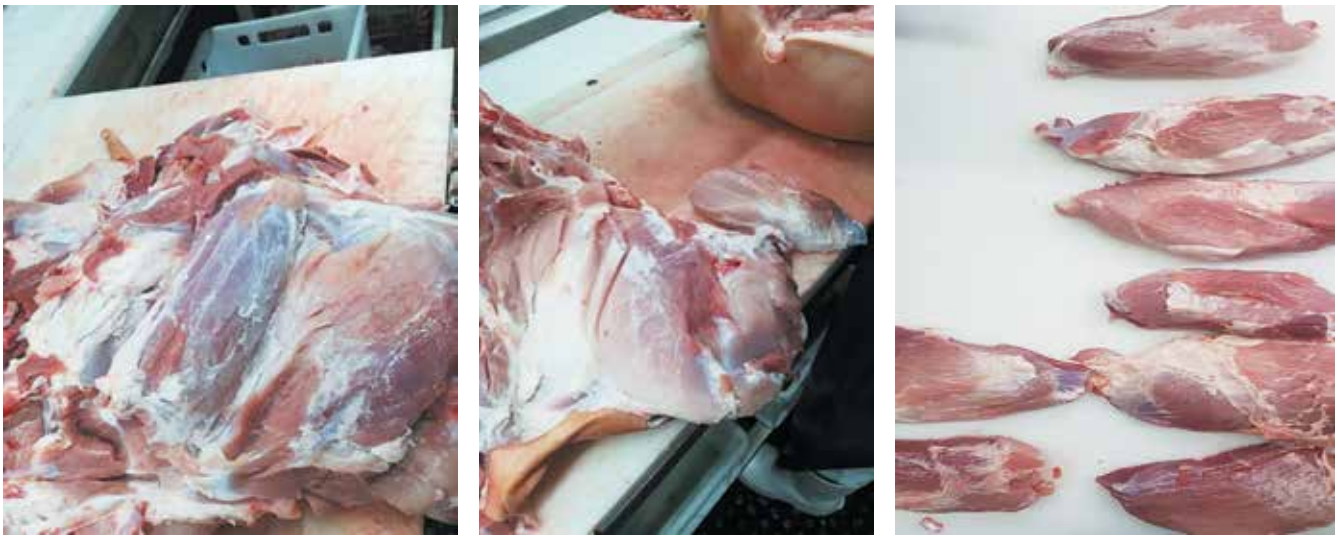
Sampling of muscle tissue was carried out in an industrial enterprise that slaughtered hybrid pigs (LWxDxL) in the quantity of 800 heads/day. Immediately after slaughter, 60 hot porcine carcasses with a weight of 85 ± 3 kg were taken for investigations. Twenty carcasses were randomly selected from 60 chilled porcine carcasses 24 hours after slaughter before cutting and deboning. Their leg cuts were sent to deboning to excise *m. semitendinosus*. To this end, a leg cut was opened by cutting intermuscular connective tissue interlayers with a knife providing access to *m. semitendinosus* (Figure 1a). Then, *m. semitendinosus* was excised without compromising muscle integrity (Figure 1b). After the muscle was excised, it was additionally cleaned from adjacent tissues (Figure 1c). Two muscles were excised from each carcass from the left and right leg parts, respectively.

When sampling, each muscle was cut in half for visibility of the boundary between the dark and light parts (Figure 2a). To this end, a knife was moved at first through "red" meat and then through "white" meat. After that, pieces with a size of about (2.5–3.0) × (2.5–3.0) × (2.5–3.5) cm were cut out from the dark and light parts of each sample (Figure 2b) for histological investigations. The rest of flesh was divided into "red" and "white" meat and minced by forcing meat through a grinder plate with 3 mm holes. The obtained minced meat (Figure 2c) was used for investigations of the functional-technological characteristics.

Histological investigations

To study the microstructure, muscle tissue samples taken from the dark and light parts of each muscle were fixed in the 10% neutral buffered formalin solution (BioVitrum, Russia) for 72 hours at room temperature (21 ± 1 °C). Two pieces (1.5 × 1.5 × 0.5 cm) with the longitudinal and cross orientation of muscle fibers were taken from each sample for the following study. The pieces were washed with cold running water for four hours and, after that, they were embedded in gelatin solutions (AppliChem GMBH, Germany) in an ascending concentration (12.5%, 25%) at a temperature of 37 ± 1 °C for 8 hours in each solution using a thermostat TS-1/20 SPU (Smolensk SKTB-SPU, Russia).

Preparation of serial sections with a thickness of 16 μm was carried out on a cryostat «MIKROM–HM525» (Thermo Scientific, USA). Three sections were made from each piece. The prepared sections were mounted on Menzel-



a) b) c)

Figure 1. Sampling during deboning of the leg cut:
a) opening the cut to excise *m. semitendinosus* (the oval marks the location of the muscle);
b) the leg cut after excision of *m. semitendinosus*; c) samples of *m. semitendinosus* after cleaning from adjacent tissues

a) b) c)

Figure 2. Sampling: a) appearance of cut *m. semitendinosus* (a boundary between the dark and light parts is well seen);
b) pieces cut out from the dark and light parts of *m. semitendinosus* for histological investigations;
c) appearance of minced meat obtained from meat of the dark and light parts of *m. semitendinosus*

Glaser slides (Thermo Scientific, USA) and stained with Ehrlich's hematoxylin and 1% aqueous-alcoholic solution of eosin (BioVitrum, Russia) by the conventional method [15]. Investigation of the histological preparations and their photographing were carried out using an Axio Imager A1 light microscope (Carl Zeiss, Germany) with the connected AxioCam MRc-5 camera (Carl Zeiss, Germany).

Morphometric investigations were carried out using the image analysis system AxioVision 4.7.1.0 (Carl Zeiss, Germany). The general scheme of the investigations corresponded to the methodology published earlier [9]. The diameter of muscle fibers, sarcomere length, and cross-sectional area of giant fibers were measured in the interactive mode. No less than 100 objects were calculated for each section. A fiber diameter was measured with an accuracy of ± 1.0 μm. A sarcomere length was determined with an accuracy of ± 0.1 μm. In addition, the number of giant fibers/1 cm^2 of the section and packing density of muscle fibers / 1 mm^2 were also calculated.

When studying cross sections of histological preparations, investigations were performed to determine a shape of muscle fibers, their packing density, condition of nuclei, thickness and condition of connective tissue layers, as well as to reveal giant fibers. In longitudinal sections, investigations were aimed to determining a condition and shape of muscle fibers, condition of sarcolemma, presence of striation (cross-striation and longitudinal striation), presence of destructive changes (ruptures, cracks, fragmentation) as well as to revealing knots of hyper-contraction.

To determine muscle fiber types, sections with cross orientation of muscle fibers were additionally stained with Sudan B according to MR001–00496254/00419779–2021[1] and PAS reaction (in Shabadash's modification) was performed

[1] MR001–00496254/00419779–2021. Performance of histological investigations on determination of myopathy. Approved by the director of L. K. Ernst Federal Science Center for Animal Husbandry N. A. Zinovieva and the director of V. M. Gorbatov Federal Research Center for Food Systems of RAS O. A. Kuznetsova, 2021, Moscow, 11 p.

according to [15]. Determination of muscle tissue types, their diameter and packing density was carried out.

Investigation of the functional-technological characteristics

Minced samples of *m. semitendinosus* obtained from its dark and light parts were investigated by the following methods:

— pH measurement by the potentiometric method using a pH-meter Testo 205 (Testo, Germany) with a measurement error of ±0.02; measurement was carried out in raw and cooked minced meat (minced meat was cooked as described below);

— determination of color characteristics CIELab — lightness (L*), redness (a*) and yellowness (b*) — with a spectrocolorimeter Konika Minolta CM-2300d (Konika Minolta, Japan). Before investigation, a spectrophotometer was calibrated using standard white and black plates;

— cooking losses in minced meat: before thermal treatment, 2% of nitrite salt with the a mass faction of sodium nitrite of 0.4% was added and mixed, cured samples with a weight of 100 g were placed into polymer packages, polymer packages were tightly tied, then packages with minced meat were placed into a water bath with an initial water temperature of 78 ± 1 °C. Thermal treatment was carried out at 76 ± 1 °C until reaching a temperature of 75 ± 1 °C in the geometrical center of a sample. After that, a package was opened and a moisture was removed; a piece of minced meat was placed on filter paper for chilling and draining. After chilling samples to room temperature, the samples were weighed. Weight losses were calculated in% to the initial weight of samples by a difference between weights before and after thermal treatment;

— structural-mechanical characteristics were determined using a texture analyzer "Structurometr ST-2" (Quality laboratory, Russia). For each test, a test portion with a size of no less than 2.5 × 2.5 × 3.9 cm was cut out from the core of the cooked sample; the test portion was placed in the testing field of a texture analyzer and subjected to compression between the lower unmovable platform and Bloom indenter fixed on the upper movable platform. Load force was registered upon introducing the indenter into the test portion to a depth of 20 mm at its speed of movement (introduction) of 1.0 mm/s, after touch force of 7 g, using the software of the texture analyzer. The maximum value of load force on the indenter expressed in grams was taken as a result of the test.

All investigations of functional-technological characteristics were carried out in triplicate.

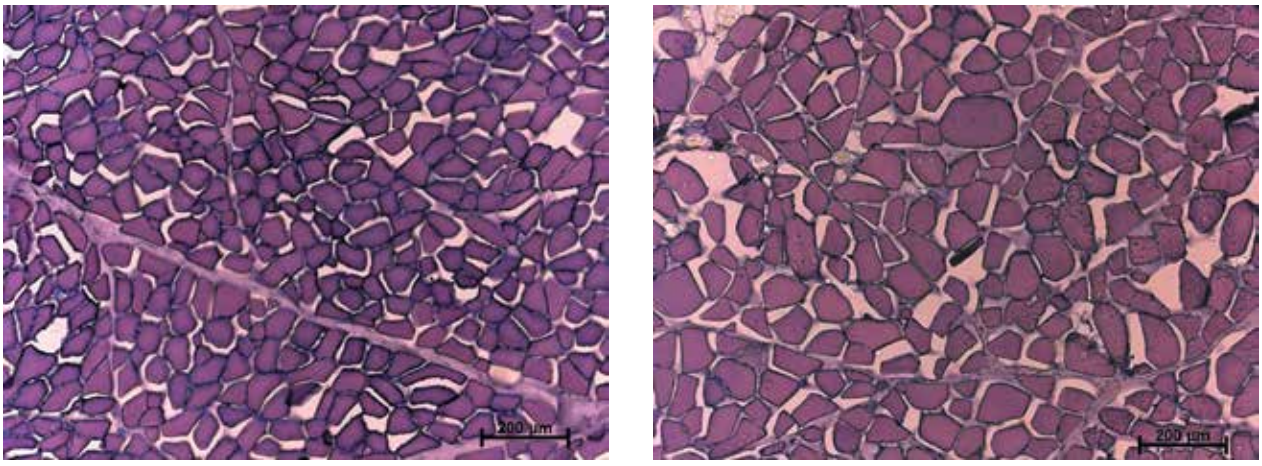
Statistical analysis

Statistical analysis of the experimental data was carried out using the software R (version 4.3.0). Quantitative data are presented as the arithmetic mean (Mean), standard deviation (SD), standard error of the mean (SE), minimum and maximum values (Min/Max), interquartile range (P 25/75), confidence interval (CI) и median. The Kolmogorov–Smirnov test was applied to assess the normality of distribution of parameters of quantitative variables. The coefficient of variation (CV) was used as the main method for assessing parameters of distribution. Correlation between indicators of muscle fibers and data of standard investigations was evaluated by the Pearson parametric method. Differences were considered significant and the presence of a relationship between parameters was recognized at a probability level of not higher than 0.05.

Results and discussion

Results of the histological investigations

All studied samples both from the dark part and from the light part of *m. semitendinosus* (Figure 3) exhibited the uniform condition of muscle tissue. On the cross section, muscle fibers had the polygonal or weakly round shape. The interlayers of the endomysium were well defined; the boundaries between individual muscle fibers were revealed without major difficulties. Giant fibers with a round-oval shape and large diameter were observed.



a) b)

Figure 3. Typical microstructure of the samples of the dark (a) and light (b) parts of *m. semitendinosus* (cross section, staining with hematoxylin and eosin; magnification: 10 x, scale bar = 200 µm)

In the longitudinal section, most muscle fibers showed clearly defined cross-striation and straightened shape. Individual wavy fibers with longitudinal striation were revealed, which pointed to the presence of contraction zones.

The nuclei in muscle fibers were well stained, had the oval shape and were located directly under the sarcolemma.

The connective tissue interlayers of the perimysium were wavy, tightly adjacent to the bundles of muscle fibers. The nuclei in the connective tissue interlayers were clearly seen in the histological preparations. Individual adipocytes or their small groups having the typical histological structure were found between the bundles of muscle fibers in the areas of the perimysium.

The functional condition of muscle tissue in each group of samples was quite uniform. Sporadic cross microcracks and ruptures of sarcolemma were noticed. Destruction of myofibrils, multiple ruptures and fragmentation of "normal" muscle fibers were not found. Destruction of sarcomeres and appearance of cracks and ruptures of fibers were observed in the knots of hyper-contraction (giant fibers on the longitudinal section).

Statistical processing of the morphometry results for the samples taken from the dark and light parts of *m. semitendinosus* is presented in Tables 1–3.

The dark part of the muscle was characterized by a higher packing density of fibers on the cross section, higher number of capillaries and sarcomere length. It is necessary to note that the sarcomere length is interrelated in a complicated way with biochemical reactions of proteolysis and metabolism of glycogen. The sarcomere length also influences the palatability of prepared meat and water holding capacity of meat products [16].

On the contrary, the light part was characterized by a higher diameter of muscle fibers with higher variability of this indicator, although high variability of this indicator (CV higher than 20%) was also observed in the dark part of the muscle. All differences by the main morphometric indicators were significant ($p < 0.05$).

Figure 4 presents a histogram and "heat map" for the distribution of muscle fibers of the dark and light parts of *m. semitendinosus* created for each of the studied samples (N = 20). The character of distribution of muscle fibers by diameter in the dark and light parts of *m. semitendinosus* differed in all samples. Comparison of the histogram data (Figures 4a, 4b) showed that the scatter by fiber diameter was shifted to the left and a range of variability was less pronounced in the dark part compared to the samples from the light part of the muscle. Such a narrow range of variability of the attribute can be associated with a lower effect of the internal and external factors on the muscle tissue condition before and after animal slaughter.

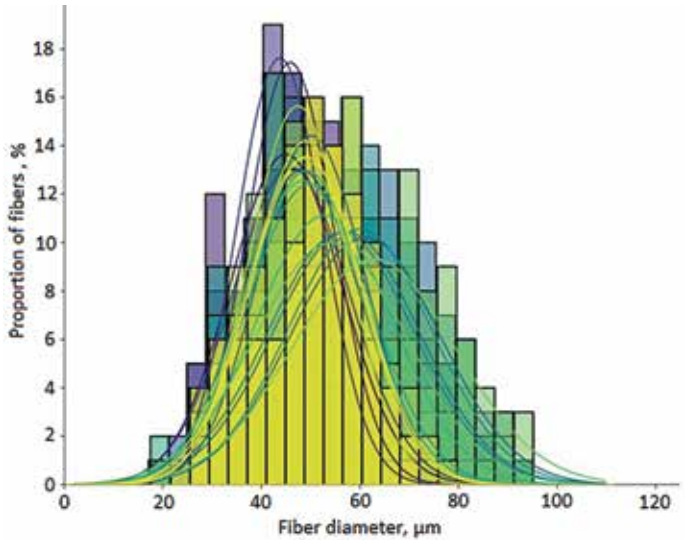
For pork, fibers with a lower diameter are especially desirable as they exert a beneficial effect on its quality and are considered an indicator of its tender structure [17]. An increase in the muscle fiber diameter reduces tenderness and water holding properties of meat [18]. Therefore, the dark part of the muscle represented by muscle fibers of lower diameter is more desirable in terms of technological characteristics.

The composition of muscle fibers is the main criterion for classifying muscles as "red" and "white". The study of the composition of muscle fibers allows predicting biochemical changes in muscle tissue and, consequently, meat quality as the rate of post mortem metabolism depends on a ratio between quantities of muscle fibers of different types (oxidative and glycolytic) [11].

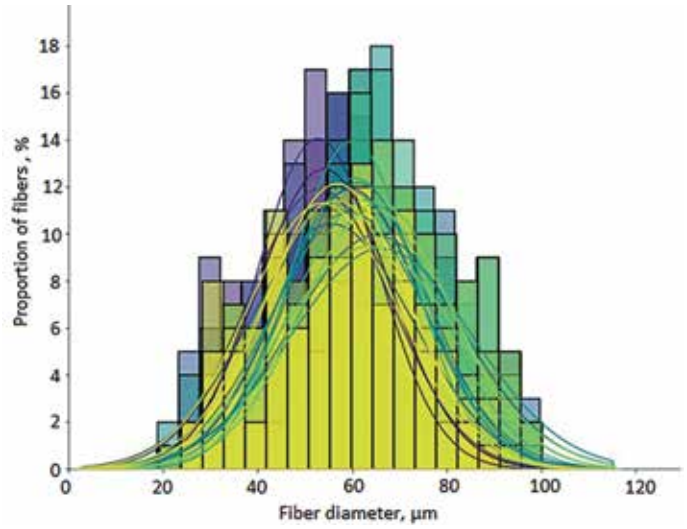
Investigation of muscle fiber types (Table 2) in the *m. semitendinosus* samples showed that the proportion of type I, intermediate and type IIb fibers was higher by 9.3, 5.2 and 4.1%, respectively, in the dark part.

Table 1. Results of the statistical processing of the main morphometric characteristics of muscle tissue in the dark and light parts of *m. semitendinosus*

Statistical indicator	Value of the statistical indicator for			
	fiber diameter, μm	fiber density fibers/mm^2	sarcomere length, μm	Number of capillaries, capillaries/mm^2
Dark part				
Mean (Mean ± SE)	51.17 ± 0.31	273 ± 6	2.81 ± 0.03	34.0 ± 0.3
Min/Max	17.00/94.50	162/359	2.15/3.58	30/39
Interquartile range (P25/75)	41.50/60.11	239/310	2.58/3.06	33/36
Median	50.56	295	2.78	35
Confidence interval (CI)	0.60	12.80	0.07	0.59
Coefficient of variation (CV), %	26.79	18.49	12.20	6.80
Light part				
Mean (Mean ± SE)	59.54 ± 0.36	190 ± 4	2.12 ± 0.02	29.7 ± 0.4
Min/Max	18.43/99.37	128/253	1.74/2.88	24/38
Interquartile range (P25/75)	48.30/70.90	168/215	1.96/2.30	28/31
Median	60.20	188	2.01	30
Confidence interval (CI)	0.71	7.56	0.05	0.77
Coefficient of variation (CV), %	27.33	15.72	11.10	10.21



a)



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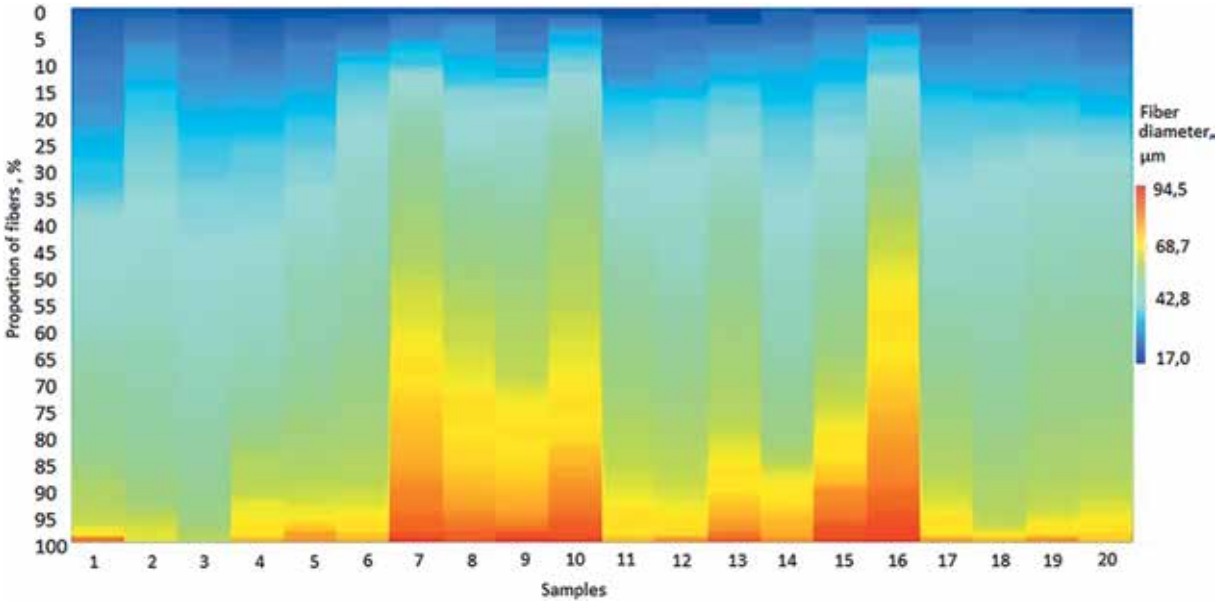

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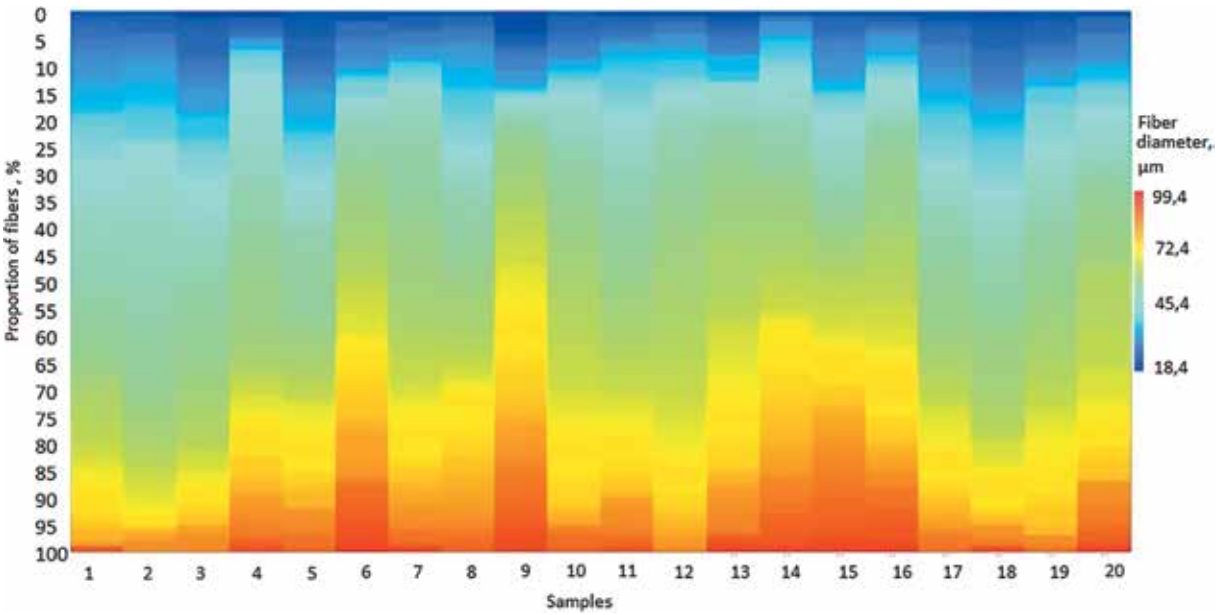

d)

Figure 4. Distribution (a, b — histogram, c, d — "heat map") of muscle fibers by diameter in the samples of *m. semitendinosus*: a, c — dark part and b, d — light part

Table 2. Differences in the dark and light parts of *m. semitendinosus* by muscle fiber types

Statistical indicator	Value of the indicator for			
	Dark part		Light part	
	Proportion, %	Diameter, μm	Proportion, %	Diameter, μm
Type I (oxidative) muscle fibers				
Mean (Mean ± SE)	19.4 ± 1.0	32.69 ± 0.24	10.1 ± 0.6	30.79 ± 0.27
Min/Max	11.0/25.0	17.01/43.26	5.0/16.0	18.44/38.77
Interquartile range (P25/75)	14.8/23.0	29.61/36.41	8.0/11.25	28.30/33.60
Median	20.5	33.35	10.0	30.87
Confidence interval (CI)	1.98	0.48	1.20	0.53
Coefficient of variation (CV), %	23.31	14.74	27.05	12.38
Type IIa (intermediate) muscle fibers				
Mean (Mean ± SE)	15.8 ± 1.1	42.35 ± 0.14	11.0 ± 1.0	41.32 ± 0.20
Min/Max	8.0/25.0	36.12/48.92	5.0/17.0	34.06/48.08
Interquartile range (P25/75)	0.0/0.0	45.92/43.98	6.8/15.3	39.09/43.72
Median	15.00	42.44	10.0	41.70
Confidence interval (CI)	2.19	0.27	2.01	0.40
Coefficient of variation (CV), %	31.77	5.74	41.85	7.32
Type IIb (glycolytic) muscle fibers				
Mean (Mean ± SE)	64.9 ± 2.0	58.82 ± 0.28	79.0 ± 1.5	65.75 ± 0.30
Min/Max	52.0/81.0	42.46/94.50	67.0/89.0	44.23/99.37
Interquartile range (P25/75)	57.0/74.0	51.04/64.96	73.5/84.3	56.42/73.98
Median	64.5	56.89	80.5	64.34
Confidence interval (CI)	3.91	0.55	2.87	0.59
Coefficient of variation (CV), %	13.74	17.17	8.29	18.13

Type I and intermediate type fibers did not have significant differences in terms of diameter in the samples from the dark and light parts. At the same time, light muscle tissue demonstrated not only the relatively high content of IIb type fibers but also their larger size in terms of diameter (by about 12%).

Significant variability in the proportion of type I muscle fibers was observed both in the dark part and in the light part of the muscle (CV was 23.31 and 27.05%, respectively). Even higher variability (CV more than 30%) was revealed for intermediate (IIa) type fibers. With that, CV of this indicator in the light part of *m. semitendinosus* was more than 10% higher than the corresponding value in the dark part, which suggests heterogeneity of the muscle tissue samples from the light part of the muscle by the number of type IIa muscle fibers. On the contrary, moderate variability with regard to the proportion of IIb muscle types was observed both in the samples from the dark part and light part of the muscle. However, the light part was characterized by a higher value of the CV coefficient than the dark part (18.13 and 13.74%, respectively).

These differences are also clearly seen on the histograms of distribution of muscle fibers of different types by diameter that were built using the results of data analysis for each sample (Figure 5). Fiber diameters varied in all samples depending on their type; with that, the maximum individual variability was observed in the dark and light parts of the muscle for the diameter of type IIb fibers.

Difference in the ratio of muscle fiber types can be the main reason for different meat quality, including color and its stability, pH, water holding capacity. It is known that even the high content of glycogen in dark skeletal muscles of the oxidative type does not lead to the sharp post mortem drop in pH due to the low content (or absence) of

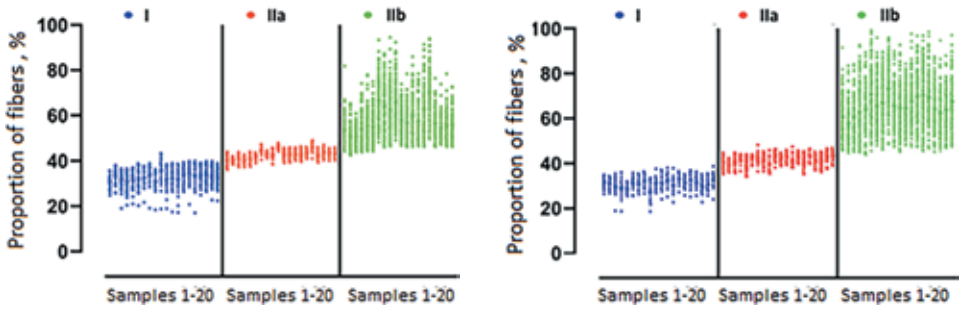

Figure 5. Distribution of muscle fibers by diameter in the samples of *m. semitendinosus*: a — dark part, b — light part

fibers of the glycolytic type [2]. With that, it is type I fibers that are considered to be linked with the high quality of pork [11,19].

The development of giant fibers is regarded as one of significant reasons for a decrease in the technological quality of pork. These structural abnormalities are linked with the fast growth of muscles and stress loads. Hypercontraction (giant fibers) is usually accompanied by a decrease in the capillary density and myoplasmic calcium loading. Typically, anaerobic fast-twitch muscles with low lactate metabolism but accelerated onset of rigor mortis are most prone to the development of giant fibers [20].

In our study, significant differences were established between the dark and light parts of *m. semitendinosus* by the quantity and size of giant fibers (Table 3).

As can be seen from Table 3, the light part was characterized by a larger quantity (more than 5 times) of giant fibers with the fibers of a larger size (almost by 11%). The high variability of the quantity of giant fibers (84.14%) in the dark part of the muscle apparently suggests that the presence of a significant quantity of giant fibers is not typical of the dark part: giant fibers were revealed only in three samples (5–6 fibers/cm^2 each). In the light part, the quantity of giant fibers was up to 20 fibers/cm^2. With that, the giant fibers in the light part of the muscle were characterized by an insignificant variability of sizes (CV < 20%). Appearance of giant fibers in the porcine muscle tissue due to the enhanced and long post mortem glycolysis is closely linked with the PSE defect [21,22] and suggests that the light part of *m. semitendinosus* is more prone to show poor functional-technological characteristics.

Results of the study of the functional-technological characteristics

An effect of histological indicators on pork quality is underestimated. Various studies continue to reveal interrelation between the microstructure of muscle tissue and functional-technological indicators, such as pH, cooking losses, sensory tenderness, color and others [23].

Meat color is one of the most important indicators of meat quality that influences a desire to consume it [24]. The color of the final product remains to be a criterion of freshness and quality for consumers, as well as a stimulus to make a buying decision [5]. The minced meat samples made from the dark part and light part of *m. semitendinosus* had significant differences in color both in the raw (Figure 2c) and in the cooked (Figure 6) state.

Results of the instrumental determination of color (Table 4) confirmed color differences between the samples in the raw and cooked state. In the raw samples, significant ($p<0.05$) differences were recorded in the mean values of lightness, redness and yellowness by 6.00, 4.68 and 3.01 units, respectively. Color differences (by 6.53, 2.99 and 1.8, respectively) also retained in the cooked samples after chilling for mean values of L*, a* и b* ($p<0.05$).

Table 3. Quantity of giant fibers and their area in the dark and light parts of *m. semitendinosus*

Statistical indicator	Value of the indicator for			
	Dark part		Light part	
	Quantity, fibers/cm^2	Area, μm^2	Quantity, fibers/cm^2	Area, μm^2
Mean (Mean ± SE)	2.1 ± 0.4	13,415.74 ± 398.18	11.9 ± 1.0	15,062.39 ± 190.53
Min/Max	0.0/6.0	7,621.51/20,102.66	5.0/20.0	9,233.93/23,737.56
Interquartile range (P25/75)	1.0/2.8	11,306.31/14,906.29	9.0/14.0	12,952.09/16,807.18
Median	2.0	13,051.34	12.5	14,947.44
Confidence interval (CI)	0.80	644.10	0.19	369.99
Coefficient of variation (CV), %	84.14	20.35	36.59	18.50

a) b)

Figure 6. Cooked minced meat samples from the dark and light parts of *m. Semitendinosus*:
a) appearance in packages immediately after thermal treatment; b) appearance on the cut surface after chilling
(left – samples from the dark part; right – samples from the light part of the muscle)

Table 4. Color characteristics of the *m. semitendinosus* samples in the raw and cooked state

Indicator	Value of the indicator for					
	Dark part			Light part		
	L	a*	b*	L	a*	b*
Raw minced meat						
Mean (Mean ± SE)	57.76 ± 0.80	7.92 ± 0.25	20.34 ± 0.14	63.76 ± 0.40	3.24 ± 0.17	17.33 ± 0.32
Min/Max	49.90/60.96	6.65/10.06	19.22/21.25	61.00/67.97	2.08/4.49	14.49/19.37
Interquartile range (P25/75)	56.22/60.23	7.17/8.31	19.99/20.88	62.38/64.90	2.58/3.83	16.14/18.66
Median	59.15	7.69	20.38	63.29	3.35	17.44
Confidence interval (CI)	1.56	0.48	0.28	0.79	0.32	0.64
Coefficient of variation (CV), %	6.02	13.49	3.01	2.74	22.28	8.15
Cooked minced meat						
Mean (Mean ± SE)	69.61 ± 0.39	8.08 ± 0.07	10.05 ± 0.10	76.44 ± 0.51	5.09 ± 0.17	11.86 ± 0.10
Min/Max	67.28/70.99	7.84/8.63	9.50/10.47	74.49/79.57	4.07/5.69	11.52/12.84
Interquartile range (P25/75)	69.29/70.52	7.89/8.15	9.78/10.30	75.39/76.89	4.53/5.61	11.66/11.94
Median	70.02	8.04	10.20	76.02	5.32	11.74
Confidence interval (CI)	0.76	0.14	0.20	1.01	0.34	0.2
Coefficient of variation (CV), %	1.93	3.11	3.51	2.33	11.65	2.96

Color differences between the minced meat samples point to the different content of muscle pigments in the dark and light parts [24] of *m. semitendinosus* and different amounts of nitrosomyoglobin formed upon curing with nitrite salt.

