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ENTOMOPHAGY — AN EVALUATION OF QUALITY AND ACCEPTABILITY OF RAPHIA PALM WEEVIL LARVAE (*RHYNCHOPHORUS PHOENICIS)* AS INFLUENCED BY THERMAL PROCESSING METHODS

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Keywords: *Consumers, cooking methods, enhancement, microbial load, sensory characteristics*

Abstract
In this study, the quality and acceptability factor of Raphia palm weevil larvae (Rhynchophorus phoenicis) as influenced by different thermal processing methods were investigated. Raphia palm weevil larvae (n=1000) were randomly distributed into four groups of 250 larvae per group according to a treatment, namely: T1 = boiling (100 °C), T2 = roasting (120 °C) T3 = frying (160 °C) and T4 = oven-drying (180 °C). All treatments lasted 20 minutes. Analyses were carried out to determine the physical, chemical, vitamin and mineral composition, and microbial load. In addition, sensory characteristics were evaluated. Weevil larvae processed by the boiling method had the highest cooking yield (97.59%), water holding capacity (21.78%) and the lowest cooking loss (2.41%). The protein and fat content was higher in weevil larvae processed by frying (37.63% and 17.70%, respectively), while moisture was lowest (18.68%) in oven-dried larvae. The calcium, magnesium and phosphorus content was higher in oven-dried larvae, while there were no significant differences in iron, manganese, zinc and vitamins in the processed larvae irrespective of the methods. Boiled larvae had a higher microbial load, while fried and oven-dried larvae had the lowest microbial load. Fried larvae elicited highest sensory characteristics except tenderness, which was higher in boiled larvae, but fried larvae had higher overall acceptability than those processed by other methods. Therefore, it has been shown that the frying method is an appropriate method of processing Raphia palm weevil larvae for enhanced quality and acceptability.

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Introduction

The most pressing nutritional problem in the developing countries is the shortage of protein in the diets of a large section of the population. The acute shortage, especially of animal protein, has been attributed to the phenomenal rise in the price of conventional animal protein sources such as meat, milk and eggs [1]. This led to malnutrition in most developing nations, hence the call for more studies on alternative or unconventional animal protein sources such as snails, rodents, frogs and insects [2]. Insects have been presented as an attractive alternative source of protein as they serve as natural food for many vertebrates including humans [3]. Although insects are not traditional food in many cultures, there is a growing public interest and demand for them due to their nutritional importance [4,5,6,7]. Among insect species that are used for food, *Rhynchophorus phoenicis* (Raphia palm weevil) larvae are considered the major source of dietary lipids and protein, especially in developing and under-developed countries where consumption of animal protein is limited as a result of economic factors [8,9]. Palm weevil larvae have been shown to be high in crude protein, fatty acids, minerals and vitamins [10,11,12,13,14]. However, the nutritional value of palm weevil larvae changes according to preparation and a processing method adopted [15,16]. Hence, there is a need to explore nutrient potentials and processing methods for palm weevil larvae exploitation to bridge the gap between animal protein production, supply and consumption [17]. This line of reasoning evoked this study interest to investigate the quality and acceptability factors of *Rhynchophorus phoenicis* as influenced by thermal processing methods.

Materials and methods

Collection and preparation of weevil samples

Live palm weevil larvae (n = 1000) were purchased from Itokin in Epe local government area of Lagos State. They were transported to the Meat Science Laboratory of the Department of Animal Production, Olabisi Onabanjo University, Ayetoro Campus in a well-ventilated container within 24 hours.

Preparation

Larvae were killed and prepared following the methods outlined by [18,19]. The killing of larvae was carried out by immersing them is 15 liters of warm water at 37 °C and stirred

for five minutes with a wooden stick. The water was drained from the larvae and the process was repeated twice in order to remove volatile anti-nutritional compounds (Pentacosane) that are known to cause temporary blindness [18]. The larvae were chilled in a refrigerator at 4 °C for 24 hrs prior to further processing and analysis [20].

Processing of larvae

Larvae were removed from the refrigerator, washed, and divided into four groups of 250 larvae each according to the processing method as follows:

T1 = Boiling method (250 larvae)
T2 = Roasting method (250 larvae)
T3 = Frying method (250 larvae)
T4 = Oven-drying (250 larvae)

The processing methods were applied in triplicate with the following regimes:

Boiling: Larvae were wrapped in cellophane papers and boiled in water at 100 °C for 20 minutes.

Roasting: Larvae were sticked and roasted on a charcoal glowing fire at 120 °C for 20 minutes.

Frying: Larvae were fried at 160 °C for 20 minutes.

Oven-drying: Larvae were dried at 180 °C for 20 minutes.

In the roasting, frying, and oven-drying methods, larvae were turned over at intervals of 5 minutes.

Measurement of parameters

Physical factors

Cooking loss

Larvae in each group were weighed before and after thermal processing. Larvae were taken out when the temperature at the center of the test larvae reached 65 °C and then cooled. After that, the cooking loss was calculated by the following equation [21].

$$\text{Cooking loss (\%)} = \frac{W_0 - W_1}{W_0} \times 100 \quad (1)$$

Where:

W_0 = weight of larvae before processing;
W_1 = weight of larvae after processing.

Cooking yield

The cooking yield of larvae in each group was calculated by the equation according to [22].

$$\text{Cooking yield (\%)} = \frac{W_1}{W_0} \times 100 \quad (2)$$

Where:

W_0 = weight of larvae before processing;
W_1 = weight of larvae after processing.

Thermal shortening

The thermal shortening of larvae in each group was calculated by the equation prescribed by [20]:

$$\text{Thermal shortening (\%)} = \frac{L_0 - L_1}{L_0} \times 100 \quad (3)$$

Where:

L_0 = Length of larvae before processing;
L_1 = Length of larvae after processing.

Water holding capacity (WHC)

The water holding capacity (WHC) of larvae was determined according to the procedures of [21]. The larvae were minced and 5g was taken and heated at 70 °C in a water bath for 30 minutes, cooled and centrifuged at 1,000 rpm for 10 minutes. Total moisture was measured and WHC was calculated using the following equation:

$$\text{WHC (\%)} = \frac{(T - S) \times 0.951}{W_0} \times 100 \quad (4)$$

Where:

T = Total water content;
S = Separated water content;
0.951 = Pure water amount for larvae moisture that was separated under 70 °C.

Chemical factors

Moisture content

The moisture content of processed larvae was determined following the procedures described in [23] by weighing 2 g of ground larva samples from each group into crucible of the known weight and drying at 100–105 °C for 24 hrs in an oven until a constant weight was attained. The moisture content was calculated as follows:

$$\text{MC (\%)} = \frac{W_1 - W_0}{W_2 - W_0} \times 100 \quad (5)$$

Where:

W_0 = *Wt* of empty crucible;
W_1 = *Wt* of crucible + dried sample *Wt*;
W_2 = *Wt* of crucible + wet sample *Wt*.

Crude Protein

Crude protein of processed larvae was determined using the Kjehdahl method as described in [23]. Samples of minced larvae each weighing 700 mg were placed in a Kjehdahl digestion tube. The digesting catalysts (5g of K_2SO_4 + 0.5g of $CuSO_4$) and 25ml of concentrated H_2SO_4 were added. The samples were digested for 1 hr, cooled and 20ml of deionized water and 25ml of 40% NaOH were added. The samples were distilled and NH_3 liberated was collected into boric acid, and titrated with 0.1 HCl. A blank titer was prepared and titrated alongside the tested samples, and the crude protein was calculated as follows:

$$\text{CP (\%)} = \frac{(St - Bt) \times 14 \times 6.25}{Sw} \times 100 \quad (6)$$

Where:

St = sample titer;
Bt = blank titer;
Sw = sample weight;
14 = molecular weight of N_2;
6.25 = N_2 conversion factor.

Crude fat (Ether Extract)

The fat content of processed larvae was determined following the procedures described in [23] by weighing 5g of dried minced larva samples into thimbles and putting into an Allihn Condenser of the Soxhlet extraction apparatus.

Petroleum ether (250 ml) as an extractant was poured into a pre-dried boiling flask and the solvent was heated for 14 hours to extract the fat. The solvent was evaporated using a vacuum condenser. The boiling flask with the extracted fat was dried in an oven at 100 °C for 30 minutes, cooled in a desiccator at 27 °C and weighted. The flask containing oil/fat was weighed and dried in an oven to a constant weight and fat content was calculated as follows:

$$\text{Crude fat (\%)} = \frac{Wo}{Ws} \times 100 \quad (7)$$

Where:
Wo = weight of oil;
Ws = weight of sample.

Ash content

The total ash content of processed larvae was determined according to [23]. Test portions (1 g) from samples of each group were weighted into pre-heated crucibles and incinerated overnight in a Muffle furnace at 550 °C until white ash free of carbon was obtained. The crucibles were removed from the Muffle furnace, cooled in a desiccator at a room temperature of 27 °C and reweighed. The ash content of the samples was calculated using the equation:

$$\text{Ash (\%)} = \frac{Wa}{Ws} \times 100 \quad (8)$$

Where:
Wa = weight of ash;
Ws = weight of sample.

Crude fiber

This was determined according to the procedures of [23] by weighting 1 g of minced larva samples from each treatment into a flask, adding 200 ml of 1.25% H_2SO_4 and heating the mixture under reflux for 1 hr. The mixture was filtered through a Buckner funnel, the residues were washed back into the flask with 200 ml of 0.31M NaOH and the mixture was heated for another 1hr. An amount of 2 ml of 180 isoamyl alcohol was added and the mixture was filtered through fiber sieve cloth. The residues were washed with hot water twice and were transferred into dried weighed crucibles which were oven dried at 550 °C for 4 hrs. The crucibles with the residues were cooled in a desiccator at 27 °C and weighed. The percentage of crude fiber was obtained using the equation as follows:

$$\text{CF (\%)} = \frac{Wt_0 - Wt_1}{Ws} \times 100 \quad (9)$$

Where:
Wt_0 = *Wt.* of oven dried sample;
Wt_1 = *Wt* of ash dried sample;
Ws = weight of sample.

Carbohydrate value

The carbohydrate (CHO) values of larva samples from each treatment were calculated as the difference between 100 and the sum of the percentages of moisture, protein, ash and total fat (proximate composition) according to the procedures of [23]. Thus, CHO values were obtained by this equation.

$$\text{CHO (\%)} = 100 - \text{Proximate composition.}$$

Vitamins and minerals

Vitamins and minerals of processed larva samples from each treatment were determined following the procedures described by [23].

pH value

The pH values of processed and minced larva samples from each treatment were measured using a pH meter (Model H-18424 Micro-Computer, Hanna Instruments, Romania) as described by [24].

Sensory evaluation

Sensory evaluation of processed larvae was carried out using a 10-member semi-trained panel following the procedures of [25]. Processed larva samples were cooled at a room temperature of 27 °C and were served to panelists on clean saucers. The larva samples were evaluated one after the other. The panelists were provided with unsalted crackers and clean water to clear the condition of the palate between tasting samples. The panelists rated the processed larva samples using a 9-point hedonic scale, wherein 1 = dislike extremely and 9 = like extremely for color, flavor, tenderness, juiciness, texture and overall acceptability.

Experimental design and statistical analysis

This study was conducted using completely randomized design (CRD). The results from this study were applied to analysis of variance (ANOVA) using [26] and significant means were verified at a level of 50% ($p < 0.05$) with the Duncan multiple range test of the same software.

Results and discussion

Physical characteristics

The change in physical properties of processed palm weevil larvae is shown in Table 1. The cooking loss after processing showed significant differences depending on the processing method ($p < 0.05$). The cooking loss was lowest (2.41%) in boiled larvae (T1) and highest in oven-dried larvae (T4) followed by that in fried larvae (T3). The results showed that the cooking yield was lowest (88.85%) in oven-dried larvae (T4) and highest (97.59%) in boiled ones (T1). The results of the larva thermal shortening demonstrated a similar trend with the cooking loss. The thermal shortening was highest (19.63%) in oven-dried larvae (T1) and lowest (11.99%) in boiled ones (T1). The results of the water holding capacity (WHC) of processed larvae reflected the similar trend with the cooking yield. WHC was highest (21.78%) in boiled larvae (T1) and lowest (17.13%) in oven-dried larvae (T4). Both the results of the cooking loss and thermal shortening showed the same trends, the values for both variables increased from boiling to oven-drying indicating the fact that both variables increased as the temperature applied in

the processing methods rose. The results observed for both variables agree with the findings of [27], who reported that when insects/larvae underwent thermal processing both the cooking loss and thermal shortening increased due to the temperature growth depending on the intensity of heat because moisture was lost in the process. The same line of thought is applied to WHC, which is the ability of any edible larvae to retain some appreciable moisture after processing. This variable differed between the treatments decreasing with an increase in temperature as more moisture was retained in the larvae that were processed by the method that required lower degree of temperature according to [28].

Table 1. Physical characteristics of Raphia palm weevil larvae depending on the processing method

	Treatments				
	T1	T2	T3	T4	
Variable	Boiling	Roasting	Frying	Oven-drying	SEM
Cooking loss (%)	2.41^{d}	5.95^{c}	9.66^{b}	11.15^{a}	0.02
Cooking yield (%)	97.59^{a}	94.05^{b}	90.34^{c}	88.85^{d}	0.01
Thermal shortening (%)	11.99^{d}	14.53^{c}	15.28^{b}	19.63^{a}	0.02
WHC (%)	21.78^{a}	20.42^{b}	18.34^{c}	17.13^{d}	0.03

abcd: Means in the same row with different superscripts are statistically significant ($p < 0.05$)
WHC = Water Holding Capacity

Table 2 shows the results of the chemical composition and pH of processed larvae. There were significant differences in the values of all variables with the exception of pH values ($p < 0.05$). Boiled larvae had the highest moisture content compared to larvae processed by other methods (26.43%), while oven-dried larvae showed the lowest moisture content (18.63%). Crude protein was lowest in oven-dried larvae (27.17%), while fried larvae had the highest protein content (37.63%) followed by those larvae that were boiled (32.60%). The fat content was highest in fried larvae (17.70%) followed by boiled ones (15.57%), while it was lowest in over-dried larvae (11.43%). The ash content was highest (5.63%) in oven-dried larvae and lowest in boiled ones (3.37%), while roasted and fried ones had similar values. The carbohydrate (CHO) content was highest in oven-dried larvae (37.04%) and lowest in fried ones (19.63%), while roasted larvae had the value (27.29%) close to that in oven-dried ones. The oven-dried larvae had significantly higher crude fiber content, while it was lower and similar in boiled and roasted larvae. There was no significant difference in the pH of processed larvae across the four treatment methods ($p<0.05$). The result obtained for the moisture content of processed larvae in this study agrees with [29] who reported 26% moisture, but is different from [28,30] who recorded lower values in boiled larvae. Although boiling and roasting treatments are generally known to coagulate protein, the protein content in fried larvae was higher than in boiled and roasted larvae. The reason for this could be the contribution of protein in the oil used for frying and the inherent protein in the larvae according to [31] who reported that palm weevil larvae elicited higher protein when fried than when boiled or roasted. The same explanation goes for the fat content of processed larvae, but the crude fiber content was higher in oven-dried larvae probably due to dehydration as a result of high temperature, which was not so high in other treatment methods, especially boiling and roasting. The values of the crude fiber content obtained in this study corresponded to those reported by [29] for edible insect larvae. The values of ash and pH obtained in this study are close to the values reported by [32] who recorded a range of 3–6% for the ash content and 7–9 for pH of processed larvae.

The carbohydrate content was highest in oven-dried larvae as a result of high dehydration and denaturation of protein with higher protein coagulation in oven-dried larvae.

Table 2. Chemical composition and pH of Raphia palm weevil larvae depending on the processing method

Variable	Treatments				
	T1	T2	T3	T4	
	Boiling	Roasting	Frying	Oven-drying	SEM
Moisture (%)	26.43^{a}	24.57^{b}	20.23^{c}	18.63^{d}	0.32
Crude protein (%)	32.60^{b}	30.20^{c}	37.63^{a}	27.27^{a}	0.40
EE (fat) (%)	15.57^{b}	13.37^{c}	17.70^{a}	11.43^{d}	0.36
Ash (%)	3.37^{c}	4.57^{b}	4.77^{b}	5.63^{a}	0.29
CHO (%)	22.03^{d}	27.29^{c}	19.63^{b}	37.04^{a}	0.73
Crude fiber (%)	3.40^{c}	3.67^{c}	4.63^{a}	5.7-^{a}	0.24
pH	7.30	7.26	6.90	7.10	0.19

abcd: Means in the same row with different superscripts are statistically significant ($p < 0.05$)
CHO = Carbohydrate

The results of the content of some vitamins and minerals in processed palm weevil larvae are shown in Table 3. The level of ascorbic acid was highest (0.60 mg/100 g) in boiled larvae and lowest (0.39 mg/100 g) in oven-dried samples of larvae, while the level of ascorbic acid in roasted larvae (0.57 mg/100 g) was close to that of boiled larvae. The niacin level was highest (2.66 mg/100 g) in boiled larvae and lowest (2.21 mg/100 g) in oven-dried larvae. Also, the thiamine level was highest in the boiled larva samples (0.15 mg/100 g) and lowest in oven-dried larvae with a value of 0.08 mg/100 g. A sequential decrease in the content of vitamins in larvae treated by different processing methods was observed for all vitamins except riboflavin, which content increased irrespective of the level of heat applied in processing of larvae.

The results obtained for vitamins in this study agree with [33], who reported that ascorbic acid, niacin and thiamine were susceptible to heat destruction, while riboflavin was not. Hence, the numerical decrease in the levels of ascorbic acid, niacin and thiamine was observed in the larva samples treated by different processing methods, while riboflavin significantly increased ($p < 0.05$), because it was not affected by heat. The levels of some minerals such as calcium, magnesium, phosphorus and sodium increased significantly in this study as heat increased due to processing methods, while others such as manganese and zinc increased insignificantly under

heat. Dobermann et al. [6] showed that generally minerals in insects are not susceptible to heat depending, though, on a level and type of thermal processing that is employed. The same observation was recorded in this study as none of the processing methods employed affected the mineral composition of the weevil larvae adversely.

Table 3. Content of some vitamins and minerals of Raphia palm weevil larvae depending on the processing method, (mg/100g)

Variable	Treatments				
	T1	T2	T3	T4	
	Boiling	Roasting	Frying	Oven-drying	SEM
Vitamins					
Ascorbic acid	0.60	0.57	0.43	0.39	0.19
Niacin	2.66	2.56	2.32	2.21	0.04
Riboflavin	0.14^{d}	0.18^{c}	0.22^{b}	0.25^{a}	0.03
Thiamine	0.15	0.12	0.10	0.08	0.02
Minerals					
Calcium	260.00^{c}	280.00^{b}	290.00^{a}	290.00^{a}	8.15
Magnesium	90.00^{d}	93.30^{c}	96.70^{b}	100.00^{a}	7.69
Manganese	0.03	0.03	0.04	0.04	0.02
Phosphorus	153.30d	160.00^{c}	168.30^{b}	185.00^{a}	7.69
Sodium	52.20^{d}	61.00^{a}	54.36^{c}	58.67^{b}	0.11
Zinc	15.80	16.62	16.01	16.03	0.04

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

Table 4 shows the results of the microbial load of palm weevil larvae processed by different thermal methods. The total viable count was highest in boiled larvae (2.2×10^5 cfu/g) compared with the microbial load of larvae processed by roasting (1.1×10^5 cfu/g), frying (1.0×10^5 cfu/g) and oven-drying (1.0×10^5 cfu/g), respectively. Similar results were observed for the total yeast count with boiled larvae having the highest load (2.0×10^2 cfu/g) compared to larvae processed by other three methods, namely, roasting (1.1×10^2 cfu/g), frying (1.0×10^2 cfu/g) and oven drying (1.1×10^2 cfu/g). There were no significant differences in the fungal and staphylococcal loads of processed larvae. The trend of microbial contamination of processed larvae followed a degree of heat applied. The number of microbes decreased as thermal methods with higher temperatures were employed. Boiled and roasted larvae carried higher ($p < 0.05$) levels of microbes than fried and oven-dried larvae as a result of lower degrees of temperature involved in their processing. The results observed in this study agree with the findings of [15] who studied the effect of processing palm weevil larvae by cooking and drying as well as storage and reported that higher temperature reduced the number of microbes on the larvae processed. The larvae processed in this study were not stored; however, the level of asepsis of the product was assessed in relation to handling during processing, therefore a degree of contamination of the product (larvae) was not higher than the recommended level of tolerance and consumerism [34].