The highest variability of color characteristics of the samples was observed in raw minced meat for the redness values. On the contrary, the cooked samples were characterized by insignificant variability of color (CV from 1.93 to 3.51 excluding redness (11.65) for the samples from the light part of *m. semitendinosus*).

When processing meat, it is extremely important to reveal raw materials with lower technological quality. Measuring pH values is the main and the most often used criterion of quality [25,26].

Measurement of pH and statistical processing of the results (Table 5) show that the dark part of *m. semitendinosus* was characterized by higher pH values; with that, differences in pH between the dark and light parts were significant ($p < 0.05$) both for the raw and cooked samples.

It is believed that a pH range at 24 hours after slaughter of 5.7 ~ 6.1 is the most suitable for consumers and processors [26]. As can be seen from Table 5, the minimum values of pH of raw meat both in the dark and light samples were higher than 5.7, which indicated the absence of the PSE defect. For the samples from the light part of *m. semitendinosus*, the mean and maximum pH values were lower than 6.1, which is characteristic for pork with the normal course of autolysis. On the contrary, the samples from the dark part of *m. semitendinosus* showed the mean and maximum values of pH higher than 6.1, which is typical of DFD meat.

A slight increase (by 0.48 and 0.43, respectively) in the mean value of pH was observed in the cooked samples from the light and dark parts of *m. semitendinosus* as a result of thermal treatment. In general, however, the cooked samples from the dark part had a higher mean value of pH (by 0.30 units). The highest variability of pH values was characteristic of the samples from the dark part of the muscle both before and after thermal treatment. A pH value of a product also determines the shelf life of foods [27]. The understanding of the variability of this indicator in a product and reduction of its values due to the choice of raw materials can be used in combination with other hurdles to increase shelf life of pork products.

Cooking test is of great importance in pork quality assessment [28]. The dark and light samples did not show significant differences in cooking losses (Table 6). However, the tendency ($p = 0.052$) was observed toward higher losses in the samples of muscle tissue from the light part of *m. semitendinosus*: the mean values differed by 1.83% and medians by 1.43%. At the same time, high variability

Table 5. Results of pH changes in the samples of *m. semitendinosus* in the raw and cooked condition

Indicator	Value of the indicator for			
	Dark part		Light part	
	Raw minced meat	Cooked minced meat	Raw minced meat	Cooked minced meat
Mean (Mean ± SE)	6.15 ± 0.05	6.58 ± 0.03	5.80 ± 0.02	6.28 ± 0.01
Min/Max	5.74/6.63	6.23/6.98	5.69/5.93	6.15/6.38
Interquartile range (P25/75)	6.01/6.22	6.52/6.65	5.72/5.89	6.23/6.33
Median	6.16	6.58	5.79	6.29
Confidence interval (CI)	0.09	0.07	0.03	0.02
Coefficient of variation (CV), %	4.06	2.88	1.50	1.02

was observed for the value of cooking losses in all samples: 28.45% and 26.04%, respectively.

Table 6. Cooking losses (%) in the dark and light parts of *m. semitendinosus*

Indicator	Value of the indicator for	
	Dark part	Light part
Mean (Mean ± SE)	6.67 ± 0.60	8.50 ± 0.70
Min/Max	3.54/9.13	6.71/13.83
Interquartile range (P25/75)	5.76/7.94	6.83/8.76
Median	6.76	8.19
Confidence interval (CI)	1.17	1.37
Coefficient of variation (CV), %	28.45	26.04

Higher moisture losses upon cooking did not influence tightening of consistency of the samples made from the light part of *m. semitendinosus*. Tactile evaluation demonstrated that their consistency was less elastic, looser and more crumbly than that in the samples made from the dark part of *m. semitendinosus*.

The results of the structural-mechanical investigations of the samples of cooked minced meat confirmed this observation, and statistical data analysis showed that the differences in the structural-mechanical characteristics between the samples from the dark and light parts of *m. semitendinosus* were significant ($p < 0.05$) (Table 7).

Table 7. Results of determination of load force (g) in the cooked samples from the dark and light parts of *m. semitendinosus*

Indicator	Value of the indicator for	
	Dark part	Light part
Mean (Mean ± SE)	1,369.74 ± 112.54	1,984.78 ± 97.53
Min/Max	869.67/2,259.98	1,515.03/2,599.88
Interquartile range (P25/75)	1,030.73/1,712.12	1,666.64/2,333.49
Median	1,202.95	1,878.77
Confidence interval (CI)	220.57	191.15
Coefficient of variation (CV), %	31.82	19.03

It should be noted that the samples taken in an enterprise in our study were characterized by pH values higher than 5.7, which points to the absence of the PSE defect. Nevertheless, the light part demonstrated a reduction in the functional-technological characteristics of meat. Heat stress in pigs can lead to an increase in differences in the quality of the dark and light parts due to the differences between the dark and light parts of *m. semitendinosus* in the rate of the metabolic response to external factors [29].

At the same time, it is well known that some diets have a positive effect on reduction of the proportion of glycolytic muscle fibers in *m. semitendinosus*. Such diets include, for example, addition of microalgae [30], low content of amylose/amylopectin [31].

For the time being, however, it is acknowledged that the strongest effect on the microstructure of muscle tissue is exerted by a breed rather than a raising system. Less intensive growth of pigs shows lower quantity of glycolytic muscle fibers also influencing quality of *m. semitendinosus* [10].

Conclusion

Pork is the most consumed meat after poultry meat both in Russia and in the world. Consumers and processors are interested in increasing pork quality. This interest should stimulate animal husbandry to improve characteristics of industrially raised slaughter animals. Apparently, some special features of pork muscles, in particular, *m. semitendinosus*, must be taken into account in breeding work to produce meat raw materials with required technological characteristics for production of delicacy whole-muscle (whole-piece) products. Our study of *m. semitendinosus* showed significant differences in the microstructure of the dark and light parts of this muscle. The dark part was characterized by a higher density of muscle fibers, higher length of sarcomeres and higher number of capillaries. On the contrary, the light part was characterized by a larger diameter of muscle fibers with predominance of type IIa and type IIb fibers, as well as by a higher quantity of giant fibers, which presence is linked with the PSE defect of meat quality and myopathy. A significant range of variability of microstructural indicators in the light part, in particular, by fiber diameter, can be associated with less conservative heredity and a possibility of the further breeding work. Differences in the microstructure are the main reason for different quality of meat from the dark and light parts of *m. semitendinosus*. The dark part was characterized by higher pH values, better color development with nitrite and better consistency after cooking. With that, there was a tendency toward lower cooking losses. It should be noted that even at pH in a range of 5.7 ~ 6.1, which is optimal for pork, the special features of the microstructure of the light part of *m. semitendinosus* from domestic hybrid pigs can cause problems with quality of final products with regard to the development of color and consistency. Therefore, it is expedient to include *m. semitendinosus* along with *m. longissimus dorsi* into the plan of investigations. The results of such investigations can be useful for animal breeders and meat processors for the further improvement of quality of Russian pork and pork products.

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COMPARATIVE ASSESSMENT OF BEEF CHARACTERISTICS FROM YOUNG BULLS OF DIFFERENT BREEDS AND THE INFLUENCE OF STORAGE CONDITIONS ON MEAT QUALITY INDICATORS

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Keywords: *beef, quality indicators, storage conditions*

Abstract
Comparative assessment results for quality indicators of meat samples obtained from Black Pied, Simmental and Aberdeen Angus young bulls and the influence of different temperature conditions on the quality of vacuum-packed beef during refrigerated storage are presented. The general chemical composition, physicochemical properties, and biological value of the samples based on the content of nonessential and essential amino acids were determined, as well as the protein quality index (PQI) and amino acid score. Analysis of the general chemical composition revealed higher moisture and protein content and the lowest fat content in Black Pied beef compared to other breeds. The amino acid composition of the protein showed a higher content of essential amino acids and the highest PQI value in Simmental beef. According to the calculation results, higher amino acid scores for lysine (149.1% and 129.1%) and tryptophane (200.0% and 240.0%) were noted in meat from Simmental and Aberdeen Angus young bulls, respectively. For the process of storing vacuum-packed meat in a cooled (at a temperature of 2.0 ± 0.5 °C) and superchilled state (minus 2.0 ± 0.5 °C) at a relative air humidity of 85%, a comparative analysis of changes in free amino acids and dynamics of hydrolytic and oxidative spoilage of meat samples from various breeds was conducted. After 21 days of storage in a superchilled state, the content of free amino acids in Black Pied, Simmental and Aberdeen Angus beef was lower by 13.1% ($P > 0.05$), 24.1% ($P \leq 0.05$) and 17.0% ($P \leq 0.01$) compared to storage in a cooled state, respectively. For all studied samples stored in a cooled state, the acid number values were 40% to 41% ($P \leq 0.01$) higher than in a superchilled state and peroxide number values were 20% to 23% ($P \leq 0.05$) higher than in a superchilled state. It has been established that lowering the storage temperature of vacuum-packed beef helps to better preserve quality and ensure safety for meat from all breeds studied.

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Introduction

Currently, one of the most important strategic goals of state policy is to increase domestic production of meat raw materials and products under international restrictions. Achieving this goal will ensure food security, solve the problems of import substitution, increase the competitiveness of the Russian economy and meet the needs of the population for high-quality meat products [1–2].

The concept of sustainable development of meat livestock breeding in the Russian Federation for the period until 2030[1] provides for the necessary measures of socio-economic, legal and administrative nature in solving key problems in the development of meat livestock breeding including ensuring expanded reproduction of meat and crossbred livestock and increasing beef production.

Beef is in growing demand in the consumer market not only in our country, but also abroad. Due to its high protein content, low fat content, and good taste, this type of meat is able to effectively satisfy the body's need for all essential nutrients, vitamins and microelements [3].

According to the National Meat Association, the share of beef in the total volume of meat consumption in the Russian Federation has decreased to a reasonable level due to the affordability of poultry and pork [4]. However, it is impossible to reduce beef production because demand for it in the world is not decreasing. One of the new poten-

[1] Ministry of Agriculture of the Russian Federation. National Beef Producers Union. Federal State Budgetary Scientific Institution "Federal Research Center for Biological Systems and Agricultural Technologies of the Russian Academy of Sciences". (2017). Concept of sustainable development of meat livestock breeding in the Russian Federation for the period until 2030. Retrieved from http://fncbst.ru/wp-content/uploads/2018/10/Концепция-мясного-скотоводства-версия-27.02.18.pdf Accessed April 10, 2023. (In Russian)

tial markets is China, which opens up broad prospects for increasing exports. At the same time, there is greater interest in high-quality beef obtained from specialized meat breeds, which are currently in short supply.

Currently, the share of specialized beef and crossbred cattle in the Russian Federation is about 20%, and the main backup for increasing beef production is its production through the use of dairy and combined productivity cattle [5]. Breeding specialized meat breeds and their crosses should become one of the strategic directions for the formation of a large-scale industry of specialized beef cattle breeding capable of compensating for this deficiency in the future until 2030.

The biological value of meat mainly depends on the quantity and quality of protein and is determined by its structural features, composition and degree of absorption. Meat proteins are distinguished by a high content of essential amino acids, the ratio of which in meat is especially favorable [6].

As can be seen at Figure 1, the amino acid composition of meat proteins is close to the amino acid composition of proteins that are officially accepted as ideal ones, i. e. human milk and chicken egg proteins [7]. Beef is a valuable addition to a healthy diet. It satisfies human need for essential amino acids, and is a rich source of vitamins B6 and B12 and essential microelements, i. e. potassium, phosphorus, iron and zinc [8,9].

One of the effective ways to preserve meat and meat products in order to preserve their structure, biological and nutritional value is to cool them to a temperature not lower than cryoscopic one. However, during the period of refrigerated storage, autolytic processes in tissues only slow down, but do not stop [10].

The quality of chilled meat deteriorates over time, not only during storage, but also as it moves through the all stages of the cold supply chain. At the same time, the intensity of negative changes from the chemical reactions and microbiological activity largely depends on strict adherence to the required temperature conditions during the process of distribution, storage, transportation and sale [11].

The most common method for significantly increasing shelf life is freezing technology, since it inhibits microbiological spoilage and significantly slows down autolytic processes in products of animal origin. However, the use of low temperatures irreversibly affects meat quality and taste, especially when the freezing process is slow, which leads to a high concentration of dissolved substances in the unfrozen matrix and loss of nutrients after thawing [11].

Assuming the maintenance of meat consumer properties, the shelf life of chilled meat may be increased by storing it in a controlled temperature range below the water freezing point while maintaining storage conditions that prevent the product itself from freezing [12–14]. In recent years, NFTS technology (near-freezing temperature storage) has been widely used for various products of plant origin (blueberries, spinach, green beans, pears, peaches, and cherries) [15–19].

The shelf life of poultry and pork meat may also be significantly increased when using this technology [20,21]. Research results [22] showed that fresh meat was stored for approximately 14 days in a superchilled state, which exceeded the shelf life of chilled meat due to slower growth of pathogenic microorganisms. At the same time, such a quality parameter as drip loss has shown that superchilling has advantages over freezing [22]. Thus, superchilling method is not as effective for long-term storage as freezing, but it allows for longer quality maintenance compared to storage in a cooled state.

A number of studies have confirmed that when stored in a superchilled state at a temperature of minus 2 °C, the shelf life of rabbit and broiler meat may be increased by 3 to 5.5 times [20,23]. The shelf life of beef steaks stored at cooling medium temperatures down to minus 4 °C was 2.4 times longer (up to 12 weeks) than for steaks stored at 2 °C [24].

The superchilling process in combination with the use of film packaging under vacuum or in a modified atmosphere increases product shelf life by 1.5 to 4 times compared to traditional cooling. In [12], the authors studied the effect of various storage conditions on beef texture and concluded that storage at freezing point using vacuum

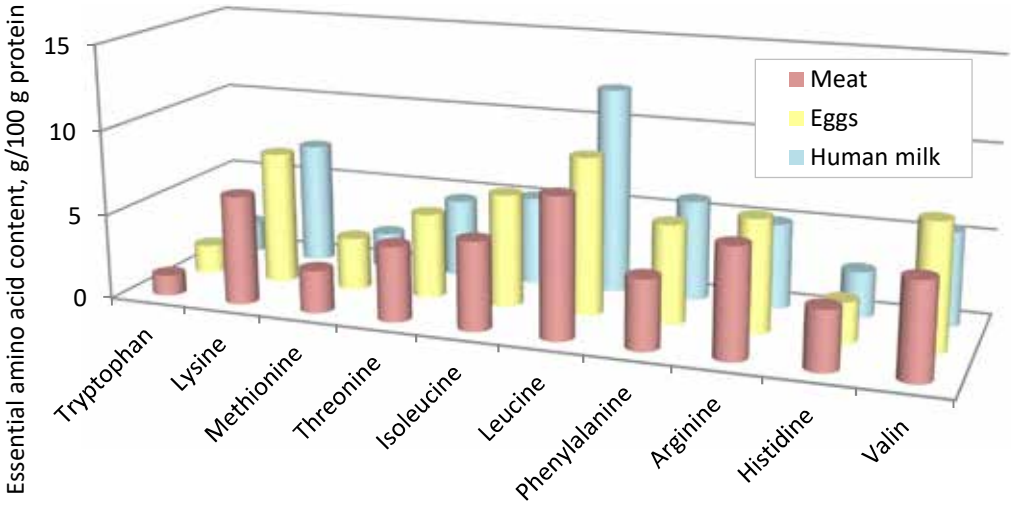


Figure 1. Content of essential amino acids in meat and the most valuable foods, g/100 g protein [7]

packaging may effectively delay the processes of microbiological spoilage, as well as maintain quality for a long time. Meat quality may be assessed by the following indicators: pH, water-binding capacity, amount of lactic acid and volatile fatty acids, protein solubility, color and tenderness. Nutritional value is the content of amino acids, fatty acids, vitamins, minerals and other components, which are important quality indicators of meat. Quality factors perceived by consumers are related to sensory properties (color, tenderness and taste), nutritional properties (caloric content, vitamin content and fatty acid profile) and appearance (exudation, marbling and visible amount of fat). Meat quality also depends on muscle fiber structure, including internal structure (sarcomere length, myofilament diameter, and fiber types) and chemical composition (mass content of moisture, protein, fat, and ash) [3].

An analysis of existing refrigerated storage technologies shows that the superchilling method is the most attractive in terms of increasing shelf life while preserving the structure of chilled meat as far as possible. The use of this method involves freezing a small part of the free moisture, but eliminates significant fluctuations in the temperature of the cooling medium and creates conditions for stabilizing the temperature of the food product during storage, transportation and retail trade [25–27]. The degree of superchilling is one of the most important parameters that determine the quality of stored products [25–27], which depends on the portion of frozen water. The lower the degree of superchilling, the better the taste of chilled products; the degree of superchilling over 30% may lead to a greater loss of moisture from the product [25,28–30].

The main reason for the introduction of superchilling technology may be its ability to combine the beneficial effect of low temperature along with the conversion of some free water into ice [31], thereby making it less accessible to the processes of product structure destruction by ice crystals. A number of studies have shown that the optimal proportion of frozen moisture is 5% to 30% when it is remains in the structure of the food product [28–30]. The advantage of superchilling technology is its ability to maintain the structural "freshness" of muscle tissue, as opposed to freezing. Therefore, its potential for industrial application has been noted by many authors [20,23,32–34].

The purpose of the study was a comparative assessment of the quality indicators for meat obtained from Black Pied, Simmental and Aberdeen Angus young bulls and the influence of different temperature conditions on the quality of vacuum-packed beef from these breeds during refrigerated storage.

Objects and methods

The object of the study was the samples of *Longissimus dorsi* muscle obtained from Black Pied, Simmental and Aberdeen Angus young bulls. Twenty-four hours after slaughter and cooling of half-carcasses at air temperature of 0 to 4 °C, *L. dorsi* from the lumbar part was isolated in the form of a rectangular layer, removed from the lumbar vertebrae 1 to 2 cm below the transverse processes without membranes and tendons for the preparation of test samples.

Before vacuum packaging and storage, for the initial samples of meat from various breeds, the chemical composition and physicochemical properties were studied, protein quality index (PQI) was determined, and the amino acid score was calculated.

The study of the chemical composition and physicochemical parameters of muscle tissue was carried out in order to determine the nutritional value of meat as a source of complete proteins of animal origin. The chemical composition of meat was determined for the following indicators:

— moisture content according to GOST 9793–2015[2];
— protein content according to GOST 25011–2017[3];
— fat content according to GOST 23042–2015[4];
— ash content according to GOST 31727–2012[5].

PQI (tryptophane to oxyproline ratio) according to GOST R70149–2022[6] and GOST 23041–2015[7];

The active acidity of meat (pH) was measured with Testo 205 portable pH meter (Germany). The water-holding capacity of meat was determined by the planimetric pressing according to Grau-Hamm method, modified by Volovinskaya-Kelman.

The amino acid composition of the protein was assessed by comparing the results obtained with a standard (reference) protein based on the amino acid score:

$$AC = \frac{C_{test} \cdot 100}{C_{std.}}$$

where AC is amino acid score, %; C_{test} is the content of essential amino acids in 1 g of tested protein, mg; $C_{std.}$ is the content of the same amino acid in 1 g of standard protein, mg; 100 is conversion factor.

Storage studies were carried out on an experimental bench with the ability to precisely control the temperature of the cooling medium in the refrigeration chamber. Two modes of vacuum-packed meat storage in a cooled and superchilled state were selected: at cooling medium temperatures of 2.0 ± 0.5 °C and minus 2.0 ± 0.5 °C, respectively. At

[2] GOST 9793–2015. "Meat and meat products. Method for determination of moisture content". Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/1200144231 Accessed February 8, 2023. (In Russian)

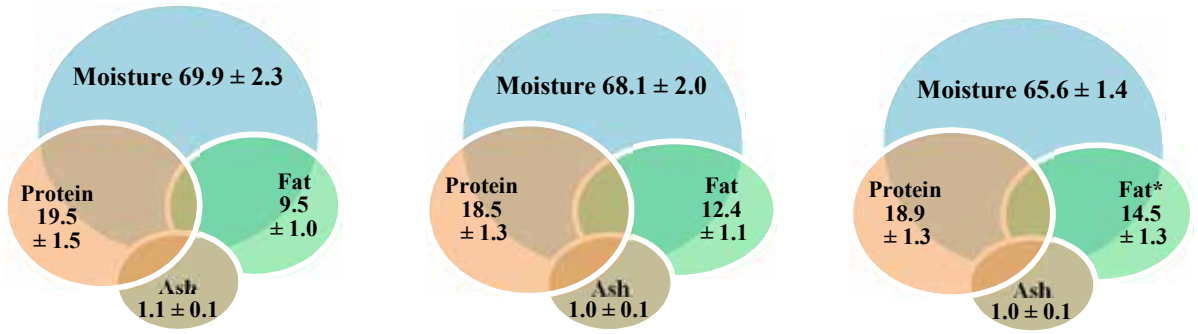
[3] GOST 25011–2017. "Meat and meat products. Protein determination methods". Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/1200146783 Accessed February 8, 2023. (In Russian)

[4] GOST 23042–2015. "Meat and meat products. Methods of fat determination". Moscow: Standartinform, 2017. Retrieved from https://docs.cntd.ru/document/1200133107 Accessed February 8, 2023. (In Russian)

[5] GOST 31727–2012. "Meat and meat products. Determination of total ash". Moscow: Standartinform, 2013. Retrieved from https://docs.cntd.ru/document/1200098742 Accessed February 8, 2023. (In Russian)

[6] GOST R70149–2022." Meat and meat products. Determination of the mass fraction of tryptophane by spectrophotometric method". Moscow: Standartinform, 2023. Retrieved from https://docs.cntd.ru/document/1200184801 Accessed February 8, 2023. (In Russian)

[7] GOST 23041–2015. "Meat and meat products. Method for determination of oxyproline". Moscow: Standartinform, 2016. Retrieved from https://docs.cntd.ru/document/1200123926 Accessed February 8, 2023. (In Russian)



a) b) c)

Figure 2. Chemical composition of meat, %: a) Black Pied; b) Simmental; c) Aberdeen Angus

* $P \leq 0.05$ — compared to Black Pied.

the selected temperature conditions, the meat was stored for 21 days at a relative humidity of 85%. Air temperature and humidity were measured with DV2TSM-R instruments (Research and production company "Microfor", Russia), temperature measurement range: minus 40 to 60 °C, permissible absolute measurement error limit: ± 0.5 °C, relative humidity measurement range: 0% to 98% (±1.0%).

During storage, control samples were taken on days 10 and 21. Quality indicators of the initial and control samples were determined during storage using the following methos:

— total amino acid analysis according to GOST 34132–2017[8];
— free amino acid content according to measurement procedure MVI-02–2002[9];
— acid and peroxide numbers according to GOST R55480–2013[10] and GOST 34118–2017[11], respectively.

Data obtained were statistically processed using the MS Office application package. The research was repeated in triplicate. The significance of the difference was accepted at a significance level of $P \leq 0.05$; $P \leq 0.01$.

Results

Study results for the chemical composition and physicochemical properties of beef

The chemical composition makes it possible to determine the nutritional and caloric value of meat as a source of complete animal proteins, as well as fats and minerals. Figure 2 shows that in terms of moisture and protein content, the highest values were obtained in beef from Black Pied young bulls. Before storage, these indicators were higher for moisture content by 1.8 abs.% and 4.3 abs.% ($P > 0.05$) and for protein content by 1.0 abs.% and 0.6 abs.% ($P > 0.05$) compared to Simmental and Aberdeen Angus, respectively.

The highest fat content was found in meat of Aberdeen Angus young bulls, 14.5%. Comparing this indicator in Aberdeen Angus with Black Pied and Simmental young animals, it was found that it was higher by 5.0 abs.% ($P \leq 0.05$) and 2.1 abs.% ($P > 0.05$), respectively. In the meat of the studied breeds, no differences were found in minerals content. Chemical analysis results for meat obtained from young bulls of different productivity types show that the beef of the studied breeds was characterized by high nutritional value.

After the slaughter of animals, the process of meat autolysis begins, and its physicochemical properties change significantly. During autolysis, intensive breakdown of glycogen occurs and lactic acid is formed, which softens meat and, after ageing, it acquires a specific taste and smell characteristic of aged meat.

In the context of meat processing and storage technology, active acidity (pH) is an important quality indicator. Measured 24 hours after slaughter, this indicator is a direct result of muscle glycogen (energy) levels. Its final value determines meat color, water-holding capacity, texture and, accordingly, the quality of the raw material. Analysis results of the data shown in Figure 3 indicate that the active acidity decreased after 24 hours ageing compared to 1 hour after slaughter by 1.6% to 3.3% due to the conversion of muscle glycogen into lactic acid. The highest values of pH_1 and pH_{24} were in Aberdeen Angus young bulls, and the lowest values were in Black Pied young bulls. According to this indicator, beef from young bulls of all breeds had no defects and was characterized as NOR beef.

Water-holding capacity depends on the composition and properties of proteins, pH level and structure, which is important during meat processing and affects the sensory properties of finished meat products made from it. The ability to retain water in relation to the total moisture in the meat of the studied breeds varies from 60.4% to 66.5%. Black Pied beef had the highest water-holding capacity: higher by 3.7 abs.%. and 6.1 abs.% ($P > 0.05$) compared to the meat of Simmental and Aberdeen Angus young bulls, respectively.

Figure 3 shows that Black Pied beef is characterized by the best values.

[8] GOST 34132–2017. "Meat and meat products. Determination of amino acids composition of animal protein". Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200146930 Accessed February 8, 2023. (In Russian)

[9] Measurement procedure No. 02–2002. "Mass concentration of basic amino acids in aqueous solution. Methodology for performing measurements using high-performance liquid chromatography". Irkutsk, 2002. Retrieved from new.econova.ru Accessed February 8, 2023. (In Russian)

[10] GOST R55480–2013. "Meat and meat products. Method for determination of acid value". Moscow: Standartinform, 2014. Retrieved from https://docs.cntd.ru/document/1200103311 Accessed February 8, 2023. (In Russian)

[11] GOST 34118–2017. "Meat and meat products. Method for determination of peroxide value". Moscow: Standartinform, Retrieved from https://docs.cntd.ru/document/1200146654 Accessed February 8, 2023. (In Russian)

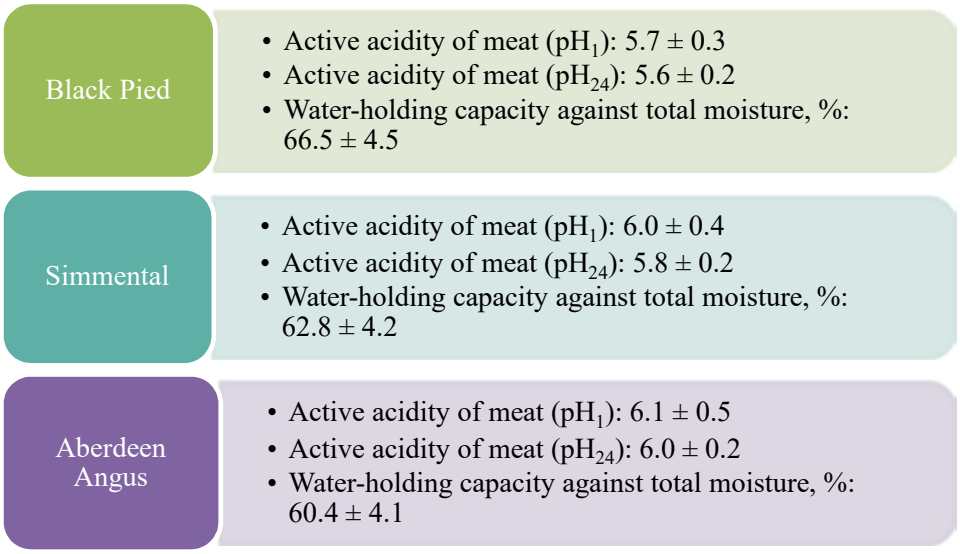


Figure 3. Physicochemical parameters of meat ($\overline{X} \pm S\overline{x}$)

Study results for meat biological value

The biological value of meat is determined by the presence of amino acids in its composition, as well as their quantitative ratio (Table 1).

Table 1. Amino acid composition of muscle tissue, g/100 g protein, ($\overline{X} \pm S\overline{x}$)

Parameter	Black Pied	Simmental	Aberdeen Angus
	Essential amino acids		
Isoleucine	4.05 ± 0.15	4.59 ± 0.11*	4.22 ± 0.16
Leucine	6.92 ± 0.31	7.41 ± 0.27	7.33 ± 0.26
Lysine	7.23 ± 0.31	8.22 ± 0.32	7.10 ± 0.26
Methionine + Cystine	3.33 ± 0.15	3.89 ± 0.10*	3.64 ± 0.16
Valine	5.38 ± 0.15	5.08 ± 0.22	5.03 ± 0.16
Tryptophane	1.54 ± 0.21	2.00 ± 0.05	2.39 ± 0.26
Phenylalanine + Tyrosine	8.82 ± 0.20	8.43 ± 0.22	8.24 ± 0.27
Threonine	5.77 ± 0.21	4.32 ± 0.22**	5.56 ± 0.16
Total	43.04 ± 5.44	43.94 ± 5.51	43.51 ± 5.34
	Nonessential amino acids		
Aspartic acid	8.97 ± 0.36	9.08 ± 0.32	9.63 ± 0.32
Serin	4.67 ± 0.21	4.65 ± 0.22	3.75 ± 0.16*
Glutamic acid	19.79 ± 0.62	19.78 ± 0.32	16.77 ± 0.53*
Glycine	5.08 ± 0.26	4.38 ± 0.16	5.89 ± 0.21**
Alanine	4.56 ± 0.26	4.11 ± 0.16	5.89 ± 0.21**
Histidine	3.79 ± 0.15	3.46 ± 0.05	3.39 ± 0.11
Arginine	6.46 ± 0.36	5.84 ± 0.38	6.95 ± 0.21
Proline	3.28 ± 0.15	4.43 ± 0.16**	3.82 ± 0.16
Oxyproline	0.36 ± 0.10	0.33 ± 0.22	0.40 ± 0.26
Total	56.96 ± 5.74	56.06 ± 6.05	56.49 ± 4.91
Amino acid index,%	75.6	78.4	77.0
PQI	4.28 ± 0.1	6.06 ± 0.2**	5.98 ± 0.2**

* P ≤ 0.05; ** P ≤ 0.01 — compared to Black Pied

The biological value of meat may be identified using the protein quality index (PQI) determined by the ratio of tryptophane (an essential amino acids) to oxyproline (a typical representative of nonessential amino acids). The biological value of meat is lower the more oxyproline it contains, the content of which determines the amount of connective tissue proteins.

In general, meat of Simmental and Aberdeen Angus young bulls had a high protein quality index; in Black Pied young bulls this indicator was lower by 1.78 and 1.70 units (P ≤ 0.01), respectively. Meat of Simmental young bulls was characterized by the highest biological value due to the high content of tryptophane, which is essential amino acid.

For each group, the amino acid score was calculated and the limiting amino acids were determined. The calculation results are presented in Table 2.

Table 2. Amino acid score, %

Amino acids	Breed		
	Black Pied	Simmental	Aberdeen Angus
Valine	108.0	102.0	100.0
Isoleucine	102.5	115.0	105.0
Leucine	98.6*	105.7	104.3
Lysine	130.9	149.1	129.1
Methionine + Cystine	94.3*	111.4	102.9
Tryptophane	160.0	200.0	240.0
Threonine	145.0	107.5	140.0
Phenylalanine + Tyrosine	146.7	140.0	108.3

* Limiting amino acids.

The data in Table 2 show that when calculating the amino acid score in the meat of Black Pied young bulls, the limiting amino acids are: leucine (98.6%) and methionine + cystine (94.3%). For other amino acids, this value was more than 100%.

In Simmental and Aberdeen Angus beef, the amino acid score exceeds 100% for all limiting (essential) amino acids as follows:

— for lysine content, 149.1% and 129.1%, respectively;

— for tryptophane content, 200.0% and 240.0%, respectively.

According to the results of the studies (Table 1 and Table 2), the meat of the studied breeds is characterized by high biological value.

Study results for meat quality indicators depending on storage conditions

During storage for 10 and 21 days at different temperatures, the dynamics of changes in free amino acids, hydrolytic and oxidative spoilage of fats in vacuum-packed young bull meat of various breeds were assessed.

In the initial samples (before storage), the lowest content of free amino acids was observed in Black Pied young bulls' meat (0.404 mg/100 g), which is lower compared to Simmental and Aberdeen Angus breeds by 27.7% and 25.9%, respectively (P > 0.05) (Table 3).

Table 3. Change in free amino acids during storage of vacuum-packed meat, mg/100 g muscle tissue ($\overline{X} \pm S\overline{x}$)

Breed	Storage period	Temperature, °C	Free amino acids, mg/100 g
Black Pied	Initial samples	—	0.404 ± 0.050
	10 days	2.0 ± 0.5	0.431 ± 0.046
		minus 2.0 ± 0.5	0.406 ± 0.018
	21 days	2.0 ± 0.5	0.615 ± 0.097
		minus 2.0 ± 0.5	0.544 ± 0.096
Simmental	Initial samples	—	0.516 ± 0.079
	10 days	2.0 ± 0.5	0.773 ± 0.066*
		minus 2.0 ± 0.5	0.698 ± 0.095*
	21 days	2.0 ± 0.5	0.887 ± 0.006*
		minus 2.0 ± 0.5	0.715 ± 0.014
Aberdeen Angus	Initial samples	—	0.511 ± 0.015
	10 days	2.0 ± 0.5	0.641 ± 0.016*
		minus 2.0 ± 0.5	0.524 ± 0.016**
	21 days	2.0 ± 0.5	0.745 ± 0.015
		minus 2.0 ± 0.5	0.637 ± 0.016

* P ≤ 0.05; ** P ≤ 0.01 — compared to Black Pied.

During the first 10 days of storage at cooling medium temperature of 2.0 ± 0.5 °C and minus 2.0 ± 0.5 °C, the content of free amino acids increases. At air temperature of 2.0 ± 0.5 °C, the content of free amino acids is higher compared to storage at air temperature of minus 2.0 ± 0.5 °C: for Black Pied by 0.025 mg/100 g or 5.8% (P > 0.05), for Simmental by 0.075 mg/100 g or 9.7% (P>0.05), and for Aberdeen Angus by 0.117 mg/100 g or 18.3% (P ≤ 0.01) (Table 3).

After 21 days of storage in a cooled and superchilled state, the lowest content of free amino acids was observed in Black Pied young bulls' meat (0.615 ± 0.097 and 0.544 ± 0.096 mg/100 g, respectively). For Simmental and Aberdeen Angus beef, this indicator (Table 3) was higher by:

— 0.272 mg/100 g or 44.23% (P ≤ 0.05) and 0.13 mg/100 g or 21.1% (P > 0.05) at a temperature of 2.0 ± 0.5 °C (Simmental breed);

— 0.171 mg/100 g or 31.4% (P > 0.05) and 0.093 mg/100 g or 17.1% (P > 0.05) at a temperature of minus 2.0 ± 0.5 °C (Aberdeen Angus breed).

Thus, when stored for 21 days in a superchilled state, the content of free amino acids in beef was lower compared to a cooled state: in Black Pied by 0.071 mg/100 g or 13.1% (P > 0.05), in Simmental by 0.172 mg/100 g or 24.1% (P ≤ 0.05) and in Aberdeen Angus by 0.108 mg/100 g or 17.0% (P ≤ 0.01).

It should be noted that the cooling process allows to preserve higher consumer properties of both meat itself and meat products, such as flavor, taste, texture, color of the product. The acid number of fats in meat and meat products is a measure characterizing the degree of fat hydrolysis, which allows to predetermine shelf life. Thus, after 10 days of storage in a refrigerator with a temperature of 2.0 ± 0.5 °C (Figure 4), the acid number in Black Pied, Simmental and Aberdeen Angus beef increased compared to initial values by 3.50, 2.63, and 2.56 (P ≤ 0.01) times, respectively, while at minus 2.0 ± 0.5 °C, the changes in this indicator were insignificant. After 21 days of storage in a cooled state, the acid number increased by 7.33, 5.63, and 5.44 (P ≤ 0.01) times, respectively, while in a superchilled state, there was an increase by 4.33, 3.38, and 3.32 (P ≤ 0.01) times, respectively.

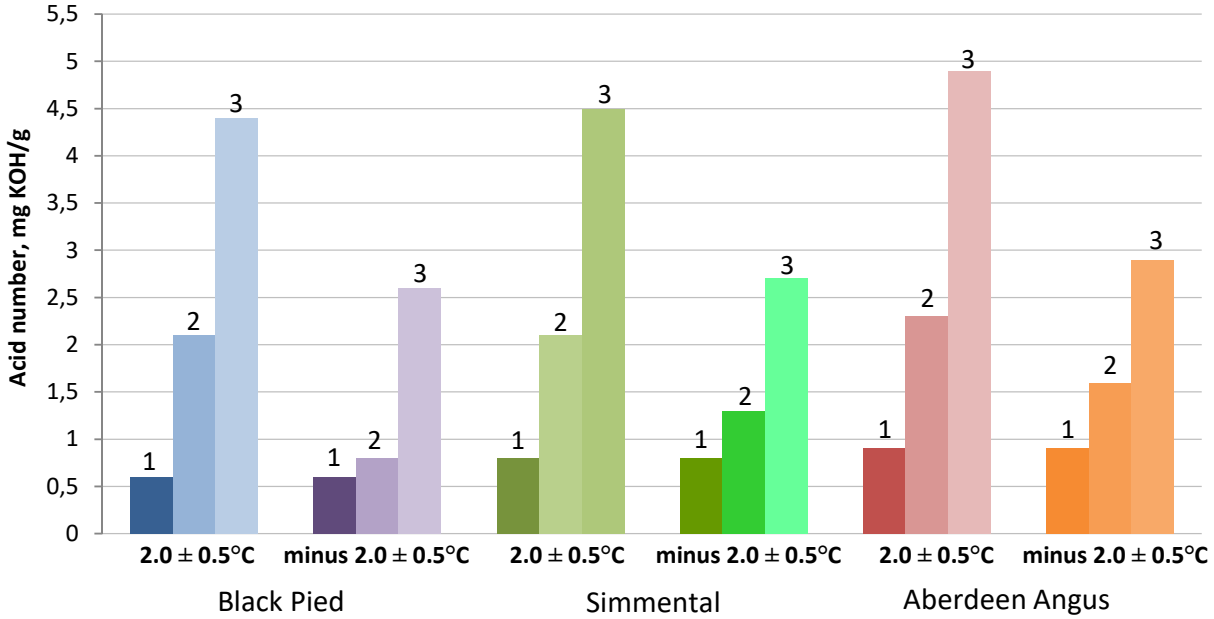


Figure 4. Dynamics of changes in acid number during storage of vacuum-packed meat, mg KOH/g:
1 — initial samples; 2–10 days storage; 3–21 days storage

For all studied samples stored in a cooled state for 21 days, the increase in acid number was 40% to 41% ($P \le 0.01$) higher than in a superchilled state.

The data in Figure 4 show that the greatest increase in acid number during storage for 21 days at a temperature of 2.0 ± 0.5 °C was observed in Aberdeen Angus beef (5.0 to 5.4 times, $P \le 0.01$). The smallest increase in acid number was found in the meat of Black Pied breed (3.0 to 3.3 times, $P \le 0.01$)).

During the same period of meat storage at a temperature of minus 2.0 ± 0.5 °C for 21 days, the lowest acid number of 2.6 mg KOH/g was observed in meat of Black Pied breed, which is lower compared to Simmental and Aberdeen Angus by 0.1 mg KOH/g and 0.3 mg KOH/g, respectively ($P > 0.05$). This may be explained by the fact that during storage, the acid number of fats in fattier meat samples increases faster, resulting from the hydrolysis of triglycerides and accompanied by the formation of free fatty acids.

After 10 days of storage, an increase in peroxide number in Black Pied, Simmental and Aberdeen Angus beef was also observed as a result of fat oxidation (Figure 5):

— for storage at 2.0 ± 0.5 °C, by 2.3, 2.7, and 3.0 times, respectively ($P \le 0.01$);
— for storage at minus 2.0 ± 0.5 °C, by 1.7, 2.1, and 2.0 times, respectively ($P \le 0.01$).

After 21 days of storage in a cooled state, this indicator exceeded the values obtained for 21 days of storage in a superchilled state: for Black Pied and Simmental by 0.3 mmol active oxygen/kg and for Aberdeen Angus by 0.4 mmol active oxygen/kg ($P \le 0.05$).

Discussion

During the research, data was obtained and a comparative analysis of the chemical composition and physicochemical parameters of meat from Black Pied, Simmental and Aberdeen Angus young bulls of different productivity type was carried out. The biological value of meat samples from various breeds was determined based on the content of nonessential and essential amino acids, as well as the protein quality index and amino acid score were calculated. Based on the results of experimental studies, it was concluded that beef obtained from young bulls of the studied breeds has high nutritional and biological value and contains all the essential amino acids for protein synthesis, which is consistent with the results of studies by other scientists [35–37].

As a result of the comparative assessment, it was concluded that in terms of physical and chemical indicators (active acidity and water-holding capacity of meat), Black Pied beef is characterized by the best indicators.

It was established that in muscle tissue (samples of the *Longissimus dorsi* muscle) of Simmental and Aberdeen Angus young bulls, the amino acid score for all limiting (essential) amino acids exceeds 100%.

For storage processes of vacuum-packed meat at a relative air humidity of 85% and a temperature of 2.0 ± 0.5 °C (cooled state) and minus 2.0 ± 0.5 °C (superchilled state), a comparative analysis of the changes in free amino acids and the dynamics of hydrolytic and oxidative spoilage of meat samples from the studied breeds was conducted. Thus, analysis of the data in Table 3 indicates a general tendency to increase the amount of free amino acids during meat storage compared to the original samples, both in a cooled and superchilled state. The results obtained may be explained by changes in amino acid content as a result of enzymatic processes. Decomposition of amino acid decreases their content in meat and is catalyzed by the activity of oxidases and decarboxylases, which is highest during the initial period of refrigeration and storage. An increase in the activity of cathepsins is promoted by proteolysis and, accordingly, an increase in the content of amino acids occurs later in the process of refrigerated storage, as they are released from degrading lysosomes. The initial decrease and subsequent increase in the content of free amino acids in meat at different storage times may be explained by differences in the rates of these enzymatic processes [7].

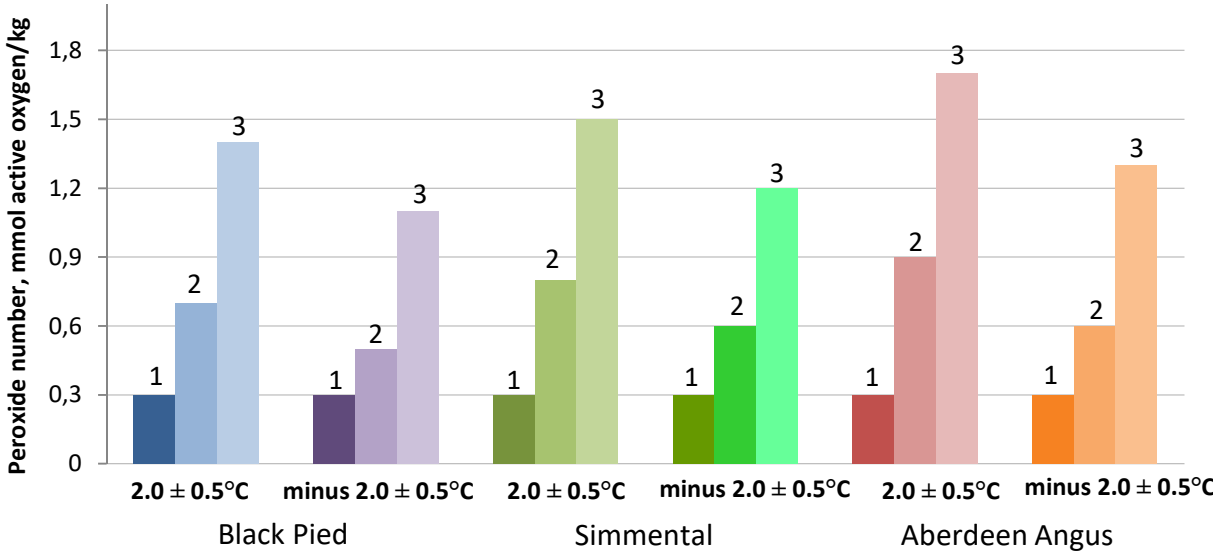


Figure 5. Dynamics of changes in peroxide number during storage of vacuum-packed meat, mmol active oxygen/kg: 1 — initial samples; 2–10 days storage; 3–21 days storage

It was found that lowering the storage temperature of vacuum-packed beef contributes to better preservation of meat quality for all breeds studied, which is consistent with research data from other authors [38, 39]. After 21 days of storage in a superchilled state, the content of free amino acids in the meat of Black Pied, Simmental and Aberdeen Angus breeds was lower compared to storage in a cooled state by 13.1% ($P > 0.05$), 24.1% ($P \leq 0.05$), and 17.0% ($P \leq 0.01$), respectively. For all samples stored in a cooled state, the acid number values were 40% to 41% higher ($P \leq 0.01$) and peroxide number values were 20% to 23% higher ($P \leq 0.05$) than in a superchilled state.

It has been confirmed that at a lower temperature of the cooling medium, hydrolytic and oxidative changes in lipids slow down, as well as the accumulation of free amino acids in vacuum-packed meat, which has a positive effect on maintaining quality and is consistent with research data from other authors [38, 39].

A comparative analysis of the data obtained and the results of similar studies shows that the predicted shelf life of vacuum-packed beef stored in a superchilled state may be increased by 20% to 40% [10, 14, 40].

Conclusions

Beef obtained from young bulls of the studied breeds was generally characterized by a balanced chemical composition. According to physical and chemical indicators, it belonged to NOR quality group, contained all essential amino acids and had high nutritional and biological value.

The results of experimental studies show that the superchilling better maintains the quality of vacuum-packed meat compared to cooling method.

To determine the maximum shelf life of vacuum-packed beef, further experimental studies are needed, taking into account not only changes in free amino acids, hydrolytic and oxidative dynamics, but also microbiological parameters during storage.

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Completely prepared the manuscript and is responsible for plagiarism.

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MICROALGAE AS ALTERNATIVE PROTEINS FOR THE SUSTAINABLE FOOD INDUSTRY: A REVIEW

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Keywords: *food resources, meat alternatives, alternative proteins, sustainable diets, food consumption standards*

Abstract

This paper reviews the nutritional properties and consumer perceptions of microalgae foods through various recent studies on alternative protein sources. Food choices, including meat consumption, are a common concern for humanity. Thus, we focused on whether microalgae foods have a sufficient value as a protein source and what nutritional benefits they have. Based on existing papers, we conducted a systematic review using Web of Science, Google Scholar, and Scopus to comprehensively investigate and summarize the nutritional characteristics of microalgae, sustainable diets, and awareness of microalgae as an alternative protein source. Research has shown that microalgae have been consumed by humans as a protein source since ancient times, and contain enough protein to be used as an alternative protein source. They also have many other nutritional benefits, such as vitamins. We have found that consumers are increasingly interested in alternative protein sources, and the more they learn about microalgae, the more accepting they become. These results may suggest a need for further research to improve microalgae as an alternative protein source in the long run and develop them into a variety of foods.

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Introduction

Present-day meat consumption and manufacturing patterns are far from being sustainable and impose a heavy burden on the environment. Animal agriculture is one of the leading causes of greenhouse gas emissions and anthropogenic climate change. Enormous meat consumption also has adverse health effects. To compensate for these downsides of meat consumption, an increasing number of consumers are turning to meat alternatives [1]. A sustainable diet means reducing meat intake or using alternative protein sources [2]. Previous research on meat substitutes has primarily focused on consumer acceptance and preference of meat replacers [3]. De Boer et al. [3] found that a very limited percentage of non-vegetarian consumers reported using meat substitutes. However, acceptance and attractiveness of meat substitutes increase when they are similar to common meals with meat (for example, meals with minced meat) [4].

Compared to meat, plant-based meat alternatives are considered to be a healthier source of protein and offer a range of environmental, social and health advantages. They can play an essential role in cutting down meat consumption. However, previous studies lack information on what role plant-based meat alternatives may play. And it is not known how the characteristics of specific meat alternatives can influence consumers' ability to moderately replace meat in their diets. Carbon footprint, production methods, and brands play a secondary role in the choice of meat alternatives [5].

Compared to meat proteins, plant proteins are preferable because they have a lower environmental impact. Cereals (*e. g.*, barley, wheat) and legumes (*e. g.*, peas, soybeans, lupins, beans) are considered the most important sources of plant protein. However, there are also controversies associated with legumes, namely soybeans, and genetically modified organisms [6].

Another study involves consumer analysis of the acceptability of insects as a meat alternative. The researchers found that general acceptance was low due to food aversion, but high concern for the environment and the use of minced insects in ready-to-eat foods to reduce aversion can lead to increased consumer acceptance of insect-based meat alternatives [7].

The term microalgae refers to a wide range of photosynthetic organisms. The cell structure is eukaryotic in microalgae and prokaryotic in cyanobacteria. In the context of microorganisms such as microalgae and cyanobacteria, "eukaryotic" and "prokaryotic" refer to fundamental differences in their cellular structures. Eukaryotic microorganisms, such as microalgae, have cells with a well-defined nucleus enclosed within a membrane. This nucleus houses their genetic material (DNA) and is surrounded by various membrane-bound organelles, including mitochondria and chloroplasts. These organelles perform specialized functions within the cell. On the other hand, prokaryotic microorganisms, such as cyanobacteria, lack a distinct nucleus enclosed within a membrane. Instead, their

genetic material floats freely in the cell's cytoplasm. They also lack membrane-bound organelles like mitochondria and chloroplasts. Prokaryotic cells are generally smaller and simpler in structure compared to eukaryotic cells. Thus, in summary, when discussing microalgae and cyanobacteria, the terms "eukaryotic" and "prokaryotic" refer to whether these microorganisms have cells with a defined nucleus and membrane-bound organelles (eukaryotic) or cells without a defined nucleus and such organelles (prokaryotic). Microalgae are capable of synthesizing carbohydrates, accumulating sugars, and storing other important organic substances and lipids through a process known as photosynthesis and other metabolic pathways. Regarding carbohydrate synthesis, microalgae are photosynthetic organisms, meaning they receive energy from sunlight to convert carbon dioxide and water into carbohydrates (primarily in the form of sugars such as glucose). This process occurs in the chloroplasts of microalgal cells, where chlorophyll and other pigments capture sunlight and convert it into chemical energy. The synthesized carbohydrates are used as an energy source for the microalgae. In the case of sugar accumulation, during photosynthesis, microalgae produce more sugars than they immediately need for energy. Microalgae can store these extra sugars as a reserve energy source in the form of starch or other polysaccharides. This stored energy can be mobilized when the microalgae need it, such as when light is scarce or nutrients are scarce. Microalgae also have the ability to accumulate lipids, including triglycerides and fatty acids, within their cells. This lipid accumulation can occur under certain growth conditions, such as nutrient limitation, and is often induced to enhance the production of biofuels or other valuable lipids. Lipids act as energy stores and can be harvested for a variety of uses. Finally, microalgae can synthesize and accumulate other organic materials, including proteins and pigments. Proteins are essential for the growth and maintenance of microalgal cells, and pigments such as chlorophyll, carotenoids, and phycobiliproteins are involved in photosynthesis and light harvesting [8]. The consumption of microalgae has a long history. Spanish chroniclers described local fishermen gathering blue-green masses of microalgae from the lake and preparing them into dry cakes known as "*tecuitlatl*". For centuries, Chadians have harvested spirulina (also known as "*dihé*") from Lake Kossorom on the northeastern edge of Lake Chad for daily consumption. *Nostoc* is traditionally used in South America, Mongolia, and China to make foods known as "*fa cai*" and "*lakeplum*". Another edible blue-green alga, *Aphanotheca sacrum* (formerly *Phylloderma sacrum),* is used in Japan for a special delicacy called "suizenji-nori" [9].

What makes microalgae perfect candidates for modern-day "nutraceuticals" or "functional foods" is their ability to synthesize products of useful value for human nutrition. Nutritional components of microalgae include long-chain fatty acids, vitamins, minerals, essential and non-essential amino acids, enzymes, and carotenoids [10]. As a food innovation, alternative protein foods based on microalgae are promising, because microalgae can be produced on uncultivated land and have high yields per square meter. This is eco-friendly food production. The high nutritional value and high protein content are also characteristics that make consumers regard microalgae as healthy food [1]. Against this background, this study aims to review the nutritional value and related criteria for a specific meat substitute called microalgae and analyze consumer preferences for it.

Objects and methods

Search strategy

For this review, we searched PubMed, Scopus, ResearchGate, and Google Scholar using the following search term chains: meat substitutes, alternative proteins, functions of microalgae, microalgae food standards, and microalgae food consumer preferences, following PRISMA flow guidelines. Figure 1 is a flowchart depicting the process of selecting studies for inclusion in this review.

Eligibility criteria

Articles included in this review had to meet the eligibility criteria for this review, which included selecting studies related to the function of microalgae, the nutritional properties of microalgae, and microalgae as an alternative protein.

Screening and data extraction

For inclusion criteria, we considered a variety of article types, including original articles, full-length articles, Internet articles, summary reports, and series. We did not impose restrictions on publication date or language. Exclusion criteria included inaccessible full texts, full texts not containing raw data, inappropriate topics, university theses and dissertations, and topics irrelevant to the main focus of the review.

Selecting studies and extracting data

We used a literature review approach. A total of 215 references were selected using the PRISMA flowchart from the leading journal search sites such as PubMed, Google Scholar, ResearchGate, and Scopus. As a result, a total of 51 articles from 2016 to 2023 were finally selected. The PRISMA flowchart is shown in Figure 1.

Results

Microalgae-based foods

Algae are unicellular or multicellular organisms that vary in size and shape, and are classified into microalgae (micro) and macroalgae/algae (macro) groups. Microalgae have the ability to self-organize into clusters and filaments. Colonies of microalgae are often formed by individual cells clustered together. These clusters can provide a number of benefits, including protection from predators, improved nutrient uptake, and better access to light. These clusters can vary in size from small groups to large visible colonies,

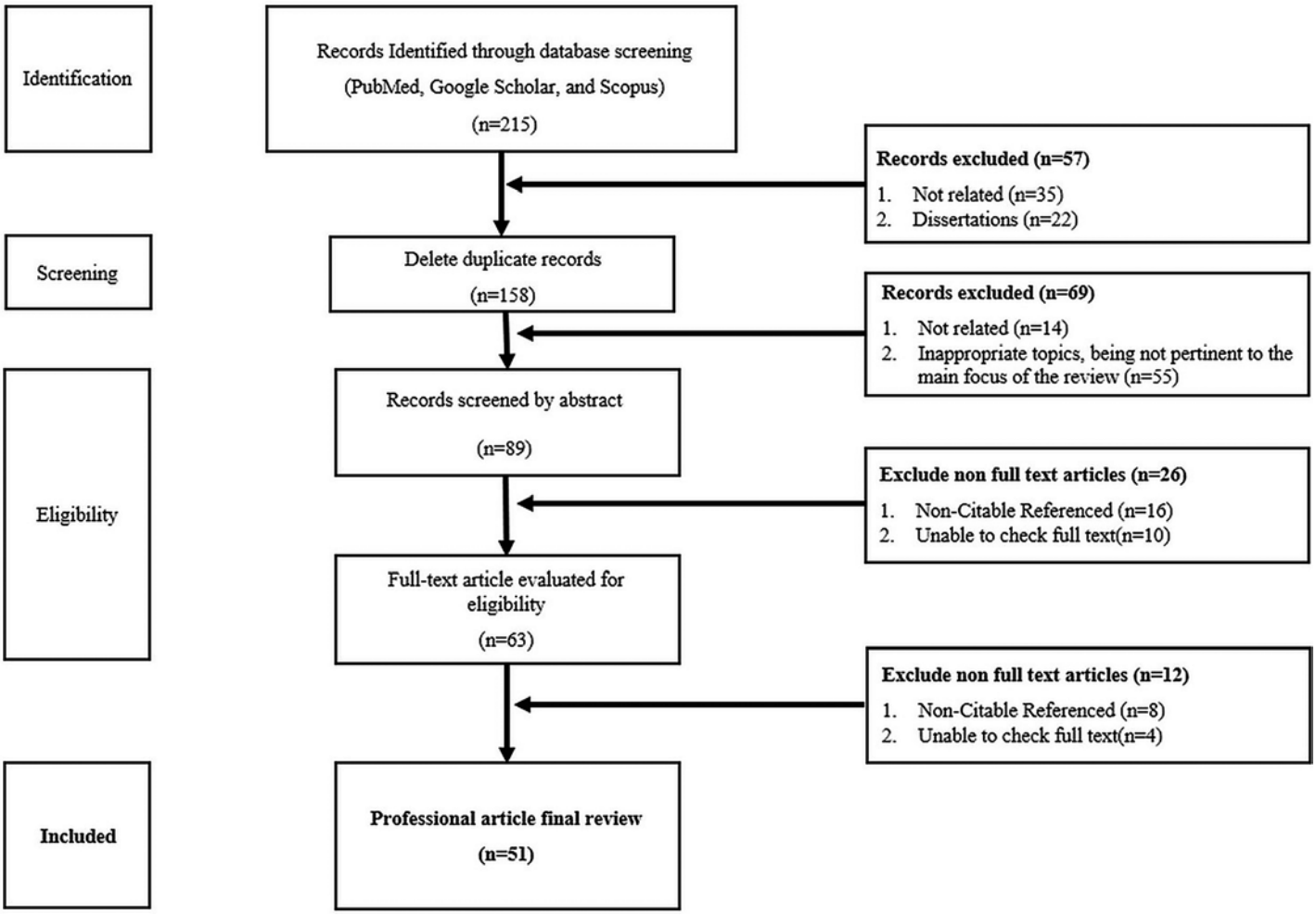

Figure 1. PRISMA flow chart for literature review search results

depending on the species and environmental conditions. Some microalgae can also be organized into filamentous structures. These filaments are chains of interconnected cells that can form elongated structures. Filaments can help microalgae effectively navigate their environment, utilize nutrients, and optimize their position for light absorption. Filamentous microalgae are adapted to life in aquatic environments because they can float on water or attach to substrates by extending their filaments. As a result, these self-organizing structures of microalgae show adaptability and resourcefulness in response to their surroundings, and contribute to their ecological success in a variety of aquatic ecosystems. If you look at microalgae, you will see clusters and filaments that allow them to organize themselves [11]. Microalgae are a smaller, more heterogeneous group with organisms ranging in size from 1 μm to 1 mm and are found primarily in freshwater or soil [12].

Increasing demands for water, food, and energy are putting pressure on sustainability. Natural resources are finite. Climate change is threatening ecosystems and societies. Biodiversity conservation is especially important. This requires planning for the efficient use of food resources [13]. Food production primarily depends on soil resources because there are limited alternative sources of sustenance. Therefore, dwindling food resources are one of the major concerns for the future [14]. In the 20th century, the need to use biomass for food spurred the use of microalgae. However, with the growth of traditional agriculture, the alternative of growing microalgae on a large scale was pushed to the back burner [11]. Nevertheless, the moment has already arrived for traditional agriculture to make the transition from the current food system to sustainable production options. By 2050, climate change is expected to increase by 50–90%. The main driver is an increase in the world population [15,16].

Microalgae may seem like the food of the future, but they are not. Microalgae are already longtime allies of human nutrition, with *Nostoc* and *Spirulina* having been used as food since before 1900 [17]. Microalgae biomass has been mainly used as foodstuff supplements and additives. In recent decades, it has been considered a foodstuff with health advantages. (Figure 2).

For example, certain microalgae species are rich in docosahexaenoic acid (DHA), which is often found in juices, drinks, or milk for infants and children [19–20].

Approximately 13,090 new products containing algae or algae-derived ingredients were developed globally from 2015 to 2019. Among these new products, 79% were foods and 21% were beverages [21]. Microalgae are an excellent source of a wide variety of compounds. They have the high protein content (*Atrosphaera platensis*) and low fiber content (*Chlorella*) [22,23]. Microalgae species such as *Pyro-*

Figure 2. Classification of products based on algae and microalgae [18]

cystis lunula, *Nannochloropsis gaditana*, and *Atrospera platensis* have the potential for the production of commercial carbohydrates such as monosaccharides, disaccharides, and polyalcohols [24].

Microalgae protein composition and nutritional value

Microalgae have become an important ingredient in recent years for a variety of applications, from the global food and beverage industry and aquaculture to human nutrition and animal feed. This is due to the following reasons: (1) long-term sustainability, as microalgae have the lowest carbon, water, and arable land footprint compared to any crop (2) high content of healthy nutrients such as protein, essential amino acids, vitamins, antioxidants, omega-3 PUFAs, and minerals; (3) high productivity compared to terrestrial crops and animal foods; and (4) environmental remediation (*e. g.*, ecosystem services) [25–27].

With an estimated 200,000 species, microalgae are as diverse as they are numerous. Among the microalgae, we are most familiar with *Cyanophyceae* (blue-green algae), *Chlorophyceae* (green algae), *Bacillariophyceae* (including the diatoms), and *Chrysophyceae* (including the golden algae) [28]. The protein content of microalgae is highly dependent on the environment, in which they live, and the protein composition varies between species and strains. However, many microalgae species typically contain high levels of protein. This can range from 40 to 60 percent on a dry matter basis [29]. Most microalgal species have been found to have a crude protein content greater than 40% based on dry mass. The distribution of the crude protein content in microalgal biomass ranges from 6 to 63% [27]. We analyzed the protein content of 16 species of microalgae and found that the protein content (% of dry cellular material) ranged from 12% (*Chaetoceros gracilis*, a diatom) to 35% (*Nannochloropsis oculata*, a eustigmatophyte).

Sustainable consumerism is driving a demand and preference for new alternative protein sources that can reduce meat consumption. As a result, plant-based alternative protein sources, such as pulses and microalgae, have received increasing attention in recent years [30,31]. When measuring protein quality, microalgae such as *Chlorella sorokiniana* and *Chlorella vulgaris* have been shown to have higher Protein Digestibility-Corrected Amino Acid Score (PDCAAS) levels than legumes such as lentils, peas, chickpeas, and soy, which are commonly used as plant-based protein alternatives [32]. Microalgae have also traditionally been consumed in a dried state, which helps to provide a rich source of nutrients and health benefits [33]. However, some species can have low digestibility, so using various techniques to disrupt the cell wall and selecting species that are inherently less recalcitrant is important for species consumed as whole cells. When consuming microalgae, the use of purified forms can significantly improve digestibility and final protein quality compared to protein concentrates or protein isolates. The downside is that they are more expensive to produce [34].

Microalgae have a variety of health benefits, including antioxidant, anti-cancer, and anti-inflammatory properties. These are all effects that can be synthesized through the metabolism of microalgae. *Spirulina*, in particular, has been found to be safe and has no toxic effects, according to reports submitted to the US Food and Drug Administration (FDA) [35]. Microalgae have a high nutritional value. They are also rich in PUFAs and have a high content of bioactive peptides [36].

Consumer attitudes toward microalgae as an alternative protein source

As a novel food, algae can contribute to healthier and more sustainable food consumption. Because of this potential, it is thought to be a promising alternative protein source. Therefore, it is very necessary to analyze the preferences and perceptions of consumers who may accept microalgae as food [37]. In addition to positive drivers for microalgae as a food, previous studies have found barriers including meat preference habits when considering microalgae-based meat substitutes [1], and an unsafe image and lack of information about microalgae as a food [38,39].

Drivers of consumers' positive acceptance of microalgae foods included perceiving microalgae as healthy food and believing that they have health benefits [40,41], knowledge of microalgae and experience with them as a food [42], and consumers' belief that they do not need meat in their diet [1]. When consumers were given enough infor-

mation about how microalgae were produced, they were less averse to microalgae foods [43].