Table 4. Microbial counts of Raphia palm weevil larvae depending on the processing method, (cfu/g)

Variable	Treatments			
	T1	T2	T3	T4
	Boiling	Roasting	Frying	Oven-drying
Total viable count	$2.2^{a} \times 10^5$	$1.1^{b} \times 10^5$	$1.0^{b} \times 10^5$	$1.0^{b} \times 10^5$
Total fungal count	1.2×10^4	1.3×10^4	1.0×10^4	1.0×10^4
Total Staphylococcus count	0.10×10^3	0.10×10^3	0.01×10^3	0.01×10^3
Total yeast count	$2.0^{a} \times 10^2$	$1.1^{b} \times 10^2$	$1.0^{c} \times 10^2$	$1.1^{b} \times 10^2$

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

The results of sensory evaluation of palm weevil larvae processed differently are shown in Table 5. All eating quality variables significantly differed across the processing treatments ($p < 0.05$). Weevil larvae processed using the frying method showed the highest values ($p < 0.05$) of color, (7.00) flavor (7.50), juiciness (6.70), texture (6.80) and overall acceptability (7.50), while tenderness was highest (6.30) in boiled larvae. Larvae processed by oven-drying had the lowest eating quality values. For meat and meat products to be palatable and acceptable to consumers, the palatability factors must be significantly high coupled with the reasonable water holding capacity, moisture, protein, and fat contents of a piece of meat [35,36]. The sensory scores of fried palm weevil larvae were high, as well as the WHC, moisture, protein and fat content, which are needed to enhance flavor, juiciness, and hence, high acceptability. Overall acceptability of fried larvae was high due to the aforementioned characteristics, which in addition to high color and texture scores may attract consumers to accept fried weevil larvae more than those processed by other methods. The results of sensory evaluation of larvae processed by frying were in agreement with the findings of [37] who reported that consumers preferred fried meats rather than those processed using other thermal processing methods.

Table 5. Sensory scores for Raphia palm weevil larvae depending on the processing method

Variable	Treatments				
	T1	T2	T3	T4	
	Boiling	Roasting	Frying	Oven-drying	SEM
Color	5.00^{c}	6.00^{b}	7.00^{a}	4.00^{d}	0.96
Flavor	4.10^{d}	6.30^{b}	7.50^{a}	5.20^{c}	0.93
Tenderness	6.30^{a}	5.40^{b}	3.90^{c}	2.80^{d}	0.86
Juiciness	3.10^{c}	5.50^{b}	6.70^{a}	3.00^{c}	1.01
Texture	4.30^{c}	5.40^{b}	6.80^{a}	3.20^{d}	1.12
OA	3.30^{d}	6.10^{b}	7.50^{a}	4.50^{c}	1.34

abcd: Means in the same row with different superscripts are statistically significant ($p<0.05$)
OA = Overall Acceptability

Conclusion

Thermal methods have been applied to process insects, especially palm weevil larvae, to enhance their eating factors. This study was carried out to investigate the quality and

acceptability factors of *Rhyncophorus phoenicis* processed by different thermal methods — boiling, roasting, frying and oven-drying. This study showed that palm weevil larvae processed by the frying method had the low cooking loss, thermal shortening, microbial load, and high WHC, cooking yield, content of protein, fat, minerals, vitamins and sensory characteristics. Therefore, it has been shown that the frying method is an appropriate method to process *Rhyncophorus phoenicis* for better eating quality factors and higher consumer acceptability.

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EVALUATION OF APPROACHES TO INCREASE THE EFFECTIVENESS OF VARIOUS DISINFECTANTS AGAINST BIOFILM COMMUNITIES OF DIFFERENT AGES

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Abstract
Disinfectants are used as the main agents against microorganisms circulating on the surfaces of food enterprises. However, the adaptive ability of microorganisms to form biofilms complicates the process of surface cleaning and reduces the effectiveness of disinfectants. Modern disinfectants act against freely circulating microflora, but it is known that they are not always effective against biofilms. The purpose of this study was to investigate effective disinfectant compositions with bactericidal effect on binary bacterial biofilms of different ages. The article describes the effects of disinfectants based on chlorine, peracetic acid and quaternary ammonium compounds with enzymes in concentrations recommended by the manufacturer and increased several times on Salmonella 38, Brochothrix thermosphacta 2726 and Staphylococcus equorum 2736 planktonic cultures and binary biofilms. Binary biofilms of different ages (2 and 9 days old) were exposed to disinfectants with various active ingredients in combination with adjuvants, i. e. hydrogen peroxide 6% and various concentrations of isopropyl alcohol (30%). All products in concentrations recommended by the manufacturer did not have a disinfectant effect against the studied biofilm cultures. As a result of the work, it was found that the most effective disinfectants against multispecies biofilms were quaternary ammonium compounds in combination with enzymes and chlorine in combination with isopropyl alcohol (30%). The results obtained allow to expand knowledge about effective methods for controlling biofilms.

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Introduction

Food contamination causes great economic losses to society. Hygiene in the food industry is therefore of great importance and requires consideration of all types of microbiological hazards that may arise in the facilities (conveyor belts or slicing and packaging machines) and in the processing environment, as well as in raw materials throughout the process [1].

In food processing plants, failure to follow sanitation and disinfection procedures may result in the formation of bacterial niches that are not properly disinfected, so the bacteria are exposed to subinhibitory levels of disinfectants [2].

At the same time, dried organic matter and biofilms, if they are not removed during the washing process, may prevent the penetration of the disinfectant and reduce the effectiveness of surface cleaning [3].

In the food industry, pathogenic and spoilage microorganisms form biofilms on food contacting surfaces [4]. This results in equipment contamination, microorganism growth in drinking water systems, and post-processing contamination, which contributes to food spoilage and outbreaks of foodborne infections [5,6].

A number of studies have shown that multispecies biofilms commonly found in meat processing plants increase the resistance of bacteria to antibacterial treatment [7,8]. Non-lethal concentrations of antibiotics or disinfectants may stimulate the formation of bacterial biofilms [9,10], which are tolerant to aggressive external factors, including antimicrobial substances [11,12]. Over the years, this process has become better understood through researches; however, its potential risk and impact on food safety have not yet been fully established [13,14]. At the same time, complete inactivation and removal of mature biofilms formed on food contacting surfaces is difficult. Meat processing equipment with scratches, cracks or dents, and other hard-to-reach areas such as the underside of conveyor belts are potential niches for biofilm development. *Escherichia coli* O157: H7 and *Salmonella enterica* may form biofilms in meat processing plants [15,16], and in previous studies [17,18], many common disinfectants failed to

completely kill these biofilms due to the three-dimensional (3D) structure of the biofilm and the presence of bacterial extracellular polymeric substances (EPS) [19].

Biofilm formation provides significant benefits to bacterial cells by providing protection from physical and chemical stress. Multiple layers of surface-colonizing biofilms may prevent the penetration and diffusion of disinfectant solutions into the inner layers of biofilms. Thus, bacteria in these layers may be better protected and survive treatment with disinfectants. Surviving bacteria may multiply and contaminate food. Bacterial resistance to disinfectants also involves a nonspecific change in cell wall or membrane structure that affects cell permeability or increases efflux pump activity that helps biofilm cells to block and remove disinfectants outside the matrix. To counteract such resistance mechanisms, the ability of a disinfectant to dissolve the biofilm matrix, penetrate multiple layers of the biofilm, and disrupt the integrity of the cell membrane is critical to inactivate and remove the biofilms [20].

Given the growing interest in studying the resistance of biofilms to chlorine, quaternary ammonium compounds (QAC) and peracetic acid (PAA), many studies have been conducted [21]. Bacteria in mature biofilms are 10 to 1000 times more resistant to antibiotics than planktonic forms of bacteria [22], and resistance to biocides also exists. However, the etiology of this natural resistance is poorly understood and likely depends on many factors, mainly biofilm structural barriers and genetic adaptation factors. To explain this resistance, several authors [23] have proposed three possible causes under three hypotheses. The first is based on slow or incomplete diffusion of antibiotics into the inner layers of the biofilm. The second is the changes that occur in the biofilm microenvironment, as some biofilm bacteria enter a slow growth phase due to lack of nutrients or accumulation of harmful metabolites and therefore survive [24]. Finally, the third hypothesis indicates the presence in the biofilm of a subpopulation of cells, whose differentiation resembles the process of sporulation. They have a unique and highly resistant phenotype that protects them from the effects of antibiotics [25].

Therefore, the meat industry requires effective disinfectants for biofilm removal with an easy-to-implement protocol that can be applied to the production environment and various equipment, including hard-to-reach areas [26].

Improper disinfection of food contacting surfaces leads to the formation of biofilms, which puts food safety and public health at risk [27].

To overcome the problems posed by potent biofilm-forming bacteria with various complex resistance mechanisms, a comprehensive approach using multiple disinfectants or combining disinfectants with other cleaning methods has been proposed [28].

The purpose of this study was to investigate effective disinfectant compositions with bactericidal effect on binary bacterial biofilms of pathogenic and opportunistic bacteria of different ages.

Objects and methods

The objects of the study were the microorganisms *Brochothrix thermosphacta* 2726 and *Staphylococcus equorum* 2736 isolated from pork carcass swabs, and *Salmonella* sp. 38 isolated from a food product (pork steak). Strains were stored at –70 °C in Lennox broth (LB; Acumedia, Baltimore, MD) containing 15% glycerol. Before use, each strain was inoculated from the original glycerol solution into liquid LB medium and grown during the night time at 30 °C.

In the experiments, we used disinfectants approved for use in food production for treating working and production surfaces:

(1) Dimax Chlorine (INTERSEN-plus LLC, Russia). Dimax Chlorine is based on the sodium salt of dichloroisocyanuric acid. The product is in form of round white tablets with a characteristic odor of chlorine. The active ingredient is active chlorine, which is formed in water when the tablets are dissolved. The recommended concentration of active substances for sterilization of working surfaces is 0.015% in the working solution, exposure time is at least 5 minutes.

(2) BFR Biocid Enzym (BFR Labs LLC, Russia). BFR Biocid Enzym contains the following active ingredients: didecyldimethylammonium chloride (6.0%), NN-bis(3-aminopropyl) dodecylamine (3.0%), benzalkonium chloride (8.0%), as well as Enzumix multiple enzyme preparation containing a mixture of carbohydrases 3% to 5%, anticorrosion additives, technological and functional components. The recommended concentration of active substances for sterilization of working surfaces is 0.5% in the working solution, exposure time is at least 5 minutes.

(3) Peracetic acid as part of P3-Oxonia Active 150 product (Ecolab Production France SAS, France). Composition of the disinfectant: peracetic acid — PAA (15.5% to 17.0%), hydrogen peroxide (15.8% to 18.0%), acetic acid, functional additives. The recommended concentration of active substances for sterilization of working surfaces is 0.05% in the working solution, exposure time is at least 5 minutes.

Substances with different mechanisms of action were selected as *adjuvants* enhancing the biocidal effect of the main disinfectant:

— Hydrogen peroxide (6%) (Lega LLC, Russia) is a strong oxidizing agent; when interacting with catalase-positive microorganisms it forms gaseous oxygen.

— Isopropanol (30%) (Chemical line, Russia) has a protein-denaturing and coagulating effect on polymer solutions.

All these substances are approved for use in food production as disinfectants.

Preparation of microorganisms

The selection of strains for the formation of binary biofilms was based on the similarity of growth dynamics. Growth dynamics were assessed and reproduced on a CLARIO star device (BMG Labtech, Germany) for 36 hours (Figure 1).

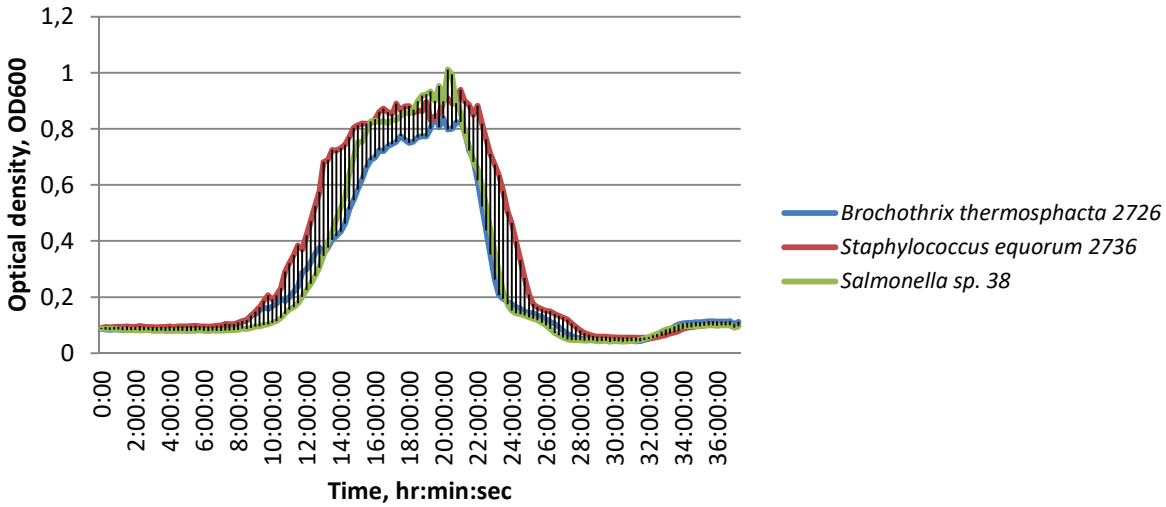

Figure 1. Growth dynamics for planktonic cultures of the studied microorganisms

Inoculation on a solid medium revealed the absence of an antagonistic effect on bacterial growth in binary cultures.

Effects of disinfectants on planktonic bacterial cultures

To assess the effectiveness of disinfectants on planktonic cultures of *Brochothrix thermosphacta* 2726, *Staphylococcus equorum* 2736, *Salmonella* sp. 38 using the suspension method in laboratory environment, the technique described in Guideline R4.2.3676–20 was used [29]. Working concentrations of disinfectant were selected in accordance with the manufacturers' recommendations, a well as increased concentrations (P3-Oxonia Active 150: 1%, 0.5%, and 0.05%; Dimax Chlorine: 0.30%, 0.15%, and 0.015%; BFR Biocid Enzym: 0.5%, 5.0%, and 10%).

Obtaining models of biofilms at the solid surface/air interface

Biofilms of this type were obtained using easily dispersible fiberglass materials as substrates according to previously described method [30]. Fiberglass filters (Whatman GF/F) were cut into 15x15 mm squares and sterilized by autoclaving (for 20 minutes, at a temperature of 120 °C), then laid out on the surface of LB agar medium in plates.

Bacterial cultures were grown separately in LB broth until the stationary growth phase. Turbidity was reproduced in pure LB0.5 broth according to McFarland method using DEN-1B Densitometer (Biosan, Latvia). To form binary biofilms, *Brochothrix thermosphacta 2726/Salmonella* sp. 38 and *Staphylococcus equorum* 2736/*Salmonella* sp. 38 cultures were mixed 1:1 in separate tubes. Next, the resulting bacterial suspensions were applied in amount of 40 µl onto previously prepared sterile fiberglass filters in sterile plates with PCA agar medium (bioMérieux, France) in duplicate. Then they were grown in a thermostat for nine days at a temperature of 30 °C.

Effects of disinfectants on biofilms

On days 2 and 9 of biofilm growth, they were treated with disinfectants with and without adjuvants. Disinfectant solutions in sterile water were prepared immediately before application to the filters. Biofilms were removed from the surface of the growth medium, transferred to sterile plates, and disinfectant solutions in amount of 100 µl were applied to each plate until the filter was completely wetted. The disinfectant exposure time was 10 minutes.

Then the fiberglass filter was placed in a Falcon tubes with sterile saline solution (10 µl). A sterile glass mortar and beads were used to homogenize the fiberglass filter. The resulting contents of the Falcon tubes were considered as the first dilution. Aliquots of the resulting homogenates (100 µl) were diluted in 900 µl of sterile saline solution and a series of decimal dilutions was prepared. In each dilution, the number of viable cells (CFU/ml) was determined by the cultural method and then the CFU titer in the primary filter homogenate was calculated.

The plates were incubated in a thermostat at 30 °C for 24 hours followed by counting the colonies on the plates. Experiments were performed in two independent biological replicates.

Statistical analysis

All studies were carried out in duplicate; each replication included two parallel experiments. When calculating CFU titers, mean values and experimental errors were determined using the average deviation of experimental values from the mean function of 5–7 independent samples using Microsoft Office Excel 2010. Differences between values were considered significant if they exceeded the level of experimental error (typically 20% or less) according to Student's t-test for $p = 0.05$. In the figures, data from typical experiments are presented as means ± experimental errors.

Results and discussion

Effects of disinfectants on planktonic bacterial cultures

In order to correct the concentrations of disinfectants recommended by the manufacturer, at the first stage of work, their biocidal effect was tested on planktonic bacterial cultures. Microorganisms in the planktonic state were not resistant to the recommended concentrations of the disinfectants. Antimicrobial activity suppressed the

viability of microorganisms, reducing growth by 7 orders of magnitude (Table 1).

Table 1. Cell viability of planktonic cultures (lg CFU/ml) after exposure (10 minutes) to disinfectants in concentrations recommended by the manufacturer

Planktonic cultures	Disinfectants			
	Control (no treatment)	Dimax Chlorine (0.015%)	P3-Oxonia Active 150 (0.05%)	BFR Biocid Enzym (0.5%)
	Cell count, lg CFU/ml			
Brochothrix thermosphacta 2726	9.04 ± 0.08	< 2	< 2	< 2
Staphylococcus equorum 2736	9.53 ± 0.10	< 2	< 2	< 2
Salmonella sp. 38	9.45 ± 0.06	< 2	< 2	< 2

A change in antimicrobial effect occurs when microorganisms form mono and binary biofilms, thereby increasing their resistance to disinfectants. Results for the antimicrobial effect of different concentrations of disinfectant working solution on binary biofilm of *Brochothrix thermosphacta* 2726/*Salmonella* sp. 38 are presented in Figure 2.

BFR Biocid Enzym at a concentration of 0.5% recommended by the manufacturer did not have disinfectant activity at either 2-day-old or 9-day-old biofilm. When the concentration of the solution was increased by 10 times, the antimicrobial effect was observed only on 9-day-old biofilm, where there was a decrease by log 5.44 compared to the control. The greatest antimicrobial effect was observed when the concentration of BFR Biocid Enzym was increased by 20 times. However, differences in the effects of the disinfectant depending on biofilm age were not observed in this case. A similar pattern was observed when exposed to P3-Oxonia Active 150 and Dimax Chlorine, where the greatest antimicrobial effect was noted for a concentration increased by 20 times. When exposed to the above-mentioned agents in the studied concentrations, a complete antimicrobial effect was not detected. At the same time, a 20-fold increase in concentrations did not show significant differences in the antimicrobial effect depending on the age of the biofilm.

Results for resistance of binary biofilm of *Staphylococcus equorum* 2736/*Salmonella* sp. 38 to disinfectants are presented in Figure 3.

The concentrations recommended by the manufacturer for disinfection (BFR Biocid Enzym, 0.5%; P3-Oxonia Active 150, 0.05%; and Dimax Chlorine, 0.015%) had no antimicrobial effect on the biofilms studied. The concentration of P3-Oxonia Active 150 increased by 10 times (0.5%) had a greater antimicrobial effect against 9-day-old biofilm of *Staphylococcus equorum* 2736/*Salmonella* sp. 38 compared to 2-day-old biofilm. Increasing the concentration by 20 times did not lead to a complete antimicrobial effect, but reduced the number of CFU by $\log_{10}$ 6.64 compared to the control.

Dimax Chlorine with 10-fold increased concentration (0.15%) showed a decrease in CFU for 2-day-old and in 9-day-old biofilms of *Staphylococcus equorum* 2736/*Salmonella* sp. 38 by $\log_{10}$ 3.08 and $\log_{10}$ 6.64 compared to the control respectively. Exposure to a concentration increased by 20 times contributed to a decrease in CFU for 2-day-old and in 9-day-old biofilms by up to $\log_{10}$ 6.64 compared to the control.

In order not to go beyond the recommended concentrations of antimicrobial agents, but at the same time, to maintaining their effectiveness, a technique was used to increase the activity of the active substance due to the synergistic effect of an additional compound, an adjuvant [30,31].

Results for sensitivity of binary biofilms to enzymatic disinfectant based on QAC (BFR Biocid Enzym) in combination with adjuvants (isopropyl alcohol and peroxide) are presented in Figures 4 and 5.

The concentration recommended by the manufacturer for BFR Biocid Enzym of 0.5% was enhanced by hydrogen peroxide adjuvant at a concentration of 6%, but the combination had no antimicrobial effect on either 2-day-old or 9-day-old biofilms and did not significantly reduce CFU.