We reviewed a study that measured consumer attitudes toward microalgae as a real food (hereafter referred to as microalgae attitudes). The study developed four items to measure consumers' varying attitudes toward microalgae in terms of health, nutrients, and sustainability. The four items are: (1) Algae are a more sustainable source of protein than meat", (2) Algae contain a lot of protein", (3) Algae are very healthy", and (4) Algae contain a lot of vitamins and nutrients". Consumers participated in the study by indicating their opinions on the four items above on a 5-point Likert scale (1 = completely disagree to 5 = completely agree). The average of the four items for each consumer was used to derive a final measure of microalgae attitudes. The reliability of the multiple-item manipulation of the dependent variable was high across all responding consumers. Overall, consumers had an average microalgae attitude of 3.38 to 3.65 [37].

The time-saving aspect can be helpful when introducing meat alternatives such as microalgae-based foods to consumers. For example, compared to grilling your own meat or making your own food after work, meat alternatives are largely processed and marketed as easy to prepare, requiring only heating and eating [39]. Increasingly, consumers prefer foods that meet health and sustainability standards. In addition, economics will be a critical factor in the adoption of microalgae as a protein source alternative to meat. These findings provide food producers and marketers with insights that are critical to increasing microalgae's share of the food market. It is best to achieve low prices through economies of scale before going to market. In addition, recipes should be provided so that consumers can utilize microalgae when making familiar foods [37].

Microalgae product quality standards and legislation

Studies have shown that the risks associated with microalgae include toxins, pathogens, allergens, heavy metals, and pesticides. However, toxins have not been found in spirulina and chlorella [8]. Microalgae are considered non-traditional foods and must undergo a series of toxicity tests to prove they are harmless. Toxins are categorized into biological and abiotic. Biological toxins are algal toxins and nucleic acids, while abiotic toxins are environmental pollutants, with heavy metals being a typical example [10].

Companies that want to sell products containing microalgae must comply with regulations. Relevant safety and regulatory requirements vary by country or region. This usually means applying for a license and submitting scientific information and health and safety assessments to the appropriate authorities (Figure 3).

In Europe, three levels of regulation oversee microalgae-related ingredients and marketing: (1) general food safety regulations, (2) new food ingredients, and (3) nutrition and health claims for foods [44]. Of these, the closest regulation relevant to microalgae is Regulation (EC) No 2015/2283 on novel foods, which repealed the earlier Regulation No 258/97. One of the criteria for food to be considered a novel food continues to be the absence of use for human consumption to a significant degree within the EU before May 15, 1997. The scope of Regulation No 2015/2283 in principle remains the same as that of Regulation (EC) No 258/97. Nevertheless, it was necessary to review, clarify and update categories of foods that constitute new foods in the light of scientific and technological developments that occurred after 1997. According to Regulation (EC) No 2015/2283, these categories should include whole insects and their parts; foods with novel or intentionally modified molecular structures; foods from cell or tissue cultures derived from animals, plants, microorganisms, fungi or algae; foods from microorganisms, fungi or algae; and foods derived from mineral substances. The definition of novel foods can also include foods composed of certain micelles or liposomes [45].

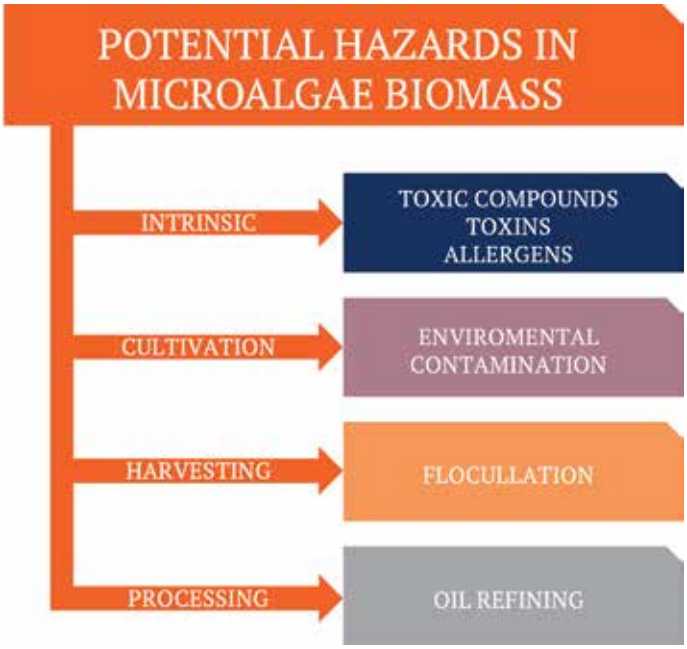

Figure 3. Classification of hazards linked to using algal biomass [18]

Even the few microalgae known so far have the potential to be used in a wide range of applications because they produce a wide range of nutrients (fatty acids, pigments, proteins, vitamins, and precursors to vitamins). Currently, the use of algae requires a permit. The strict legal provisions are intended to protect consumers from insufficiently researched or potentially unsafe foods. With the discovery and general approval of the potential of microalgae, approving additional species of algae with specific nutrient profiles will open up new opportunities for alternative proteins [46].

Discussion

Microalgae products come in a variety of flavors and colors, which is enough to maintain interest toward them [38]. However, the public still has questions about the safety of these new products; thus, there are as many challenges for microalgae and microalgae-infused food supple-

ments as growth prospects. Therefore, food regulations need regular updates to ensure that consumers are well informed about the risks and safety of new foods. To benefit consumer health, the more ingredients included, the more stringent the maximum allowable concentration [47].

Meanwhile, policies are proposed to integrate microalgae cultivation into agricultural systems, with the aim of increasing consumption demand: good production practices, advances in production technologies, and quality monitoring. Finally, extensive knowledge of production in local growing realities is required, with a strategy designed with strong planning across the entire food chain [48,49].

The high production cost of microalgae, technical challenges associated with extraction and purification, and palatability issues have hindered their application in food despite their nutritional similarities to other plant-based proteins [50]. A cost-benefit analysis and review of production costs are important and necessary considerations for utilizing microalgae. Microalgae also need to be proven to be a functionally better alternative to animal proteins [51].

Conclusion

The negative effects of meat consumption on the environment and health have led to a growing interest in sustainable diets. Meat alternatives are being developed, and microalgae are one of them. Microalgae foods are high in protein and have many nutritional benefits that support health. The quality of their protein is also higher than that of legumes, a well-known alternative protein source. Consumer interest in new protein sources to replace animal protein has also increased. Microalgae, in particular, are generally accepted as a healthy and sustainable source of protein that contains many vitamins and nutrients. They are also less aversive to consumers than foods made from insects, another alternative protein source. As standards and regulations for microalgae foods are gradually refined, the market for alternative proteins using microalgae is expected to grow.

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COMPARATIVE ANTIOXIDANT EFFECT OF ASCORBIC ACID AND ROSEMARY EXTRACT

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Keywords: *antioxidant, minced meat, color indicators, fat oxidation, thiobarbituric acid value, peroxide value*

Abstract

The aim of the work was to study an effect of ascorbic acid and the extract of rosemary on fat oxidation, color characteristics, pH and moisture binding capacity of minced pork during cold storage. The antioxidants were introduced into minced pork in an amount of 0.05%. After addition of the antioxidant, minced meat was packed in the modified atmosphere with the high oxygen content and stored at a temperature of 4 ± 2 °C for 15 days. The indicators of the hydrolytic (acid value) and oxidative (peroxide value and thiobarbituric acid value) spoilage, color characteristics, pH and moisture binding capacity (MBC) were determined during the whole storage period (0, 5, 8, 12, 15 days). An increase in the acid value was recorded in all minced meat samples during storage without a significant difference between the control and experimental samples. Addition of the antioxidants led to a decrease in the peroxide value after 12 days of minced meat storage. Malonaldehyde began to accumulate in the control and the sample with ascorbic acid on the 8th day of storage and in the sample with the rosemary extract on the 12th day. The results obtained point to inhibition of fat oxidation in the minced meat samples with the antioxidants. Addition of the antioxidants facilitated an increase in redness compared to the control. Contrary to the rosemary extract, addition of ascorbic acid led to a decrease in pH and MBC of minced meat. Therefore, the use of the rosemary extract exerted more effective action of minced pork stability during storage compared to the same dose of ascorbic acid.

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Introduction

Lipids play a key role in formation of food product quality. An effect of fat on the sensory properties of products is linked with two main components — triglycerides and phospholipids. Triglycerides are located in adipose tissue and intermuscular adipose cells being solvents for many aromatic substances. Phospholipids are located in the myofibril membranes [1]. In the process of meat processing, fats are subjected to two main transformations — lipolysis and oxidation. The intensity of these changes depends on many factors including a raw material type, presence of non-meat recipe ingredients, packaging method, type and duration of technological processing [2–4].

The oxidative processes have a significant effect on formation of food product safety and quality, and cause changes that influence their nutritional value, sensory characteristics and shelf life. Prevention of fat oxidation has the utmost importance for the meat industry facilitating an increase in production of high-quality foods and shelf-life extension.

The self-oxidation processes can be retarded using natural and synthesized substances — antioxidants. Antioxidants are compounds that inhibit oxidation suppressing generation of free radicals as a result of the following mechanisms: scavenging species that initiate peroxidation, chelating metal ions so that they are unable to generate reactive species or decompose lipid peroxides, quenching $\bullet O^{2-}$ preventing formation of peroxides [5].

Multiple studies show that lipid oxidation in meat products can be minimized by introducing not only food additives but also substances isolated from natural sources having the antioxidant activity [6–8]. Substances with the simultaneous antioxidant and antimicrobial activities and capable of improving sensory characteristics are of special interest. For example, the rosemary extract can be used not only to improve aroma but also as a potential antioxidant and preserving agent [9–11]. Several studies point to the antibacterial activity of rosemary against *E. coli*, *Bacillus cereus*, *Staphylococcus aureus*, *Clostridium perfringens*, *Aeromonas hydrophila*, *Bacillus cereus* and *Salmonella choleraesuis* [12]. The antibacterial and antioxidant effects of the rosemary extract were noticed both in the experiments on raw meat [12,13] and processed meat products [12,14].

The mechanism of the action of the rosemary extract as an antioxidant is extensively discussed in several publications [15,16]. Cui et al. [17] and Kontogianni et al. [18] believe that the antioxidant activity of the rosemary extract can be explained by the presence of carnosol. According to Loliger [19], carnosic acid and carnosol have an ability to scavenge peroxyl radicals. However, rosmarinic acid and hesperidin also act as scavengers of free radicals [20,21].

Aruoma et al. [22] believe that more than 90% of the antioxidant properties of the rosemary extract are conditioned by the presence of carnosic acid and carnosol. Schwarz et al. [23] reported that carnosic acid degraded less compared to other phenolic diterpenes in the conditions of the high temperature. Houlihan et al. [24] are of the opinion that the antioxidant properties of rosemary are explained by the high content of isoprenoid quinones, which scavenge free radicals and act as chelating agents for the reactive oxygen species (ROS). According to Hwang et al. [25], the antioxidant activity of rosemary is explained by the presence of the phenolic compounds that prevent free radicals as well as the spread of the free radical reaction due to chelation of ions of transition metals such as iron. Moreno et al. [26] found that the rosemary extract was similar to butylhydroxytoluene and α-tocopherol in terms of the high radical scavenging activity.

Therefore, the majority of researchers agree that carnosic acid is the main factor responsible for the antioxidant activity of the rosemary extract. Several studies point to a positive effect of the rosemary extract on a decrease in the TBA value during storage of both raw and thermally processed meat products [27–29]. On the contrary, Jin et al. [30] reported that addition of the rosemary powder did not change the TBA value in sausages compared to the control; however, rosemary showed an ability to scavenge DPPH radicals during storage. According to several studies, besides inhibiting fat oxidation, the rosemary extract facilitated an improvement in the color of meat products during storage, which was manifested, first of all, as an increase in redness stability [31–33]. However, the available data are contradictory and several authors reasoned that rosemary does not exert a significant effect on the color of meat products [34,35]. The results of the influence of the rosemary extract on the product sensory characteristics are also ambiguous. Karpińska-Tymoszczyk [36] reported that addition of the rosemary extract allowed improving sensory quality of turkey meat balls during cold storage. Racanicci et al. [37] also indicated that introduction of rosemary reduced the TBA value and enabled longer preservation of consumer-acceptable sensory characteristics of pre-cooked meat balls made from chicken breast. On the contrary, Jin et al. [30] reported about lower scores in taste and aroma assessment of sausages with rosemary. Differences in the results can be linked with a form of used rosemary (extract, powder), different types of meat raw materials, the use of thermal treatment, presence of other recipe components — salt, phosphates, sodium nitrite and others. An important method for preservation of safety and quality of meat and meat products is the use of modified atmosphere packaging (MAP) [38–40]. However, at high concentrations of CO_2 in the gas mixture, a reduction in pH can be observed, which is accompanied by changes in the natural meat color, moisture separation and appearance of off-taste. Martínez et al. [41] found that an increase in the CO_2 concentration in the gas mixture facilitated oxidation of myoglobin and lipids. Preservation of proper quality of packed sausages was achieved when using not more than 20% CO_2. Oxygen is widely used as a component of gas mixtures for meat due to the fact that myoglobin oxidation with the oxymyoglobin formation imparts the red color to meat and makes a product more attractive for consumers. The use of modified atmosphere containing the high oxygen concentration (80%) and low CO_2 concentration for meat packaging facilitates myoglobin oxygenation maintaining the attractive red color of meat [42–44]. According to Jakobsen and Bertelsen [45] the stability of meat color characteristics was achieved when using oxygen in MAP gas mixtures in amounts of 55–80%. On the other hand, Martinez et al. [46] found that the presence of O_2 in MAP gas mixtures caused a significant increase in the oxidation rate, reduction of the shelf life due to discoloration and deterioration of aroma in packed pork sausages. An improvement of color during 8-day storage was observed at an O_2 concentration of 80%. Several other studies also suggest a negative effect of O_2 in the MAP gas mixtures on oxidation of lipids and proteins [47,48]. To preserve a meat color and inhibit oxidative changes in fat and proteins, the combined use of antioxidants and modified atmosphere packaging (MAP) containing oxygen is of interest. Thus, within the framework of the present study, we carried out the comparative assessment of the antioxidant properties of the rosemary extract compared to the most widely used antioxidant ascorbic acid for pork packed in MAP with the high oxygen concentration.

Objects and methods

Samples

The objects of research were samples of m. *Longissimus dorsi* taken from carcasses of hybrid pigs (Large White × Duroc × Landrace) with a live weight of 115 ± 15 kg three days after slaughter. Meat was ground through a grinder plate with a hole diameter of 8 mm and divided into three groups: 1) control (without antioxidants); 2) experimental sample (AS) with addition of ascorbic acid in an amount of 0.05%; 3) experimental sample (RE) with addition of the rosemary extract in an amount of 0.05%. The prepared minced meat samples with a weight of 500 ± 5 g were packed in MAP (80% O_2 + 20% CO_2) and stored for 15 days at a temperature of 4 ± 2 °C. Minced meat samples were analyzed on 0, 5, 8, 12 and 15 days of storage.

Methods

The acid value, peroxide value and thiobarbituric acid were determined according Tunieva et al. [49]. The acid value was determined by the method based on titration of free fatty acids in the ether-alcohol solution of fat with the aqueous solution of alkali; peroxide value by the method based on oxidation of iodhydric acid by peroxides contained in fat with the following titration of liberated iodine with sodium thiosulphate. Determination of the thiobarbituric acid value was carried out by the method based on

the development of stained substances as a result of interaction of fat oxidation products with 2- thiobarbituric acid and measurement of the color intensity on a spectrophotometer.

Color characteristics were measured on a spectrophotometer CM-2300d (Konica Minolta, Japan). The moisture binding capacity (WBC) was determined by the pressing method consisting of pressing a sample with a load of 1 kg and the following calculation by the difference between masses before and after pressing and the area of the wet spot measured using a planimeter. The results were expressed in percent of the total mass of moisture in a product.

The pH value was registered by a potentiometric method using Testo 205 portable pH-meter (Testo, Germany).

The experiments were performed in triplicate, representing the findings as the mean ± SD. A one-way ANOVA was performed to assess the analysis. Duncan's multiple range test was used for comparison at the 95% confidential level ($p < 0.05$)

Results and discussion

Oxidation of lipids

The analysis results for the indicators of hydrolytic and oxidative spoilage of minced pork during storage are presented in Table 1.

Table 1. Changes in the acid value, peroxide value and TBARS of minced pork during storage

Indicators	Time of storage (d)	Control	AS	RE
Acid value (mg KOH/g fat)	0	1.69 ± 0.13^{ax}	1.60 ± 0.16^{ax}	1.64 ± 0.15^{ax}
	5	2.07 ± 0.14^{ax}	1.83 ± 0.18^{ax}	1.94 ± 0.18^{ax}
	8	2.44 ± 0.15^{abx}	2.33 ± 0.16^{abx}	2.36 ± 0.16^{abx}
	12	2.84 ± 0.18^{bx}	2.61 ± 0.14^{bx}	2.41 ± 0.17^{abx}
	15	3.71 ± 0.20^{cx}	3.38 ± 0.19^{cx}	3.20 ± 0.22^{bx}
Peroxide value (mmol active O_2/kg fat)	0	2.27 ± 0.11^{ax}	1.90 ± 0.14^{ax}	2.20 ± 0.18^{ax}
	5	2.98 ± 0.17^{bx}	2.58 ± 0.18^{ax}	2.24 ± 0.20^{ax}
	8	3.89 ± 0.19^{cx}	3.73 ± 0.21^{bx}	3.36 ± 0.24^{bx}
	12	5.24 ± 0.15^{dx}	4.65 ± 0.28^{by}	4.13 ± 0.21^{by}
	15	6.96 ± 0.26^{ex}	5.82 ± 0.21^{cy}	5.11 ± 0.20^{cy}
TBARS (mg MA/kg)	0	0.000^{ax}	0.000^{ax}	0.000^{ax}
	5	0.000^{ax}	0.000^{ax}	0.000^{ax}
	8	0.078 ± 0.008^{bx}	0.039 ± 0.004^{by}	0.000^{az}
	12	0.195 ± 0.020^{cx}	0.094 ± 0.009^{cy}	0.042 ± 0.008^{bz}
	15	0.284 ± 0.025^{dx}	0.158 ± 0.014^{dy}	0.097 ± 0.012^{cz}

a-e-Values with different letters within a column are significantly different (P < 0.05); x-z-Values with different letters within a row are significantly different (P < 0.05), n=3/

According to the data obtained, an increase in the acid value was observed in all minced meat samples during storage; with that, a significant difference between the control and experimental samples was not found during 8 days of storage. On the 12th day, lower acid values were noticed in the minced meat samples contained the rosemary extract. Apparently, this was conditioned by the manifestation of the inhibitory action of this antioxidant on the hydrolytic changes in fat in the sample. Similar data were obtained by Karpińska-Tymoszczyk [50] who recorded lower acid values in the samples of turkey meat balls contained the rosemary extract compared to the control. The peroxide values in the samples of minced meat did not have significant differences from the control up to 8 days of storage. Addition of the antioxidants led to a decrease in the peroxide value after 12 days of minced meat storage. By the end of storage, the highest accumulation of peroxides was noticed in the control sample. Significant differences in the peroxide value of the sample with ascorbic acid and the rosemary extract were not observed during the whole storage period. The antioxidants exerted more significant impact on accumulation of the secondary oxidation products. For example, malonaldehyde began to accumulate in the control and the sample with ascorbic acid on the 8th day of storage and in the sample with the rosemary extract on the 12th day. The results obtained indicate inhibition of fat oxidation in the minced meat samples with the antioxidants. With that, the use of the rosemary extract had the higher effect compared to ascorbic acid at the same dose. The data obtained correspond to Al-Hijazeen & Al-Rawashdeh [51] who established that the rosemary extract in an amount of 350 ppm had the higher effect on the TBA value and the level of carbonyls in chicken meat parties compared to the same dose of ascorbic acid. Manhani et al. [52] also reported that addition of the rosemary extract led to the more effective decrease in the amount of malonaldehyde during storage of frozen beef hamburgers compared to addition of the oregano extract and sodium erythorbate. Similar data were obtained by Perlo et al. [29] who did not find a significant effect of ascorbic acid on the TBARS value in pork steaks, while spraying the rosemary extract enabled significant reduction of the TBA value. On the contrary, Lund et al. [53] found that the rosemary extract (0.05%) and the mixture of ascorbate (0.05%) and citrate (0.05%) allowed the equal reduction of TBARS in beef patties during 6-day storage. Jin et al. [30] reported that addition of rosemary in amounts of 0.1 and 0.2% did not change the TBA value in sausages compared to the control; however, rosemary showed an ability to scavenge the DPPH radicals during storage. It is worth noting that a rosemary powder and not an extract was used in this study. Moreover, other recipe components in the sausage composition (salt, nitrite, phosphates) could affect the obtained results.

Color characteristics

According to several studies the rosemary extract besides inhibition of fat oxidation also facilitated an improvement in a meat product color during storage, which was manifested, first of all, as an increase in the stability of redness. However, Resurreccion & Reynolds reported that rosemary oleoresin inhibited oxidation without improving a color of frankfurters [34]. McCarthy et al. demonstrated the antioxidant action of rosemary on pork

patties; however, an effect on the product color was insignificant [35].

The results of the instrumental assessment of the minced pork color are presented in Tables 2 and 3.

Table 2. Changes in redness of minced pork

Storage duration, days	Redness (a*)		
	Control	AS	RE
0	3.28 ± 0.34^{ax}	4.59 ± 0.37^{axy}	4.97 ± 0.31^{ay}
5	3.36 ± 0.23^{ax}	4.29 ± 0.27^{axy}	5.15 ± 0.44^{ay}
8	3.35 ± 0.31^{ax}	4.90 ± 0.37^{ay}	4.75 ± 0.21^{ay}
12	3.84 ± 0.43^{ax}	5.14 ± 0.31^{ax}	4.95 ± 0.25^{ax}
15	3.23 ± 0.27^{ax}	4.72 ± 0.38^{ay}	4.84 ± 0.32^{ay}

a-e-Values with different letters within a column are significantly different (P < 0.05); x-z-Values with different letters within a row are significantly different (P < 0.05), n = 3.

Table 3.Changes in yellowness of minced pork

Storage duration, days	Yellowness (b*)		
	Control	AS	RE
0	10.34 ± 1.09^{ax}	12.37 ± 0.73^{ax}	12.23 ± 1.19^{ax}
5	11.53 ± 1.17^{ax}	12.21 ± 0.51^{ax}	12.86 ± 0.38^{ax}
8	11.83 ± 0.81^{ax}	12.83 ± 0.88^{ax}	12.51 ± 0.90^{ax}
12	12.24 ± 1.15^{ax}	13.07 ± 1.04^{ax}	12.91 ± 0.80^{ax}
15	14.18 ± 1.08^{ax}	14.01 ± 1.22^{ax}	12.98 ± 0.78^{ax}

a-e-Values with different letters within a column are significantly different (P < 0.05); x-z-Values with different letters within a row are significantly different (P < 0.05), n = 3.

According to the data obtained during minced meat storage, there were no significant changes in redness and yellowness in the internal part of minced meat in all samples. Apparently, the absence of color changes during storage is explained by oxygen dissolution and myoglobin oxygenation, which inhibited its oxidation with metmyoglobin formation. Addition of antioxidants facilitated an increase in redness compared to the control; however, it did not have a significant effect on yellowness. With that, significant differences between color indicators in minced meat with ascorbic acid and the rosemary extract were not observed. The data obtained correspond to the study of Al-Hijazeen & Al-Rawashdeh [50], who reported that addition of the rosemary extract and ascorbic acid positively affected color characteristics of chicken meat; however, significant differences between the experimental samples with the antioxidants were not revealed. Fernández-López et al. [54] demonstrated inhibition of the metmyoglobin formation by rosemary in cooked pork; with that, an effect of rosemary addition increased with an increase in product storage duration. On the contrary, Perlo et al. [29] reported an absence of a significant effect of spraying ascorbic acid and the rosemary extract on color characteristics of vacuum packed pork steaks. Lee et al. established that ascorbic acid (0.1%) significantly inhibited the metmyoglobin formation on the surface of minced beef, but not in the depth of the product; while carnosin (1.0%) significantly inhibited the metmyoglobin formation and the brown color development throughout the whole product [55].

pH value

There are different data about pH changes during meat and meat product storage. Blixt & Borch pointed to a decrease in meat pH during storage [56]. A reduction of pH during storage is reported to a greater extent for vacuum packed products. For example, the absence of oxygen in vacuum packaging promotes the development of *Lactobacillus* [57], and accumulation of their metabolites leads to pH drop [58]. In contrast, Apple et al. [59] reported about an increase in pH during storage of vacuum packed pork after 6 weeks. Perlo et al. found that ascorbic acid and the rosemary extract did not exert a significant effect on pH of vacuum packed pork steaks [29]. However, a decrease in pH was noticed during cold storage for 45 days.

According to the results of this study presented in Table 4, addition of ascorbic acid led to a reduction of pH, while the rosemary extract did not significantly affect a change in the pH value. Teruel et al. also revealed that the rosemary extract did not influence pH of frozen chicken nuggets [60]. On the contrary, Jin et al. reported that addition of the rosemary extract in amounts of 0.1 and 0.2% led to a reduction of pH in sausages [30]. During storage for four weeks, pH of sausages reduced and then increased by the 6th week of storage.

Table 4.Changes in the pH value of minced pork

Storage duration, days	pH		
	Control	AS	RE
0	$5.52 \pm 0{,}04^{ax}$	5.25 ± 0.02^{ay}	5.51 ± 0.03^{ax}
5	5.51 ± 0.02^{ax}	5.31 ± 0.02^{aby}	5.55 ± 0.01^{ax}
8	5.48 ± 0.02^{ax}	5.32 ± 0.04^{ab}	5.56 ± 0.03^{ax}
12	5.50 ± 0.04^{ax}	5.34 ± 0.01^{by}	5.58 ± 0.02^{ax}
15	5.54 ± 0.04^{ax}	5.35 ± 0.02^{by}	5.61 ± 0.03^{ax}

a-e-Values with different letters within a column are significantly different (P < 0.05); x-z-Values with different letters within a row are significantly different (P < 0.05), n=3.

Changes in pH were not revealed during storage of the control sample and minced meat sample with the rosemary extract. In contrast, pH decreased in the sample with ascorbic acid. The data obtained correspond to Ozer & Sariçoban, who reported that addition of ascorbic acid in an amount of 300 mg/kg led to a reduction of pH in chicken patties [61]. During storage, the pH values decreased initially and then increased. The initial decrease can be linked with addition of acid and an increase can be a result of the production of microbial metabolites. Fernández-López et al. reported that pH in cooked pork increased as a result of cold storage reaching the ultimate value of 5.92, while the pH values in the samples with rosemary did not have significant changes during storage [54]. These differences are, possibly, linked with the use of different packages and analysis of different meat product types including those that contained other food ingredients and additives.

Moisture-binding capacity

It is known that pH affects moisture-binding capacity (MBC) of meat. Taking into account a decrease in pH in the presence of ascorbic acid, MBC was measured in the analyzed minced meat samples (Table 5).

Table 5.Changes in MBC of minced pork

Storage duration, days	MBC		
	Control	AS	RE
0	62.7 ± 2.0^{ax}	55.0 ± 1.1^{ay}	61.6 ± 1.6^{ax}
5	56.2 ± 1.5^{abxy}	53.2 ± 1.9^{ay}	62.8 ± 2.2^{ax}
8	54.5 ± 2.1^{bxy}	51.3 ± 1.7^{aby}	61.6 ± 1.5^{ax}
12	50.6 ± 1.3^{bcy}	49.7 ± 0.7^{by}	59.2 ± 1.4^{ax}
15	52.4 ± 1.6^{by}	47.0 ± 2.8^{by}	59.8 ± 0.8^{ax}

a-e-Values with different letters within a column are significantly different ($P < 0.05$); x-z-Values with different letters within a row are significantly different ($P < 0.05$), n=3.

Addition of ascorbic acid led to a decrease in MBC of meat by 12.2% ($p < 0.05$) at the initial stage of storage compared to the control. Apparently, lower MBC values of meat in the samples with ascorbic acid are linked with a shift in pH towards the acid side, which in turn caused a reduction of the moisture binding capacity of proteins. It was established that at the initial stage of storage up to 12 days, the experimental samples with the rosemary extract did not have significant differences in the MBC value with the control ($p > 0.05$). A significant decrease ($p > 0.05$) in the MBC value of the control was found upon an increase in storage duration, which probably can be explained by a shift in pH towards the acid side during storage due to production of microbial metabolites. The MBC value in the samples with the rosemary extract was relatively stable during the whole storage period ($p > 0.05$).

Conclusions

Addition of the antioxidants ascorbic acid and the rosemary extract inhibited hydrolysis and oxidation of fat, which was especially manifested at the late period of storage after 8 days. With that, the use of the rosemary extract exerted the higher antioxidant effect compared to ascorbic acid at the same dose. Addition of the antioxidants promoted an increase in redness; however, significant differences between color indicators in minced meat with ascorbic acid and the rosemary extract were not found. Contrary to ascorbic acid, the rosemary extract did not lead to a decrease in pH and MBC compared to the control and during storage. Therefore, the use of the rosemary extract exerted more effective impact on minced pork stability during storage compared to the same dose of ascorbic acid.

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THE EFFECT OF MAGNETIC AND ELECTRIC FIELDS ON THE PROCESSES OF FOOD FREEZING

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Keywords: *food products, cooling, freezing, phase transition, magnetic fields, electric fields, frozen food quality*

Abstract
The article summarizes the results of studies based on scientific publications on the effect of magnetic fields (MF) and electric fields (EF) on the kinetics of freezing processes applied onto biological tissue and on their properties. The processes of freezing food media on installations equipped with the Cells Alive System (CAS) magnetic system manufactured by ABI Co., Ltd., Japan are considered in this article. It is shown that the majority of researchers did not confirm the benefits claimed by the CAS system developers in comparison with the processes of fast freezing in the chambers without the magnetic field. In the case of using the alternating magnetic fields (AMF) with high field induction values, the effect is more pronounced. The application of strong static or alternating EF contributes to the creation of a fine-grained structure of ice, reduces the freezing duration and decreases mass loss during the food thawing.

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Introduction

Freezing as a way to preserve raw food materials, semi-finished food and ready-to-eat food is widely applied in the world. The main disadvantages of these technologies are irreversible violation of the biological tissues integrity during the cycle of freezing — thawing, which lead to a decrease of food quality parameters compared to a non-frozen food.

Lots of studies have been published in the scientific literature on the processes of water crystallization in food products [1,2] and it has been found that the shape and size of ice crystals mainly depend on the rate of freezing. If the freezing occurs at high speed, then heat is quickly removed from the object, the cell does not have time to lose moisture, and crystallization occurs both in the intercellular space and inside the cell, which reduces the risk of cell damage. As far as real size objects are concerned (meat, fish, poultry), it is practically not possible to maintain a high rate of phase transition of water into ice throughout the volume of the food, because the heat from the inside of the product is transferred due to the thermal conductivity of the flesh, therefore, in the central part the freezing process develops more slowly compared to the periphery and the reduced freezing rate forms larger ice crystals, thus causing tissue damage. In this regard, there is a growing interest in the search for new freezing technologies, including the use of additional aids to cold, which would help reduce the negative effect of low temperatures on changes of the food quality parameters and provide longer shelf life.

In a number of countries over the past 15 years the researches have been actively carried out to develop methods for controlling the process of water crystallization during freezing. Those methods include: super chilling under high pressure [3,4], ultrasound [5,6], various types of cryoprotectors, as well as electric and magnetic fields. This article focuses on the discussion only the results of research on the effect of magnetic and electric fields on freezing.

In the scientific community, patents for inventions by Owada N. [7,8], describing new technologies for freezing food products in a freezer using magnetic fields, induced a great response. The principal design of this installation, according to the description [9], look like (Figure 1) a refrigerating chamber (1) equipped with a refrigerating unit (2), fans (3) and a control system. To place food a number of shelves (4) are provided, around the shelves the several vertical rectangular coils frames are installed along the depth of the cabinet (5), in which coils generate alternating magnetic field.

The freezer can also be equipped with a certain number of permanent magnets (6) embedded into the door, top, bottom and walls of the freezer. The chamber runs various temperature conditions (down to –50 °C) and stepwise regulation of alternating magnetic field induction from 0 up to 100%, while at the 0% position of the regulator only the static magnetic field (SMF) is applied onto the food. The exact values of the air flow rate, the values of the magnetic field induction are not specified by the manufacturer of the freezer. The author [7,8] states that in the installation equipped with a CAS system, a food which quality differs

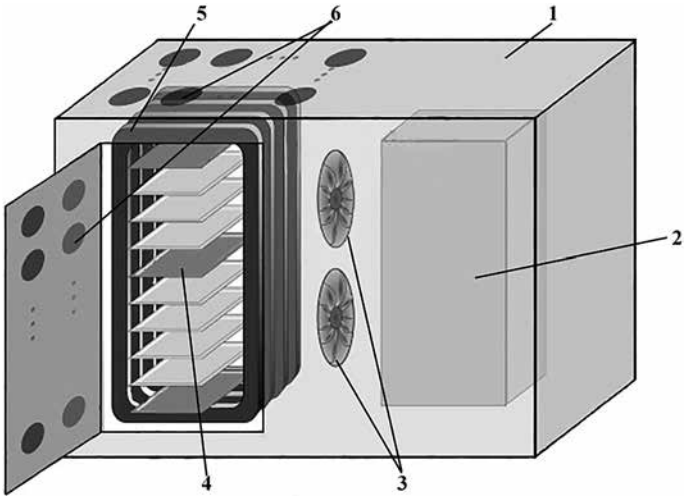

Figure 1. Schematic drawing of a freezer with a CAS magnetic system (model CAS-30B, ABI Co., Ltd., Chiba, Japan): 1 — refrigerator; 2 — refrigeration unit; 3 — fans; 4 — shelves for food placement; 5 — frame-coils for alternating magnetic field generation; 6 — permanent magnets [9]

little from the original can be obtained after thawing. However, these statements have not received proper scientific justification and confirmation by other research laboratories. Below there is an analysis of the results of studies on the use of magnetic and electric fields in the processes of freezing various food media, obtained both in installations equipped with the CAS system and on special research test-benches, in order to establish trends in the development of new methods for freezing food that provide maximum preservation of original properties after thawing.

Objects and methods

Systematic search of scientific literature was run onto the search engines: www.elibrary.ru, www.sciencedirect.com, www.springer.com and FriDoc of the International Institute of Refrigeration (www.iifiir.org) by the key phrases: food freezing, freezing in magnetic field, freezing in electric field, phase transition of water into ice. In total, the search inquiry gave 1,940 sources, which were grouped into thematic areas of the review. Articles were included based on a preliminary analysis of titles and abstracts. A total of 165 scientific studies were analyzed, including the reviews.

Inclusion criteria:

- researches publication within 2001–2023, and the work of leading scientists of other years devoted to the food refrigeration and food storage;
- the source must be indexed;
- the sources are predominantly foreign. The works published before 2001 were accepted and included in case of absence of new sources according to the specified search criteria.

Exclusion Criteria:

- publications published before 2001;
- works devoted to the study of the processes of freezing non-food media, including the use of additional aids to cold in the form of mechanical, chemical and other effects;
- publications duplicating research results;

Full texts of articles matching the search criteria were reviewed, while the majority of the used sources were published from 2017 to 2022.

Studies of processes of food media freezing in refrigerating chambers equipped with a CAS magnetic system

James et al. [10] studied the processes of freezing garlic bulbs on an experimental installation manufactured with the assistance of specialists from ABI Co., Ltd and equipped with a CAS system by the above-specified company with factory settings of magnetic field modes. The studies were run at an air temperature of –30 °C, an air velocity of 5 m/s, and at the values of the AMF induction equal to: 0.418 mT; 0.155 mT and 0.098 mT (oscillation frequency was not specified). The authors did not find a noticeable effect of magnetic fields either on the processes of super chilling or on the quality parameters of the food samples. Similar studies on the same installation, that had been used by James et al. [10], were run by Purnell et al. [11] for freezing the apples and potatoes samples at an air temperatures of –30 °C and –45 °C, an average air velocity of 0.9 m/s, and factory settings of the CAS system. The values of the AMF induction measured by the authors ranged from 0 to 0.4 mT when the frequency of the alternating magnetic field (AMF) varied from 60 to 6.5 Hz. The authors found that when potatoes and apples were frozen at –30 °C, the duration of the process using the CAS system did not differ significantly from the control time when freezing without the use of magnetic fields. In the case of freezing apples at a temperature of –45 °C, the duration of freezing significantly increased with the CAS within the entire range of magnetic field induction, while for potato samples at this temperature this dependence was not confirmed — the duration of freezing with the CAS system and without the CAS system showed no significant differences.

In general, the results of this study are consistent with those reported by Yamamoto et al. [12], which also found no effect of the CAS system on the freezing characteristics of food samples, contrary to the claims of the authors [7,8]. Almost in the same range of MF induction values and in the same chamber as James et al. [10] used, Rodríguez et al. [13] ran the experiments with freezing of pork loin samples. Freezing was run at a temperature of –30 °C and an air velocity of 1–2 m/s with a change of alternating magnetic field induction from 0 to 0.53 mT, the frequency of AMF was not specified. The authors of [13] did not find any significant effect of MF on the degree of super chilling in any of the samples; no correlation was found between the values of MF induction and the characteristic freezing time or the freezing rate. The magnetic field also did not affect the weight loss during thawing, the change in color characteristics and quality parameters. The higher values of the AMF induction (B = 0...2 mT; f = 59...6 Hz), were used by Otero et al. [9] in the CAS-30B freezer, ABI Co., Ltd., for

freezing the crab sticks. The experiments were run at the stepwise factory settings for controlling the AMF induction of 10%, 50% and 100% at an air temperature of –25 °C in the freezing chamber. The authors [9] noted a significant non-uniformity of the magnetic field induction values over the inner volume of the chamber and did not establish any benefits compared to the control sample. The same values were observed in reference to the duration of the phase transition, weight loss during thawing, water-holding capacity, color characteristics. Despite the different freezing conditions, the number of authors: Fernández-Martín et al. [14,15] when freezing egg white and yolk; Suzuki et al. [16] and Watanabe et al. [17] when freezing radishes, sweet potatoes and spinach in the CAS chamber, found no differences in the duration of the freezing in comparison with the control samples, also they found no noticeable effect of magnetic fields on the microstructure, weight loss of the food during thawing, color, texture and organoleptic characteristics of the food products.

Unlike most researchers who found no benefits from the use of CAS for freezing, Okuda et al. [18] obtained pretty positive results on freezing mackerel muscle tissue at low temperatures (–30 °C and –50 °C). The authors state that samples frozen in CAS chambers and thawed in ice water show virtually no tissue damage caused by the ice crystals. To a large extent, the positive result may have been induced probably by the speed of freezing at low temperatures, the thickness of the product and the thawing conditions.

Studies of the food media freezing processes with application of SMF and AMF on the specialized test-benches

A number of researchers have studied the effect of magnetic fields in a wider range of changes of the magnetic induction SMF and AMF. Tang et al. [19,20] studied their effect on the freezing processes and the microstructure of blackberries and cherries. The objects of study were blackberries and cherries slices, which were placed in the center of a microscope cooling stage chilled with nitrogen at a rate of 4 °C/min to a temperature of –30 °C. The system of magnetic field generation consisted of permanent magnets and AC coils that generated AMF. When being exposed to SMF, the size of ice crystals decreased by 33.6%, and when AMF was applied — by 53.8%. The AMF values were in the range of 0.05...1.74 mT at the frequency f = 50 Hz. The phase transition became shorter compared to the control experiment. When freezing cherries with SMF, the size of ice crystals decreased by 67%, and using AMF — by 78%. Freezing carrot slices in SMF gave the following values: B = 1.8 and 3.6 mT. Chen et al. [21] also obtained a positive effect, expressed in the homogeneity of the structure and minimal damage of cell. The authors believe that the magnitude of the field strength should be individually selected for each type of vegetable and fruit. The results obtained by Tang et al. [19,20] and Chen et al. [21] significantly contradict the data of other authors. One of the reasons of this difference may be different experimental conditions. When freezing thin slices, their freezing was implemented at high rate, which rather could lead to a decrease in the ice crystals size than the effect of the magnetic field. This is supported indirectly by Tang et al. [22] in the experiments with freezing pork samples of larger sizes, cut into cubes 6 × 6 × 6 mm in size.

The effect of stronger fields with induction above 7 mT was studied by Leng et al. [23] during freezing of channel catfish. This mode decreased the freezing process duration and reduced weight loss after thawing. However, these data contradict the results of Otero et al. [24], who ran series of experiments at even stronger SMF (B = 40–200 mT) to freeze potato samples prepared at a temperature of –25 °C. The results obtained in this study showed that exposure to SMF with an induction of up to 150 mT provided no significant effect on nucleation, kinetics of the freezing process, the quality of the thawed product, moisture loss, color change and structural changes.

Baryshev et al. [25] studied the effect of a low-frequency alternating magnetic field on the formation of ice crystals in the beef muscle tissues during freezing in an alternating magnetic field (B = 0.5 mT, f = 18 Hz). During the experiments, it was found that AMF provided a positive effect on the meat structure. In the control samples frozen in conventional way an uneven structure with randomly located centers of crystallization was observed, while in the samples frozen under the AMF exposure those crystallization centers were more ordered. The efficiency of AMF has been shown by Panayampadan et al. [26] while freezing samples of purified guava. The field was generated with a ring inductor with an induction of 2.4; 5.6 and 8.8 mT, with frequency from 2 to 40 kHz. The duration of the freezing process with AMF reduced by 27%, 44% and 62% was found for samples with side sizes of 2, 3 and 4 cm, respectively. The authors note that there is a possibility that the obtained results were generated by the electric field generated in the inductor.

The technical devices for freezing food products using SMF and AMF are described and specified in the patents CN1054860A [27], CN111520948 [28] and CN113819701 [29]. The authors believe that application of permanent magnets can reduce the size of ice crystals in the food product during its freezing and will help maintain the original quality of the food. The technical solutions for applying the combined and multidirectional fields are proposed. However, the patents do not provide any examples of the practical implementation of the proposed devices, nor the modes of processing the food product, which does not allow us to judge their technical performance.

In a number of works, the effect of SMF on the processes of freezing water and salt solutions as more homogeneous media has been studied.

Zhao et al [30] studied the effect of SMF on the freezing of deionized water, 0.9% NaCl aqueous solution, and 5% ethylene glycol aqueous solution placed in 5 ml glass vials. The solutions were frozen at –16 °C under the effect of SMF

(B = 0.5 mT). When freezing a 0.9% aqueous solution of NaCl, the use of SMF allowed super chilling the solution by 3 °C compared to freezing conditions without the use of the magnetic field. The authors attribute this effect to the presence of ions in the solution, as they increase their mobility in a magnetic field, and increase of the coefficient of diffusion. It was shown that the duration of freezing of the NaCl solution was reduced by 53% compared to the control sample, however, when the ethylene glycol solution was frozen, the duration of freezing increased. When freezing deionized water, SMF provided almost no effect on the kinetics of the process and the temperature of super chilling. Cai et al. [31], while studying the change in the properties of water in magnetic fields, found that SMF (B = 0.5 mT) in a flow water system significantly affected the surface tension and viscosity of water. So Cai et al. [31] came to the conclusion that the effect of SMF on aqueous hydrogen bonds is similar to the effects of temperature reduction.

Otero et al. [32] noted the absence of univocal position of the researchers on estimation of the SMF effect on water super chilling and freezing kinetics. They studied the freezing of water and 0.9% NaCl solutions. The magnetic field induction ranged from 107 to 359 mT in the experiments with "attraction of the poles", and ranged from 0 to 241 mT in the experiments with "repulsion of the poles" (opposite switching of the magnets). As a result of the research, no effect was found from the SMF, regardless of the induction values and the field orientation, neither on the magnitude of super chilling, nor on the kinetics of freezing, both for pure water samples and 0.9% NaCl solutions.

The freezing kinetics and physical properties of pure water and cucumber tissue fluid were studied by Yang et al. [33] when the samples were exposed to a pulsed magnetic field (PMF) with an induction of 2.4–6 mT with fixed duty factor ratio of 0.5 and a frequency of 25 Hz. It was established that PMF exposure increased the degree of super chilling of water and cucumber tissue fluid, reduced the duration of freezing of cucumber tissue fluid and its super chilling. Compared with the control sample, PMF exposure allowed reducing the freezing temperature of water and cucumber tissue fluid by 0.59 °C and 0.74 °C, respectively. The authors believe that PMF's effect on the charged particles in the processed sample, that inhibited the growth of ice crystals. The results of studies of effects of alternating magnetic fields (AMF) and static magnetic fields (SMF) are systematized below in the Tables 1 and 2.

Table 1. Effect of an alternating magnetic field (AMF)

Type of effect	Product type. Sample's shape and size	Experiment conditions	Result	Source
AMF	Apples. Cylinder: d=17mm; l=20mm	Installation with the CAS system. B = 0...0.4 mT; f = 8...60 Hz; T= –30 °C and T= –45 °C	*At T = –30 °C no effect was observed. At T= –45 °C, the duration of freezing increased.*	Purnell et al. [11]
AMF	Potato. Cylinder: d=17mm; l=20mm	Installation with the CAS system. B = 0...0.4 mT; f = 8...60 Hz; T = –30 °C and T = –45 °C	At T= –30 °C, the duration of the phase transition shortened. At T= –45 °C no effect was observed	Purnell et al. [11]
AMF	Pork loin. Cylinder: d =13.6; 21,8 and 30,3 mm; l =22mm	Installation with the CAS system T= –30 °C; B =0... 0.53 mT	*Weak effect*	Rodriguez et al. [13]
AMF	Crab sticks. Sticks: 15x15x38 mm	Installation with the CAS system. B = 2 mT; f = 6...59 Hz; T= –25 °C	*No changes found*	Otero et al. [9]
AMF	Mackerel. Carcasses: l=37.9 mm; of which fillets 20.6 mm thick	Installation with the CAS system. B =0.1–0.5 mT; T= –30 °C и T= –50 °C.	Inhibition of histological damage, reduction of losses during thawing in ice water.	Okuda et al. [18]
AMF	Egg white and egg yolk	Installation with the CAS system. B = 1.66 mT; f = 6 Hz; T= –50 °C	*No benefits found*	Fernandez et al. [14,15]
AMF	Blackberry. Slices: 10x3x0.1 mm	Specialized test bench. B =0...1.74 mT; f = 50Hz; T= –30 °C	Reducing the size of ice crystals by 53.8%, decreasing of the total duration of freezing, increasing the duration of the phase transition	Tang et al. [19]
AMF	Cherry. Slices: 10x3x0,1 mm	Specialized test bench. B = 0...1,74 mT, f = 50 Hz; T= –30 °C	Reducing the size of ice crystals by 53.8%, reducing the total duration of freezing, increasing the duration of the phase transition	Tang et al. [20]
AMF	Pork. Cubes: 6x6x6 mm	Specialized test bench. B = 0...1,74 mT, f = 50Hz; T = –30 °C	Increase in the phase transition time, an increase in the nucleation temperature	Tang et al. [22]
AMF	Meat. Slices of 10 mm thick	Specialized test bench. B = 0,5 mT, f = 18 Hz	Reduced damage to the structure, increased plasticity	Baryshev et al. [25]
AMF	Sweet potato potato, fish. Cylinder: d=40 mm; l=40 mm,	Specialized test bench. B = 0.5mT, f = 50Hz ...10кHz T = –35 °C	*No clear effect was found on either the degree of super chilling or the duration of freezing.*	Suzuki et al. [16]

End of Table 1

Type of effect	Product type. Sample's shape and size	Experiment conditions	Result	Source
AMF	Daikon, sweet potato.	B = 0–100 mT, T = –35 °C	*No clear effect was found on either the degree of super chilling or the temperature and duration of freezing.*	Watanabe et al. [17]
AMF	Garlic bulb, weight 40,4 g	Installation with the system CAS. B = from 0 to 0.418 mT; T = –35 °C	*No clear effect was found on either the degree of super chilling or the temperature and duration of freezing.*	James et al. [10]
IMF (impulse magnetic field)	Water and tissue fluid of cucumber. Test tube, 3.5 ml of capacity	Specialized test bench. B = 2.4 and 6 mT, porosity 0.5; f = 25 Hz; T= –15 °C	Decreasing the freezing point, Changing the freezing time: ➢ at B = 6 mT increased; ➢ at B =2...4 mT has not changed. Freeze time reduced by up to 62%	Yang et al. [33]
AMF	Guava. Cubes of the sides sizes: 2, 3 and 4 cm	Specialized test bench. B = 2.4; 5.6; 8.8 mT; f = 2...40 кHz. T = –25 °C.	Freeze time reduced by up to 62%	Panayampadan et al. [26]

Table 2. Effect of static magnetic field (SMF)

Type of effect	Product type. Sample's shape and size	Experiment conditions	Result	Source
SMF	Blackberry, slices: 10x3x0.1 mm	Specialized test bench. B = 0...10 mT, T = –30 °C	Reducing the duration of freezing; reduction in the size of ice crystals	Tang et al. [19]
SMF	Cherry, slices: 10 × 3 × 0.1 mm	Specialized test bench. B = 0...10 mT, T = –30 °C	Reducing the duration of freezing; reduction in the size of ice crystals	Tang et al. [20]
SMF	Pork, cubes: 6x6x6 mm	Specialized test bench. B = 0...16 mT; T = –30 °C	Reducing the size of ice crystals. Raising up the degree of super chilling	Tang et al. [22]
SMF	Deionised water; 0,9% NaCl. Vials, V=5 ml.	B =11.4 mT	When freezing water, no effect was found. Freezing 0.9% NaCl: ➢ reduction of freezing duration by 53%; ➢ of super chilling by 3 °C	Zhao et al. [30]
SMF	Pure water; 0,9% NaCl Vial, capacity = 10 ml	B = 107...359 mT and 0...241 mT	*No effect found*	Otero et al. [32]
SMF	Carrot, slices	B = 0.46 and 0.92 mT	*No effect found*	Chen et al. [21]
SMF	Carrot, slices	B = 1.8 and 3.6 mT	Homogeneous crystals, minimal tissue damage	Chen et al. [21]
SMF	Potato. Cylinder: d= 20 mm, l = 45 mm	B = 40–55 mT B = 150–200mT	*No significant effect was found either on the kinetics of the freezing process or on the quality of the food product.*	Otero et al. [24]

Studies of the processes of freezing food media using electric fields

It is known that the quality of frozen food and the preservation of tissue microstructure largely depend on the rate of nucleation. At the same time, the nucleation process has a stochastic character and in practice, with conventional freezing methods, this metastable state is difficult to control [34]. Orlowska et al. [35] proposed a new approach to controlling of the ice nucleation within the frozen objects using an electrostatic field and creating appropriate freezing conditions. This effect has been experimentally confirmed by water freezing under the effect of low temperatures and a static electric field with a strength of 0 to 6.0×10^6 V/m. The experiments were carried out at electrostatic voltage ranging from 2.0 to 12 kV. The creation of higher voltages (over 12.6 kV) led to the ionization of air. It was found that the use of static electric field (SEF) allowed to significantly reduce the duration of the phase transition in the water sample and increased the nucleation temperature, while the highest degree of super chilling was obtained at a field strength of 5×10^6 V/m. The authors have shown that SEF exposure makes it possible to change the degree of super chilling and initiate the nucleation of the crystals. Similar studies were run by Dalvi-Isfahan et al. [36] on freezing mutton when exposed to SEF. The experimental setup consisted of 2 horizontal copper plate electrodes, which were installed in a freezer with forced air circulation at a temperature of minus 20 °C. The applied voltages were 0; 4; 8 and 12 kV. It was found that during the freezing with an increase in the SEF voltage from 0 to 12 kV, the equivalent diameter of ice crystals decreased by almost 3 times and their sphericity increased (Table 3).

Table 3. Results of microscopic analysis of ice crystals

Tension of SEF, (V/m)	0.0	1.9×10^5	3.9×10^5	5.8×10^5
Equivalent ice crystals diameter, μm	30.15 ± 5.12	17.64 ± 7.05	14.97 ± 4.2	11.95 ± 3.54
Sphericity, (0–1)	0.42 ± 0.05	0.59 ± 0.1	0.49 ± 0.03	0.56 ± 0.06

The effect of SEF on some parameters of the food products was evaluated by Fallah-Joshaqani et al. [37] when freezing distilled water, an aqueous solution of NaCl, an extract of a fungus and mushrooms. The field strength varied from 0.0 to 9.6×10^5 V/m at voltage of 0; 4.5; 9 and 13.5 kV. The results also confirmed the correlations [35] on the decrease of water super chilling on the increase in field strength. The highest nucleation temperature in pure water was observed at an electric field with strength of 6.4×10^5 V/m, and a further increase in the field strength to 9.6×10^5 V/m provided no significant effect on the nucleation temperature. For the NaCl solution, a decrease of the super chilling degree was observed only with an increase in the field strength to 3.2×10^5 V/m; a further increase in the field strength led to its increase also. The similar studies on the effect of a pulsed electric field (PEF) on the parameters of the rice flour gel freezing process were run by Roujia [38]. The results showed that the application of a pulsed electric field in the range from 0 to 25 kV led to a decrease of duration of the phase transition, in formed the smaller and more spherical ice crystals. The effect of PEFs on the freeze-thaw quality of the Atlantic salmon was studied by Li et al. [39]. The salmon was frozen at the electric field with strength of 1 kV/cm, current voltage of 5 kV, frequency of 50 Hz, and pulse width of 200 μs. As a result of the research, it was found that exposure to PEF under the conditions of air ionization made it possible to preserve the structure of the food product better, significantly reduce the duration of the thawing process and reduce the loss of moisture during thawing to 6%. The authors draw attention to an increase of the shelf life of this product after its thawing due to additional surface treatment with ionized air. At the same time, the authors point to a slight increase in losses during cooking and this is associated with a possible denaturation of the protein under the effect of ozone generated by the PEF. Babakin et al. [40,41] obtained experimental data on the effect of electrostatic fields on the processes of meat freezing. The object of the study was beef samples of various quality groups (PSE, NOR and DFD), pieces with size of $120 \times 30 \times 30$ mm. the beef was frozen in a refrigerating chamber at a temperature of minus 25 °C in an electrostatic field with a strength of $(7.5 \ldots 9) \times 10^5$ V/m under electroconvection conditions. The electroconvection was generated by a corona discharge which initiated a turbulent flow of ionized air [42], the velocity of the "electric wind" was 2–3 m/s. The control samples were frozen without their exposure to the field, at the same air velocity provided by the cooling fans. Figure 2 below shows the kinetic curves of the samples' freezing.

The frozen beef was stored at a temperature of minus 12 °C. It was found that the use of electroconvection reduced the duration of the freezing process by 27–41%, depending on the meat quality groups, and reduced the weight loss of unpacked meat during its freezing and storage.

The effect of microwaves (UHF) on the processes of freezing apples and potatoes was studied by Jha et al. [43]. The conducted studies have shown that the use of microwaves during freezing of these products did not affect the parameters of the freezing process, the forms of the obtained freezing curves during conventional freezing of the apples and the potatoes exposed to the microwaves were almost identical. It was found that the microwaves exposure did not reduce the duration of the freezing process, but led to a more uniform distribution of ice crystals, which reduced the loss of juice during thawing, and ensured better preservation of the food hardness. Xanthakis et al. [44] also studied the process of food freezing under microwave exposure using pork as an example. In contrast to [43], it was shown that the use of a microwave field increased the duration of the freezing process compared to the control mode, and reduced the degree of super chilling as the power increases. Data that confirmed the formation of smaller ice crystals have been obtained. Zhang et al. [45] proposed a device for quick freezing of a cooked rice dish (patent CN114009660A). The device contains a moving conveyor belt on which the food to be frozen is placed. The pulsed electric field is formed by two electrodes, which are fed with a current voltage of 5 to 20 kV, a frequency of 200 to 600 Hz, with a pulse width of 150 ... 300 μs. An alternating magnetic field is generated using Helmholtz coils

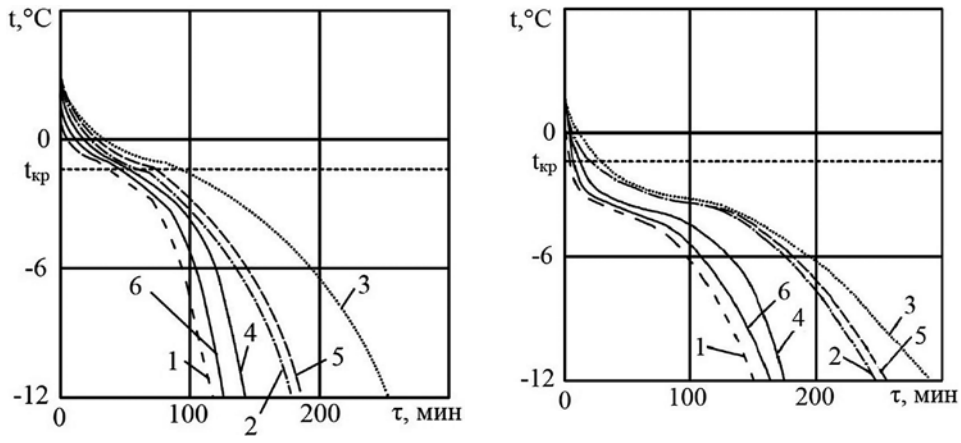

Figure 2. Change of temperature of the beef meat samples: a — on the surface, b — in the center (1,4,6, — experimental sample; 2,3,5 — control sample; 1,2 — DFD meat, 3,4 — PSE meat, 5,6 — NOR meat)

with a frequency of 50 to 150 Hz and an induction of 0.6 to 1.2 mT. PEF and AMF act together synergistically during the freezing process. The authors believe that this technical invention will increase the freezing rate, provide finer crystalline structure in the food product and maintain its original properties. However, the authors do not provide any experimental data confirming the above-specified parameters. The results of studies on the effect of variable and static magnetic fields are systematized below in the Table 4.

Discussion

The results of studies on the MF efficiency in the process of freezing food are controversial. This is predominantly explained by the fact that experiments are run by different researchers on heterogeneous objects under different freezing conditions and with different induction of magnetic fields, which does not allow unambiguous comparison of the obtained results. Most of the studies were run on installations equipped with the CAS system; the results proved that magnetic fields with induction from 0.4 to 2 mT provided no significant effect on either the freezing kinetics or the change in product properties. However, Okuda et al. [18] applied a field induction of no more than 0.5 mT and obtained the positive results in preserving the original structure of the mackerel fillet after its freezing and subsequent thawing in ice water. To a large extent, the positive result could be affected by the properties of the product being frozen, by freezing conditions at low temperatures, as well as by the thawing conditions.

Studies run on the specialized test benches allowed obtaining the results in a wider range of changes in the magnetic fields induction; however, most researchers who ran experiments on the real sizes samples of food did not find a clear effect of magnetic fields on either the freezing kinetics or the food properties. Positive results were obtained by Tang et al. [19,20] who froze blackberries and cherries samples at 10 mT while exposing the samples to SMF induction, the same was observed by Chen et al. [21] when freezing carrots. However, it should be noted that these studies were run on thin-sliced samples that were frozen at a high rate, therefore it is incorrect to extend the results obtained to objects of real sizes that are processed in the food industry, but they may be of interest for the implementation of special freezing technologies. It will be necessary to study the processes of freezing food media at higher values of SMF induction, taking into account the obtained results.

In general, the evidence base on the effect of SMF on the kinetics of freezing processes and food quality parameters currently is still insufficient. The articles do not answer to the question of what exactly reacts in a biological tissue or in water to a weak static magnetic field and what effect can be expected when strong fields are applied to it.

Research results show that AMFs can be more efficient than the static ones, especially at the stage of the water crystallization process. In some cases, they reduce the size of ice crystals, inhibit the histological damage, decrease of the nucleation temperature, and reduce the moisture losses after thawing.

It is shown that low-intensity AMFs practically have little effect on the freezing processes, but at high field induction values (more than 100 mT) and at frequencies (20 kHz and more), their effect can be observed, and it is apparently associated with the generating of an alternating magnetic field of the electric field, which strength is proportional to the amplitude and frequency of the AMF. At frequencies within the range of several tens of kHz, intense

Table 4. Effect of the electric fields

Type of effect	Product type. Sample's shape and size	Experiment conditions	Result	Source
SEF	Distilled water. Measuring cell, V = 1.6 ml	U = from 0 to 12 kW E = (0...6) × 10^6 V/m T = –16 °C	Phase transition reduction, nucleation temperature increase, nucleation initiation.	Orlowska [35]
SEF	Mutton. Cylinder: d = 10mm; l = 10mm	U = from 0 to 12 kW E = (5.8 × 10^5) V/m T = –20 °C	The effect on the kinetics of the process is weak. Reducing the size of ice crystals by 2.5–3 times, sphericity of crystals, reducing losses.	Dalvi-Isfahan [36]
SEF	Distilled water, NaCl solution, mushroom extract, mushrooms. Measuring cell. Cylinder: d = 20 mm; h = 20 mm	U = from 0 to 13.5 kW E = from (0 to 9.6) × 10^5 V/m, T = –30 °C	Increasing the nucleation temperature	Fallah-Joshaqani [37]
IEF (electric convection)	Meat, bar 130 × 30 × 30 mm	E = (7.5...9) × 10^5 V/m. Temperature T = –12 °C	Reduced freezing time, reduced wastage	Babakin et al. [41]
IEF (electric convection)	Atlantic salmon, cubes 50 × 40 × 20 mm	U = 5 kW; *f* = 50Hz Width of impulse 200 ms Temperature: of freezing –18 °C; of thawing 10 °C	Reduced ice crystal size, reduced thawing time, 6% reduction in wastage	Li, J. et al. [39]
UHF	Apple and potato	P = 167 and 222 Wt/kg, width of impulse 10 s, interval 20 s. T = –30 °C	Preservation of microstructure, even distribution of ice crystals, reduction of loss during thawing	Jha [43],
UHF	Pork, cylinder: diam. d = 7.5 mm; h = 6 mm	P = 700 Wt, T = –30 °C	Reducing the size of ice crystals, reducing the duration of freezing	Xanthakis [44]

dielectric losses are observed in biological tissues, which can improve the freezing kinetics as Kolodyazhnaya et al. [46] found, and, as a consequence, it leads to the destruction of the dendrites during the phase transition.

The research results show that exposure to the strong SEF with strength of (105...107) V/m leads to a decrease in the degree of super chilling, accelerates the beginning of water crystallization in the food product, reduces the duration of freezing. This indicates that the effect of reorientation of polar water molecules along the field direction (so called ordering) is achieved, which in its turn counteracts the thermal fluctuations and accelerates the freezing process. In addition, the authors [39,40] note that when salmon and meat are being frozen in an electric field under ionization, an electroconvective movement of the air medium take place, which intensifies the process of heat transfer. To achieve the positive results on the use of EF in laboratory conditions, it was necessary to create electric fields with strength of up to 10^6 V/cm. To ensure the same values of specific tension in the real workshop conditions at the place of food production and storage, it is necessary to achieve even stronger SEFs, which can be dangerous for the working personnel. In this regard, it is difficult to scale this method in the real industry. A number of authors have used a high-frequency field in freezing processes: Jha et al. [43] froze potatoes and apples, while Xanthakis et al. [44] froze pork. It was found that exposure to the microwave field can increase the duration of the freezing process compared to the control sample, but it decreases the degree of super chilling as the power increases, thus contributing to a significant reduction in the ice crystals size. These results are consistent with those obtained by freezing meat under exposure to the high-frequency radio frequency waves by Anese et al. [47]. One of the main limitations of this method is the generation of heat during the food processing; therefore, optimization of plant power is necessary. The same effect is also observed when radio frequency affects a frozen object, but in this case, scaling and safety are provided easier at any power of radio signals, since a radio signal can be introduced into the refrigerating chamber in a non-contact way, both using external high-frequency electromagnetic frames, and by using radio antennas. Many authors studied the food products with various thermo-physical properties, at various values of field induction and field frequencies, which makes it difficult to reproduce the results, to scale them and to compare. It is advisable to keep on researches in a wider range of the field induction values and it would be useful to introduce the additional unifying requirements for the researchers to test their own research results in certain standard modes, including the samples of comparable geometric shape, weight and size, with the same freezing conditions and the same control methods.