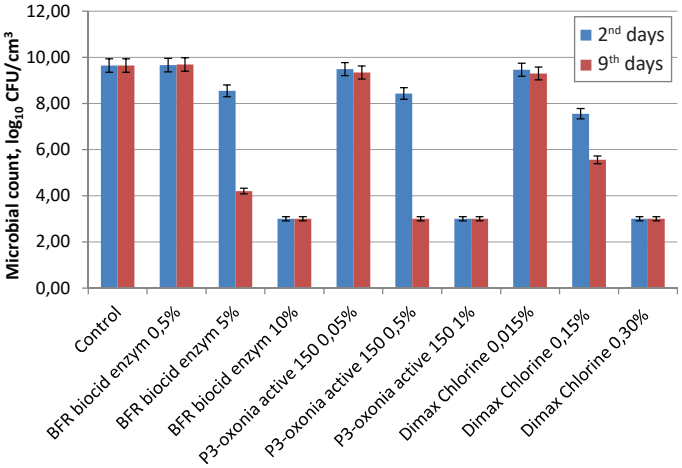

Figure 2. Sensitivity of binary biofilms of *Brochothrix thermosphacta 2726/Salmonella* sp. 38 of different ages to different concentrations of disinfectants

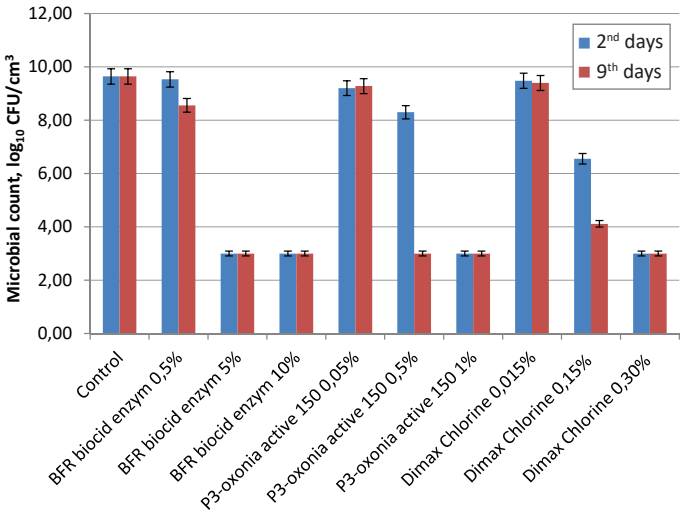

Figure 3. Sensitivity of binary biofilms of *Staphylococcus equorum 2736/Salmonella* sp. 38 of different ages to different concentrations of disinfectants

The addition of isopropyl alcohol adjuvant in varying concentrations had an antimicrobial effect depending on the concentration of isopropyl alcohol. Adding isopropyl alcohol 10% to the disinfectant did not have a significant antimicrobial effect on 2-day-old biofilm, but reduced CFU in 9-day-old biofilm by $\log_{10}$ 1.98 compared to the control. The combination of isopropyl alcohol 20% and BFR Biocid Enzym 0.5% showed a similar effect and reduced CFU in 9-day-old biofilm by $\log_{10}$ 1.9 compared to the control. A decrease in CFU of 2-day-old and 9-day-old biofilms by $\log_{10}$ 6.64 compared to the control was noted with the addition of isopropyl alcohol adjuvant at a concentration of 30%. At the same time, the combination of BFR Biocid Enzym with isopropyl alcohol 30% had the same effect on both 2-day-old and 9-day-old biofilms.

Results for sensitivity of binary biofilms of *Staphylococcus equorum* 2736/*Salmonella* sp. 38 of different ages to enzymatic disinfectant based on QAC with different adjuvant concentrations are presented in Figure 5.

Hydrogen peroxide adjuvant at a concentration of 6%, added to the working concentration of enzymatic disinfectant based on QAC, did not have a strong antimicrobial effect on binary biofilm of *Staphylococcus equorum* 2736/*Salmonella* sp. 38. The addition of isopropyl alcohol at concentrations of 10% and 20% led to a decrease in CFU only in older 9-day-old biofilm by $\log_{10}$ 2.01 and $\log_{10}$ 1.89 compared to the control respectively. Exposure to a working concentration of disinfectant with the addition of isopropyl alcohol 30% contributed to CFU decrease in 2-day-old biofilm by $\log_{10}$ 6.64, and in 9-day-old biofilm by $\log_{10}$ 6.04 compared to the control.

The results for the antimicrobial effect of disinfectant based on peracetic acid in combination with various adjuvants on binary biofilms are presented in Figures 6 and 7.

Disinfectant based on PAA with a working concentration of 0.05% did not have a pronounced biocidal effect on either 2-day-old or 9-day-old biofilms; a decrease in CFU was only by $\log_{10}$ 0.15 and $\log_{10}$ 0.3 respectively. Addition of isopropyl alcohol adjuvant at concentrations of 10% and 20% did not reduce CFU in 2-day-old biofilm of *Brochothrix thermosphacta* 2726/*Salmonella* sp. 38 compared to the control, but reduced cell count in 9-day-old binary biofilm by $\log_{10}$ 1.34 and $\log_{10}$ 0.74 compared to the control respectively. The addition of isopropyl alcohol 30% as an adjuvant had no complete antimicrobial effect on 2-day-old biofilm, but contributed to CFU decrease in 9-day-old biofilm by $\log_{10}$ 4.64.

Results for sensitivity of binary biofilms of *Staphylococcus equorum* 2736/*Salmonella* sp. 38 of different ages to disinfectant based on PAA with different adjuvant concentrations are presented in Figure 7.

The effect of disinfectant based on PAA with a working concentration of 0.05% on biofilm of *Staphylococcus equorum* 2736/*Salmonella* sp. 38 had no significant antimicrobial effect; there was a decrease in the number of microorganisms by only $\log_{10}$ 0.44 and $\log_{10}$ 0.36 in 2-day-old

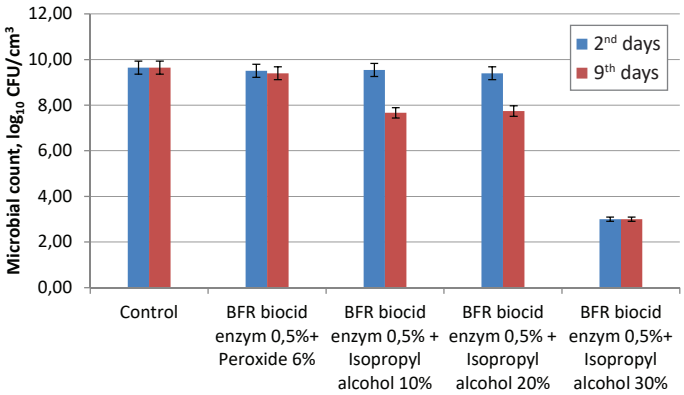

Figure 4. Sensitivity of binary biofilms of *Brochothrix thermosphacta 2726/Salmonella* sp. 38 of different ages to enzymatic disinfectant based on QAC with different adjuvant concentrations

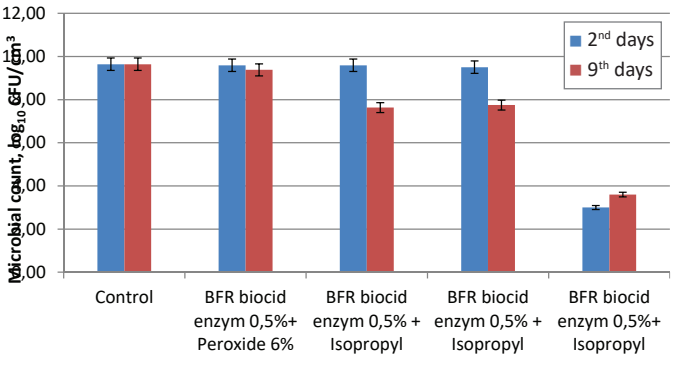

Figure 5. Sensitivity of binary biofilms of *Staphylococcus equorum 2736/Salmonella* sp. 38 of different ages to enzymatic disinfectant with different adjuvant concentrations

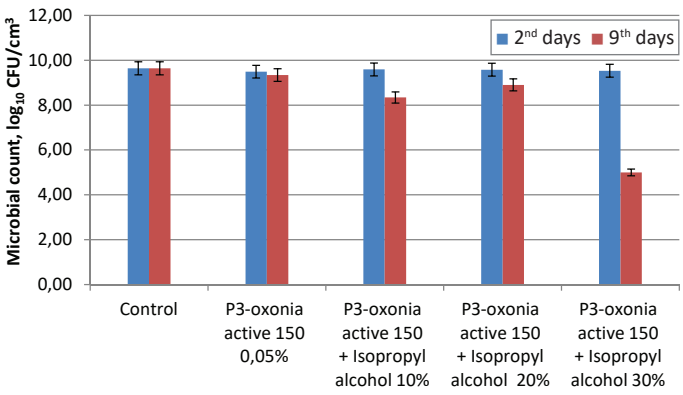

Figure 6. Sensitivity of binary biofilms of *Brochothrix thermosphacta/Salmonella* of different ages to disinfectant based on PAA with different adjuvant concentrations

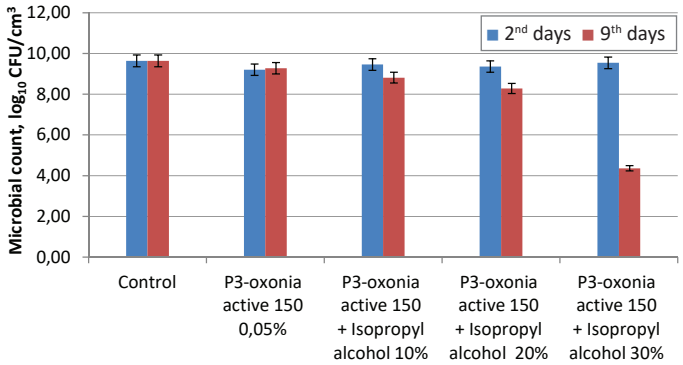

Figure 7. Sensitivity of binary biofilms of *Staphylococcus equorum 2736/Salmonella* sp. 38 of different ages to disinfectant with different adjuvant concentrations

and 9-day-old biofilms respectively. The combination of exposure to the recommended concentration of disinfectant based on PAA with an adjuvant in the form of isopropyl alcohol at concentrations of 10%, 20% and 30% slightly reduced CFU in 2-day-old biofilm by $\log_{10}$ 0.1 to $\log_{10}$ 0.28, and in 9-day-old biofilm by $\log_{10}$ 0.83 to $\log_{10}$ 1.36. The addition of isopropyl alcohol 30% as an adjuvant had an antimicrobial effect exclusively on 9-day-old biofilm of *Staphylococcus equorum* 2736/*Salmonella* sp. 38 with a decrease in viable cell count by $\log_{10}$ 5.28 compared to the control.

The results for the effect of chlorine-based disinfectant, Dimax Chlorine, with adjuvants on binary biofilms are presented in Figures 8 and 9.

Exposure to disinfectant based on active chlorine with a working concentration of 0.015% had no antimicrobial effect on binary biofilm of *Brochothrix thermosphacta* 2726/*Salmonella* sp. 38. The combined effect of isopropyl alcohol adjuvant at concentrations of 10% and 20% did not significantly reduce CFU in biofilm of *Brochothrix thermosphacta* 2726/*Salmonella* sp. 38 of two ages studied. Addition of isopropyl alcohol 30% to the concentration of active chlorine disinfectant recommended by the manufacturer led to a significant cell count reduction by $\log_{10}$ 5.72 exclusively in 9-day-old biofilm.

Results for sensitivity of binary biofilms of *Staphylococcus equorum* 2736/*Salmonella* sp. 38 of different ages to chlorine-based disinfectant with different adjuvant concentrations are presented in Figure 9.

The working concentration of disinfectant based on active chlorine recommended by the manufacturer had no antimicrobial effect on binary biofilm of *Staphylococcus equorum* 2736/*Salmonella* sp. 38. The combined effect of disinfectant and isopropyl alcohol adjuvant at concentrations of 10%, 20% and 30% showed no antimicrobial effect on 2-day-old binary biofilm of *Staphylococcus equorum* 2736/*Salmonella* sp. 38. However, a clear decrease in CFU was observed in 9-day-old biofilm when exposed to disinfectant with the addition of isopropyl alcohol 30%, where the decrease in cell count was by $\log_{10}$ 6.64 compared to the control, while with the addition of 10% and 20%, cell count decreased by $\log_{10}$ 0.76 and $\log_{10}$ 1.26 respectively.

The main theoretically important and practically focused result of the research is the expansion of our knowledge about the biofilm development and methods for their effective control. The work includes a comparative analysis of binary biofilm resistance to disinfecting agents depending on the biofilm age and the type of disinfectants used individually or together with agents that enhance the antimicrobial effect (adjuvants).

The results obtained on the pronounced synergistic effect of adjuvants and traditional disinfectants significantly complement the knowledge about methods for combating biofilms. To control biofilms, several dozens of special substances with different types of action on biofilms have been proposed (inhibiting the synthesis or destroying matrix components and cellular structures of the biofilm phenotype, hydrolases disrupting signal transmission, inhibitors of cellular metabolism, etc.) [32,33]. A possible problem for the practical use of new substances and approaches is the lack of data on their use in real production environment or in disease treatment, as well as the lack of safety reports and approvals.

An important approach to control biofilms is the creation of complex disinfectants from those known and used, which, in our opinion, is more effective, because it relies on the use of substances with known mechanisms of action that are already approved for practical use. Many examples of such combinations are known [34,35,36]. Disinfectants with physical [37,38] and biological factors [39] have been successfully used. The effect of antimicrobial agents on biofilms has been demonstrated to be enhanced in the presence of ultrasound [37], rotating magnetic field [38], or antagonistic bacteria (*Pseudomonas aeruginosa*) [39].

In addition to obvious effectiveness of the simultaneous use of two or more antimicrobial factors [40], there is an approach that includes the use of additional effects, not necessarily biocidal, but enhancing the effectiveness of the biocide used. Such additional substances are called adju-

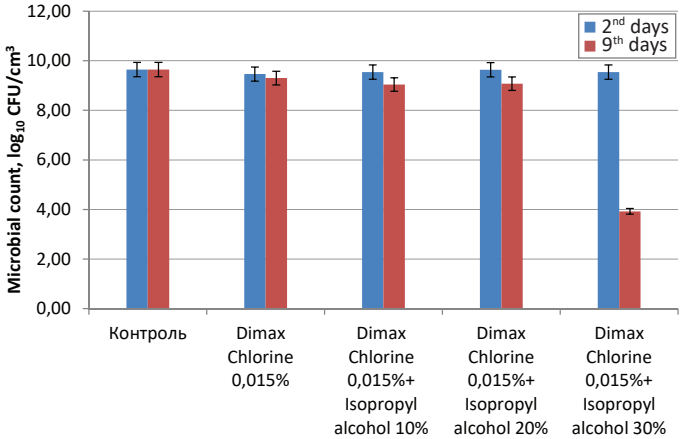

Figure 8. Sensitivity of binary biofilms of *Brochothrix thermosphacta 2726/Salmonella 38* to disinfectant with different adjuvant concentrations

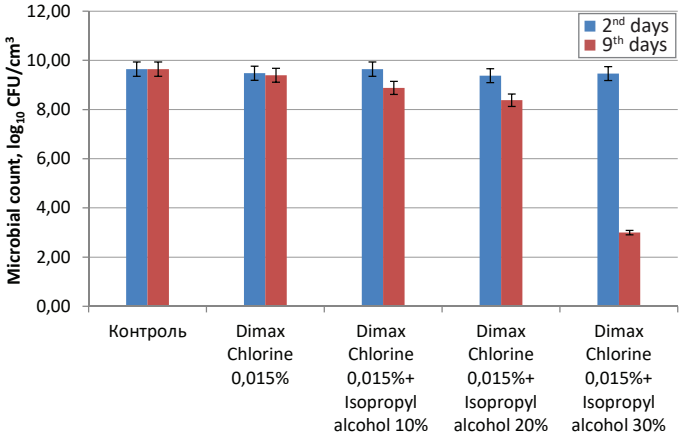

Figure 9. Sensitivity of binary biofilms of *Staphylococcus equorum 2736/Salmonella* sp. 38 to disinfectant with different adjuvant concentrations

vants (enhancers). This term was originally used in medicine for enhancers of the immune response [41]. Some substances that enhance the effect of a disinfectant on biofilms are also adjuvants (hydrolases).

In [42], the authors showed a sterilizing effect on binary biofilms of non-pathogenic bacteria when exposed to a disinfectant with the addition of adjuvants. The biocidal activity of BFR Biocid Enzym was increased to the greatest extent by adjuvants that additionally disrupt the structure of the matrix, i. e. H_2O_2 (due to rupture of the matrix by the resulting oxygen) and alcohols, which cause coagulation of matrix biopolymers [42]. Whereas in this work, on binary biofilms of pathogenic and opportunistic bacteria, it was shown that the most effective enhancement of BFR Biocid Enzym and Dimax Chlorine based on active chlorine occurred when they were combined with isopropyl alcohol (30%) disrupting the structure of the matrix, as it was noted above.

Resistance of microorganisms to disinfectants in multispecies biofilms is also related to biofilm age. Mature biofilms are generally more resistant to stress conditions than newly formed biofilms due to the robust three-dimensional structure of bacterial cell layers forming a physical barrier that restricts and prevents the penetration of disinfectants or other chemicals [43]. In our study, 9-day-old binary biofilms demonstrated greater sensitivity to the studied antimicrobial compositions than 2-day-old ones. This may be due to the fact that by the 9th day of cultivation, the biofilms were already at the stage of disintegration and release of free microorganisms.

Conclusion

Biocidal concentrations of disinfectants used in production environment (Dimax Chlorine, PAA and BFR Biocid Enzym) were established in relation to binary biofilms of pathogenic and opportunistic strains formed on fiberglass carriers. For all products, the concentrations recommended by the manufacturer had no disinfectant effect against the studied biofilm cultures. An increase in concentration by 20 times (with the same exposure time of 10 minutes) had a complete biocidal effect (6 orders of magnitude) on the studied binary biofilms. Chlorine-containing disinfectant at recommended concentrations and in combination with isopropyl alcohol was less effective than QAC-containing disinfectant (BFR Biocid Enzym). It may be assumed that agents whose action is based on oxidative reactions (Dimax Chlorine, P3-Oxonia Active 150) form multiple concentration resistance of biofilm strains due to constant surface treatment in production environment. Even with the addition of a non-oxidizing agent (isopropyl alcohol), a synergistic disinfectant effect was not established. We observed a biocidal effect when combining Dimax Chlorine and isopropyl alcohol 30% only in old biofilms. Whereas, when biofilms were exposed to QAC-containing biocide (BFR Biocid Enzym) with the addition of protein coagulating agent (isopropyl alcohol 30%), a decrease in the number of viable cells by 6 orders of magnitude was observed. At the same time, the combination of BFR Biocid Enzym with oxidizing agent (peroxide 6%) had no biocidal effect.

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EFFECT OF INCORPORATING FOOD-GRADE LACTIC ACID IN MINCED BEEF ON STORAGE STABILITY AND SENSORY EVALUATION OF THE PRODUCED PATTIES

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Keywords: *fresh beef, longissimus dorsi, incorporation process, meat color, total plate count*

Abstract

The objective of this research is to evaluate quality properties and storage stability of beef patties formulated from fresh beef incorporated with food-grade lactic acid (LA). Fresh beef was purchased from the local market immediately after slaughter, minced and formulated using water incorporated with food-grade lactic acid in concentrations of 0.0% (control); 0.5%; 0.75% and 1.00%. The LA-incorporated formulations were used in the preparation of the patties. The prepared patties were stored at a refrigeration temperature of 5 °C for 12 days. pH, instrumental color, texture profile analysis (TPA), water activity and total viable count (TVC) were investigated. At the end of the storage period, the patties were cooked and sensory evaluated. The results revealed a significant ($p \leq 0.05$) decrease in pH of the control patties from 6.2 ± 0.1 to 5.1 ± 0.2–5.4 ± 0.2 from day 8 to day 12 of the storage time. The same trend was observed in the LA-incorporated patties. The LA-incorporated patties did not show any significant differences ($p \geq 0.05$) in the water activity values through all storage time. At the end of the storage time, the control had the TVC value of almost near the spoilage limit, while all LA-incorporated patties had significantly ($p \leq 0.05$) lower TVC compared with the control. The results revealed high stability in the physicochemical properties as well as total microbial growth during the storage period. The hardness of the LA-incorporated patties was significantly ($p \leq 0.05$) lower than that of the control sample. There was no significant ($p \geq 0.05$) difference in overall sensory acceptability of the patties made from beef incorporated with food-grade lactic acid compared to the control. This study suggests that incorporating fresh beef with food-grade lactic acid in the mentioned concentrations could result in great benefits of increasing the storage life of fresh beef products with no effect on sensory quality attributes.