Conclusion

The declared efficiency of the freezers with a magnetic CAS system, proposed by Japanese scientists, used for freezing food products and based on the food exposure to the weak static magnetic fields and alternating magnetic fields in the vast majority of tests received no experimental confirmation and no proper scientific justification. To a large extent, the positive effects in some cases were obtained not due to the use of magnetic fields, but due to the use of rational freezing modes and subsequent storage of products at the lower temperatures compared to the stage of their freezing. These techniques make it possible to slow down the processes of moisture recrystallization, compared with technologies when the food is stored at higher air temperatures than the temperature used during the freezing.

The presented results of studies were obtained from the experiments run on the specialized test benches. The results confirm that weak static magnetic fields provide practically no effect on the kinetics of the process of freezing the real sizes samples. Fields with high induction values can have an effect, but there are not enough databases of these processes.

It is shown that AMFs of low intensity also provide very little effect on the freezing processes, however at the high field induction values the effect manifests itself, and it is probably associated with the generating of the alternating magnetic field of the alternating electric field, which affects the inner kinetics of the freezing process.

The application of strong electric field and microwave field during freezing of food media decreases the degree of water super chilling so that it's easier to achieve, it accelerates the beginning of the water crystallization, decreases the ice crystals size, and reduces the losses during thawing of the food product. For a number of reasons, these methods will require a lot of effort when scaling up in the food processing industry.

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NONPARAMETRIC STATISTICS. PART 3. CORRELATION COEFFICIENTS

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Keywords: *correlation, association coefficient, contingency coefficient, rank correlation, grading scales, test of significance*

Abstract

A measure of correlation or strength of association between random variables is the correlation coefficient. In scientific research, correlation analysis is most often carried out using various correlation coefficients without explaining why this particular coefficient was chosen and what the resulting value of this coefficient means. The article discusses Spearman correlation coefficient, Kendall correlation coefficient, phi (Yule) correlation coefficient, Cramér's correlation coefficient, Matthews correlation coefficient, Fechner correlation coefficient, Tschuprow correlation coefficient, rank-biserial correlation coefficient, point-biserial correlation coefficient, as well as association coefficient and contingency coefficient. The criteria for applying each of the coefficients are given. It is shown how to establish the significance (insignificance) of the resulting correlation coefficient. The scales in which the correlated variables should be located for the coefficients under consideration are presented. Spearman rank correlation coefficient and other nonparametric indicators are independent of the distribution law, and that is why they are very useful. They make it possible to measure the contingency between such attributes that cannot be directly measured, but can be expressed by points or other conventional units that allow ranking the sample. The benefit of rank correlation coefficient also lies in the fact that it allows to quickly assess the relationship between attributes regardless of the distribution law. Examples are given and step-by-step application of each coefficient is described. When analyzing scientific research and evaluating the results obtained, the strength of association is most commonly assessed by the correlation coefficient. In this regard, a number of scales are given (Chaddock scale, Cohen scale, Rosenthal scale, Hinkle scale, Evans scale) grading the strength of association for correlation coefficient, both widely recognized and not so well known.

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Introduction

Correlation (from the Latin *correlatio*), or correlation dependence is a statistical relationship between two or more random variables (or values that may be considered as such with some acceptable degree of accuracy), while changes in the values of one or more of these variables are accompanied by a systematic change in the values of other variable(s) [1].

The term "correlation" was first used by the French paleontologist Jean Cuvier (1769–1832) in 1806: he developed the "law of correlation" for parts and organs of living organisms to restore the appearance of fossil animals. The law of correlation helps to reconstruct the appearance of the entire animal and its place in the system using skulls, bones, etc. from excavations: if the skull has horns, then it was an herbivore, and its legs had hooves; if legs have claws, then it was a carnivore without horns, but with large cuspids [2]. The following story is known about Cuvier and the "law of correlation". During a university holiday, students decided to play a prank on Professor Cuvier. They dressed one of the students in a goatskin with horns and hooves and lifted him in Cuvier's bedroom window. The student stomped his hooves and yelled: "I'll eat you!" Cuvier woke up, saw a silhouette with horns and calmly answered: "If you have horns and hooves, then according to the law of correlation, you are an herbivore, and you cannot eat me. And for not knowing the law of correlation, you'll get a bad mark!" [3].

However, in statistics, the term "correlation" (in relation to Spearman correlation) was first used by the English biologist and statistician Galton F. (1822–1911) at the end of the 19th century. In 1892, he was the first to propose principles on how to calculate the correlation coefficient. His work was greatly influenced by the papers of Charles Darwin, who was his cousin. At a meeting of the Royal Society in 1888, Galton F. has presented a report "Correlations and their measurement, mainly from anthropometric data", which was devoted to the correlation between the length of arms and legs in a well-proportioned person. An article based on the 1888 report was published next year [4]. "*Two variable organs are considered correlated when a change in one of them is accompanied, in general, by a greater or lesser change in*

the same direction in the other organ. Thus, the length of the arm is considered to be correlated with the length of the leg, because a person with a long arm usually has a long leg, and vice versa" [4].

Galton F. calculated the correlation coefficient in anthropometry and in heredity studies. At University College London, Galton F. was the supervisor of Pearson K. (1857–1936), and then they worked together for many years. Pearson K. subsequently became a brilliant mathematician and biographer of Galton F.

Pearson K. is the founder of mathematical statistics, in particular the theory of correlation. He improved mathematical tools for calculating correlation. As a result, widely recognized Pearson correlation coefficient appeared, or analysis using Pearson method. In addition to Pearson K., Francis Ysidro Edgeworth and Walter Frank Raphael Weldon also worked on Pearson correlation coefficient [5]. He also developed nonparametric xi-squared coefficient. These coefficients are widely used in psychodiagnostics studies. Due to them, a tradition of using quantitative methods in the development and use of psychological tests was established.

Along with this, the following scientists made a significant contribution to the development of correlation analysis: Charles Edward Spearman (1863–1945), Maurice George Kendall (1907–1983), Alexander Tschuprow (1874–1926), George Udny Yule (1871–1951) and many others.

There are two types of association between phenomena, i. e. functional and correlation ones.

Correlation relationships between attributes may arise in different ways.

The first (most important) **way** is the causal dependence of the resulting attribute (its variation) on the variation of the factor attribute. For example, attribute x is a score for assessing soil fertility, and attribute y is the yield of an agricultural crop. Here it is completely clear which attribute acts as an independent variable (factor) x, and which attribute acts as a dependent variable (result) y.

The second way is contingency, which arises in the presence of a common cause. There is a well-known classic example given by the largest statistician in Russia at the beginning of the 20th century, Tschuprow A.: if we take the number of fire brigades in the city as attribute x, and the amount of losses from fires per year in the city as attribute y, then there is a direct correlation between attributes x and y in the Russian cities; on average, the more firefighters in a city, the greater the losses from fires! Did the firefighters set fires for fear of losing their jobs? No, the point is different. This correlation cannot be interpreted as an association between cause and consequence; both attributes are consequences of a common cause, i. e. the size of the city. It is quite logical that in large cities there are more fire departments, but there are more fires and losses from them per year than in small cities.

The third way correlation arises is a relationship of attributes, each of which is both a cause and a consequence. This is, for example, the correlation between the levels of labor productivity of workers and the level of wages for 1 hour of labor (rate). On the one hand, the level of wages is a consequence of labor productivity: the higher it is, the higher the payment. But, on the other hand, established rates play a stimulating role: with the right payment system, they act as a factor on which labor productivity depends. In such an attribute system, both formulations of the problem are permissible; each attribute can act as an independent variable x and a dependent variable y.

The publication provides an overview and systematizes information on the conditions for using various correlation coefficients and their grading scales.

Objects and methods

The research materials are monographs, manuals, articles, educational documents on statistics.

The authors searched for publications using key phrases: "correlation coefficients", "rank correlation coefficients", "association coefficient and contingency coefficient", "grading scales for correlation coefficients" in Scopus, PubMed, MEDLINE, Web of Knowledge, Google Scholar, IEEE Xplore, Science Direct, eLibrary (RSCI) databases.

The identified publications were preliminarily analyzed in the context of abstracts. The authors selected the following exclusion criteria:

1. works, scientific publications, textbooks devoted to "classical" methods of statistics;
2. publications not related to food and agricultural products.

Inclusion criteria:

1. scientific articles, textbooks, monographs devoted to nonparametric statistics;
2. publications predominantly in English.

Main part

Correlation coefficients

Typically, correlation coefficient is a measure of correlation (or strength of association) between random variables. The following correlation coefficients exist: Pearson coefficient, Spearman coefficient, Kendall coefficient, etc. [6]. Table 1 presents the types of correlation coefficients and describes the scales in which variables vary.

Pearson correlation coefficient (r)

Pearson coefficient is used quite often by researchers. But before choosing this criterion you need to: 1) know the data type; 2) know the distribution of the studied attributes in the general population, and if this is unknown, you need to check the distribution of both variables in the sample; 3) construct scattergrams in order to make sure that the association between variables is linear, and also to check the homoscedasticity [8].

With skewed distributions, as well as in the presence of true outliers (if researchers decide to include them for analysis), it is better to use nonparametric correlation coefficients, i. e. Spearman coefficient, Kendall coefficient, Cramér's coefficient, etc. It is worth noting that in foreign publications, Spearman correlation coefficient is found much more often [9,10].

Table 1. Types of correlation coefficients [7]

Correlation coefficient	Types of scale	
	variable X	variable Y
Pearson coefficient (r)	Interval scale with normal distribution	Interval scale with normal distribution
Spearman coefficient (ρ)	Interval scale with normal distribution	Ordinal scale
	Interval scale with normal distribution	Interval scale with normal distribution
Kendall coefficient (τ)	Ordinal scale	Ordinal scale
Phi correlation coefficient (φ) for tables 2 × 2	Nominal scale	Nominal scale
Cramér's coefficient (V) for tables more than 2 × 2	Nominal scale	Nominal scale
Rank-biserial correlation coefficient (r_{rb})	Nominal scale	Ordinal scale
Point-biserial correlation coefficient (r_{pb})	Nominal scale	Interval scale with normal distribution
Matthews correlation coefficient (MCC)	Nominal scale	Nominal scale
Fechner correlation coefficient (r_{Φ})	Interval scale with normal distribution	Interval scale with normal distribution
Tschuprow contingency coefficient (r_{ch})	Nominal scale	Nominal scale

Spearman rank correlation coefficient (ρ)

Rank correlation coefficients are used to measure relationships between attributes, the values of which may be ordered or ranked according to the decrease (or increase) of a given indicator in the objects under study.

Spearman rank correlation coefficient is a quantitative assessment of the association between phenomena, which is used in nonparametric methods. It determines the strength and direction of the correlation association between two attributes or two profiles of attributes. In this case, the actual degree of parallelism between the two quantitative series of observations being studied is determined and an assessment of the established association strength is given using a quantitatively expressed coefficient [11]. The criterion was developed and proposed for correlation analysis in 1904 by Charles Edward Spearman, an English psychologist, professor at the Universities of London and Chesterfield.

$$\rho = 1 - \frac{6\sum_{i=1}^{n} d_i^2}{n^3 - n} \quad (1)$$

where $d_i = x_i - y_i$ is the difference between ranks;
n is the number of attribute values observed.

Rank is the position of an element in a variation series. A variation series is a series whose elements are arranged in ascending or descending order.

The significance of Spearman correlation coefficient may be determined by Student's test (t-test) with the number of degrees of freedom equal to $n - 2$.

$$t = \rho \cdot \sqrt{\frac{n-2}{1-\rho^2}} \quad (2)$$

The criteria for applying the nonparametric Spearman rank correlation coefficient are described in detail in [10], and are as follows:

1. Examination of quantitative data distributions for normality is not required. It can be used for samples whose data partially or completely does not follow the law of normal distribution.

2. If the data from one of the samples can be presented on an ordinal scale, the data from the second sample must be quantitative.

3. If the sample size exceeds 5 observations.

4. If there are a large number of identical ranks for one or both compared variables, then Spearman correlation coefficient gives rough values. Ideally, both correlated series should represent two sequences of divergent values.

Kendall correlation coefficient (τ)

Kendall rank correlation [12] is an alternative to Spearman correlation in the case of two ordinal scales. This method is a measure of the strength of a nonlinear association and uses an increase or decrease in the resultant attribute as the factor attribute increases. Thus, the calculation of Kendall correlation coefficient involves counting the number of coincidences and inversions. To use Kendall correlation coefficient, there is only one requirement: the scales of the X and Y variables must be ordinal.

$$\tau = \frac{4Q}{n(n-1)} - 1 \quad (3)$$

where Q is the minimum number of exchanges of neighboring elements in one of the rankings for its coincidence with another ranking.

The statistics for the test of significance of this coefficient have a normal distribution $N(0, 1)$.

$$T_{kr} = z_{kr} \cdot \sqrt{\frac{2\cdot(2n+5)}{9n\cdot(n-1)}} \quad (4)$$

where n is a sample size; z_{kr} is a critical point of the two-sided critical region determined by Laplace function $\Phi(z_{kr}) = \frac{1-\alpha}{2}$ [13,14,15,16].

If $|\tau| < T_{kr}$, rank correlation between attributes is insignificant.

If $|\tau| > T_{kr}$, there is a significant rank correlation between attributes.

Same as for Spearman correlation coefficient: when ranks coincide $\tau = 1$, with opposite ranks $\tau = -1$. Kendall rank correlation coefficient has some advantages over Spearman coefficient. In particular, it may also be used for multivariate analysis. With a sufficiently large number of objects ($n \geq 10$), there is a simple association between the values of rank correlation coefficients $\rho = 1.5 \cdot \tau$ [17].

Example. Two panelists conduct a sensory analysis of 10 cooked sausage samples: ranked in descending order.

Ranks by the first panelist: 2, 3, 1, 6, 5, 4, 8, 7, 10, 9

Ranks by the second panelist: 3, 1, 2, 6, 7, 4, 5, 9, 10, 8

Using Spearman rank correlation coefficient and Kendall rank correlation coefficient, it should be determined whether the ratings of the panelist are consistent.

Solution.

I.1. To determine Spearman rank correlation coefficient, let's find the difference between the ranks d_i, and the square of the difference between the ranks d_i^2. The results are presented in Table 2.

Table 2. Calculation results

No. of the cooked sausage sample	1	2	3	4	5	6	7	8	9	10
Panelist 1	2	3	1	6	5	4	8	7	10	9
Panelist 2	3	1	2	6	7	4	5	9	10	8
d_i	–1	2	–1	0	–2	0	3	–2	0	1
d_i^2	1	4	1	0	4	0	9	4	0	1

2. Let's determine the sum of squares of the rank difference. The number of samples is 10, i.e. n=10.

$$\sum_{i=1}^{n} d_i^2 = 1 + 4 + 1 + 0 + 4 + 0 + 9 + 4 + 0 + 1 = 24$$

3. According to the formula (1), let's calculate Spearman rank correlation coefficient

$$\rho = 1 - \frac{6 \cdot 24}{10^3 - 10} = 0.8545$$

4. Test of significance is carried out according to the formula (2). Let's calculate Student's test (t-test)

$$t = 0.8545 \cdot \sqrt{\frac{10 - 2}{1 - 0.8545^2}} = 4.6537$$

5. Let's calculate the critical values of Student's test, significance level $p = 0.05$, the number of degrees of freedom in our case will be equal to $v = n - 2 = 10 - 2 = 8$. We can use statistical tables [13,14,15,16] or the function in MS Excel, TINV (Figure 1).

6. Let's plot "the axis of significance" (Figure 2) for our example.

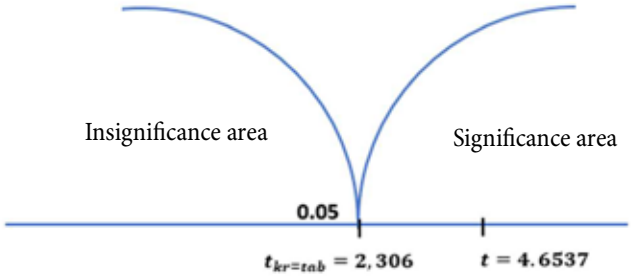

Figure 2. The axis of significance

Since 4.6537 > 2.306, the correlation is statistically significant.

II.1. To determine Kendall rank correlation coefficient, let's find the minimum number of exchanges of neighboring elements in one of the rankings for its coincidence with the other ranking. The results are presented in Table 3.

Table 3. Calculation results

No. of the cooked sausage sample	1	2	3	4	5	6	7	8	9	10
Panelist 1	2	3	1	6	5	4	8	7	10	9
Panelist 2	3	1	2	6	7	4	5	9	10	8
Q_i	7	8	7	4	3	4	3	1	0	0

$Q_1 = 7$, since in the line "Panelist 2" to the right of 3 (the values of samples that are to the right of the sample under consideration), there are 7 values larger than 3 (samples 4, 5, 6 ,7, 8, 9, 10).

$Q_2 = 8$, since in the line "Panelist 2" to the right of 1, there are 8 values larger than 1 (samples 3, 4, 5, 6, 7, 8, 9, 10).

$Q_3 = 7$, since in the line "Panelist 2" to the right of 2, there are 7 values larger than 2 (samples 4, 5, 6, 7, 8, 9, 10).

$Q_4 = 4$, since in the line "Panelist 2" to the right of 6, there are 4 values larger than 6 (samples 5, 8, 9, 10).

We filled in further in the same way.

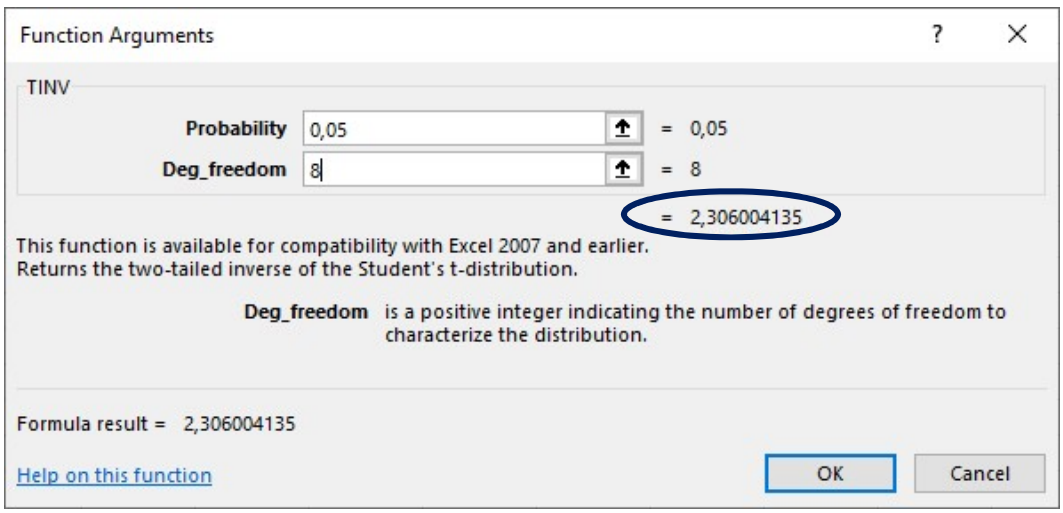

Figure 1. Calculation of the critical (reference) value of Student' s test

2. Let's calculate the sum of the values Q_i

$$\sum_{i=1}^{n} Q = 7 + 8 + 7 + 4 + 3 + 4 + 3 + 1 + 0 + 0 = 37$$

3. According to the formula (3), let's calculate Kendall rank correlation coefficient

$$\tau = \frac{4 \cdot 37}{10 \cdot (10 - 1)} - 1 = 0.6444$$

4. Test of significance is carried out according to the formula (4). Let's calculate the criterion T_{kr}

$$T_{kr} = z_{kr} \cdot \sqrt{\frac{2 \cdot (2n + 5)}{9n \cdot (n - 1)}}$$

Critical point z_{kr} in Laplace table [13,14,15,16] is equal to 1.96 at $\Phi(z_{kr}) = \frac{1-\alpha}{2} = \frac{1-0.05}{2} = 0.475$

$$T_{kr} = z_{kr} \cdot \sqrt{\frac{2 \cdot (2n + 5)}{9n \cdot (n - 1)}} = 1.96 \cdot \sqrt{\frac{2 \cdot (2 \cdot 10 + 5)}{9 \cdot 10 \cdot (10 - 1)}} =$$

$$= 1.96 \cdot \sqrt{\frac{50}{810}} = 0.017$$

5. Since $|\tau| > T_{kr}$, rank correlation between scores in two tests is significant.

Phi (φ) correlation coefficient and Cramér's V-coefficient

To study the strength of association between variables measured on a nominal scale, phi correlation coefficient and Cramér's coefficient are used.

In statistics, phi correlation coefficient (or root mean square contingency coefficient) is a measure of association between two binary variables. In machine learning, it is known as Matthews correlation coefficient (MCC) introduced by biochemist Brian W. Matthews in 1975 and is used as an indicator of the quality of binary (two-class) classifications [18]. Phi correlation coefficient was introduced by Pearson K. [19], also known as Yule phi coefficient introduced by George Udny Yule in 1912 [20].

The criteria for applying phi (φ) correlation coefficient:

1. Variables X and Y must be measured on a dichotomous scale.

2. The number of attributes in the compared variables X and Y must be the same.

Two binary variables are considered positively associated if the majority of the data is in the diagonal cells. Otherwise, if most of the data falls off the diagonal, then the binary variables are considered negatively associated.

Phi correlation coefficient may be calculated using fourfold contingency table 2×2 (Table 4).

Table 4. Fourfold contingency table 2×2

	Y=1	Y=0	
X=1	a	b	m1=(a+b)
X=0	c	d	m2=(c+d)
	n1=(a+c)	n2=(b+d)	n=(a+b+c+d)

$$\varphi = \frac{a \cdot d - b \cdot c}{\sqrt{m1 \cdot m2 \cdot n2 \cdot n1}} \quad (5)$$

where *a, b, c, d* are non-negative values of the number of observations, which add up to *n*, the total number of observations.

Phi (φ) correlation coefficient is related to point-biserial correlation coefficient and Cohen's *d* and estimates the degree of relationship between two variables (2 × 2) [21].

Matthews correlation coefficient (MCC) is defined identically to phi correlation coefficient and is widely used in the fields of bioinformatics and machine learning. The coefficient is considered as a balanced measure that can be used even if the classes have very different sizes [22].

Matthews correlation coefficient (MCC) returns a value between -1 and +1. Coefficient of "+1" represents a perfect prediction, "0" is no better than a random prediction, and "-1" indicates a complete discrepancy between the prediction and observation.

Cramér's V-coefficient is a modified phi correlation coefficient for tables larger than 2×2. This indicator of association between two nominal variables varies from 0 to + 1 (inclusive). It is based on Pearson chi-square statistics and was published by Harald Cramér in 1946 [23].

The criteria for applying Cramér's V-coefficient:

1. Variables X and Y must be measured on a nominal scale, where the number of codings is more than two (not dichotomous scales).

2. The number of attributes in the compared variables X and Y must be the same.

Like phi correlation coefficient, Cramér's V-coefficient is calculated using contingency tables (larger than 2×2).

$$V = \sqrt{\frac{\chi^2}{n \cdot min(row-1, column-1)}} \quad (6)$$

Chi-square test is calculated according to the formula:

$$\chi^2 = \sum \frac{(n_i - \hat{n}_i)^2}{\hat{n}_i} \quad (7)$$

where n_i is the actual number of observations in *ij* cells;
$\hat{n}_i$ is the expected number of observations in *ij* cells.

A general overview of the expected values is presented in Table 5.

Table 5. A general overview of the table of the expected values

	There is an outcome (1)	There is no outcome (0)	Total
There is a risk factor (1)	$\frac{(A+B)\cdot(A+C)}{A+B+C+D}$	$\frac{(A+B)\cdot(B+D)}{A+B+C+D}$	A+B
There is no risk factor (0)	$\frac{(C+D)\cdot(A+C)}{A+B+C+D}$	$\frac{(C+D)\cdot(B+D)}{A+B+C+D}$	C+D
Total	A+C	B+D	A+B+C+D

Example. A study is being conducted on the effect of smoking on the risk of developing arterial hypertension. For this purpose, two groups of subjects were selected: the first group included 70 people who smoke at least 1 pack of cigarettes daily, the second group included 80 non-smokers of the same age. In the first group, 40 people had high

blood pressure. In the second group, arterial hypertension was observed in 32 people. Thus, in the group of smokers, normal blood pressure was in 30 people (70 – 40 = 30), and in the group of non-smokers, normal blood pressure was in 48 people (80 – 32 = 48)."

Solution. Let's generate a contingency table (Table 6).

Table 6. Fourfold contingency table 2×2

	There is an arterial hypertension (1)	There is no arterial hypertension (0)	
Smokers (1)	A=40	B=30	A+B=70
Non-smokers (0)	C=32	D=48	C+D=80
	A+C=72	B+D=78	A+B+C+D=150

A general overview of the expected values is presented in Table 7 according to the formulas in Table 6.

$$\frac{(A+B)\cdot(A+C)}{A+B+C+D}=\frac{70\cdot 72}{150}=33.6$$

$$\frac{(C+D)\cdot(A+C)}{A+B+C+D}=\frac{80\cdot 72}{150}=38.4$$

$$\frac{(A+B)\cdot(B+D)}{A+B+C+D}=\frac{70\cdot 78}{150}=36.4$$

$$\frac{(C+D)\cdot(B+D)}{A+B+C+D}=\frac{80\cdot 78}{150}=41.6$$

Table 7. A general view of the expected values

	There is an arterial hypertension (1)	There is no arterial hypertension (0)	
Smokers (1)	33.6	36.4	70
Non-smokers (0)	38.4	41.6	80
	72	78	150

Let's calculate chi-square test:

$$\chi^2=\sum\frac{(n_i-\hat{n}_i)^2}{\hat{n}_i}=4.3956$$

We determine the number of degrees of freedom $v=(row-1)\cdot(column-1)=(2-1)\cdot(2-1)=1$, where row is the number of rows (in our example row=2), column is the number of columns (in our example column=2).

We find the critical value of Pearson chi-square test at significance level of p=0.05. We can use statistical tables [13,14,15,16] or the MS Excel function, CHIINV (Figure 3).

At significance level of p=0.05 and number of degrees of freedom equal to 1, $\chi^2_{kr(tab)}=3.8415$.

Let's plot "the axis of significance" (Figure 4).

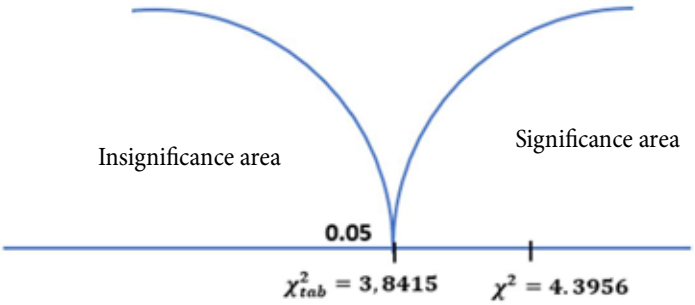

Figure 4. The axis of significance

Since 4.396 > 3.841, the dependence of the arterial hypertension incidence on smoking is statistically significant. The significance level of this relationship corresponds to p<0.05.

Cramér's V-coefficient:

$$V=\sqrt{\frac{\chi^2}{n\cdot min(row-1,column-1)}}=0.1712$$

Fechner correlation coefficient (r_ϕ)

The simplest indicators of strength of association include the sign correlation coefficient, which was proposed by the German physicist, philosopher and psychologist, founder of psychophysics, Gustav Theodor Fechner (1801-1887). In his posthumously published collective measurement theory (Kollektivmasslehre, 1897) [24], Fechner introduced the concept of the two-sided Gauss' law (Zweiseitige Gauss'sche Gesetz) or two-part normal distribution to account for the asymmetries he observed in empirical frequency distributions in many areas.

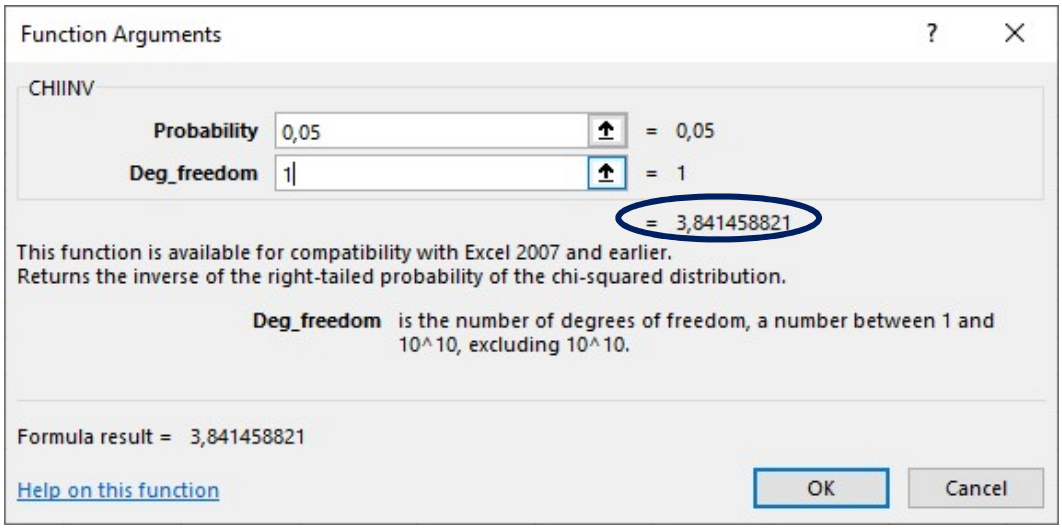

Figure 3. Calculation of the critical (reference) value of Pearson chi-square test

Fechner correlation coefficient is based on assessing the degree of consistency in the directions of deviations in individual values of factor attribute and resulting attribute from the corresponding averages. To calculate it, the average values of the resulting attribute and factor attribute are calculated, and then deviation signs for all values of correlated pairs of attributes are assigned.

$$r_{\phi} = \frac{C-H}{C+H} \quad (8)$$

where C is the number of coincidences of identical difference signs, both positive and negative $(x_i - \bar{x})$ and $(y_i - \bar{y})$;
H is the number of non-coincided difference signs $(x_i - \bar{x})$ and $(y_i - \bar{y})$;
$\bar{x}, \bar{y}$ are the average values of vectors (samples) x_i, y_i.

Like Pearson correlation coefficient, Fechner correlation coefficient may be in the range from –1 to +1. With a positive correlation, it has a positive sign, and with a negative correlation, it has a negative sign.

When using Fechner correlation coefficient, it should be noted that the distribution law of Fechner coefficient is unknown. Therefore, the question of assessing reliability remains.

Example. Based on the data accumulated on the milk fat content for cows at the farm and their 12 daughters of the same age (Table 8), we need to determine the relationship between the milk fat content for cows of the maternal generation and their offspring of the same age [25].

Table 8. Given data

Fat percentage in milk			
cows x_i	daughters y_i	cows x_i	daughters y_i
3.10	3.65	3.80	3.61
3.17	3.11	3.65	3.98
3.76	3.57	3.34	3.36
3.61	3.61	3.65	3.89
3.27	3.44	3.45	3.45
3.61	3.71	4.05	3.79

Solution.

1. First let's calculate arithmetic averages of the vectors of milk fat content for cows (x_i) and their daughters (y_i).

$$\bar{x} = (3.10 + 3.17 + 3.76 + 3.61 + 3.27 + 3.61 + 3.80 + +3.65 + 3.34 + 3.65 + 3.45 + 4.05)/12 = 3.5383$$

$$\bar{y} = (3.65 + 3.11 + 3.57 + 3.61 + 3.44 + 3.71 + 3.61 + +3.98 + 3.36 + 3.89 + 3.45 + 3.79)/12 = 3.5975$$

2. Then let's calculate the difference $(x_i - \bar{x})$ and $(y_i - \bar{y})$; data are presented in Table 9.

3. We calculate the number of coincided signs C = 10, and the number of non-coincided signs H = 2 (highlighted in green in Table 9).

4. Let's calculate Fechner correlation coefficient

$$r_{\phi} = \frac{10-2}{10+2} = 0.6667$$

Table 9. Deviations from the average values

Fat percentage in milk		Deviations from the average values		Signs of deviations from the average values	
cows x_i	daughters y_i	$(x_i - \bar{x})$	$(y_i - \bar{y})$	$(x_i - \bar{x})$	$(y_i - \bar{y})$
3.10	3.65	–0.4383	0.0525	–	+
3.17	3.11	–0.3683	–0.4875	–	–
3.76	3.57	0.2217	–0.0275	+	–
3.61	3.61	0.0717	0.0125	+	+
3.27	3.44	–0.2683	–0.1575	–	–
3.61	3.71	0.0717	0.1125	+	+
3.80	3.61	0.2617	0.0125	+	+
3.65	3.98	0.1117	0.3825	+	+
3.34	3.36	–0.1983	–0.2375	–	–
3.65	3.89	0.1117	0.2925	+	+
3.45	3.45	–0.0883	–0.1475	–	–
4.05	3.79	0.5117	0.1925	+	+

Thus, it can be stated that there is a moderate association between the milk fat content from cows of the maternal generation and their offspring of the same age.

Rank-biserial correlation coefficient (R_{rb})

In cases where one variable is measured on a dichotomous scale (variable X), and the other variable is measured on a rank scale (variable Y), rank-biserial correlation coefficient is used. Variable X measured on a dichotomous scale have only two values (codes), 0 and 1. It should be especially emphasized: despite the fact that this coefficient varies in the range from –1 to +1, its sign does not matter for the interpretation of the results. This is another exception to the general rule.

This coefficient is calculated according to the formula:

$$R_{rb} = \frac{(\bar{x}_1 - \bar{x}_0)\cdot 2}{N} \quad (9)$$

where $\bar{x}_1$ is the average rank for those elements of variable Y that correspond to code (attribute) 1 in variable X;
$\bar{x}_0$ is the average rank for those elements of variable Y that correspond to code (attribute) 0 in variable X;
N is the total number of elements in variable X.

Example. A psychologist tests a hypothesis about whether there are gender differences in verbal ability.

Solution. To solve this problem, 15 teenagers of different genders were ranked by a literature teacher according to the degree of expression of verbal abilities. The data obtained are presented in the form of a table (Table 10).

In this case, the correctness of the ranking need not be checked, since there are no coincided ranks and the ranking is carried out in order. In Table 10, boys are designated by code 1 (green), and girls are designated by code 0. In our case, there are 9 boys and 6 girls.

1. Let's calculate the average rank values separately for boys and girls.

$$\bar{x}_1 = \frac{1+6+9+7+4+3+5+12+2}{9} = \frac{49}{9} = 5.44$$

$$\bar{x}_0 = \frac{10+15+8+13+11+14}{6} = \frac{71}{6} = 11.83$$

Table 10. Verbal abilities of teenagers

No. of the subject	Gender	Verbal ability ranks
1	1	1
2	0	10
3	1	6
4	1	9
5	0	15
6	1	7
7	0	8
8	0	13
9	1	4
10	1	3
11	1	5
12	0	11
13	1	12
14	1	2
15	0	14

2. Let's calculate rank-biserial correlation coefficient R_{rb} according to the formula (9):

$$R_{rb} = \frac{(\bar{x}_1 - \bar{x}_0) \cdot 2}{N} = \frac{(5.44 - 11.83) \cdot 2}{15} = -0.852$$

3. Let's check the significance of the resulting correlation coefficient using the formula

$$T_\Phi = |R_{rb}| \cdot \sqrt{\frac{N-2}{1-R_{rb}^2}} \quad (10)$$

at $\upsilon = N - 2 = 15 - 2 = 13$ (υ is the degree of freedom by which the reference (critical) value is found and compared with the calculated value obtained according to the formula (10).

$$T_\Phi = |R_{rb}| \cdot \sqrt{\frac{N-2}{1-R_{rb}^2}} = |-0.852| \cdot \sqrt{\frac{15-2}{1-(-0.852)^2}} =$$

$$= 0.852 \cdot \sqrt{\frac{13}{1-0.725904}} = 0.852 \cdot \sqrt{\frac{13}{0.274096}} =$$

$$= 0.852 * 6.88684529674 = 5.87$$

In our case, the number of degrees of freedom will be equal to υ=13. To calculate the critical (reference) value of Student's test, we can use statistical tables [13,14,15,16] or the MS Excel function, TINV (Figures 5 and 6).

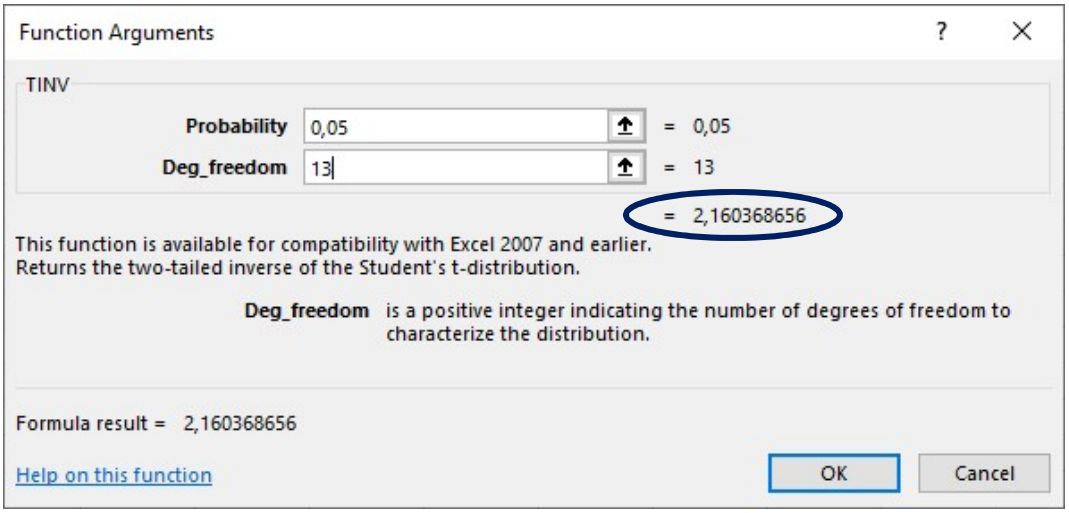

Figure 5. Calculation of the critical (reference) value of Student's test at significance level of $p<0.05$

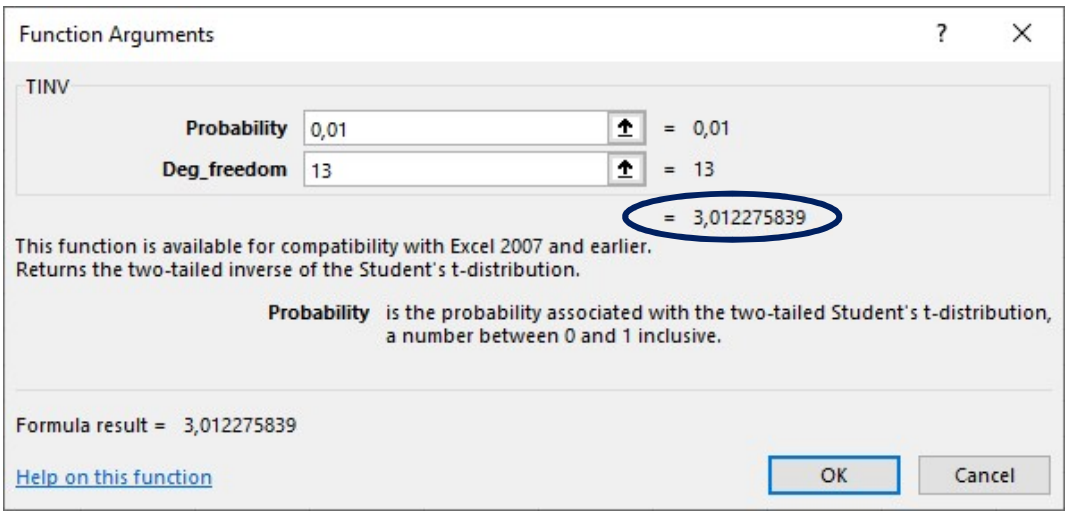

Figure 6. Calculation of the critical (reference) value of Student's test at significance level of $p<0.01$

4. The critical (reference) value of Student's test for P<0.05 is equal to $t_{kr=tab} = 2.16$ and for P<0,01 is equal to $t_{kr=tab} = 3.01$. In the accepted notation form it looks like this:

$$t_{kr=tab} = \begin{cases} 2.16 \; at \; P \leq 0.05 \\ 3.01 \; at \; P \leq 0.01 \end{cases}$$

5. Let's plot "the axis of significance" (Figure 7):

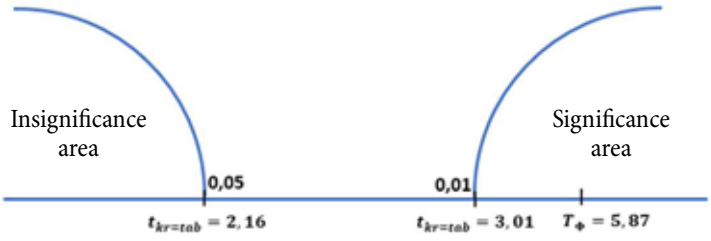

Figure 7. The axis of significance

The result is in significance area. Therefore, hypothesis H_1, according to which the resulting rank-biserial correlation coefficient is significantly different from zero, is accepted. In other words, in this sample of teenagers, significant gender differences were found in the degree of expression of verbal abilities.

To use the rank-biserial correlation coefficient, the following criteria must be met:

1. The variables being compared must be measured on different scales: X on a dichotomous scale; Y on a rank scale.

2. The number of varying attributes in the compared variables X and Y must be the same.

3. To assess the level of reliability of the rank-biserial correlation coefficient, we need to use the formula to determine $T\phi$ and a table of critical values for Student's t-test at $\upsilon = N - 2$.

Tschuprow contingency coefficient ($K_\text{ч}$) is calculated according to the formula

$$K_\text{ч} = \sqrt{\frac{\varphi^2}{(K_1-1)\cdot(K_2-1)}} \qquad (11)$$

where K_1 and K_2 the number of groups in the columns and the number of groups in the rows.

The result of assessing the strength of association obtained by Tschuprow contingency coefficient is more accurate, since it takes into account the number of groups for each of the studied attributes.

It is also beneficial to use when there is a greater division of population into groups according to correlated attributes. Pearson contingency coefficient is used mainly in the case of a square table, while Tschuprow contingency coefficient is suitable for measuring association in rectangular tables also.

It is believed that already with contingency coefficient value of 0.3, we can talk about a strong association between the variation of the studied attributes.

Example. Using Tschuprow contingency coefficient, it is necessary to determine the strength of association between the grain yield in agricultural enterprises of the region and their legal form according to Table 11.

Table 11. Grouping of agricultural enterprises with different grain yields according to legal form

Grain yield, dt/ha (x_i)	Number of enterprises, units (f_{i0})	including		
		State enterprises (f_{i1})	collective enterprises (f_{i2})	Farming enterprises (f_{i3})
15.80-18.97	3	2	1	–
18.97-22.14	4	–	4	–
22.14-25.31	11	3	8	–
25.31-28.48	7	1	3	3
28.48-31.65	4	–	1	3
31.65-34.82	1	–	–	1
Total:	30	6	17	7

Let's transform the table into more convenient form for calculating Tschuprow contingency coefficient (Table 12).

Table 12. Distribution of agricultural enterprises in the region by their legal form and level of grain yield

Group of enterprises	By grain yield (dt/ha)						Total	Average yield by group, dt/ha
By legal form	15.80-18.97	18.97-22.14	22.14-25.31	25.31-28.48	28.48-31.65	31.65-34.82		
Average value of the range	17.4*	20.6	23.7	26.9	30.1	33.2		
State enterprises	2	–	3	1	–	–	6	22.14**
Collective enterprises	1	4	8	3	1	–	17	23.54
Farming enterprises	–	–	–	3	3	1	7	29.16
TOTAL:	3	4	11	7	4	1	30	24.57

* $\frac{15.8+18.97}{2} = 17.38 \approx 17.4$; $\frac{18.97+2\ .14}{2} = 20.55 \approx 20.6$, etc.

** $\frac{17.4\cdot2+23.7\cdot3+26.9\cdot1}{6} \approx 22.14$; $\frac{17.4\cdot1+20.6\cdot4+23.7\cdot8+26.9\cdot3+30.1*1}{17} \approx 23.54$, etc.

According to the formula

$$\varphi^2 = \sum \frac{f_{ij}^2}{F_i F_j} - 1 \qquad (12)$$

where $F_i = \sum_i f_{ij}, F_j = \sum_j f_{ij}$

the mean square contingency is equal to

$$\varphi^2 = \left(\frac{2^2}{3\cdot6} + \frac{1^2}{3\cdot17} + \frac{4^2}{4\cdot17} + \frac{3^2}{11\cdot6} + \frac{8^2}{11\cdot17} + \frac{1^2}{7\cdot6} + \frac{3^2}{7\cdot17} + \right.$$

$$\left. + \frac{3^2}{7\cdot7} + \frac{1^2}{4\cdot17} + \frac{3^2}{4\cdot7} + \frac{1^2}{1\cdot7}\right) - 1 =$$

$$= \left(\frac{4}{18} + \frac{1}{51} + \frac{16}{68} + \frac{9}{66} + \frac{64}{187} + \frac{1}{42} + \frac{9}{119} + \frac{9}{49} + \frac{1}{68} + \frac{9}{28} + \frac{1}{7}\right)$$

$$- 1 = 0.718$$

According to the formula

$$K_{\text{ч}} = \sqrt{\frac{\varphi^2}{(K_1-1)\cdot(K_2-1)}} = \sqrt{\frac{0.718}{(6-1)\cdot(3-1)}} = 0.268$$

Tschuprow contingency coefficient is = 0.268. Since this value verges towards 0.3, we can talk about the presence of a fairly strong association between the yield of grain crops and the legal form of enterprises.

Tschuprow correlation coefficient (r_{ch})

Tschuprow correlation coefficient (r_{ch}) is calculated using the following formula:

$$r_{ch} = \pm\sqrt{\frac{\chi^2}{N\sqrt{(a-1)\cdot(b-1)}}} \quad (13)$$

where χ^2 is the empirical value of the chi-square test;
N is the sample size (number of objects for which both attributes were taken into account);
a, b is the number of modalities of both attributes.

The reliability of Tschuprow correlation coefficient is assessed by the value of the chi-square test. Chi-square test is calculated according to the formula:

$$\chi^2 = \sum\frac{(n_i-\hat{n}_i)^2}{\hat{n}_i} \quad (14)$$

Number of components added when calculating χ^2 is equal to the product $a \times b$.

The null hypothesis is that there is no reliable association between the variables. If $\chi^2 > \chi^2_{0.05}$, the null hypothesis is rejected (the association between variables is significant); if $\chi^2 < \chi^2_{0.05}$, the null hypothesis is accepted (the association between variables is insignificant).

If it is proven that the association is insignificant, Tschuprow correlation coefficient is not calculated and is set to **0**.

Example. To establish the association between the shape of the glands on the leaf petioles and the degree (score) of powdery mildew damage to peach, 1319 cultivars were studied. The frequencies of occurrence of peach cultivars by combination of modalities of these attributes are as follows (Table 13). What is the correlation between the shape of the glands on the petioles and powdery mildew in peach?

Table 13. Given data: powdery mildew damage on petioles

Powdery mildew damage	Shape of the glands	
	reniform	rounded
Absent or minor	453	40
Medium or severe	46	780

Solution. The attribute "shape of the glands" is nominal, since the modalities "reniform" and "rounded" cannot be ranked. The attribute "powdery mildew damage" can be considered as an ordinal attribute, since its states, i.e. "absent or minor" and "medium or severe" are easily ranked. If at least one of the attributes is nominal, then Tschuprow correlation coefficient is used to estimate the correlation between it and other attributes.

1. First, we generate a table for frequencies of occurrence of cultivars based on the two studied attributes (Table 14) and calculate the theoretically expected frequencies, provided that there is no correlation between these attributes:

Empirical and theoretically expected frequencies of occurrence of peach cultivars based on the combination of modalities "shape of the glands" and "powdery mildew damage", provided that there is no correlation between these attributes.

Table 14. Frequency of cultivar occurrence by two attributes

Powdery mildew damage	Shape of the glands				Sum
	reniform		rounded		
	n_i	$\hat{n}_i$	n_i	$\hat{n}_i$	
Absent or minor	453	186.51	40	306.49	**493**
Medium or severe	46	312.49	780	513.51	**826**
TOTAL:	**499**		**820**		

$$\hat{n}_{11} = \frac{499\cdot 493}{1319} = 186.51$$

$$\hat{n}_{12} = \frac{820\cdot 493}{1319} = 306.49$$

$$\hat{n}_{21} = \frac{499\cdot 826}{1319} = 312.49$$

$$\hat{n}_{22} = \frac{820\cdot 826}{1319} = 513.51$$

2. Let's calculate chi-square test value:

$$\chi^2 = \sum\frac{(n_i-\hat{n}_i)^2}{\hat{n}_i} = \frac{(453-186.51)^2}{186.51} + \frac{(46-312.49)^2}{312.49} + \frac{(40-306.49)^2}{306.49} + \frac{(780-513.51)^2}{513.51} = 978.04$$

3. We find the critical value of Pearson chi-square test at significance level of p=0.05 and the degrees of freedom equal to $\upsilon = 2-1 = 1$. To calculate the critical (reference) value of Pearson chi-square test, we can use statistical tables [13,14,15,16] or the MS Excel function, CHIINV.

The critical (reference) value of Pearson chi-square test at significance level of $p = 0.05$ and the degrees of freedom equal to $\upsilon = 1$ is 3.84 ($\chi^2_{0.05} = 3.84$).

$\chi^2 = 978.03 > \chi^2_{0.05} = 3.84$

Statistical conclusion: the correlation between powdery mildew damage and the shape of the glands is significant.

4. Let's calculate Tschuprow correlation coefficient:

$$r_{ch} = \pm\sqrt{\frac{\chi^2}{N\sqrt{(a-1)\cdot(b-1)}}} = \pm\sqrt{\frac{978.04}{1319\sqrt{(2-1)\cdot(2-1)}}} = \pm\sqrt{0.7415} = \pm 0.86$$

Conclusion: The correlation between the powdery mildew score and the type of glands is reliable and strong. However, it is impossible to establish which variable is an argument and which is a function. Though, it may be logically assumed that the shape of the glands is an independent variable (argument), and the powdery mildew damage

is a dependent variable (function). After all, it is difficult to say that the degree of powdery mildew damage changes the cultivar of peaches and the shape of the glands on their leaves. Conversely, the assumption that the degree of damage to peach leaves by powdery mildew depends on the shape of the leaf glands is reasonable.

Spearman rank correlation coefficient and other non-parametric indicators are independent of the distribution law, and that is why they are very useful. They make it possible to measure the contingency between such attributes that cannot be directly measured, but can be expressed by points or other conventional units that allow ranking the sample. The benefit of rank correlation coefficient also lies in the fact that it allows to quickly assess the relationship between attributes regardless of the distribution law.

To determine the strength of association between two attributes, each of which consists of only two groups, *association coefficient and contingency coefficient are used*.

If there is a relationship between the variation of attributes, this means their association, or relationship. If the association was formed randomly, this means contingency. To evaluate association in this case, a number of indicators are used.

To calculate them, Table 15 is generated, which shows the association between two phenomena, each of which must be alternative, i.e. consisting of two different attribute values (for example, a product is good or defective).

Table 15. For calculation of association coefficient and contingency coefficient

a	c	a+c
b	d	b+d
a+b	c+d	a+b+c+d

The coefficients are calculated using the formulas:

Association coefficient:

$$K_a = \frac{ad-bc}{ad+bc} \quad (15)$$

Contingency coefficient:

$$K_k = \frac{ad-bc}{\sqrt{(a+b)\cdot(b+d)\cdot(a+c)\cdot(c+d)}} \quad (16)$$

Contingency coefficient is always less than association coefficient.

Association is considered confirmed if

$$K_a \geq 0.5 \text{ or } K_k \geq 0.3$$

Example. We study the association between the participation of the population of one of the cities in environmental actions and their level of education. The survey results are characterized by the following data (Table 16). Let's define: 1) association coefficient; 2) contingent coefficient.

Solution.

1. Example calculation of association coefficient

$$K_a = \frac{ad-bc}{ad+bc} = \frac{78\cdot 68 - 22\cdot 32}{78\cdot 68 + 22\cdot 32} = \frac{5304-704}{5304+704} = \frac{4600}{6008} = 0.7656$$

Table 16. Dependence of the participation of the city population in environmental actions on educational level

Groups of workers	Population of the city, persons	among them	
		Participants in the actions, persons	Not participants in the actions, persons
With secondary education	100	78	22
Without secondary education	100	32	68
TOTAL:	200	110	90

2. Example calculation of contingency coefficient

$$K_k = \frac{ad-bc}{\sqrt{(a+b)\cdot(b+d)\cdot(a+c)\cdot(c+d)}}$$

$$= \frac{78\cdot 68 - 22\cdot 32}{\sqrt{(78+22)\cdot(22+68)\cdot(78+32)\cdot(32+68)}}$$

$$= \frac{4600}{\sqrt{100\cdot 90\cdot 110\cdot 100}} = \frac{4600}{\sqrt{99000000}} = \frac{4600}{9949.87437106}$$

$$= 0.4623$$

Thus, there is an association between the participation of the city population in environmental actions and its educational level.

When measuring the strength of association between qualitative alternative attributes and a continuously varying quantitative attribute, *biserial correlation coefficient* (r_{bs}) is used. The coefficient is calculated according to the formula:

$$r_{bs} = \frac{\bar{x}_1 - \bar{x}_2}{s} \cdot \sqrt{\frac{n_1 \cdot n_2}{N\cdot(N-1)}} \quad (17)$$

where $\bar{x}_1$ and $\bar{x}_2$ are the average values for alternative groups;

s is the standard deviation;

n_1 and n_2 are sizes of alternative groups;

$N = (n_1 + n_2)$ is the total number of observations.

Biserial correlation coefficient varies from **–1** to **+1**; at $x_1 = x_2, r_{bs} = 0$. As for association coefficient, the sign of biserial coefficient has no meaning.

Example. We study the effect of tops affected by buck eye rot on the yield of "Priekulsky ranny" potato (Table 17). It is necessary to determine whether there is a correlation between potato yield and tops affected by buck eye rot.

Table 17. Given data

Yield, kg per bush (X)		0.7	0.6	0.5	0.4	0.3	0.2
Number of bushes, pcs.	total (f)	12	15	18	13	9	6
	incl. affected (f_1)	0	4	9	10	7	6

Solution.

1. We generate calculation table (Table 18).

2. Let's calculate average values for alternative groups:

$$\bar{x}_1 = \frac{\sum_{i=1}^{n_1} f_{1i}X}{n_1} = \frac{14.2}{36} = 0.3944;$$

$$\bar{x}_2 = \frac{\sum_{i=1}^{n_2} f_{2i}X}{n_2} = \frac{21.3}{37} = 0.5757;$$

Table 18. Calculation table

X	f_1	f_2	$f = f_1 + f_2$	f_1X	f_2X	fX	X^2	fX^2
0,7	0	12	12	0	8.4	8.4	0.49	5.88
0,6	4	11	15	2.4	6.6	9.0	0.36	5.40
0,5	9	9	18	4.5	4.5	9.0	0.25	4.50
0,4	10	3	13	4.0	1.2	5.2	0.16	2.08
0,3	7	2	9	2.1	0.6	2.7	0.09	0.81
0,2	6	0	6	1.2	0	1.2	0.04	0.24
Sum	**36**	**37**	**73**	**14.2**	**21.3**	**35.5**	**1.39**	**18.91**

3. Let's calculate standard deviation:

$$s = \sqrt{\frac{\sum fX^2 - \frac{(\sum fX)^2}{N}}{N-1}} = \sqrt{\frac{18.91 - \frac{35.5^2}{73}}{73-1}}$$

$$= \sqrt{\frac{18.91 - 17.26}{72}} = \sqrt{0.0229} = 0.15$$

4. Let's calculate biserial correlation coefficient:

$$r_{bs} = \frac{\bar{x}_1 - \bar{x}_2}{s} \cdot \sqrt{\frac{n_1 \cdot n_2}{N \cdot (N-1)}} =$$

$$= \frac{0.3944 - 0.5757}{0.15} \cdot \sqrt{\frac{36 \cdot 37}{73 \cdot (73-1)}} = \frac{-0.1813}{0.15} \cdot \sqrt{\frac{1332}{5256}}$$

$$= \frac{-0.1813}{0.15} \cdot \sqrt{\frac{1332}{5256}} = -1.21 \cdot \sqrt{0.2534} = -0.6091$$

5. Let's calculate biserial correlation coefficient error:

$$s_{r_{bs}} = \sqrt{\frac{1 - r_{bs}^2}{N-2}} = \sqrt{\frac{1 - (-0.6091)^2}{73-2}} = \sqrt{\frac{1 - 0.3710}{71}} =$$

$$= \sqrt{0.0089} = 0.094$$

6. Let's calculate the criterion for the significance of biserial correlation coefficient:

$$t_r = \frac{r_{bs}}{s_{r_{bs}}} = \frac{-0.6091}{0.094} = -6.48$$

Since the sign of the criterion does not have any meaning, we discard it.

Using a statistical table or using the MS Excel TINV function, we find the value of Student's test at a 5% significance level and the number of degrees of freedom equal to $v = N - 2 = 73 - 2 = 71$. The critical (reference) value of Student's test is $t_{0.05} = 1.99$.

The criterion is greater than Student's test, therefore there is a significant correlation between the attributes.

Conclusion: With an increase in the incidence of buck eye rot on tops, the yield of "Priekulsky ranny" potatoes decreases significantly.

We presented a description of correlation coefficients and demonstrated examples of their application, so it is interesting to further discuss what grading scales exist for interpreting these coefficients.

Thus, we know that Pearson correlation coefficient is in the range from –1 to 1. The closer the resulting correlation coefficient to –1 or 1, the stronger the association between the studied indicators. When assessing the strength of association for correlation coefficients, various scales are used.

Chaddock scale

In 1925, the American statistician Robert Emmet Chaddock (1879–1940) introduced a scale for Pearson correlation coefficient [26]. This scale is the first gradation of correlation strength: 1) 0.1–0.3, poor association; 2) 0.3–0.5, fair association; 3) 0.5–0.7, good association; 4) 0.7–0.9, very good association.

Cohen scale 1960–1988

In the 1960s, statistician in the field of psychology and sociology Jacob Cohen (1923–1998, USA) proposed his "statistical power" scale for use in cases where the effects were small [27].

The power (of a test or research) is influenced by: 1) effect size, i. e. the degree of its manifestation; 2) the selected level of statistical significance (α, the probability of erroneously rejecting the null hypothesis; for us, usually at $p < 0.05$); 3) size of sample from the general population [28,29].

According to Cohen scale, Pearson correlation coefficient has the following gradation: 1) 0.1, small association; 2) 0.3, medium association; 3) more than 0.5, large association.

Later, "Cohen's subjective standards" were brought to the logical form of ranges in very few sources [30, 31]: 1) 0.1–0.3, small association; 2) 0.3–0.5, medium association; 3) more than 0.5, large association.

However, in most sources, Cohen scale is quoted in its original form of three values.

Rosenthal scale

In the work by Rosenthal J. A. [32] published in 1996, Cohen scale was supplemented with a range of very strong association: 1) 0.1 (–0.1), weak association; 2) 0.3 (–0.3), moderate association; 3) 0.5 (–0.5), strong association; 4) 0.7 (–0.7), very strong association.

In modern publications, when using Cohen scale, Rosenthal gradation is used [33,34].

Hinkle scale 1979–2003 (versions)

Scale by D. E. Hinkle appears in publications dated 2011 to 2018 [35,36,37,38]. These publications contain references to monographs by Dennis E. Hinkle published by him in collaboration with other scientists [39,40] in the period of 1979–2003.

The following gradings are used in publications: 1) 0–0.3, little if any or negligible association; 2) 0.3–0.5, low association; 3) 0.5–0.7, moderate association; 4) 0.7–0.9, high or strong association; 5) 0.9–1.0, very high or very strong association.

A similar, but somewhat expanded scale is given on the website of Andrews University (USA, Michigan) [41]. To the

listed gradations, another association has been added: little association, <0.3. Thus, in [41] there are both 'Little' (<0.3) and 'Low' (0.3–0.5) correlation coefficient r values.

The manual "The Basic Practice of Statistics" [42] proposes the following gradation: 1) less than 0.3, very weak association; 2) 0.3–0.5, weak association; 3) 0.5–0.7, moderate association; 4) more than 0.7, strong association. Scale truncated at both ends by D. E. Hinkle et al. are presented in the manual "Statistics for Dummies" [43]: 1) 0.3–0.5, weak association; 2) 0.5–0.7, moderate association; 3) more than 0.7, strong association.

Evans scale

In 1996, the monograph by James D. Evans "Straightforward statistics for the behavioral sciences" [44] was published in the USA, in which another effect size scale was proposed. The scale is made by dividing the range of 0 to 1.0 into equal segments and does not provide for an insignificant correlation. This scale is used in publications (2012–2019) on psychology [35,45,46,47,48], programming [49], and a textbook on statistics [50]. The gradation of this scale is as follows: 1) 0–0.19, very weak association; 2) 0.2–0.39, weak association; 3) 0.40–0.59, moderate association; 4) 0.6–0.79, strong association; 5) 0.80–1.0, very strong association.

All given scales are used for grading Pearson correlation coefficient. To grade other coefficients (Spearman coefficient, Kendall coefficient, Cramér's coefficient, etc.), a search for publications in the ScienceDirect and PubMed systems gave the following information. The manual "Statistics without Maths for Psychology" [51] uses an original scale for grading Spearman correlation coefficient. The article [36] uses Hinkle scale for Spearman correlation coefficient. The review article [52] presents the original grading scale for Spearman coefficient, Kendall coefficient, Phi coefficient, Cramer's V-coefficient, and concordance correlation coefficient (CCC).

The study [53] presents a detailed overview of the effect size grading for Hill yield criterion "strength of association" according to the correlation coefficient value parameter. Koterov et al. [53] analyzed 121 sources and collected information on 19 scales. They note that Chaddock scale from 1925 is not currently used abroad, but is widely represented in the countries of the former USSR. The most well-recognized grading scales for the correlation coefficient, to which there are many references, are Cohen scale, scale by D. E. Hinkle et al., Evans scale. Along with this, it is noted that there are a number of scales by other authors published once both in educational material (including on-line), in publications, and even in manuals or monographs. Quotations from such sources were rare, and in most cases simply absent.

Conclusion

In the third part of the article "Nonparametric Statistics", Spearman correlation coefficient, Kendall correlation coefficient, phi (Yule) correlation coefficient, Cramér's coefficient, Matthews coefficient, Fechner coefficient, Tschuprow coefficient, rank-biserial correlation coefficient, point-biserial correlation coefficient, as well as association coefficient and contingent coefficient were reviewed. Scales for grading the strength of association for correlation coefficients are given, both widely known and widely used, and those found in individual publications. Examples of calculating correlation coefficients and explanations are given.

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STUDY OF THE PROCESS OF THE FROZEN RAW BEEF DEFROSTING WITH ITS SIMULTANEOUS MASSAGING IN INDUSTRIAL CONDITIONS

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Keywords: *thawing, defrosting, massaging, beef meat, vacuum, water steam, defroster massager, temperature change, mass change*

Abstract
Many meat processing enterprises use the frozen raw meat. Its defrosting and thawing is a crucial technological operation that fundamentally affects the quality of food products. The experience and knowledge obtained directly in the workshop in the process of thawing the raw materials and their using to obtain a specific finished product are of great importance. Defrosting and thawing of the frozen beef meat, as one of the stages of raw meat processing, still remains a challenging process in industrial food production. The importance of this process is constantly increasing due to the growing volumes of frozen raw materials processed in food enterprises. Scientific research shows that one of the most efficient methods of defrosting and thawing is the process of meat thawing with saturated water steam under vacuum. When applying the steam the raw materials is heated at its least and minimal losses are observed, while the duration of the process is significantly reduced. This work examines the process of beef meat defrosting and thawing with simultaneous shaking and crumpling the frozen mass, which can be called as massaging of raw materials. As studies have shown, this method of thawing makes it possible to reduce losses down to almost zero and obtain raw materials with good structural characteristics for the production of a finished product with a wide range of consumer properties. The obtained experimental curves of changes in the mass and temperature of raw materials make it possible to analyze the kinetics of heat transfer and mass transfer processes at the macro- and micro levels of the food system, which serve as the basis for modeling and controlling the technological process. This study presents the results of conventional defrosting and thawing of the raw meat but combined with massaging. Studies of the parameters of processing modes have shown that the proposed program makes it possible to use efficiently the design and technological features of the defroster-massager in order to obtain the raw beef for the production of high-quality food products. The results of experimental studies and their analysis allow making conclusion about the prospects of applying this process for the other types of raw meat materials before the main technological processing of raw materials.

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Introduction

The freezing method plays a quite significant role in preserving the quality of highly-perishable food, including meat and meat products [1,2]. The freezing as the storage method is an important way of maintaining the nutritional value and organoleptic properties of meat and meat products during its processing, storage and transportation, and it's therefore widely used in the meat industry and their cold supply chains [3,4,5].