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Introduction

Meat and meat products are considered a unique food source for human nutrition and are recognized as a source of high nutritional value protein, which includes all essential amino acids in adequate proportions, as well as long chain n-3 fatty acids, conjugated linoleic acid, nucleotides, bioactive hydrolysates, antioxidants, and connective tissue components [1–3]. Food-grade organic acids such as citric, acetic, and lactic acids are commonly used in food as antimicrobials, antioxidants, and pH adjusters for shelf-life extension. These acids are generally recognized as safe [4]. Treatment of fresh meat with food-grade organic acids can lead to the stability in physicochemical, antioxidant, and microbiological properties of beef products [5]. Earlier, food-grade organic acids were applied by different methods such as dipping [6,7] and spray washing [7] to reduce the spoilage microorganisms leading to storage life extension. The effects of food-grade organic acids on quality of different types of meat products have been reported in several research works. Ji et al. [8] reported that organic acids including citric acid (CA) and malic acid (MA) have a significant effect on cleaning and disinfection of food. They found that the extracellular alkaline phosphatase (AKP) activity of *Escherichia coli* treated with CA and MA at a concentration of 5120 μg/mL increased 8.16 and 6.95 times compared to the control and reached 3.10 U/L and 2.69 U/L, respectively. These results show that CA and MA at this concentration can have the inhibitory activity against *Escherichia coli* by damaging the cell wall [8]. In the earlier study [9], pork bologna slices were treated with organic acids (lactic acid or acetic acid at concentrations of 2.5% and 5%) or salts (2.5 or 5% sodium acetate or sodium diacetate, 5 or 10% sodium lactate, 5% potassium sorbate or potassium benzoate). The bologna slices inoculated with *L. monocytogenes* were dipped in each solution of acids or salts, and then stored vacuum-packaged at 4 °C for a period of 120 days to evaluate the growth of *L. monocytogenes*. No significant ($p > 0.05$) increase in populations of *L. monocytogenes* was observed on bologna slices treated with 2.5% or 5% acetic acid, 5% sodium diacetate, or 5% potassium

benzoate from day 0 to 120. Also, no significant ($p < 0.05$) increase in *L. monocytogenes* was recorded on bologna slices treated with 5% potassium sorbate and 5% lactic acid up to 50 and 90 days of storage, respectively [9]. According to Nkosi et al. [10] the usage of specific food-grade organic acids at lower concentrations can achieve the desired effect of reducing or killing microorganisms without influencing quality (odor, appearance, and texture) of chicken meat.

Muriana et al. [11] evaluated lactic acid at a concentration of 50,000 ppm and a combination of lactic acid with citric acid at a concentration of 2.4% of concentrate among other antimicrobials against *Escherichia coli* O157: H7 on the surface of lean beef wafers stored for 14 days. In addition, they examined the presence/absence of *Escherichia coli* O157: H7 in meat core samples after spray intervention and blade tenderization of beef. The results showed that the absence of *Escherichia coli* O157: H7 in the meat core samples correlated with the ability of the studied antimicrobials including lactic acid to reduce bacterial levels on the surface of beef before blade tenderization. For safety purpose, chemical preservatives for meat should be replaced with natural ones such as food-grade organic acids to serve as meat antimicrobial agents [12].

Manzoor et al. [13] studied an influence of lactic acid on quality characteristics of buffalo meat. Buffalo half carcasses were sprayed with 2%, 4%, and 6% lactic acid solutions after slaughter. The samples of the sirloin and tenderloin were cut from half carcasses, vacuum packed, and stored at 0 °C for 7 days. Afterward, steaks (2 cm thick) were taken from these cuts, packed under modified atmosphere, stored in a retail display-chiller for 7 days and then evaluated for an effect of lactic acid on microflora, instrumental color, shelf life and sensory parameters of meat. The study revealed that spraying buffalo meat with 2–4% lactic acid solutions after slaughter enhanced microbiological quality of meat. Furthermore, it may also improve its instrumental color.

Food-grade organic acids were also used in beef and beef products such as beef steaks to reduce microbial load without undesirable changes in meat sensory properties [14]. Organic acids are used to treat meat because they are cheap and effective in meat and meat product preservation technology and have no undesirable effects on meat quality [15]. Organic acid solutions such as acetic acid, lactic acid, ascorbic acid, citric acid, tartaric acid and fumaric acid at concentrations of 1%–5% are the most frequently used chemical interventions for beef and lamb [16]. The effect of food-grade organic acids such as citric acid on the physicochemical and sensory properties of meat was demonstrated in [5]. Using the dilute solutions of food-grade organic acids (1%–3%) is generally recognized as safe, and as a rule does not exert an effect on desirable sensory properties of meat [17].

Stamilla et al. [18] evaluated an effect of dietary supplementation of microencapsulated blend of food-grade citric and sorbic acids combined with thymol and vanillin essential oils at a concentration of 0.5% on quality and shelf-life of broiler meat. The results showed that supplementation of microencapsulated blend of food-grade organic acids and essential oils could improve the quality and shelf-life of poultry meat.

Omidi et al. [19] investigated an effect of dietary supplementation of acetic acid at a concentration of 0 and 20 g/ kg combined with *Satureja khuzistanica* essential oil (SkEO) at concentrations of 0, 200, 300, 400, 500, and 600 mg/ bird/day on the composition of fatty acids (FAs) in thigh meat of Ross 308 broiler chickens at days 34, 38, and 42 of age [14]. The acidified diet led to a decrease in MUFA, TFA, CFA and an increase in SFA and the ratio of n-6 to n-3 FAs in chicken thigh meat. The authors concluded that dietary acetic acid and its combination with SkEO inconsistently modified the concentration of certain classes of FAs in broiler thigh meat.

Previous works focused on limited treatments during meat burger processing and chilled storage. Therefore, this study aims at evaluating an effect of lactic acid incorporation at concentrations of 0.5% — 1.0% on quality properties (pH, instrumental color, texture profile analysis, water activity and sensory characteristics) and microbiological parameters (total viable count) of patties formulated using beef treated with lactic acid.

Materials and methods

Incorporation process

Fresh hot beef (m. *longissimus dorsi*) was purchased immediately after slaughter from the local fresh meat market located near the university to avoid any changes in meat quality. After that, meat was placed in ethylene vinyl acetate (EVA) bags, covered with ice, and transported fresh to the laboratory within 15 min. Meat was cut into small pieces under the hygienic condition using a filleting sterilized knife. The pieces were then minced using a meat grinder (model Sammic PS-32 Stainless Meat Mincer, Germany). One hundred grams of food-grade lactic acid with the purity of 99.9% (purchased from Melon Food Grade Company, Selangor, Malaysia) and distilled water were used to prepare LA solutions with concentrations of 0.0%, 0.5%, 0.75% and 1.00%. The prepared LA solutions were incorporated individually into minced beef batches (1 kg each). The mixing process was carried out using a silent bowl cutter (model DH901, Ding-Han Machinery Co., Ltd., Taiwan) for 5 min until complete homogenization. A dough-like mixture was obtained and used in preparation of patties. The control patties were prepared using minced beef without adding food-grade lactic acid.

Preparation of patty samples

Patties were formulated using food-grade lactic acid incorporated individually into homogenized dough batches (1kg each). The following ingredients were purchased from Melon Food Grade Company, Selangor, Malaysia and added to the formulation: 1.1% sodium chloride, 1.0% black

pepper powder, 0.5% cinnamon powder and 1.5% skimmed milk. Each formulation was mixed in a silent bowl cutter for 5 min and patty samples weighing 80 g each were shaped manually using a patty molding machine. Round-shaped patty samples approximately 85 mm in diameter and 18 mm in thickness were produced. Processed patties were stored in a refrigerator at 5 °C for 12 days. The measurements including pH, instrumental color, instrumental texture, water activity and total viable count (TVC) were taken in all patty samples on the first day of storage and then at 4-day intervals. At the end of the storage period, the patties were cooked and sensory evaluated.

pH measurement

Ten grams of each sample were individually blended with 100 mL of distilled water (in a ratio of 1:10) for 1 min using a Waring blender (model (CB15K, Puchong, Malaysia) at low speed. A pH meter (Toledo 320 pH meter, Mettler- Instrument, Germany) was standardized with two buffer solutions at pH 7.0 and 4.0 before being used. pH of patties was measured on the first day (day 0) and then at 4-day intervals.

Color measurement

Color values were measured on the first day (day 0) and then at 4-day intervals using a Hunter lab Ultrascan Sphere Spectrocolorimeter (Minol Cr- 300 Series U. S.). The instrument was calibrated before being used. Samples were placed individually into plastic Petri dishes before conducting the measurement. Good care was taken to ensure that there were no gaps between the Petri dish lids and the filled sample, and that the lens of the calorimeter touched the lid of the Petri dish in each measurement. The values of color L* (Lightness), a* (Redness), and b* (Yellowness) were measured on the surface of each sample individually through each plastic Petri dish. An average of three replications was determined.

Texture profile analysis (TPA)

The hardness measurement was carried out using the modified method of the texture measurement by Devine et al. [20]. The patty slices with a size of 15 × 6.5 mm (approximately 1 cm^2 cross-section) were sheared with a Warner-Bratzler shear blade (with a thickness of 1.0 mm and a flat edge) attached to a Stable Micro System (SMS) texture analyzer (model TA — T2 I, USA). The instrument was calibrated with a 50 kg load cell and speed at 250-mm/min. Hardness (N), springiness (mm), cohesiveness and chewiness (mJ) were calculated from the computer connected to the instrument and expressed as TPA.

Water activity measurement

Patties were chopped using a Waring chopper (Waring Products Division, New Hartford, USA) and then mixed using a glass rod before water activity determination. The instrument used for measurement of a_w was Aqua Lab model 3TE (Decagon Devices, Inc., USA). The equipment was first standardized and prepared samples were put in the sample cups individually, approximately half-full. The cup was then covered and placed individually in the sample drawer. The drawer was closed carefully and a_w of the samples was read off the instrument directly in about 40s at a temperature of 25 °C. Three replicates of each sample were obtained.

Total plate count assessment

The aerobic plate counts of patties were determined according to the method described by

Elgadir et al. [21]. Samples weighing 10 ± 0.1g were removed individually from each package using a sterilized knife and transferred aseptically to a sterilized stomacher bag which contained 90 mL of peptone water. The samples were then homogenized individually for 2 min in a Stomacher 400 blender (Seward Ltd, UK). Further dilutions of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} were made. An amount of 0.1 mL from each dilution was spread on the plate count agar and the plates were incubated at 37 °C for 48 hrs. The total aerobic plate counts were then obtained from plates with 30–300 colonies in all samples in triplicates on the first day (control sample on day 0) and then at 4-day intervals and reported as $\log_{10}$ of the numbers of colony-forming units.

Sensory evaluation

The prepared patties were cooked on a hot plate set at 170–190 °C on one side for 2 min., then they were turned over and cooked on the other side for the same time until the surface became brown. Each cooked patty was then cut into almost equal four parts and served hot to the sensory panelists. Thirty panel members performed the sensory evaluation by the hedonic scale method. The panelists were asked to evaluate patties using the nine-point hedonic scale (9=like extremely; 8=like very much; 7=like moderately; 6=like slightly; 5=neither like nor dislike; 4=dislike slightly; 3=dislike moderately; 2=dislike very much; 1 =dislike extremely), according to the attributes of color, texture, taste, flavor and overall acceptability. Scores were obtained and analyzed.

Statistical Analysis

Analysis of variance (ANOVA) was performed using the Minitab version 17 statistical package (Minitab Inc., PA, USA). Three replicates were performed for each sample. The significance of differences was defined at a p-value of ≤ 0.05.

Results and discussion

pH measurement

The change in the pH values of the samples is shown in Table 1. A gradual decrease in the pH values was observed during storage (interval measurements). The pH of the control patties decreased significantly ($p \leq 0.05$) from

6.2 ± 0.1 to 5.1 ± 0.2 from day 8 to day 12 of storage. The same trend was observed in the LA-incorporated patties. The pH of patties decreased significantly ($p \leq 0.05$) from 5.5 ± 0.2 to 5.0 ± 0.3, from 5.2 ± 0.3 to 4.9 ± 0.2 and from 5.1 ± 0.1 to 4.8 ± 0.1 in the patties incorporated with lactic acid at concentrations of 0.5%, 0.75%, and 1.0%, respectively. This is in good agreement with the finding of other studies, which found that the reduction in pH during storage may be attributed to the accumulation of lactic acid due to the growth of lactic acid bacteria in stored patties [22,23]. This disagrees with Davies and Board [24] who reported that a gradual but harmonious rise in meat pH as storage time and spoilage progressed could be attributed to the tissue breakdown and odoriferous nitrogenous compounds production.

Table 1. pH values of the control and LA-incorporated patties during storage at 5 °C for 12 days

Days of storage	Control sample	Lactic acid 0.5%	Lactic acid 0.75%	Lactic acid 1.00%
0	6.2 ± 0.1^{a}	5.5 ± 0.2^{a}	5.2 ± 0.3^{a}	5.1 ± 0.1^{a}
4	5.4 ± 0.2^{b}	5.1 ± 0.2^{a}	5.2 ± 0.1^{a}	5.1 ± 0.1^{a}
8	5.1 ± 0.2^{b}	5.1. ± 0.3^{a}	5.0 ± 0.2^{a}	4.9 ± 0.2^{a}
12	5.1 ± 0.2^{b}	5.0 ± 0.3^{b}	4.9 ± 0.2^{b}	4.8 ± 0.1^{b}

a, b: means within a column with different letters are significantly different ($p \leq 0.5$).
Means are the values obtained from triplicate readings.

Color measurement

The red color of meat, especially beef, is an important deciding factor in consumers› assessment of meat quality [25]. The red color values of the samples are presented in Table 2. As the storage time progressed, reduction in red color value (a) of the sample was observed. This fact agrees with the finding of Zhang et al. [26] who observed the same phenomenon in beef storage in the chilled conditions. In the present study, the color values were significantly ($p \leq 0.05$) different. Patties incorporated with lactic acid at all concentrations showed an increase in both L* (Lightness) and b* (yellowness) values. However, a significant ($p \leq 0.05$) decrease in the a* value was observed in the patties incorporated with lactic acid at a concentration of 1.0% compared with the control patties and the patties treated with the lactic acid at concentrations of 0.5% and 0.75%. This finding is in a good agreement with that of Abd Elgadir et al. [5], who investigated an effect of fresh beef treatment with food-grade organic acids including citric, lactic, acetic and tartaric acids at concentrations of 0.5%, 0.75% and 1.0% using the infusion process during storage at a temperature of 5 °C for 28 days. They found significant changes in the red color of the samples treated with acids at different concentrations. The control sample was reddish with the initial a* value of 3.56. The infusion of organic acids at a concentration of 1.0% led to the pale color with a* values in a range of 3.30 to 3.42. Moreover, Hunter L* values increased significantly ($p < 0.05$) upon infusion of all acids.

Table 2. Color values of the control and LA- incorporated patties during storage at 5 °C for 12 days

Days of storage	Color values	Control sample	Lactic acid 0.5%	Lactic acid 0.75%	Lactic acid 1.00%
0	L*	31.5 ± 0.1^{a}	44.9 ± 0.5^{b}	46.9 ± 0.5^{c}	47.9 ± 0.5^{d}
	a*	4.6 ± 0.02^{a}	4.3 ± 0.2^{b}	3.8 ± 0.2^{c}	3.3 ± 0.2^{d}
	b*	6.5 ± 0.02^{a}	7.5. ± 0.4^{b}	7.5. ± 0.4^{b}	7.7 ± 0.4^{c}
4	L*	34.5 ± 0.1^{a}	45.9 ± 0.5^{b}	47.9 ± 0.5^{c}	47.9 ± 0.5^{c}
	a*	4.5 ± 0.02^{a}	4.2 ± 0.2^{b}	3.6 ± 0.2^{c}	3.2 ± 0.2^{c}
	b*	6.7 ± 0.02^{a}	7.3. ± 0.1^{b}	7.5. ± 0.2^{b}	7.9 ± 0.3^{b}
8	L*	36.5 ± 0.1^{a}	47.1 ± 0.3^{b}	47.9 ± 0.1^{b}	48.8 ± 0.5^{c}
	a*	4.2 ± 0.02^{b}	4.0 ± 0.2^{a}	3.6 ± 0.2^{a}	3.2 ± 0.2^{a}
	b*	6.9 ± 0.02^{a}	7.7. ± 0.3^{b}	7.6. ± 0.1^{b}	7.8 ± 0.2^{b}
12	L*	37.5 ± 0.1^{a}	47.9 ± 0.1^{b}	48.9 ± 0.2^{b}	49.9 ± 0.1^{c}
	a*	4.1 ± 0.02^{a}	3.3 ± 0.2^{b}	3.1 ± 0.2^{b}	3.1 ± 0.1^{b}
	b*	7.5 ± 0.02^{a}	8.3. ± 0.2^{b}	8.5. ± 0.1^{b}	8.8 ± 0.4^{b}

a, b, c: means within a row with different letters are significantly different ($p \leq 0.5$).
Means are the values obtained from triplicate readings.

Water activity measurement

The water activity values for different LA-incorporated patties are presented in Table 3. A gradual increase in the water activity of all patties was observed. However, the patties prepared from beef incorporated with lactic acid at different concentrations did not show any significant differences ($p \geq 0.05$) in the water activity values through all storage time. This finding is in good agreement with previously reported investigations [5].

Table 3. Water activity (a_w) values of the control and LA-incorporated patties during storage at 5 °C for 12 days

Days of storage	Control patties	Lactic acid 0.5%	Lactic acid 0.75%	Lactic acid 1.00%
0	0.987 ± 0.001^{a}	0.985 ± 0.001^{a}	0.984 ± 0.002^{a}	0.975 ± 0.001^{a}
4	0.989 ± 0.002^{a}	0.988 ± 0.002^{a}	0.985 ± 0.002^{a}	0.979 ± 0.002^{a}
8	0.993 ± 0.001^{a}	0.990 ± 0.001^{a}	0.988 ± 0.001^{a}	0.984 ± 0.002^{a}
12	0.999 ± 0.001^{a}	0.993 ± 0.001^{a}	0.992 ± 0.001^{a}	0.989 ± 0.001^{a}

a: means within a column with the same letters are not significantly different ($p \leq 0.5$).
Means are the values obtained from triplicate readings.

Texture profile analysis (TPA)

The TPA results are presented in Table 4. The food-grade lactic acid had a significant influence ($p < 0.01$) on all texture parameters (hardness (N), springiness (mm), chewiness (mJ) and cohesiveness) of the LA-incorporated patty samples. The patties incorporated with water alone (control) were harder than patties incorporated with food-grade lactic acid. It was observed that the patties treated with LA at a concentration of 1.0% were the softest on the first day compared to other treatments. The same trend was observed at the end of the storage period. It was apparent that the increased concentration of lactic acid led to a significant decrease ($p < 0.05$) in hardness of beef patties on the first day and at the end of the storage period. Adding water incorporated with food-grade lactic acid to the patty formulation significantly ($p < 0.01$) changed both

Table 4. Texture profile analysis of the control and LA-incorporated patties during storage at 5 °C for 12 days

Texture profile analysis of patties on day 0 of storage					
Days of storage	Texture parameter	Control sample	Lactic acid 0.5%	Lactic acid 0.75%	Lactic acid 1.00%
Day 0	Hardness (N)	124.2 ± 1.6^{a}	109.3 ± 1.6^{b}	97.1 ± 1.6$^{a\,c}$	89.5 ± 1.6^{d}
	Springiness (mm)	0.76 ± 0.06^{b}	0.75 ± 0.02^{b}	0.74 ± 0.01^{b}	0.75 ± 0.03^{b}
	Cohesiveness	0.41 ± 0.01^{a}	0.42 ± 0.01^{a}	0.42 ± 0.01^{a}	0.43 ± 0.01^{a}
	Chewiness (mJ)	84.2 ± 1.3^{a}	80.1 ± 1.2^{b}	76.2 ± 1.7^{c}	74.3 ± 1.4^{d}
	Texture profile analysis of patties on day 12 of storage				
Day 12	Hardness (N)	111.5 ± 1.2^{a}	102.1 ± ^{b}	87.5 ± 1.7^{c}	81.5 ± 1.3^{d}
	Springiness (mm)	0.63 ± 0.04^{b}	0.62 ± 0.01^{b}	0.63 ± 0.02^{b}	0.62 ± 0.03^{b}
	Cohesiveness	0.38 ± 0.03^{a}	0.39 ± 0.02^{a}	0.37 ± 0.05^{a}	0.37 ± 0.02^{a}
	Chewiness (mJ)	72.2 ± 1.1^{a}	69.6 ± 1.3^{b}	68.5 ± 0.2^{c}	68.2 ± 1.4^{c}

Means with different letters within the raw are significantly different (p ≤ 0.5).
Means are the values obtained from triplicate readings.

springiness and chewiness of the samples. However, there was no significant difference (p > 0.05) between the patty samples in the cohesiveness values on day 0. The same trend was observed in samples at the end of the storage period. This finding agrees with that of Grajales-Lagunes et al. [27] who reported that an increase in the concentration of lactic acid resulted in a reduction in meat hardness. They also found that the resistance of meat decreased with an increasing lactic acid concentration during the 7-day storage period. Previous studies have already found that injecting lactic acid on beef accelerated meat tenderization and reduced hardness after two days post mortem in beef muscle [28,29].

Total plate count assessment

Total viable counts (TVC) of the samples are shown in Table 5. There was a gradual microbial growth in all samples but it was slower in the LA-incorporated samples compared with the control. By the end of the storage period, the microbial count reached 7.89 ± 0.1 $\log_{10}$ in the control sample and 3.57 ± 0.2 $\log_{10}$, 3.40 ± 0.2 $\log_{10}$, and 3.30 ± 0.1 $\log_{10}$ in the patties incorporated with lactic acid at concentrations of 0.5%, 0.75%, and 1.0%, respectively. It was reported that the value of 8 $\log_{10}$ was regarded as a cause of spoilage in meat products [30,31]. At the end of the storage period, the control had a TVC value almost near the spoilage limit, while all the LA-incorporated patties had significantly (p ≤ 0.05) lower TVC compared with the control.