Currently, the overwhelming number of meat processing enterprises run on frozen raw materials, approximately 70% of which is frozen meat (in the form of blocks and boneless meat cuts) [6]. On the one hand, it happens due to the shortage of chilled raw materials for the production of high-quality products. On the other hand, the frozen raw materials allow the enterprise to run in a stable pace.

However, storing the frozen meat has its own "disadvantage" — the process of defrosting and thawing is necessary for the subsequent processing of the meat [7]. In this regard, thawing of raw meat is becoming increasingly important as a technological process that affects the quality of the finished product due to inevitable physical and chemical changes during its defrosting [8].

The use of frozen raw meat in various technological processes implies its thawing in such a way as to get as close as possible to the quality of the raw material before thawing (chilled state) [9,10]. In practice, this is quite difficult to do, especially since long-term storage causes changes in the color of meat, intramuscular composition of fatty acids, oxidation of lipids and proteins, as well as moisture loss during defrosting [11].

In addition, as a rule, the initial characteristics of raw meat and freezing regimes when it arrives at a processing plant from various suppliers are unknown. Thus, a number of works provide research data on the quality indicators of beef under the influence of the freezing-thawing process,

depending on various characteristics of muscle fibers [3] and characteristics of cattle breeds [12]. In this regard, the experience and knowledge gained directly in production in the process of defrosting and thawing raw materials and using them to obtain a specific finished product has great practical importance.

The scale of qualitative changes in meat during its thawing depends on many factors [7], the main ones are the defrosting and thawing methods [13], duration (speed) [14] and temperature conditions of the process [15]. In this case, it is necessary to create conditions that ensure the most complete recovery of the original properties (characteristics) of the product. To do this, at a minimum, it is necessary to limit overheating of the product surface and loss of moisture, which causes the significant losses in the meat quality due to undesirable physical, biochemical and microbiological changes. Therefore, to improve the quality of defrosted and thawed meat, it is important to apply appropriate thawing methods [9].

According to the method of energy supply, defrosting processes can be divided into two large groups [1,6]. The first is classical methods based on the supply of heat to the surface of the product due to a temperature difference. This method uses warm air, water irrigation or the immersion of the frozen mass in a liquid medium [1,9]. This method is quite cheap and easy to use, but it has a range of significant disadvantages. The most modern defrosting methods of this group are: defrosting of a frozen mass in a water bath [14], exposure to the condensing saturated water steam under vacuum or vice versa at the elevated pressure [16], applying of infrasound or ultrasound [17,18,19] using ultra-high pressure, etc. [20].

The second method is the method of supplying energy through the use of physical fields: an alternating electric field [21], an electromagnetic field of a certain frequency (high-frequency and ultra-high-frequency heating, microwave radiation, infrared heating) [22,23,24].

The above specified literature sources outline the advantages and disadvantages of these defrosting and thawing methods, their effect on the quality indicators of meat and other food products, as well as the results of their comparative analysis with each other and with traditional the methods. For example, in [25] the effect of various defrosting methods using physical fields and traditional methods on frozen meat was studied. The authors, based on an analysis of the published data and the results of their own research, concluded that defrosting and thawing methods in a physical field reduce losses and preserve the color and texture of the thawed meat. In comparison to the meat samples defrosted and thawed at room temperature, the losses in pork, beef and lamb were approximately 43%, 45% and 43% lower respectively when ultrasound was used. At the same time, the oxidation of proteins and lipids in meat decreases and the content of bound and immobilized water within increases.

One of the ways to improve the defrosting process is the combined application of methods from the first and second groups [26,27,28] or their combination with other physical processes. For example, it's possible to modify the vacuum-steam method of meat thawing combining the initial stage of freeze-drying or the new vacuum-sublimation-rehydration method of thawing [29,30] with gamma irradiation for frozen beef in vacuum packaging and its subsequent thawing [31], or combining of the frozen meat exposure to a low-voltage electrostatic field together with high-moisture way of thawing of pork steaks [23].

Promising defrosting option, successfully implemented in the meat processing plants, is thawing the frozen block, lump or ground raw materials in a saturated steam environment at the reduced pressure [6,33]. Analysis of existing domestic methods of defrosting meat by the Russian [34,35] and foreign researchers have shown that it has a number of significant advantages compared to other methods [29,36,37]. For example, the duration of defrosting is reduced by 30–50% compared to the "air showering" method, and vacuum defrosters occupy way bless space compared to the air blowing chambers or industrial HF and UHF ovens, which are also more energy-consuming and require high-potential energy. At the same time, the vacuum environment has a beneficial effect on the sanitary condition of raw materials as it prevents the spread of bacterial contamination [33].

The additional positive effect can be achieved by combining the process of defrosting and thawing of raw meat with massaging [6,34]. The massaging of the raw materials ensures uniform heating, improves its quality, prevents overheating, and reduces losses. At the same time, thawing and salting raw meat in a vacuum increases protein hydration, water-holding capacity and moisture absorption of muscle tissue, which prevents the loss of meat juice [34,35]. Thus, massaging simultaneously with defrosting the raw materials in vacuum defroster-massagers is an efficient and increasingly popular method.

In the scientific works of the Russian authors devoted to the defrosting of food raw materials, the main principles of the process are shown [38,39,40] and rational modes of its implementation are proposed depending on the methods of frozen meat defrosting [41,42,43].

The analysis of data sources has shown that modern methods of defrosting and thawing meat and their technical implementation are quite well developed, and therefore the main purpose is not to find the new processing methods, but to select and define on the scientific base selection the rational modes, taking into account the characteristics of raw meat and the "history of the meat freezing". Regardless of the defrosting method, the main technological parameters of the process are the ambient temperature and duration of the process. The higher is the coolant temperature, the faster is the defrosting process and the higher the quality of the product. But from the certain threshold these two parameters conflict with the quality of raw meat. High temperature leads to denaturation of the surface layers of the product, and increasing the duration increases the percentage of losses due to the dripping of meat juice.

The search for the rational processing parameters that allow obtaining the raw materials with the required properties is a sophisticated task consisting of optimizing the thermodynamic characteristics of the coolant and the physicochemical properties of the exposed meat. Its solution is complicated by the fact that the freezing regimes for raw materials are usually unknown and it is not always possible to link these two processes into a single thermodynamic chain that guarantees the required quality of raw materials. In addition, it is necessary to take into account that thawing is an irreversible process in relation to freezing (this fact is confirmed by the nature of the curves of these processes and their different rates of occurrence [44]). The humidity of the coolant (air, steam, air-steam environment) also plays a big role. For example, defrosting in water leads to washing off the protein substances and disruption of the structure of the surface layers, while air stream forms a surface dry crust and inhibits the heat transfer process.

The peculiar feature of this study is that the process of defrosting and thawing of the raw meat by the steam vacuum method is combined with its massaging. This combined method was studied in an industrial environment at a meat processing plant according to a predetermined program. The purpose of the study of the frozen meat block behavior during its technological processing in a rotary-type mechanism was to determine the efficiency of the proposed program for this type of meat raw material and the effect of the defrosting and thawing process modes on its tested parameters.

Objects and methods

The frozen blocks of beef of the 1st quality grade weighing from 8 to 15 kg were selected as the object of research. The process of vacuum defrosting of raw meat was studied in a defroster-massager of the brand *ScanMidiTRl 10* from the company *GEA*, which is used in a number of Russian meat processing plants (Figure 1). The key features of this device are as follows:

- cylindrical drum (working volume — 10 m^3, internal diameter — 2 m, opening size of the hatch for loading and discharge the of raw materials — 0.9 m) with a volume filling degree of 66%;
- hydraulical tilting of the drum in both directions and axial mixing of the raw materials for its massaging or tumbling due to 5 asymmetrical blades and a "smart" blade at the bottom of the drum;
- to create the necessary defrosting and thawing parameters the device is equipped with systems for supplying the saturated water steam, for creating a vacuum and for adding the curing ingredients.
- this piece of machinery is equipped with the control panel with color touch screen from GEA, which allows creating and saving the variety of work programs;
- the temperature of the coolant and processed raw materials is controlled using built-in factory temperature sensors, and the control panel allows controlling and adjust the operating parameters of the program;
- the availability of precision strain gauges ensures precise control of the raw materials mass and its change during processing.

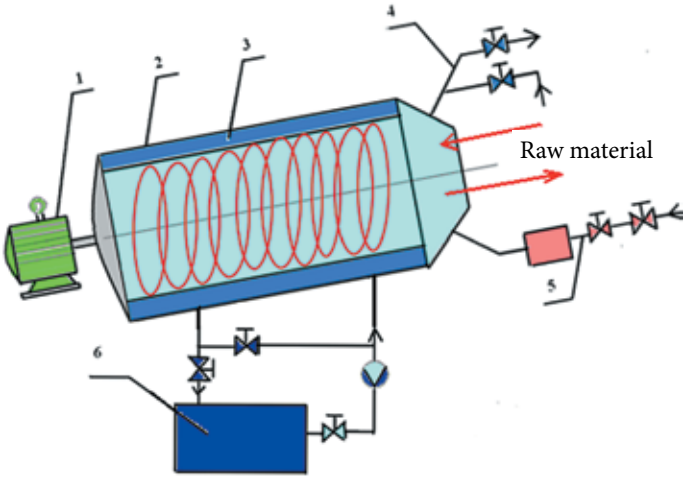

Figure 1. Schematic diagram of a vacuum defroster massager: 1 — electric motor; 2 — defroster drum; 3 — cooling jacket; 4 — system for vacuum creating and regulating; 5 — system for spraying the saturated water steam; 6 — system of cooling and supply of propylene glycol to the cooling jacket

The initial drum load during the experiments accounted for 2,510 kg. The controlled parameters of the defrosting process using the standard sensors of the device were as follows:

- temperature inside the frozen meat block (remote standard temperature sensor, rod-shaped with a screw spiral at the end) and on its surface (remote built-in pyrometer);
- water steam temperature was measured using built-in factory temperature sensors;
- the steam pressure inside the drum was measured with a standard built-in vacuum gauge;
- mass of defrosted raw materials (this parameter is measured with strain gauges installed in the defroster frame support);
- process time was measured with a timer.

The main advantage of the defroster is that, with its functional versatility, it allows full automation of the technological process, consisting of defrosting and simultaneous massaging of the raw materials. The low rotation speed of the drum eliminates surface damage to the raw meat as much as possible and creates favorable conditions for its massaging. The rotation of the drum allows even heating of the raw materials blocks. One of the advantages of this equipment is the opportunity to regulate the technological process if it deviates from the required processing parameters.

Results and discussion

Table 1 shows the program for defrosting and thawing of the raw meat in a defroster massager.

This program corresponds to the raw materials with outside temperature of the block = minus (4–6) °C, inter-

nal temperature of the block = minus (7–10) °C and a total maximum weight of raw materials up to 2,900 kg.

Table 1. Parameters of the raw meat defrosting program (recommended by the equipment manufacturer)

№ of the stage	Total time, minutes	Steam regulation		Type of the process	Vacuum value, mbar	Drum rotation speed, rpm	Method of impact, mild/hard	Angle of the drum tilt, degree	Max. steam addition, %	Steam pressure, mbar
		On, s	Off, s							
1	15	60	0	heating	50	15	mild	10		
2	90	60	0	heating	50	15	mild	10	60	800
3	90	60	0	heating	50	15	mild	10	40	800
4	45	60	0	heating	50	15	mild	10		

The technological processing parameters were determined experimentally during the research process and adjusted in accordance with the goals and objectives of the research, as well as taking into account the recommendations of the equipment manufacturer.

Structurally, the defrosting program consisted of 4 stages. Each stage has its own duration and implements certain functions. The raw materials are most affected at stages 2 and 3. At these stages of technological processing, the working environment features the highest parameters of time and thermodynamics. Despite the step-by-step differences in the impact parameters, in general the program creates a mild mode of defrosting the raw materials, including the kinematics of its movement along the ribs located on the internal surface of the drum at a low speed of rotation, which provides the necessary conditions for the homogenous heating and massaging.

The results of the process of defrosting and thawing raw meat in the form of graphs are shown in the Figures 2–4. The peculiarity of these graphs is that the given curves reflect as much as possible all real spikes and fluctuations of the parameters. This makes it possible to obtain a true picture of the process of industrial defrosting of the frozen raw materials, taking into account its characteristics at each moment in time, which does not violate the general idea of the tendency of the processes being observed.

Beef features denser structure compared to most other types of meat raw materials and lower thermo physical characteristics, therefore processes of the heat transfer and mass transfer occur in it at a low speed, as evidenced by the total duration of the defrosting process. To increase the speed, it's possible to increase the rotation speed of the defroster drum and the steam pressure up to a certain value. The consistency of raw meat at the exit of the machinery, and therefore the quality of the finished product in the form of its structure and organoleptic properties, largely depends on the results of these processes.

The kinetics of the process of defrosting and thawing of the frozen raw meat and its completion is mainly controlled by temperatures. Figures 2 and 3 show the changes in heating temperature at the inlet, outlet and inside the drum, as well as on the surface and inside the tested block of frozen beef during its defrosting.

The analysis of the above graphs, first of all, draws attention to two points: the low speed of the defrosting process and the relatively high driving force of the heat exchange process. If the speed of the defrosting process is expressed through the rate of temperature increase in the raw material, then for beef it will be on average 0.51 degrees/hour (0.0085 degrees/min). The low speed of the defrosting process is explained by the low coefficient of thermal conductivity in the raw material (depending on the grade of the raw material, the thermal conductivity coefficient is ~ 0.45 W/(m·K)) [45], which plays a key role in the heating process (Figure 2). To increase the speed of the defrosting process, it's possible to increase the driving force of the heat exchange process, in this case by increasing the parameters of the heating medium, i. e. water steam. But an uncontrolled increase in the temperature of the heating medium can lead, as mentioned above, to initial denaturation of the protein surface, which is unacceptable from the point of view of maintaining due product quality. The drawn graph (Figure 2) shows that the driving force of the heat exchange process when defrosting beef varies within the range of (20–30) °C (at a steam temperature of no higher than 20 °C) and does not have any significant fluctuations. Small fluctuations in the temperature difference between steam and raw materials ensure a relatively constant rate of the heat exchange process, which guarantees stable efficiency throughout the entire process of defrosting and allows obtaining a high-quality product.

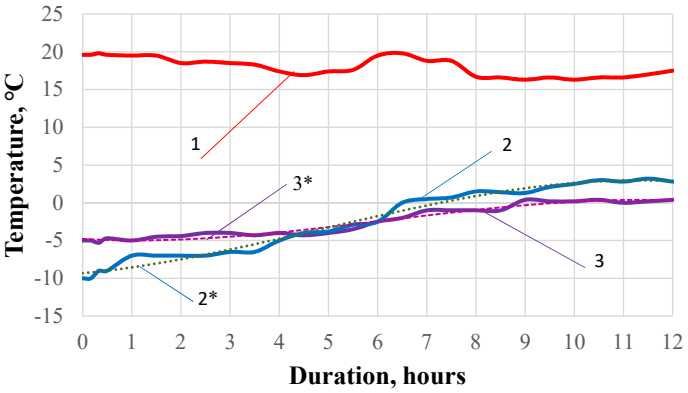

Figure 2. Change in temperature depending on the duration of the defrosting and thawing process: 1 — inside the drum; 2 — inside the beef block (2* — trend line); 3 — on the surface of the block (3* — trend line)

Mathematical processing of the experimental data in the Figure 2 allowed obtaining a correlation dependence of the temperature within the meat block and on the surface of the beef block (trend lines 2* and 3*, respectively) from the duration of the meat defrosting process (the equation (1) and the Table 2):

$$t_i = a \cdot \tau^3_{defr.} + b \cdot \tau^2_{defr.} + c \cdot \tau_{defr.} - d \quad (1)$$

Table 2. Numerical values of the coefficients included in the equation (1)

Coefficients in the equation (1)	Unit of measurement	Temperature of the meat block, °C	
		On the surface of the block, t_{ext}	Within the block, t_{int}
a	°C / h³	–0.0125	–0.0100
b	°C / h²	0.1834	0.1860
c	°C / h	0.1834	0.3671
d	—	9.3143	4.7701
Squared mixed correlation for the equation (1)			
R^2	—	0.9822	0.9623

The value of the approximation reliability (squared mixed correlation R2) when calculating the changes over time in the values of the external and internal temperatures of a block of meat indicates a high close relationship between the quantities included in equation (1) and the statistical reliability of the obtained empirical dependence for comparable conditions of the defrosting process. The mathematical model (regression equation (1) expressed in the form of a third-degree polynomial), obtained as a result of processing the experimental data presented in the Figure 2, has its advantages and disadvantages. The main advantages of the resulting model are its simplicity and good convergence with the experimental data, and its disadvantages are its discreteness and formalized nature, which initially does not reflect the physical essence of the processes that take place in the object. The resulting mathematical model has its practical value and can be considered as a certain stage in modeling of biotechnological processes in order to predict the properties of the obtained product, taking into consideration the patterns of their occurrence and mutual impact.

To provide the additional control over the temperature of the heating medium, the measurements were taken separately at the inlet and outlet of the drum machinery. The results are shown below in the graph (Figure 3).

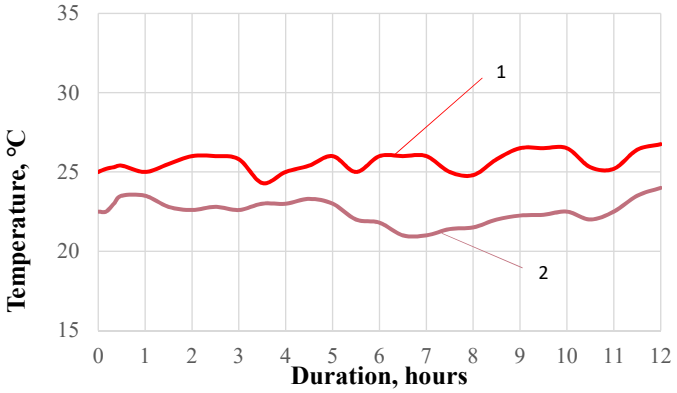

Figure 3. Change of heating temperature at the inlet (1) and outlet (2) of the drum during the frozen beef defrosting

Thermographs of the heating medium prove that the required processing conditions for raw meat are complied with and that the defrosting process occurs under relatively stable conditions, i. e. without sharp fluctuations of the heating medium parameters.

Figure 4 below shows a curve of dependence of the beef blocks mass change on defrosting time. The peculiar feature of the process in an environment of saturated water steam is the steam condensation, which gradually increases the total mass of the raw material.

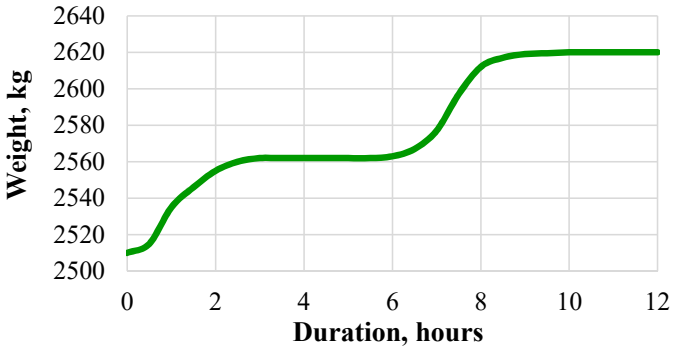

Figure 4. Change in the total mass of the beef blocks depending on the defrosting process duration

From the graph in Figure 4 it is visible that the increase in the mass of raw materials during the defrosting process occurs stepwise, incrementally. Moreover, the intervals of the raw materials mass increment are different. From this it can be concluded that the temperature-time interaction between the raw materials and steam is not smooth. In addition, this interaction implies the process of absorption of water condensate by the raw meat. The moisture formed during two parallel processes: defrosting and condensation must be absorbed by the raw materials, and only in this case a high-quality product can be obtained. But starting from a certain moment the moisture-absorbing capacity of the raw material becomes insufficient to solve this problem, and therefore a certain amount of salt is added to the defroster. For beef, the last increment of the meat mass occurs during a period of ~ (7–8) hours, when 25 kg of salt was added to the defroster so that the product absorbs all free moisture. The process of defrosting beef lasts no more than 12 hours, which is an advantage in comparison with the other methods. During the process (defrosting + massaging), the weight gain of the product amounted to 114 kg.

Thus, the defrosting modes being considered make it possible not only to defrost raw meat to cryoscopic temperature in a time not exceeding 10 hours, but also to bind free moisture released during technological processing (moisture during steam condensation and defrosting of raw materials) to a hygroscopic state. This makes the beef consistency suitable for producing high quality whole muscle food.

The considered experimental graphs allow formulating a physical model of the defrosting process based on the phenomenological parameters of the process being studied, which are the temperature and mass of the raw material. Defrosting of the raw meat is a complex process and it is accompanied by the following physical processes that can be controlled:

- heating from the initial temperature up to the temperature of the beginning of defrosting, phase transition

from a frozen state to a defrosted state, heating of the defrosted raw materials up to the final temperature;
- condensation of water steam on the surface of the raw meat;
- diffusion of moisture into the raw meat;
- massaging the raw meat.

These processes are related to each other and occur simultaneously, excluding moisture diffusion and massaging. The efficiency of this combination begins to manifest itself after the raw material reaches cryoscopic temperature.

The nature of changes in temperature and mass of the raw materials over time makes it possible to analyze the patterns of thermal and mass transfer processes at the macro level and predict phenomena that take place at the micro level, which is necessary to determine and evaluate the sustainable and costs-efficient technological processing modes.

The considered experimental graphs show that the main phenomenological parameters of the process under study are the temperature and mass of the raw material. Changing these parameters allows prediction of the heat transfer and mass transfer processes occurring in raw meat and technological equipment in general. Moreover, the most important role in forming the quality of raw materials during defrosting is played by mass transfer processes. The structure of raw meat depends on these processes. Temperature provides only the conditions for the transfer, or more precisely, the rate of mass transfer processes [39].

First of all, the mass transfer processes affect the yield of the finished product, which is one of the main economic parameters of the technological process and can serve as a criterion for its completion [38]. The yield of the finished product is understood as the ratio of the final product mass to the initial raw material (main raw material) mass. In our case, the product yield was 104%. The change in yield can occur mainly due to the moisture penetration into the product from the surrounding media during the technological processing or loss of the moisture from the meat mass.

The conditions for the mass transfer processes in the defroster-massager can be conditionally divided into two stages: the first stage takes place before reaching the cryoscopic temperature on the surface of a piece of raw meat; the second takes place after reaching the cryoscopic temperature on the surface of the meat block. The first stage is characterized by the condensation of saturated water steam and free moisture formation. Due to low temperatures and frozen moisture in the product, it is unlikely that significant mass transfer processes can occur during this period. Heating increases the speed of movement of molecules and, provided there is a temperature gradient, the process of heat transfer occurs. In turn, temperature affects the change in the molecules bond energy, which is reflected in the kinetic dependencies of chemical reactions and diffusion expressed through the kinetic constant and coefficient of the diffusion transfer, respectively. At the initial moment of heat treatment at a raw material temperature not exceeding (25–30) °C, which corresponds to the conditions of technological processing in a defroster in the second period, swelling of muscle fibers can be observed due to external moisture formed during condensation of steam.

The nature of mass change during heat exposure, depending on the type of meat raw material and production technology, can be described, essentially, by one of the curves in Figure 5, obtained by the authors and presented in the study [39].

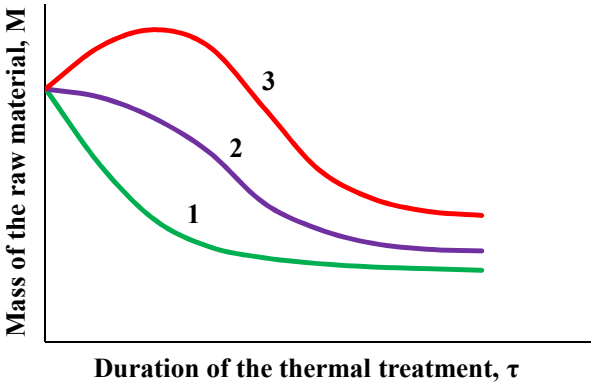

Figure 5. Change of mass of the various types of raw materials depending on the duration of the heat exposure process: 1 — raw materials with a high fat content, prone to dehydration; 2 — raw materials in which the processes of dehydration and swelling occur simultaneously; 3 — raw materials with a high content of components prone to swelling

For raw materials consisting of muscle tissue with a high fat content, curve 1 is quite distinctive and specific (Figure 5). The change of raw materials mass occurs mainly due to the release of free moisture and part of the immobilized moisture by both diffusion and filtration. Most defrosting methods are characterized by this very mass transfer process which is their serious drawback.

Meat products have become widespread in which the moisture, that increases the yield, is specially injected into the product during the pre-processing process: when preparing minced meat, when brine forcing into the meat and similar operations. Moisture introduced in a free state upon contact with the components of the raw material (mainly protein) forms more or less strong bonds, thereby passing into an immobilized state or more bound state. The part of the added moisture may remain in a free state or weakly bound state. The kinetic picture of the yield increase is directly affected only by the tightly bound part of the forced moisture. In this defroster, constant gentle massaging of raw meat and the addition of special ingredients (salt) allows firm binding of the moisture formed during steam condensation and thereby increases the yield of raw materials after its defrosting. The variety of moisture forms leads to the fact that during heat exposure, moisture transforms from one form into another without leaving the product mass. This explains the change in mass during heat treatment, corresponding to curve 2 (Figure 5). In this case, loosely bound moisture is able to leave the product faster than moisture contained within the tissues of the product itself.

The increase in mass in an atmosphere of saturated water steam can be characterized by the dependence in the form of curve 3. This mainly applies to raw materials containing a lot of components prone to swelling. Thus, the change in the yield of raw meat during heat exposure, within the accepted assumptions, is explained by the occurrence of two opposite processes: dehydration and swelling. The conditions for the heat exposure process, as well as the ratio of muscle and connective tissue proteins, determine the kinetics of the process of changing of the raw materials yield. In a defroster, the change of the raw materials mass occurs due to the swelling of muscle fibers with free moisture from the steam condensation, since dehydration of raw meat occurs only at temperatures close to (40–42) °C, when denaturation-coagulation processes begin.

In order to give the mathematic description of the output change over the time line, the basic mass transfer equation is used [39,40]:

$$\frac{M}{V \cdot \tau} = K_M \cdot \Delta \quad (2)$$

Where:

M — is the mass of the product, kg;

V — is the volume of the product, м^3;

τ — time, s;

Δ — driving force of the process, (unit of movement. f);

K_M — mass transfer coefficient, kg/(m^2·s·(unit of movement. f));

Since mass yield of raw materials increases or decreases due to the transfer of several components in different ways, the equation (2) will have the following form:

$$\frac{\Sigma M}{V \cdot \tau} = \Sigma K_M \cdot \Delta \quad (3)$$

where ΣM — total increment of the product mass, kg

It is obvious that the adopted expression for the output mass is related to the quantities included in the expression (3) as follows:

$$M' = \frac{M_0 + \Sigma M}{M_0} = 1 + \frac{\Sigma M}{M_0} \quad (4)$$

where:

M_0 — is the mass of raw materials at the beginning of the process, kg.

M' — is the yield of the raw material, fraction of a unit

In equation (2) the ratio makes sense to increase the volume concentration. In this regard, the equation (1) can be considered as an equation of chemical kinetics for the reaction of the 1st order with a shifted reference point along the ordinate axis.

To approximate the dependence of yield on process duration within the limited time interval, it is possible to use functional dependencies that include rate constants of the dehydration and swelling processes, taking into account the specifics of the initial conditions. The obtained dependencies can be used to optimize the duration of heat treatment of the newly designed types of meat products based on non-traditional sources of meat raw materials and food additives.

Using the obtained experimental data, thermal technical parameters were calculated based on balance correlations, which allowed evaluating the efficiency of steam thermal energy directly for the process of beef defrosting.

The amount of heat expended in the process of beef defrosting is determined using the modified Planck equation:

$$Q_1 = \mathrm{M} \cdot [C_{af} \cdot (t_{ct} - t_{init.}) + r_{ice} \cdot w \cdot \omega + C_{bf} \cdot (t_{fin.} - t_{ct})], \text{ kJ} \quad (5)$$

where:

C_{af} — is the specific mass heat capacity of the product after freezing, kJ/(kg·K);

C_{bf} — specific mass heat capacity of the unfrozen beef, kJ/(kg·K);

M — mass of beef before defrosting, kg;

$t_{init.}$ — temperature of beef at the beginning of the defrosting process, °C;

$t_{fin.}$ — beef temperature at the end of the defrosting process, °C;

t_{ct} — cryoscopic temperature of the product, °C;

r_{ice} — heat of phase transition (specific heat for ice melting), kJ/kg;

w — product moisture;

ω — fraction of frozen moisture;

According to scientific research, all moisture is frozen out at the freezing temperature (minus 60 °C), which is extremely rare in real practice [46]. Most often, the freezing process is run at temperatures from minus 18 °C to minus 25 °C, and under these conditions the proportion of frozen moisture is much less and can range from 0.4 to 0.8.

The total amount of heat Q (1) required for the process of defrosting of beef block weighing 2,510 kg, calculated by formula (5), is equal to 362,355 kJ. Knowing the amount of heat that steam gives off during its condensation, it is possible to determine the theoretical steam consumption that must be spent for defrosting of the raw meat based on the following equation (6):

$$Q_2 = D \cdot r_{steam}, \text{ kJ}; \quad (6)$$

where

D — is the steam consumption, kg;

r_{steam} — is the latent heat of steam condensation at 20 °C and reduced steam pressure, kJ/kg;

Assuming that $Q_1 = Q_2$, let's write it down in the following way:

$$M \cdot [C_{=af} \cdot (t_{ct} - t_{init.}) + r_{ice} \cdot w \cdot \omega + C_{bf} \cdot (t_{fin.} - t_{ct})] = D \cdot r_{steam}, \text{ kJ} \quad (7)$$

whence the theoretical steam consumption is equal to:

$$D = M \cdot [C_{af} \cdot (t_{ct} - t_{init.}) + r_{ice} \cdot w \cdot \omega + C_{bf} \cdot (t_{fin.} - t_{ct})] / r_{steam}, \text{ кг} \quad (8)$$

Thus, the performed calculations showed that the process of defrosting of beef block weighing of 2,510 kg theoretically requires spending of 142.45 kg of water steam. However, in reality, when defrosting the frozen meat in vacuum, the mass of raw materials at the end of the process increased by 114 kg. Using Planck's formula for calculation the overestimated steam consumption is obtained,

i. e. theoretically there is certain reserve of steam for the defrosting of raw materials (for this case the steam reserve is equal to 20%).

To assess the energy efficiency of the defrosting process as a whole, it is necessary to take into consideration not only the consumption of thermal energy for defrosting of the raw meat, but also other types of energy must be taken into account, i. e. energy balance should be drawn up, since there are other items of energy consumption. To a first approximation, these items include the following: heat loss to the environment; heating the equipment itself, heating the ingredients, raw meat during massaging; energy consumption for drum rotation; consumption of cold supplied to the drum cooling jacket; energy for creating a vacuum, etc. To determine them, special additional research is required.

Defrosting of frozen raw meat with the mechanical impacts in water steam environment at reduced pressure is a rather complicated and insufficiently studied process, especially in terms of kinetic impact of the raw materials massaging and its influence on the main parameters of technological processing.

Conclusions

The conducted studies showed that the proposed technological processing program (Table 1) can be successfully used for defrosting and thawing the frozen raw beef supplied in blocks along with simultaneous massaging for the production of whole muscle products. The studied modes of defrosting and thawing together with simultaneous massaging of the raw materials in an environment of saturated water steam under vacuum make it possible to increase the raw materials yield while simultaneously achieving the required organoleptic properties of the processed raw materials.

Experimental research in industrial conditions is the most rational means for improvement and refinement of the existing processes and for developing of the new processes and new types of equipment, as they allow obtaining more reliable data without such an intermediate stage as a large-scale transition from a laboratory experimental installation to industrial production machinery.

The study of the process of the raw beef defrosting combined with simultaneous massaging, in real industrial conditions made it possible to trace the patterns of complicated processing of the raw materials and to evaluate the mutual influence of heat, mass transfer and mechanical processes on the consistency of the defrosted beef. Using the opportunity to obtain data from experimental tests run on the industrial equipment, we had the chance to trace and record not only the processes occurring at the macro level based on phenomenological parameters, but to predict the phenomena occurring in the raw materials of animal origin at the micro level also.

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