Table 5. Total plate count (TVC) values of the control and LA-incorporated patties during storage at 5 °C for 12 days

Days of storage	TVC ($\log_{10}$ cfu/g)			
	Control sample	Lactic acid 0.5%	Lactic acid 0.75%	Lactic acid 1.00%
0	4.30 ± 0.4^{a}	3.15 ± 0.2^{a}	3.10 ± 0.1^{a}	3.10 ± 0.2^{a}
4	4.54 ± 0.1^{a}	3.33 ± 0.3^{a}	3.20 ± 0.2^{a}	3.11 ± 0.3^{a}
8	5.57 ± 0.2^{b}	3.34 ± 0.1^{a}	3.30 ± 0.1^{a}	3.20 ± 0.2^{a}
12	7.89 ± 0.1^{c}	3.57 ± 0.2^{b}	3.40 ± 0.2^{b}	3.30 ± 0.1^{a}

a-c: means within a column with different letters are significantly different (p ≤ 0.5).
Means are the values obtained from triplicate readings.

Sensory evaluation

Sensory evaluation of meat products is widely applied to both fresh and processed meat products and is considered an important factor affecting quality of meat and meat products [32]. Table 6 presents sensory properties of patty samples. The lower scores for taste and flavor in the patties incorporated with 0.75% and 1.0% could be attributed to the slight odor of lactic acid generated during cooking. The results of the sensory evaluation of the patties incorporated with food-grade lactic acid at different concentrations show high overall acceptability values of 8.0 ± 0.2–8.1 ± 0.1, which are comparable to the high values of 8.2 ± 0.2 in the control. This finding agrees with that of Abd Elgadir et al. [33], who performed sensory evaluation of beef burgers formulated from fresh beef infused with food-grade citric acid at a concentration of 1.00% and stored for 8 days at 4 °C. They found that sensory scores for fresh beef burgers were in a range of 6.93–8.20 on the first day (day 0) and decreased to 4.43–5.17 at the end of the storage period (day 8). The differences between scores on day 0 and day 8 of storage were significant (p >0.05). At the end of the storage period, they observed that burgers formulated from beef treated with food-grade citric acid had high overall sensory acceptability.

There was no significant difference (p ≥ 0.05) in the overall acceptability of the patties. This suggests that patties made from beef incorporated with food-grade lactic acid were comparable to the control in terms of sensory properties.

Table 6. Sensory evaluation attributes of the control and LA-incorporated patties

Lactic acid concentration	Color	Texture	Taste	Flavor	Overall acceptability
0	7.7 ± 0.2^{a}	7.4 ± 0.1^{a}	8.4 ± 0.2^{a}	7.1 ± 0.3^{a}	8.2 ± 0.2^{a}
0.5	7.5 ± 0.1^{a}	7.4 ± 0.3^{a}	7.9 ± 0.3^{a}	7.3 ± 0.2^{a}	8.0 ± 0.3^{a}
0.75	5.7 ± 0.3^{b}	5.3 ± 0.2^{b}	7.3 ± 0.1^{b}	5.6 ± 0.4^{b}	8.0 ± 0.2^{a}
1.00%	5.5 ± 0.2^{b}	5.3 ± 0.4^{b}	5.9 ± 0.2^{c}	5.4 ± 0.1^{b}	8.1 ± 0.1^{a}

a-c: means within a column with different letters are significantly different (p ≤ 0.5).
Means are the values obtained from triplicate readings.

Conclusion

High stability in physicochemical and microbiological properties during storage of patties was obtained. There was no significant difference in the overall sensory acceptability between the patties made from beef incorporated with food-grade lactic acid and the control. This suggests that incorporating beef with food-grade organic acids can have great benefits of increasing the storage life of beef products. Utilization of food-grade organic acids in meat products, such as patties, meatballs, and sausage, is highly recommended.

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ANALYSIS OF ANTIOXIDANT POTENTIAL AND STUDY OF THE FEATURES OF THE MICROSTRUCTURE IN CERTAIN TYPES OF SPICES AND HERBS USED IN THE MEAT PROCESSING INDUSTRY

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Keywords: *total antioxidant capacity, classes of antioxidants, methods of definition, histological analysis, adulteration*

Abstract

Spices and herbs are widely used in the meat processing industry to improve the taste and flavor of the food products. They contain a wide range of essential oils and biologically active components possessing antioxidant potential. Surge of spices consumption leads to their adulteration; at the same time, species identification is complex and requires increased knowledge about the peculiarities of their structure. This study researched the antioxidant potential (AOP) of six spices and three fragrant herbs, defined their structure and histological parameters of their identification. To assess AOP, total antioxidant capacity (TAC) was defined using the methods of Oxygen Radical Absorbance Capacity (ORAC) and free radical DPPH, and the main classes of AO were identified with the help of qualitative reactions, microstructure was analyzed via three staining methods. Among six classes of AO the flavonoids were found in all extracts. All of four AO classes were found in basil and allspice. Allspice extract showed the highest TAC_{DPPH} *(2,876.05 ± 19.83 μmol-eq.quercetin/l), the lowest value was found in parsley extract (157.97 ± 4.80 μmol-eq.quercetin/l). At the same time, the highest* TAC_{ORAC} *was found in the extract of dill greens and basil greens — 9,789.51 ± 433.22 μmol-eq.quercetin/l and 9,692.91 ± 203.42 μmol-eq.quercetin/l, respectively, and its lowest content was found in ginger — 956.98 ± 241.79 μmol-eq.quercetin/l. The microstructural features of cells peculiar for each sample were defined: external protective tissues, seed hulls, storage tissue, secretory and formative tissues, and their ability to perceive staining with general and specific dyes. The results obtained make it possible to test the composition of dry spices and herbs, to reveal their presence in the ready-to-consume meat products and to exclude cases of their adulteration.*

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Introduction

Spices are of great importance in nutrition and food technology all over the world, as they are natural components of plant origin that can embellish the food with an original and unique taste and flavor [1]. They have been an integral part of human diet and trade for thousands of years [2]. The wide application of spices in food products is primarily explained by their functional characteristics. Spices improve the taste and flavor of food products; they feature the bactericidal and antioxidant properties (they can be used for food preservation); benefit to the absorption of food as they are catalysts for a lot of enzymatic processes, and activate metabolism in general [3,4].

Spices are also important nutraceuticals. Recently the acknowledgement of the link between health and nutrition has increased their importance in the food industry and has attracted the interest of the researchers who attempt to define the mechanisms of the spices' action, find new bioactive compounds and the their beneficial properties with a view to apply the spices as modern functional food ingredients [5,6].

Now there are enough evidences that confirm that certain types of spices possess antioxidant, anti-inflammatory, anti-tumor, anti-carcinogenic, and glucose-lowering and cholesterol-lowering properties, as well as properties that beneficially influence cognitive function and raise vigor of mood [2,7,8]. A row of authors believe that spices are capable to protect from cardiovascular and neurodegenerative diseases, cancer and diabetes of the 2nd type [9–13].

More than 150 different types of spices are known, but not many of them are used. These are the so-called classic spices, which have the following characteristics as common [14]: they are consumed in dry form; they have specific well-pronounced aroma and pungency; they become bitter when heated strongly and when the amount of spices is increased above the recommended volume; they are used in wide scope of applications.

Depending on what part of the plant is used for production, the spices are divided into the following groups:

- seeds — mustard, nutmeg;
- fruits — pepper (black, white, allspice, red), cardamom;
- flowers, buds and their parts — cloves, saffron;

- leaves — bay leaf, rosemary;
- bark — cinnamon, cassia;
- rootstock — ginger, turmeric, galangal.

The researches over the past decade has shown the presence of essential oils and biologically active components in the spices, including sulfur-containing compounds, tannins, alkaloids, phenolic diterpenes and vitamins, flavonoids and polyphenols [8,10,15]. Essential oils are the predominant and most important antiviral components of spices [16]. Natural antioxidants in spices help prevent oxidative stress [17].

Spicy herbs include dill, parsley, coriander, anise, mint, tarragon, fennel, rue, lemon balm mint, hyssop (blue St. John's wort), basil, sweet clover, oregano, thyme, wormwood, marjoram, lovage, etc. Usually the aboveground parts of the spicy plant (stem, leaves, flowers, fruits, seeds) are used as food; the root is used comparatively rare (only in calamus, angelica, coluria). Spicy herbs are also highly valued for the biologically active compounds they contain [18]. Potential cancer prevention substances such as anetepherone, carvone and limonene have been isolated from dill oil. Dill antioxidants prevent a number of diseases, such as diabetes, Parkinson's disease, atherosclerosis, and diseases of liver [19,20]. Basil contains a lot of flavonoids, including flavonols and flavone derivatives, or phenolic acids such as rosmarinic acid and caffeic acid [21]. Parsley contains up to 0.2% ascorbic acid, carotenoids, riboflavin, thiamine, nicotinic acid, flavonoids, phytoncides [22], and its essential oil features antioxidant, anti-inflammatory, antitumor and anti-apoptotic effects [23,24,25]. Consumption of spices and fragrant herbs can contribute a significant amount of antioxidants to human nutrition.

Over the past few years the global market for spices and herbs has been constantly growing. About 50 of the 86 items produced in the world are grown in India [26]. The Russian market of spices has already been formed long time ago, and in recent years it has faced very high rates of growth. According to research [27], both the number of consumers and the frequency of spices consumption have increased. As the trade in spices has exponentially increased around the world, they have become frequently subject to adulteration, which may happen deliberately or unintentionally. Deliberate adulteration usually has underlying economic motives; it is aimed at maximizing profits and is associated, first of all, with cheating in the quantity and quality of raw materials being used, sometimes they use the plants free of any biologically active substances [5]. Unintentional adulteration is associated with improper harvesting or processing of plant material [28]. This makes quality control and safety monitoring of these components an acute and pressing issue in the industry. Species identification of the spices is very difficult and requires the involvement of advanced analytical approaches [29,30]. It is promising to use microstructural analysis methods for these purposes, which are widely used to determine the components and technology of production of various types of semi-finished and finished products [31]. Microscopic identification is often used to verify the authenticity of medicinal plant materials and components, as it allows determining the botanical origin of the plant and to assess its quality [3,32,33].

The literature does not fully describe the issues of determining the quality and composition of spices and herbs used in the meat processing industry and widely distributed in Russia. The purpose of this article was to determine their antioxidant potential, define the most common classes of antioxidants and study the microstructural features of the structure and the main indicators of authentication.

Objects and methods

Objects

The objects of research were dry spices and fragrant herbs that are most often used in the meat processing industry: red pepper (*Capsicum annuum L.*), black pepper (*Piper nigrum L.*), allspice (*Pimenta officinalis L.*), turmeric (*Curcuma longa*), ginger (*Rhizoma zingiberis*), nutmeg (*Myristica fragrans*), dried chopped basil (*Ocimum basilicum L.*), dill (*Anethum graveolens L.*) and parsley (*Petroselinum crispum*). Each sample was purchased from three different manufacturing companies in the Moscow retail chain. The total number of samples under study was 27 pieces.

Definition of antioxidant potential

Ethanol extracts were prepared to define the antioxidant potential. A weighed portion of the sample was mixed with 70% ethyl alcohol in a ratio of 1:15 (g: ml), obtaining extracts at a concentration of 66.67 g/l. Then it was infused at a temperature of 22 ± 2 °C for 24 hours. After that the extract was filtered through filter paper for quantitative analysis with a mass fraction of ash up to 0.03% (FB-III, GOST 12026–76). Before analysis, extracts were stored at 4 °C. To evaluate the AOP of the samples, the total antioxidant capacity was defined by the methods ORAC and DPPH.

Determination of the contribution to the TAC of the antioxidants acting via the mechanism of Hydrogen Atom Transfer (HAT) was defined by the ORAC method. This method was implemented on a Fluoroskan Ascent FL fluorimeter-luminometer (TermoLabsystems, Finland) with black 96-well plates (Greiner bio-one, USA). 30 µl of extract diluted with phosphate buffer depending on activity, or diluted with quercetin solution (Sigma-aldrich, India) in the concentration range 1–20 µM, was added to each well to create a calibration graph, and 75 mM phosphate buffer (pH 7.4) was used as a control sample. Then 200 µl of 0.5 µM freshly prepared sodium fluorescein (Sigma-aldrich, USA) was added to each well. After that, the plate was covered with a protective film (SSIbio, USA) and placed into an incubation device for 30 min at 37 °C, after which 30 µl of 153 µM AAPH (*2,2'-Azobis (2-methylpropionamidine) dihydrochloride*, Aldrich Chemistry, USA).

Fluorescence intensity was measured at 37 °C for 60 min with a reading interval of 5 min, while excitation and emission wavelengths were 485 nm and 535 nm, respectively. TAC_{ORAC} of the samples was calculated according to the calibration graph, which was calculated for each plate and expressed in μmol-eq.quercetin / l.

The total antioxidant capacity of the samples was determined by the DPPH radical method on the SF-2000 spectrophotometer (OKB Spektr, Russia) according to the method [34]. A stock 1 mM ethanol solution of the DPPH radical (Santa Cruz Biotechnology, USA) was prepared in a dark glass vial; the obtained solution was incubated in a special lightproof box at a temperature of 22 ± 2 °C for 12 hours. Before measurements, a DPPH working solution with a concentration of 100 μM was prepared, its optical density was not less than 1.00 optical units. To determine TAC, 1.52 ml of DPPH working solution and 80 μl of sample or 96% ethyl alcohol as a control sample, or quercetin solution (Sigma-aldrich, India) at concentrations of 100–275 μM were added to glass vials to create a calibration graph. The reaction mixture was shaken vigorously and incubated for 30 minutes in the dark at 22 ± 2 °C. The optical density of solutions was measured in cuvettes with a distance between the working faces of 1 cm at a wavelength of 517 nm. Each sample was measured in triplicate. The percentage of free radical scavenging activity (RSA,%) of DPPH was calculated by the following equation 1.

$$RSA = \frac{D_c - D_s}{D_c} \times 100\% \quad (1)$$

where D_c is the optical density of the control sample; D_s is the optical density of the sample.

TAC_{DPPH} of the samples was calculated from the calibration graph of RSA dependence of quercetin concentration ($R^2 = 0.9957$) and was expressed in μmol-eq.quercetin/l.

Analysis of the component composition of the studied samples was defined by phytochemical screening of extracts for the main classes of natural antioxidants (AO) like phenolic compounds, flavonoids, anthocyanins, coumarins, tannins and carotenoids, the reactions for which are presented below in the Table 1. The following reagents were used for the component analysis: anhydrous sodium carbonate (AppliChem, PanReac, Germany), sulfuric acid (Acros Organics, Belgium), sodium hydroxide (AppliChem, PanReac, Germany), iron (III) chloride (Sigma-Aldrich, Germany).

The analysis was run on the extracts diluted 11 times with 70% ethanol. The sample that showed a typical sign of the proceeding reaction was considered as positive; in color reactions, the color intensity of the reaction sample was associated with the quantitative content of the desired class of AO. The results of the analysis were assessed visually and recorded by photographing.

Microstructural studies

The microstructure of dry samples was analyzed on the preparations using the "crushed drop" method. The changes in cell structure during technological processing was determined and the indicators were established for identifying the spices included in the composition of finished meat products using model systems on minced beef with the addition of the samples being studied in amount of 1%. The integrity of cells was defined both in raw minced meat and after the heat processing (72 ± 2 °C).

Histological sections of model systems with a thickness of 14 μm were sliced on a microtome cryostat "MIKROM — HM525" (Thermo Scientific, USA), mounted on Menzel-Glaser glasses (Thermo Scientific, USA) and stained with Ehrlich hematoxylin and 1% aqueous-alcoholic solution of eosin (BioVitrum, Russia) according to the generally accepted method [35], Lugol's solution (for visualization of starch grains) and tolluidine blue O (for differentiation of the polysaccharide component of cells) according to the method [36].

The obtained histological preparations were studied and their photography were taken with a light microscope "AxioImaiger A1" (Carl Zeiss, Germany) using a connected video camera "AxioCam MRc 5" (Carl Zeiss, Germany). Images were processed on a computer image analysis system "AxioVision 4.7.1.0" (Carl Zeiss, Germany).

Statistical processing

To obtain reliable results, all analyzes were run in triplicate. The results of quantitative studies were presented as the mean value and standard deviation "*Mean* ± *SD*". Calculations were made in Microsoft Excel. Statistical significance was calculated in STATISTICA 10.0 by one-way analysis of variance followed by Tukey's test. A probability was assumed as significant at the level of 0.05.

Table 1. List of qualitative reactions for determining various classes of AO

Defined class of AO	Qualitative reaction	Sign of a positive reaction
Anthocyanins	1–2 ml of the sample +20 mcl Na_2CO_3 (2 M)	green staining
	1–2 ml of the sample +20 mcl H_2SO_4 (2 M)	pink-red staining
Carotenoids	1 ml of the sample +20 mcl H_2SO_4 (conc.)	dark blue staining
Coumarins	1 ml of the sample +200 mcl NaOH (10%) → (1)	yellowing (color changing) of the solution
	(1) + distilled water → (2)	Discoloration of the solution
	(2) + HCl (10%)	cloudiness / sediment precipitation
Phenols	1–2 ml of the sample +100 mcl $FeCl_3$ (1%)	violet staining
Flavonoids	1–2 ml of the sample +100 mcl $FeCl_3$ (1%)	green staining
Tannins	1–2 ml of the sample +100 mcl $K_2Cr_2O_7$ (4,4%)	Darkening of the solution

Results and discussion

At the first stage of the work the antioxidant potential of the studied samples was analyzed. The results of TAC determination by the ORAC and DPPH methods are presented below in the Table 2.

Table 2. Results of TAC analysis of spices and fragrant herbs extracts

Sample	TAC_{ORAC}, μmol-eq. quercetin/l	TAC_{DPPH} μmol-eq.quercetin/l
	Mean ± SD	Mean ± SD
Black pepper	3,629.51 ± 223.78 [a]	468.00 ± 4.88* [a]
Red pepper	2,476.18 ± 303.62 [b, c]	416.23 ± 6.87* [b, c]
Allspice	3,313.33 ± 198.83 [d, e]	2,876.05 ± 19.81* [b, d, e]
Parsley	6,265.13 ± 101.61 [b, d, f, g]	157.97 ± 4.80* [b, d, f, g]
Dill	9,789.51 ± 433.22 [b, d, f, h, k]	918.89 ± 2.02* [b, d, f, h, k]
Basil	9,692.91 ± 203.42 [b, d, f, h, m]	905.50 ± 6.35* [b, d, f, h, m]
Nutmeg	1,265.28 ± 136.92 [b, d, f, h, l, n, p]	425.29 ± 3.89* [b, f, h, l, n, p]
Ginger	956.98 ± 241.79 [b, d, f, h, l, n, s]	609.10 ± 6.53 [b, d, f, h, l, n, r, s]
Turmeric	7,757.39 ± 56.09 [b, d, f, h, l, n, r, t]	544.75 ± 5.06* [b, d, f, h, l, n, r, t]

* — statistically significant differences between TAC_{ORAC} and TAC_{DPPH} samples (P < 0,05);

a-b, c-d, e-f, g-h, k-l, m-n, p-r, s-t — statistically significant differences between TAC_{ORAC} or TAC_{DPPH} samples (P < 0,05).

During the work, it was defined that the values of TAC, defined by the ORAC method, of almost all samples were statistically different (P < 0.05), except for black pepper and allspice, dill and basil, ginger and nutmeg. These samples featured quite similar values (P > 0.05). Similar to TAC_{ORAC}, almost all the spices under study had statistically significantly different DPPH capacities (P < 0.05). Red pepper and nutmeg, dill and basil were exception, as the exhibited similar TAC_{DPPH} (P > 0.05).

As a result of the analysis, it was defined that the highest TAC_{DPPH} was found in allspice extract (2,876.05 ± 19.83 μmol-eq.quercetin/l), while the lowest value was observed in parsley extract (157.97 ± 4.80 μmol eq.-quercetin equivalent/l). The TAC_{DPPH} values of red pepper and black pepper extracts were quite close and were equal to 416.23 ± 6.87 μmol-eq. quercetin/l and 468.00 ± 4.88 μmol-eq.quercetin/l, respectively, which is 6.9 and 6.2 times lower than the same value in allspice (P < 0.05). This may be due to the high content of phenols and terpenes in allspice, which possess pronounced antioxidant properties. Black pepper and red pepper showed similar AO composition, however, they contain less of terpenes [37].

Dill (918.89 ± 2.02 μmol-eq.quercetin/l) and basil (905.50 ± 6.35 μmol-eq.quercetin/l) showed similar content of TAC_{DPPH} (P > 0.05), which may be due to the similar composition of AO. Thus, in these plants the phenolic compounds of a number of flavonoids (rutin, quercetin), flavonols (kaempferol), terpenes (carvone, carvacrol), as well as phenolic acids (caffeic, rosmarinic) are represented at their fullest. Parsley showed TAC_{DPPH} values approximately 5.5 times lower (P < 0.05) than similar values of basil and dill, which is consistent with other studies and may be explained by the lack of terpenes in its composition [38].

The discrepancies between ORAC and DPPH methods of analysis are mainly caused by the greater sensitivity of the ORAC method. It is also worth considering the fundamental differences in methods and application of various radical-generating systems. In the case of the ORAC method, the total antioxidant capacity is measured in reference to the reactive oxygen species (ROS), and in the case of the DPPH method it is measured in reference to the reactive nitrogen species (RNS).

Thus, the highest values (above the average in comparison with other samples) of TAC_{ORAC} were found in extracts of dill and basil, which amounted to 9,789.51 ± 433.22 μmol-eq.quercetin/l and 9,692.91 ± 203.42 μmol-eq.quercetin/l, respectively. Significant differences were observed in parsley extract: while having the lowest TAC_{DPPH} value of 157.97 ± 4.80 μmol-eq.quercetin/l, it exhibited a rather high TAC_{ORAC} value — 6,265.13 ± 101.61 μmol-eq.quercetin/l. This can be explained by the high content of antioxidants in parsley that act via the HAT mechanism and are more inclined to reactions with ROS than with RNS [39,40].

Ginger and nutmeg featured the lowest TAC_{ORAC} values (956.98 ± 241.79 μmol-eq.quercetin/l and 1265.28 ± 136.92 μmol-eq.quercetin/l, respectively), while the samples showed average results for TAC_{DPPH} (609.10 ± 6.53 μmol-eq.quercetin/l and 425.29 ± 3.89 μmol-eq.quercetin/l, respectively).

The component composition of the extracts was assessed by qualitative reactions to such popular classes of AO-as phenols, flavonoids, tannins, carotenoids, anthocyanins and coumarins (Table 3, 4).

As a result of the analysis, none of the samples showed positive reaction for anthocyanins. The yellow staining is associated with the presence of flavones, flavonols, and flavanones. The rich red color in the turmeric extract is associated with the presence of the antioxidant curcumin in the plant — a polyphenol, which changes color to red in an alkaline pH and remains unchanged in an acidic pH [41].

As a result of the analysis of samples for coumarin content, positive results were registered in extracts of allspice, dill, basil, ginger, nutmeg and turmeric. Unchanged color intensity when adding distilled water may be caused by the presence of water-insoluble antioxidants that reacted with sodium hydroxide.

As can be seen from the obtained data, none of the samples showed a positive result for carotenoids. However, many studies have confirmed the high carotene content in turmeric [42], also red pepper is a rich source of bright representatives of carotenoids — lutein, carotene and capsanthin [43]. This indicates the inapplicability of this method of qualitative analysis to these extracts. Negative results may also be associated with poor solubility of carotenoids in alcohols, as a result of which the studied extracts either contain them in very small quantities or do not contain them at all.

Table 3. Results of qualitative analysis of the samples for classes of antioxidants: anthocyanins, phenols and flavonoids, tannins

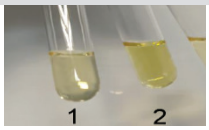
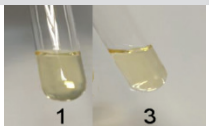
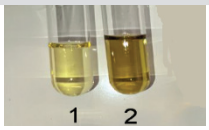
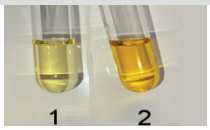
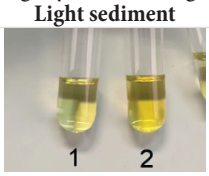
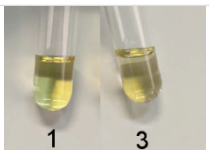
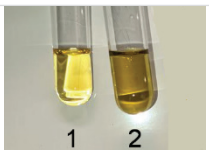
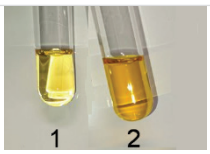
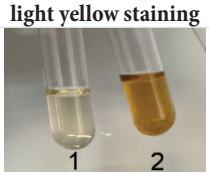
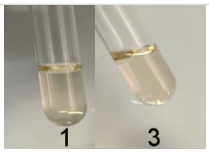
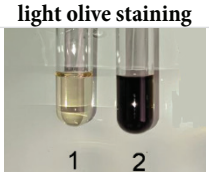
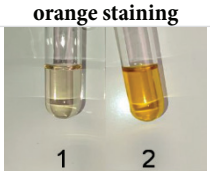
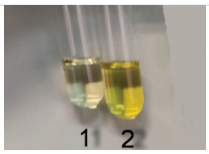
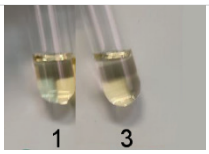
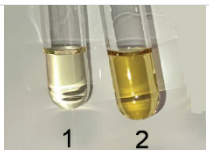
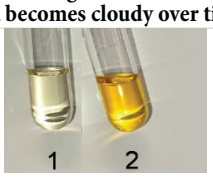
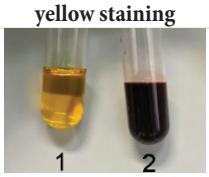
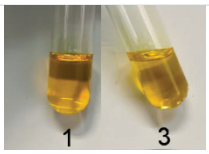
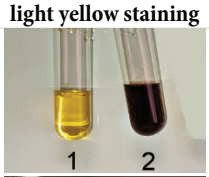
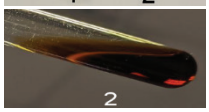
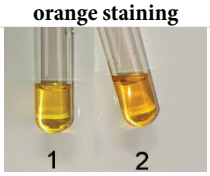
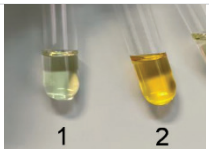
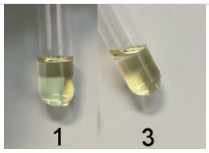
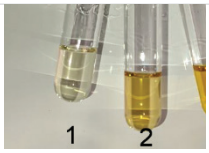
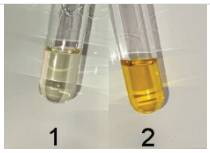
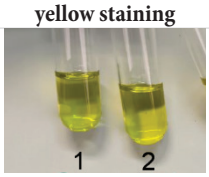
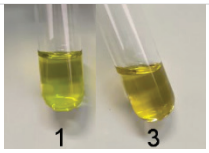
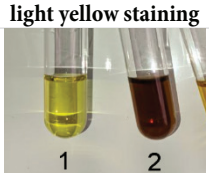
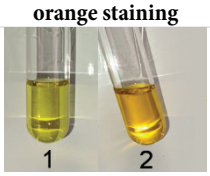
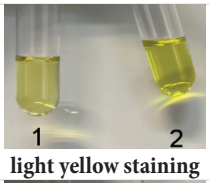
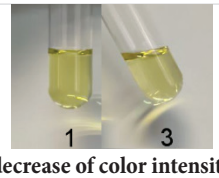
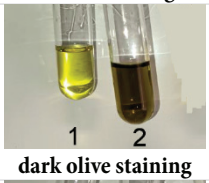
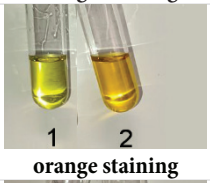
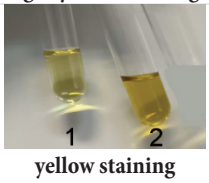
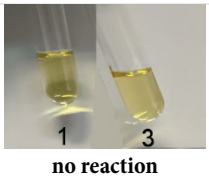
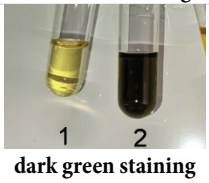
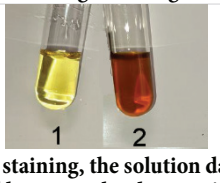
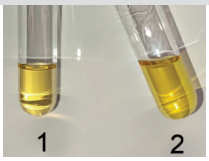
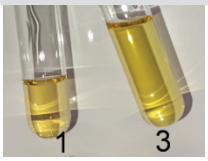
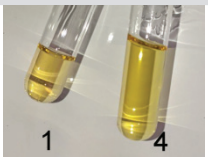
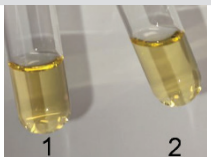
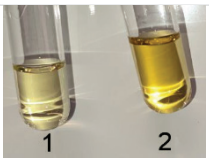
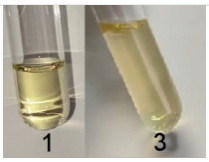
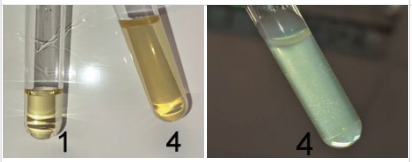
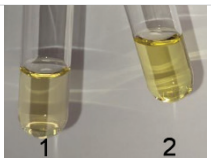
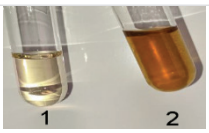
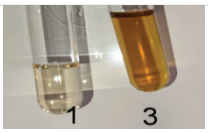
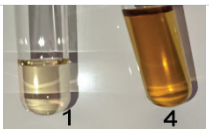
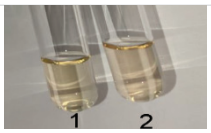
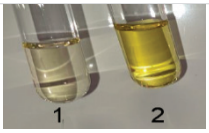
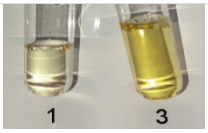
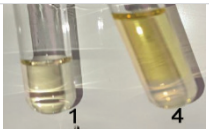
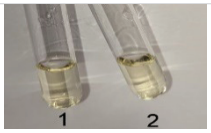
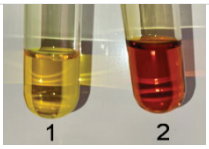
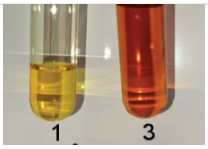
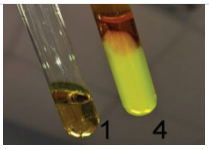
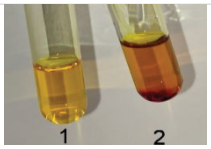
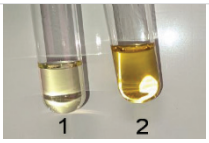
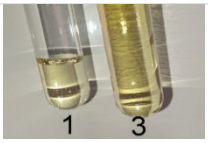
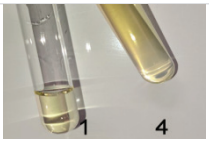
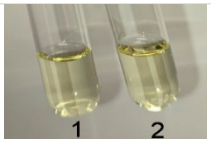
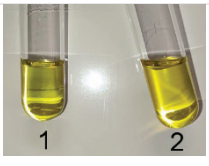
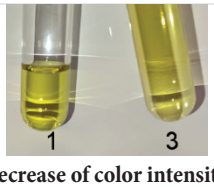
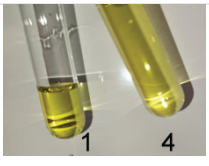
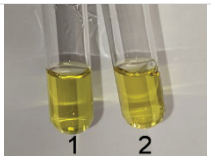
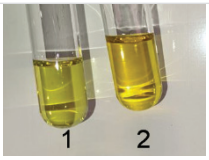
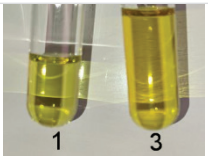
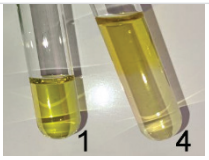
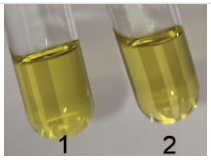
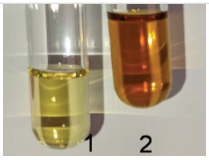
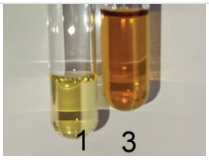
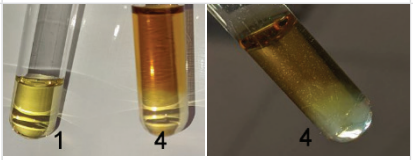
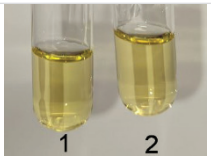
Sample	Classes of antioxidants			
	Anthocyanins	Anthocyanins	Phenols and flavonoids	Tannins
	1 — Sample 2 — Sample + Na_2CO_3	1 — Sample 3 — Sample + H_2SO_4	1 — Sample 2 — Sample + $FeCl_3$	1 — Sample 2 — Sample + $K_2Cr_2O_7$
1	2	3	4	5
Red pepper	 light yellow staining; Light sediment	 no reaction	 light olive staining	 orange staining
Black pepper	 light yellow staining	 no reaction	 light olive staining	 orange staining
Allspice	 dark yellow staining	 no reaction	 dark violet / black staining	 orange staining, the solution darkens and becomes cloudy over time
Nutmeg	 yellow staining	 no reaction	 light yellow staining	 orange staining
Turmeric	 dark red staining; cloudiness of the solution	 no reaction	  red and brown color; cloudiness of the solution	 orange staining
Ginger	 yellow staining	 no reaction	 light yellow staining	 orange staining
Parsley	 no reaction	 no reaction	 brown staining	 orange staining
Dill	 light yellow staining	 decrease of color intensity	 dark olive staining	 orange staining
Basil	 yellow staining	 no reaction	 dark green staining	 orange staining, the solution darkens and becomes cloudy over time

Table 4. Results of qualitative analysis of samples for classes of antioxidants: coumarins, carotenoids

Sample	Classes of antioxidants			
	Coumarines			Carotenoids
	1 — Sample 2 — Sample + NaOH	1 — Sample 3 — (2) + dist. water	1 — Sample 4 — (3) + HCl	1 — Sample 2 — Sample + H_2SO_4
1	2	3	4	5
Red pepper	 enhancing color intensity	 no reaction	 no reaction	 no reaction
Black pepper	 yellow staining	 decrease of color intensity	 White sediment; cloudiness of the solution	 no reaction
Allspice	 brown staining; cloudiness of the solution	 decrease of color intensity	 formation of brown flaky sediment	 no reaction
Nutmeg	 yellow staining	 decrease of color intensity	 formation of white cloudy sediment	 no reaction
Turmeric	 orange-red staining	 orange-red staining	 yellow cloudiness solution	 brown sediment formation
Ginger	 yellow staining	 decrease of color intensity	 discoloration and cloudiness of the solution	 no reaction
Parsley	 no reaction	 decrease of color intensity	 gas emission	 no reaction
Dill	 yellow staining	 no reaction	 discoloration and cloudiness of the solution	 no reaction
Basil	 orange staining	 orange staining	 discoloration and cloudiness of the solution	 no reaction

As a result of a qualitative reaction with iron (III) chloride, it was defined that allspice, parsley and turmeric contain large amounts of flavonoids, as evidenced by high color intensity. Brown coloration of parsley and turmeric extracts proved the presence of excess flavonoids with a 5-OH group. The remaining samples behaved negatively for phenols. Depending on the degree of color intensity, it is possible to conclude that the largest amount of phenolic compounds is found in the extracts of basil, turmeric and allspice. The staining of these samples featured a deep dark shade of indeterminable color, which also suggests the presence of a large amount of phenolic compounds. Black pepper and red pepper extracts showed approximately the same color intensity. Ginger and nutmeg demonstrated the weakest color changes. It cannot be stated though, that the samples with the greatest color intensity (allspice and basil) contain only one of the defined AO classes, since the possibility of color mixing should be also taken into consideration.

Analysis of the samples showed that tannins were found in basil and allspice extracts, which is consistent with several other studies [44]. The change in color of most solutions to orange can be explained by the bright orange color of the potassium bichromate solution which is the main reagent in this analysis.

Generalized data on defining of the components composition in the studied extracts for classes of antioxidants are presented below in the Table 5.

According to the authors, the amount of antioxidants in the purchased spices and herbs will be influenced by the integrity of plant cells. In addition, knowing the cells arrangement and structure of each spice, it is possible to identify it and test its purity both during the incoming control of raw materials of plant origin at the moment of their transfer to production, and during screening studies of products presented on the market. The use of light microscopy is widely used in China to identify Chinese herbal medicines according to their morphological structure [45]. In this regard, it was of interest to conduct microstructural studies of the samples and define the structural features of their cells.

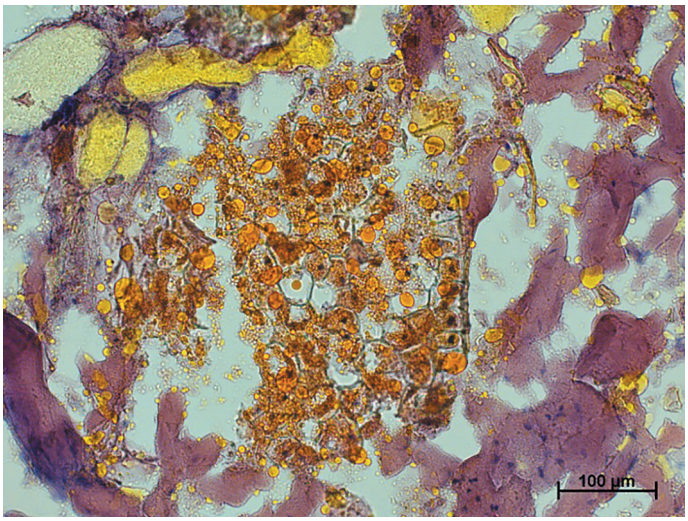
Table 5. Results of analysis of the components composition in the extracts

Sample	Phenols	Flavonoids	Tannins	Carotenoids	Anthocyanins	Coumarins
Red pepper	–	+	–	–	–	–
Black pepper	–	+	–	–	–	+
Allspice	+	+	+	–	–	+
Nutmeg	–	+	–	–	–	+
Turmeric	+	+	–	–	–	+
Ginger	–	+	–	–	–	+
Parsley	+	+	–	–	–	–
Dill	–	+	–	–	–	+
Basil	+	+	+	–	–	+

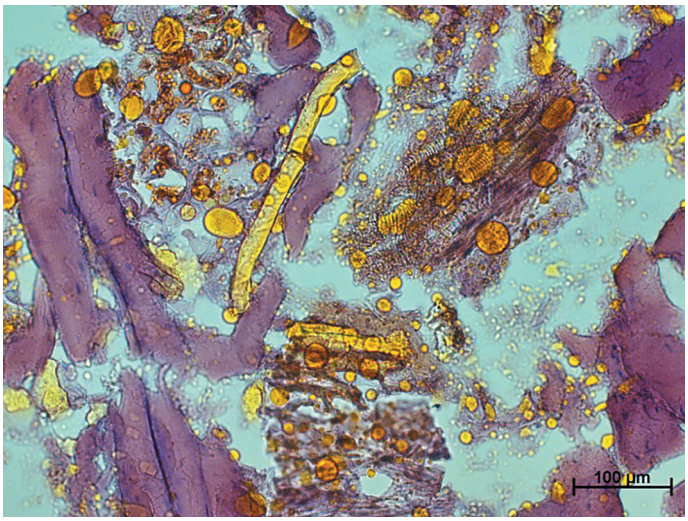
"+" — positive test for AO, "-" — negative test for AO

Histological preparations of red pepper demonstrated fragments of the external protective tissue (epidermis) with flattened, wrinkled cells. The main part consisted of colenchymatic cells of a pentagonal or hexagonal shape, with nuclei, acidophilic granules and yellow-orange oil droplets inside. The shape of large parenchyma cells looks like honeycomb. There are endocarp elements (cylindrical cells) and fragments of vascular bundles (Figure 1). After heat treatment, red pepper cells retain their shape (Figure 2).

Partial detachment of the epidermis is noticeable, expressed in an increase in interstructural spaces. In collenchyma cells, essential oils are clearly visible in the form of tiny yellow droplets, easily differentiated from each other. Drops of essential oil partially penetrate into the surrounding tissue under the influence of heat treatment and the previous process of mixing the components of the minced meat. Nuclear structures are represented by individual basophilic elements. In some cases, cell deformation and compression are observed.

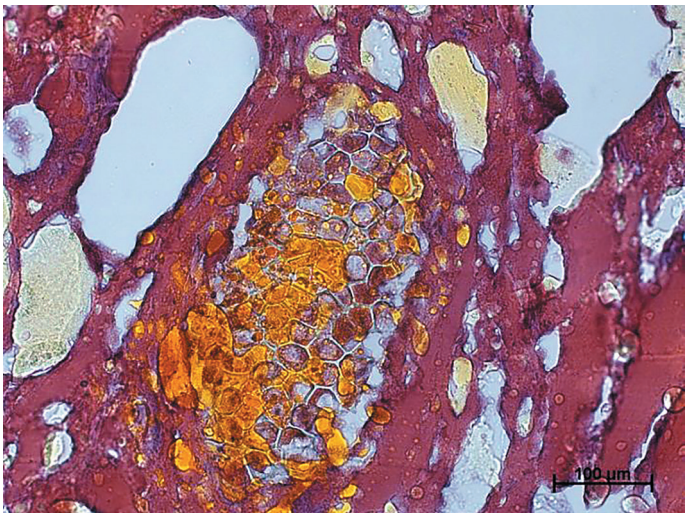


Parenchyma cells

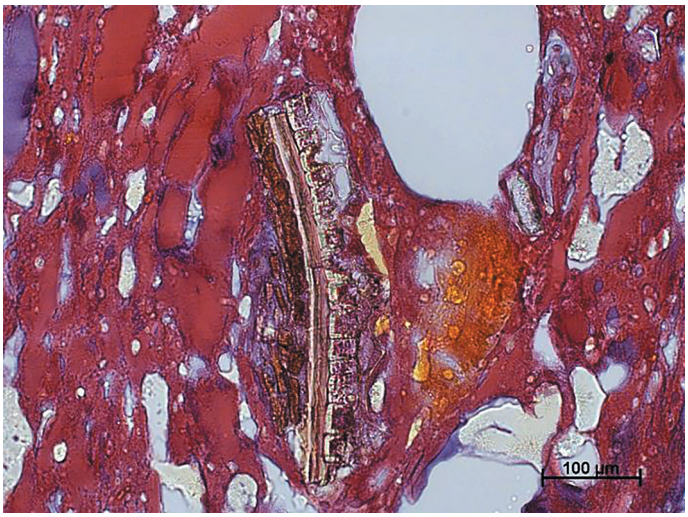


Fragments of vascular bundles

Figure 1. Red pepper. Hematoxylin and eosin staining (scale 100 µm)



Parenchyma cells



Epidermal fragment

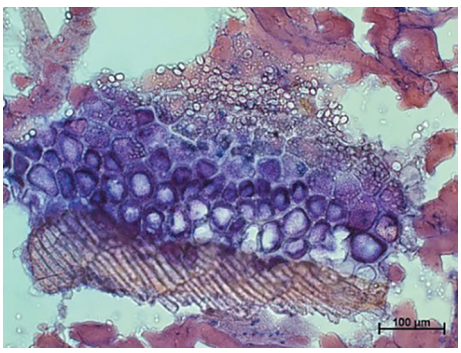
Figure 2. Red pepper after heat treatment. Hematoxylin and eosin staining (scale 100 µm)

Black pepper samples contain large fragments of the seed tissue with pronounced cell layers. Dense dark-colored fragments of the epidermis, sclereids (stony cells) layer and mesocarp parenchyma with large cells containing yellowish essential oil are visible. Multifaceted stony cells, voids and fragments of vascular bundles are clearly visible too. The elements of the seed hull include flattened prismatic epidermal cells, under which are located light-colored sclereids, almost similar in appearance to the sclereids of allspice. This is caused to the commonality of their taxonomy [46]. Moreover, in many cases these fragments are separated from the underlying tissues, possibly due to deformations during grinding of pepper seeds (Figure 3). The main part of the black pepper pea is occupied by perisperm, formed by large elongated hexagonal cells with numerous small starch grains (Figure 4), between which there are single cells with essential oil. There are small fragments of vascular bundles. After heat treatment, black pepper particles retain their microstructural characteristics and are well differentiated in histological preparations.

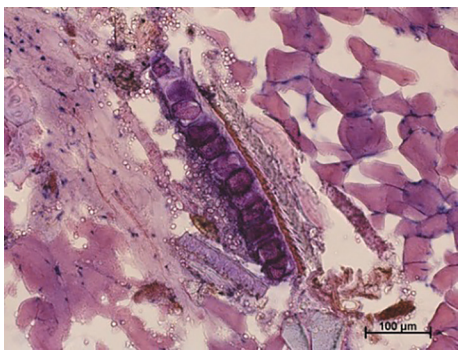
The peculiar particles of allspice samples contain variously sized, unstained "glassy" cells that form parenchymal tissue structures, as well as cylindrical, spiral-shaped conducting pathways. Fragments of the epidermis are clearly visible in the form of long strands of flattened cells. Sclereids are positioned under the epidermis, which are the unstained cells with concentrically located structural elements and a cavity in their center. Parenchyma cells are large, round, and have high content of small starch grains. In addition, secretory structures with inclusions of essential oil can be seen in the cellular elements of allspice peas (Figure 5). After heat treatment, cell complexes predominantly retain their structure (Figure 6). It can be noted that the essential oil is partially washed out during processing; on the histological specimen its drops were found only in the middle of the cell.

On histological preparations of nutmeg the fragments of the nutmeg endosperm are visible. The fragments consist of hexagonal-shaped cells containing storage substances: fats, oils, starch and aleurone (Figure 7). The brown perisperm cells are highly compressed. There are dense structures of dark brown color, consisting of small, closely spaced cells belonging to the epidermis (Figure 8). Large structural elements of external protective tissues are practically absent, since mature nutmegs have no outer pulp (the hull) and are usually dried. After heat treatment, the color of cells groups becomes lighter. Apparently, the heat treatment causes slight deformation of the cells. Within the cells small round starch grains and drops of essential oil are visible.

The special feature of turmeric is rounded conglomerates of large cells in the rootstock parenchyma, with inclu-

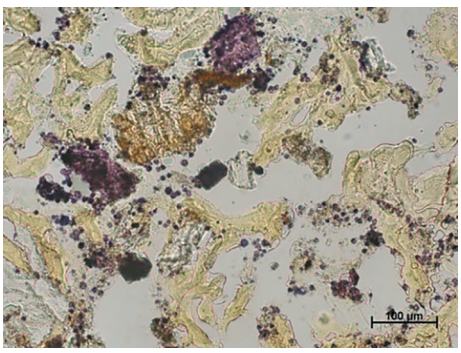


Perisperm fragment



Fragment of the epidermis

Figure 3. Black pepper. Hematoxylin and eosin staining (scale 100 µm)



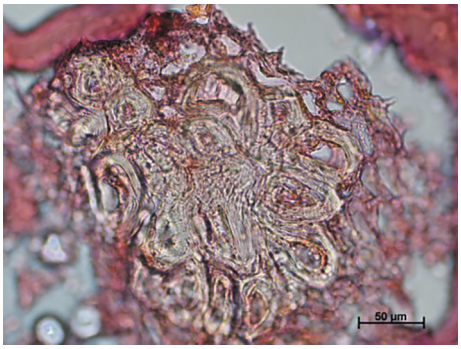
Starch grains

Figure 4. Black pepper. Staining with Lugol's solution (scale 100 µm)

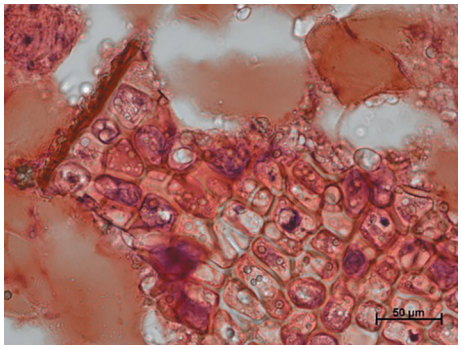
sion of elongated starch grains and a granular mass of yellowish-orange color (due to the presence of a yellow dye, curcumin, and inclusions of essential oil). The groups of epidermal type cells (elongated cells look like "columns"), fragments of vascular bundles (Figure 9) were observed. After heat treatment, inclusions of essential oils become practically indistinguishable, apparently due to their washing out during processing. The presence of parenchyma particles with wrinkled cells, sometimes with yellowish inclusions (essential oils, curcumin) should be noted. Large round cells are stained less intensely (Figure 10).

Unlike turmeric, ginger features the clusters of flattened cells in the surface layer of the rootstock, and large polyhedral parenchyma cells weakly sensitive to hematoxylin and eosin staining. These cells are characterized by a thin cellulose membrane and the high content of starch grains in the cytoplasm (Figure 11). The sample is also characterized by the presence of "columnar" tissue fragments. There are fragments of wide and thick-walled vessels bundles (Figure 12). Also on the preparation there are scattered starch grains created by grinding, which presence is a distinctive feature of ground ginger. The sizes of starch grains vary from 20 to 40 microns, they have peculiar flattened shape, narrowed at the ends. Particles of essential oils from yellow to dark yellow are observed, which are located in special cellular "secretory" structures of the parenchyma — idioblasts. The type and peculiar feature of these structural elements is one of the characteristics of this plant family.

After heat treatment, large fragments of ginger tissue are destroyed. Cover cells in the form of "columns", flattened spirals of vascular bundles, sometimes scattered, characterized by an increase in intercellular spaces, remain visible on the preparation. There are individual cells of storage tissue with the presence of yellow essential oil,

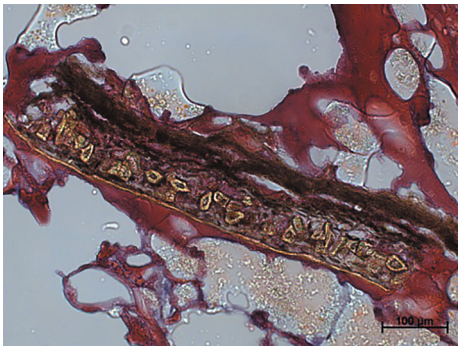


Sclereids



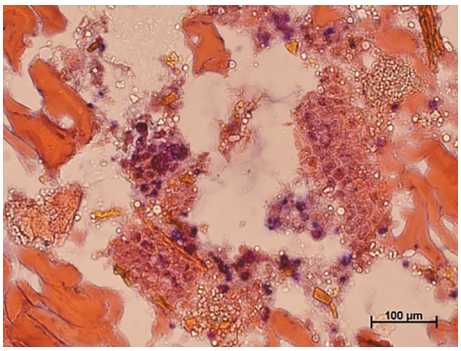
Parenchyma cells

Figure 5. Allspice. Hematoxylin and eosin staining (scale 50 µm)

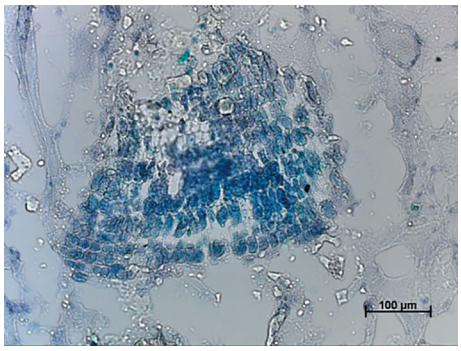


Fragment of the epidermis

Figure 6. Allspice after heat treatment. Hematoxylin and eosin staining (scale 100 µm)



Hematoxylin and eosin staining



Toluidine blue O staining

Figure 7. Nutmeg, endosperm fragments (scale 100 µm)

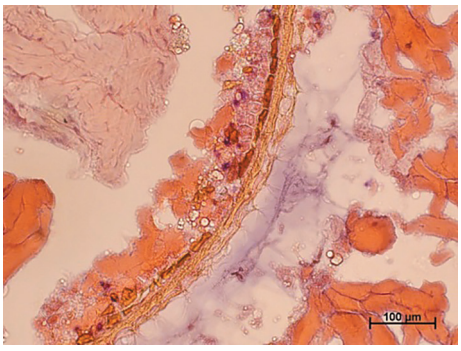
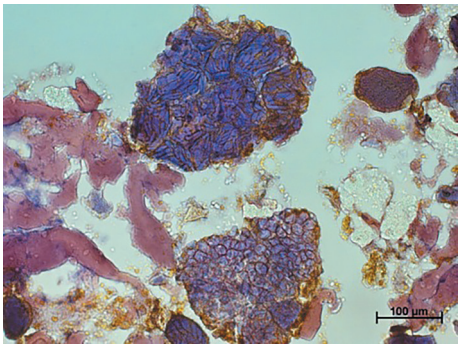
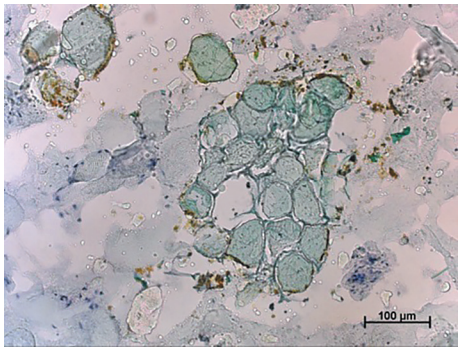


Figure 8. Nutmeg, epidermal fragment. Hematoxylin and eosin staining (scale 100 µm)



Hematoxylin and eosin staining



Toluidine blue O staining

Figure 9. Turmeric. Fragment of parenchyma. (scale 100 µm)

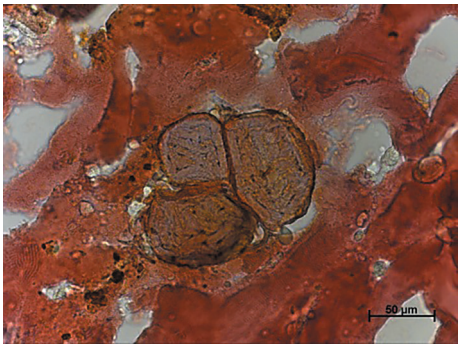
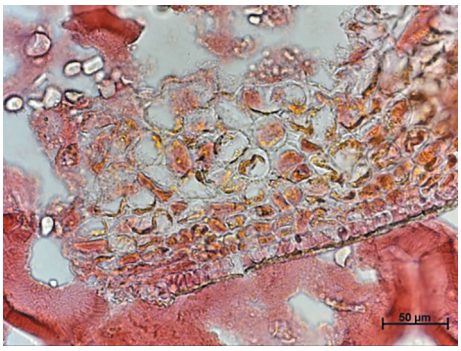
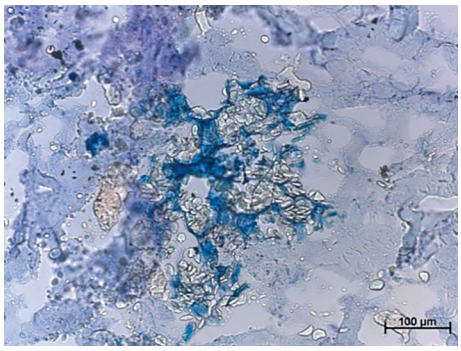


Figure 10. Turmeric. A fragment of parenchyma after heat treatment. Hematoxylin and eosin staining (scale 50 µm)



Staining with hematoxylin and eosin (scale 50 µm)



Tolluidine blue O staining (scale 100 µm)

Figure 11. Ginger. Parenchyma fragment

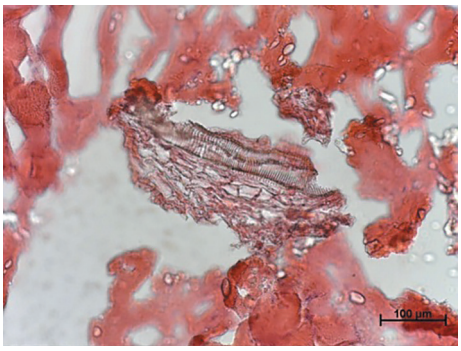


Figure 12. Ginger, fragment of a vascular bundle. Hematoxylin and eosin staining (scale 100 µm)

as well as "secretory" structures that do not perceive color well and are deformed as a result of the treatment. The membranes of parenchyma cells fit tightly to each other and are sometimes difficult to differentiate.

The samples of fragrant herbs contain a large number of particles attributed to plant leaves and having corresponding general morphological features. On the outside there are unstained closely spaced cells of the epidermis (leaf skin). The cuticle and stomata are poorly distinguishable in preparations. Mesophyll cells are located between the upper and lower epidermis. Under the top layer there are large rectangular cells of the palisade (columnar) parenchyma with numerous chloroplasts. Underneath them are several layers of cells of loose (spongy) parenchyma of irregular shape with large intercellular spaces. There are clearly visible elements of vascular-fibrous bundles: vessels, sieve-shaped tubes and collenchymal cords of mechanical tissue. On preparations of parsley and basil samples, a clear boundary is visible between the upper and lower sides of the leaf fragments (Figures 13, 14). Parenchyma cells contain starch grains, chloroplasts and small drops of essential oils. Papillary glandular hairs (essential oil) on the surface of parsley [22] and basil [47] leaves are not found.

Dill samples contain fragments of leaves in both transverse (rounded) and longitudinal sections (elongated horseshoe-shaped fragments of leaves). The epidermal cells are predominantly irregular in shape, with serpentine walls; beneath them there is a single layer of palisade tissue, which surrounds densely arranged spongy parenchyma cells. Dill does not have a clear border between the upper and lower sides of the leaf (Figure 15).

After heat treatment, fragments of fragrant herbs retain their microstructural characteristics, and their identification is not difficult in real practice. The main changes can be noted only in regard to the washing out of the essential oil: its drops remain only in the middle of the cell, mainly in the deeper layers of tissue fragments.

In a result of the studies it was found that spices and fragrant herbs at the microstructural level are characterized by the presence of cells that have a cellulose hull, peculiar for each type, due to which they are "expressively outlined" on the preparations. In this case, "transparency" of the structures can be observed due to the low density and specific tinctorial properties of plant protoplasm. Depending on the part of the plant used for production of the spice, corresponding cellular complexes and organ fragments are identified [48]. The microstructural features that play a significant role in identification include the following: characteristic parameters of the cells of the external protective tissue, seed hull, storage tissue (location and ratio of layers — endosperm and perisperm, cell color, presence of cells containing starch, essential oils, aleurone, etc.), secretory and formative tissues, and their ability to perceive staining with general and specific dyes.

The obtained results are consistent with the works [31,33], and confirm that microscopy is a practical approach for authenticating of the plant components, both in the dry mixtures composition and within the structure of ready-to-consume meat products. Currently, it is promising to combine microscopy with antioxidant analysis to determine the AOP of various plant tissues and establish a correlation between microscopic features and the active components they contain.

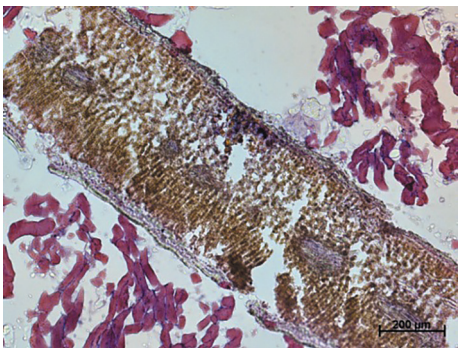


Figure 13. Basil. Hematoxylin and eosin staining (scale 200 µm)

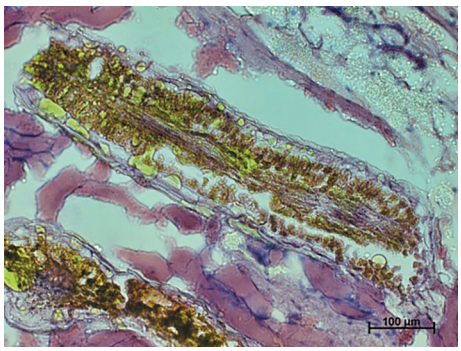


Figure 14. Parsley. Hematoxylin and eosin staining (scale 100 µm)

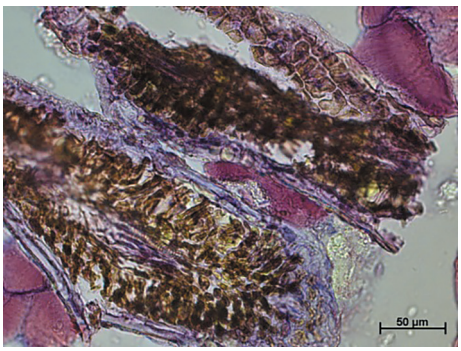


Figure 15. Dill. Hematoxylin and eosin staining (scale 50 µm)

Conclusion

The study examined the antioxidant potential of spices and herbs widespread in Russia, defined their structure and histological parameters of identification. Flavonoids were the most popular class of AO found in all samples. They were the only class of AO found in red peppers. Coumarins, which are unsaturated aromatic lactones, turned out to be quite common. Tannins and phenols were also detected in samples of allspice and basil. To expand the list of identified biologically active compounds, it is feasible to use other extraction methods and solvents.

The microstructural characteristics of the samples were studied and the main characteristic features of their cellular structure were defined. The studies have shown that accurate identification of all included plants components is a sophisticated task due to the variety of raw materials used and the complexity of cellular elements. However, in most practical cases, a significant part of them can be defined qualitatively. It should be noted that in addition to the light microscope and the general method of staining (hematoxylin and eosin), in order to increase the accuracy of spice identification it is promising to use specific staining methods, as well as polarizing microscopes and fluorescent microscopes, which allow expanding the number of morphological indicators.

The obtained results of histological analysis allow running incoming control of the dry spices and fragrant herbs compositions supplied to the meat processing plants in order to authenticate them and eliminate cases of raw materials adulteration. The established features of changes in cellular structures, that take place during technological processing, do not have fundamental nature, so it is possible to identify the spices and fragrant herbs in the ready-to-consume meat products.

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All authors made an equal contribution to the work.

The authors were equally involved in writing the manuscript and bear the equal responsibility for plagiarism.

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EVALUATION OF MEAT AND MEAT PRODUCT OXIDATION AND OFF-FLAVOR FORMATION: MANAGING OXIDATIVE CHANGES

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Keywords: *cryopreservation, oxidation of proteins, carbonyls, Maillard products, oxidation of myoglobin, cross-links*

Abstract

One of the primary issues with processed foods during heat treatment and freezing storage is fat oxidation, which causes significant changes in fats due to their interaction with reactive oxygen species (ROS). This interaction leads to the creation of various aldehydes that have a high affinity for large molecules, such as proteins, leading to the formation of final products of advanced oxidation processes that contribute to food spoilage. Co-oxidation can also result in extensive damage. Another problem affecting the quality and nutritional value of meat products is protein oxidation, which can occur during storage via freezing and thawing or as a result of heat treatment. Heat treatment can cause physical and chemical changes, such as the loss of some essential amino acids and the transformation of certain amino acids into carbonyl compounds via various mechanisms. Protein oxidation is indicated by the accumulation of these carbonyl compounds, and the heat treatment can lead to the denaturation of myoglobin, which is responsible for the brown color of cooked meat and is influenced by several factors. Active protein aggregates can interact with the oxidation products of polyunsaturated fatty acids and with carbohydrate glycation or glycoxidation to produce Maillard products. It is critical to understand the oxidative changes that occur in fats and proteins in food, particularly in meat products, since these components are among the primary constituents of food.

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Introduction

Meat and meat products (i. e., burgers, nuggets, shawarma meat) are highly valued for their nutritional and sensory properties making them popular among consumers. However, they are also particularly vulnerable to spoilage due to their chemical composition. To preserve them, several meat preservation methods are used in addition to freezing and heat treatment, such as chilling, smoking, canning, dehydration, and irradiation, which are widely used methods that help maintain the quality of meat and meat products during storage [1]. The quality of frozen meat products depends on such factors as the growth of microorganisms, physical and chemical changes, and storage temperature, which should be at or below –18 °C as indicated, for example, in Council Directive 89/108/EEC for quick-frozen foodstuffs [2]. Other factors that can affect the quality of meat and meat products include the length of the storage period, the duration of the thawing process, and the type of packaging material used. These factors also play a key role in the formation of ice crystals [3]. The size and distribution of ice crystals within the cells and spaces of frozen meat can vary depending on the rate, at which the meat is frozen. During the freezing process, many interactions can occur between the components of meat, which can alter their properties. For example, the oxidation of fats can lead to the formation of various byproducts, such as peroxides and aldehydes, including malondialdehyde (MDA), as documented in studies by [4,5,6]. These fat oxidation products can make proteins in meat more vulnerable to oxidation during cryogenic storage and heat treatment. Protein oxidation (P-OX) is a complex chemical-physical reaction that depends on various factors, such as the starting conditions of the reaction and the sensitive targets of the reaction, and involves several modifications to the peptide backbone and amino acid side chains, which are very sensitive to oxidative stress. Chemical changes associated with protein oxidation include the loss of sulfhydryl groups, loss of tryptophan fluorescence, and gain of carbonyl derivatives, as well as the formation of intra- and intermolecular cross-links [7]. The DNPH technique is commonly used to estimate the total amount of protein carbonyls in meat as a way of assessing P-OX.

The practice of heat treatment in meat and meat products has been around for a long time and has evolved into modern technologies. This process is necessary to make raw

meat suitable for consumption by eliminating pathogens and enhancing its sensory qualities. When meat is heated, the protein myoglobin undergoes a change that results in the appearance of a brown color, which varies based on the level of maturity. Although consumers often view the change in meat color from red to brown as an indication of maturity and product aesthetics, it can also be a health concern. The final color of a meat product can also be affected by browning, which can induce oxidative changes in fats that produce harmful compounds with metabolic effects on human health. This article aims to identify these oxidative changes in meat and meat products during freezing and heat treatment, and to explain how these changes are linked to color changes and the basic mechanisms that affect the final color of meat and meat products. The article provides a brief overview of freezing and heat treatment and their role in protein oxidation.

Objects and methods

The sources of information were the following scientific databases: ScienceDirect, PubMed, Scopus, ResearchGate, and Google Scholar (accessed 03/25/2023). The search strategy included the following keywords: cryopreservation, oxidation of proteins, carbonyls, Maillard products, oxidation of myoglobin, cross-links. The following acceptance criteria for research characterization were considered: meat and meat products, oxidative reactions, analytical methods to determine lipid and protein oxidation, original research. The parameters of the publications were as follows: publication from 1988 until 2023 (86 references were selected for this review); language: English. Exclusion criteria: no access to the full-text articles. Based on the review, the authors compiled information on meat and meat products, preservation of meat, oxidative reactions, formation of crosslinking, and the analytical methods to determine lipid and protein oxidation in meat and meat products.

Meat and meat products

Meat consumption, sourced mainly from herbivorous animals including cattle, buffaloes, goats, sheep, camels, and horses, is widely favored by individuals in both developed and developing countries. Meat is a very nutritious food that is widely used as a primary source of animal protein by people around the globe. Nevertheless, the American Heart Association [8] advises restricting daily meat intake to a maximum of six ounces. Meat is an essential element of human diet, and its chemical makeup might differ based on characteristics such type, breed, sex, age, animal feed, and tissue quality [9]. Meat and meat products provide vital nutrients, including premium proteins, lipids, and minerals. These goods are available in different forms, including cured meats, patties, nuggets, and meatballs. Processed meat products are food items that have been modified through methods including smoking, salting, and curing to improve their quality and increase their shelf life. The composition of meat products exhibits variations in terms of amount, type, and manufacturing procedures, as well as disparities in shape and size. However, the main ingredient is beef, which can have a fat content of up to 30%. The high fat content enhances the flavor, taste, juiciness, and emulsion of the product.

Burgers

A burger is a type of minced meat product that is flavored with spices and condiments, shaped to achieve, as a rule, a flat, disc-like shape, and cooked either by frying or baking. The name of a burger is linked to a meat type used, such as pork, beef, or chicken. Various proportions of fat are used in burger production. For example, according to the U. S. Code of Federal Regulations, hamburgers should not contain more than 30% of fat [10]. Fats are important for the sensory properties of the burger. To make a burger, meat must first be minced effectively to create a tender texture, then salted and mechanically mixed to bind the components before cooking [11].

Nuggets

Consumers readily accept restructured breaded products, such as nuggets, as a substitute for chicken meat. Chicken nuggets are made from chicken meat trimmings, skin, water, phosphate, and salt. The nugget-making process involves using small and irregular pieces of meat, then binding them together with a binder to form a larger size. To meet the increasing demand for high-quality and long-lasting nuggets, many manufacturers add additional ingredients, such as phosphate. Although the use of phosphate is still uncertain in terms of health benefits, it is added to produce a chewy and durable product [12]. The ability to bind meat particles with other ingredients is a critical factor in determining the success of a product. Finally, chicken nuggets are coated in batter and breadcrumbs, deep-fried or baked, and then quickly frozen.

Shawarma meat

Nowadays, shawarma meat is widely recognized as one of the most favored item, although it is not an indigenous product in many countries. In Sudan, for example, shawarma made its first appearance in the 1980s, originating from the Turkish region [13]. The term "shawarma" refers to thin slices of lamb, chicken, turkey, beef, veal, or a combination of meats seasoned with sauce, mixed with fat and spices, and prepared by arranging strips of fat and seasoned meat on a vertical skewer that rotates. The exterior of meat is roasted while the interior remains mostly uncooked. For serving, thin shavings are cut from the meat block, while the remaining portion of meat is kept warm on the rotating skewer [14].

Preservation of meat

Meat is vulnerable to microbial and oxidative spoilage, which makes it one of the most perishable food

items. Raw meat can harbor harmful bacteria such as *Salmonella*, *Campylobacter*, and *Escherichia coli*, and meat products derived from pork, turkey, and broiler are known to be a significant source of foodborne illnesses such as salmonellosis [15]. This has made meat preservation important for transporting meat over long distances without deterioration of its texture, color, or nutritional value, especially with the development and growth of supermarkets [16]. Preservation methods primarily aim to prevent microbial spoilage, but other preservation techniques are also used to minimize other types of deterioration such as color and oxidative changes [17]. At present, traditional preservation methods such as drying, smoking, brining, fermentation, refrigeration, and canning as well as newer methods such as high hydrostatic pressure (HHP), modified atmosphere packaging (MAP), active packaging (AP), natural antimicrobial compounds, and biopreservation have been used [18]. The goal of preservation methods is to prevent microbial spoilage as well as to minimize oxidation and enzymatic spoilage. Current meat preservation techniques fall into three broad categories: controlling temperature, controlling water activity, and using chemical or bio-preservatives. These preservation techniques can be used in combination to reduce the rate of spoilage [15,16].

Chilling

Proper chilling of meat is crucial for maintaining hygiene, safety, shelf life, appearance, and eating quality [17,19]. Chilling meat with air is effective in reducing the temperature of the surface and preventing bacterial growth. Decreasing temperature and increasing air velocity can shorten the chilling time, but cooling the deeper tissue of the carcass is challenging. Natural-convection air chilling is slower and less controlled, while forced-convection air chilling with fans is more efficient. Quick chilling improves product yield by reducing surface evaporation and bacterial growth. However, ultra-rapid chilling of pre-rigor meat can cause cold-shortening and toughening [16,17]. Spray-chilling helps oxygenate the surface myoglobin, maintain brightness, and prevent weight loss without increasing metmyoglobin [17].

Freezing

Freezing meat on a large scale began in Britain around 1880 with the arrival of frozen beef and mutton from Australia [18]. The benefits of freezing meat below the freezing point include extending its useful storage life and discouraging microbial and chemical changes. Quick freezing creates tiny ice crystals within meat cells, reducing drippings when thawed. The freezing rate depends on a size of meat, its thermal properties, a temperature of the refrigerating environment, and a method of refrigeration, as well as a type of wrapping material used for smaller cuts. To prevent quality changes in frozen meat, a temperature of –55 °C has been recommended as ideal storage conditions, where few deteriorative changes occur during storage due to minimal enzyme reactions, oxidative rancidity, and ice recrystallization [17]. Cryogenic freezing is faster than conventional air freezing because of the large temperature differences between the cryogen and the meat product, resulting in a high rate of surface heat transfer caused by boiling the cryogen. Cryogenic freezing can be achieved with a cryogen tank and suitable spray equipment, without the need for mechanical refrigeration equipment. However, the process may cause some distortion to the shape of the product, which could affect its commercial application. Additionally, the cost of cryogenic liquid is relatively high, which may limit its commercial use [17].

Curing

Various methods of curing meat are practiced, including dry cure, pickle cure, injection cure, and direct cure, using ingredients such as sodium chloride, sodium nitrate, sodium nitrite, and sugar. Preservation of meat through heavy salting has been a long-standing practice, and sodium chloride has been historically used in food preservation due to the lack of refrigeration facilities [16]. Later, the use of common salt and sodium nitrate resulted in improved products. Sodium chloride inhibits microbial growth by increasing osmotic pressure and decreasing water activity. Concentrations as low as 2% can inhibit some bacteria, and a concentration of 20% can inhibit many food spoilage yeasts. However, some microorganisms from the *Bacillus* and *Micrococcus* genera can tolerate high concentrations of salt. The current limit for nitrite in meat products is 156 ppm in the US and 200 ppm in Canada, as the use of nitrite as a food additive can form carcinogenic nitrosamines with prolonged exposure. However, there is no epidemiological evidence to support the relationship between nitrate consumption and a specific cancer or cancer risk [16,19,20].

Smoking

Smoking meat has historically been used to preserve it for extended periods of time. Smoke contains a variety of substances resulting from the breakdown of wood, such as organic acids, aldehydes, ketones, and phenols, among others. Preservation of meat through smoking is also accomplished by surface dehydration, reduction of pH of the meat surface, and the antioxidant properties of smoke components. Curing and smoking of meat are closely linked, with smoke generated in specialized smokehouses where sawdust, hardwood, or both are burned at around 300 °C. The combustion process generates numerous organic compounds and their condensation products, with aldehydes and phenols accounting for 50% of smoke components and contributing significantly to the color of smoked meat products. Phenols are the primary bactericidal compounds [16].

Canning

The process of canning meat helps maintain its sensory properties, including its appearance, flavor, and texture, to a significant degree. Additionally, canned meat products can last for at least two years at room temperature. Canning involves various stages, such as meat preparation, precooking, filling, exhausting, seaming, thermal processing, cooling, and storage [16].

Dehydration

This process of preserving meat involves removing water from a meat concentrate, which makes water-soluble nutrients inaccessible to microorganisms. In ancient times, people used sun drying of meat chunks for preservation, but rehydrating such meat was challenging. Mechanical drying involves passing hot air with controlled humidity, but it also poses difficulties for rehydration. However, freeze drying is a satisfactory process for dehydration and preservation due to better reconstitution properties, nutritive quality, and acceptability of meat products. This process involves removing water from food by sublimation from a frozen state to a vapor state by keeping it under vacuum and giving it low heat treatment. Freeze drying of meat is carried out in three steps: pre-freezing, primary drying, and secondary drying. Meat is first frozen at –40 °C, and then it is dried under vacuum for 9–12 hours at low temperature in plate exchangers at 1–1.5 mm pressure of Hg [20]. During primary drying, free and immobilized water of meat, which constitutes about 90–95% of total moisture, is removed. Secondary drying is done at high temperature to remove the remaining 4–8% bound water. After freeze-drying, meat products are packed under vacuum and have excellent storage stability. This process is commonly used for preparing dehydrated meat soup mixtures [16].

Irradiation

Irradiation, also referred to as "cold sterilization," involves the propagation of energy through materials. Electromagnetic waves are emitted in a continuous form, which can ionize molecules in their path. These waves can break down the DNA molecules of microorganisms and ionize the water inside them, thereby destroying them. It is worth noting that irradiation destroys microbes in food without significantly raising the temperature of the food. Gamma radiation only affects food during irradiation and has no impact once the source is removed. Consequently, it is extensively employed for food preservation. While UV radiation mostly kills bacteria, it lacks sufficient penetrating power and is only utilized for surface sterilization of meat [16,21].

Heat treatment

The previous studies mentioned in Table 1 show that when meat products are subjected to heat treatment, their chemical composition is affected. This means that cooking reduces the amount of moisture in a meat product, and increases the levels of fat and protein.

Oxidative reactions

An imbalance between the generation of reactive oxygen species (ROS) and the capacity of biological systems to remove them leads to oxidative stress. It is widely recognized that elevated levels of ROS can cause direct harm to proteins, lipids, and nucleic acids. Among the ROS that have a considerable impact on lipids, the hydroxyl radical (·OH) and the hydroperoxyl radical (HO_2) are the two most commonly occurring ones. The hydroxyl radical (·OH) is highly mobile and soluble in water, which makes it the most reactive ROS. As demonstrated in Figure 1, it can attack biomolecules [30].

Table 1. Influence of heat treatment (cooking) on the chemical composition of meat and meat products

Product	Chemical composition, %					References
	Moisture	Protein	Fat	Ash	carbohydrate	
Beef (lean)	75.0	22.30	1.80	1.2		[22]
Fried beef (lean)	58.4	30.40	9.20	—		
Raw beef burger	45.50	12.28	15.75	2.00		[23]
Cooked beef burger	32.74	14.55	24.00	1.94		
Raw chicken burgers	—	58.3	22.8	3.58		[24]
Cooked chicken burgers (oven)	—	54.7	20.1	2.94		
Patty burger before cooked	61.97	23.21	10.45	4.06	0.31	[25]
Patty burger after cooked	64.95	22.12	8.86	3.86	0.17	
Raw chicken burger		59.41	31.60	6.18		[26]
Cooked chicken burger		63.53	30.39	6.01		
Raw beef burgers	70.50	17.14	10.05	2.06		[27]
Cooked beef burgers	57.81	25.54	10.15	2.29		
Raw shawerma meat	69.24	23.0	3.34	2.84		[28]
Cooked shawerma meat	55.72	28.0	10.0	3.67		
Raw beef burger	59.60	14.29				[29]
After frozen storage (3 month) and cooking	48.80	19.53				

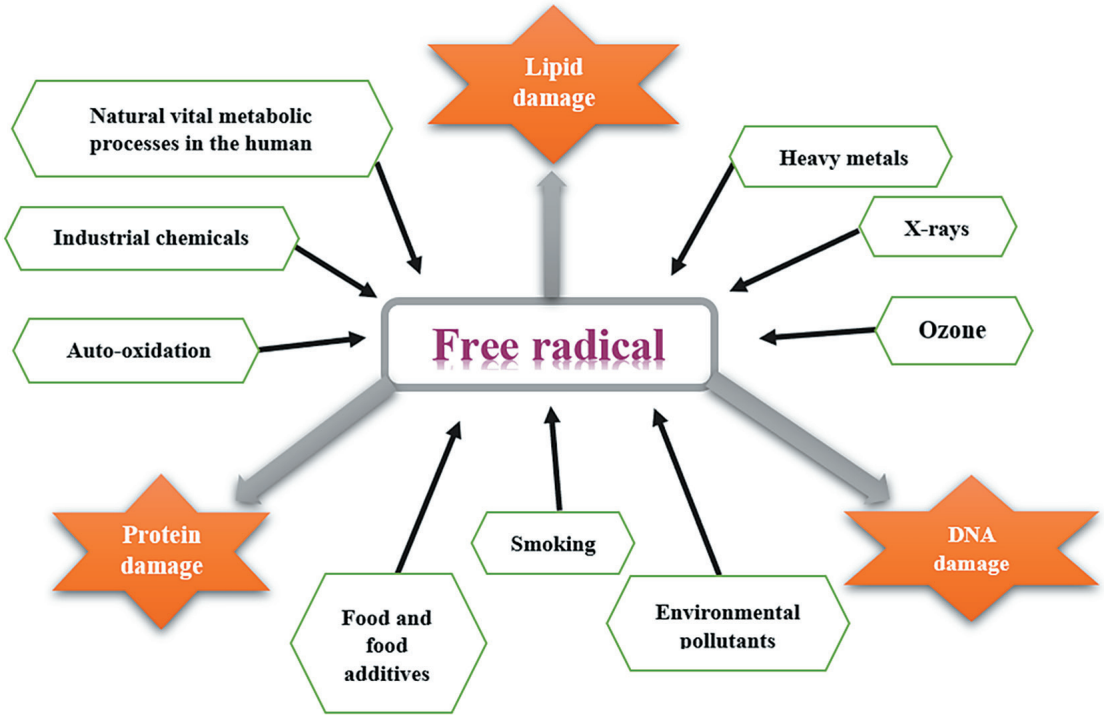

Figure 1. Sources and harmful effects of free radicals

Lipid oxidation in meat

Lipids are a vital element in all types of meat and meat-based products, as they provide desirable properties such as aroma, flavor, tenderness, and juiciness. However, the process of lipid oxidation is the primary factor responsible for quality deterioration and reduced shelf life. This natural and inevitable process affects various aspects of meat, including color, texture, nutritional value, taste, and aroma. It causes rancidity, leading to off-flavors and unpleasant taste, which often result in consumer rejection [31]. Lipid oxidation also causes the breakdown of fatty tissues in meat, ultimately affecting its commercial value [32]. Lipids are highly susceptible to oxidative reactions, which occur due to various factors such as the type of lipid structure, degree of unsaturation in fatty acids, exposure to light and heat, and presence of molecular oxygen, pro-oxidant, and antioxidant components. These reactions are quite complex and can significantly impact the oxidative stability of lipids [31].

Muscle tissue contains natural components such as iron, myoglobin, hydrogen peroxide, and ascorbic acid that can cause lipid oxidation. They act as catalysts or promote the formation of reactive oxygen species, which can damage lipids. Oxidative reactions can also be triggered by physical factors such as radiation and light. There are three primary ways lipids undergo oxidation in biological systems: photo-oxidation, enzymatic oxidation, and autoxidation. Photo-oxidation occurs when radiant energy, such as ultraviolet radiation, interacts with sensitizers like myoglobin, leading to radical reactions that form hydroperoxides different from those produced without light and sensitizers [33]. Autoxidation is a complex chemical process that involves self-propagating radical reactions and depends on such factors as temperature, pH, metal ions, and free radicals. Enzymatic oxidation is catalyzed by lipoxygenase, which oxidizes fatty acids, adding oxygen to the hydrocarbon chain. This process forms peroxides and hydroperoxides with conjugated double bonds that can undergo various degenerative reactions, forming multiple products.

Protein backbone chain oxidation

The peptide main chain and the amino acid side chains are susceptible to oxidative damage by reactive oxygen species (ROS). When metal ions and hydroxyl radicals are present, hydrogen atoms are removed from the α-carbon in proteins, creating an α-carbon radical and transforming the protein into a radical. This radical can become a peroxyl radical (POO) in the presence of oxygen, which can then transform into an alkyl peroxide (POOH) by removing a hydrogen atom from an unstable molecule. Additional reactions with ROS and metal ions such as iron and copper can result in the formation of alkoxyl and hydroxyl derivatives. When O_2 is available, the radicals centered around the carbon atom turn into a peroxyl radical RO_2·, and alkyl peroxyl reacts with H_2O_2 to form hydroperoxides. Cross-links within or between protein molecules can form under anaerobic conditions due to radical interactions [3,34,35]. Figure 2 shows the oxidative modification of protein backbone and amino acid side chains.

Moreover, the process of protein oxidation by ROS can cause the breaking of peptide bonds. This occurs when alkoxyl radicals and alkyl peroxide derivatives undergo cleavage through either the α-amidation or di-amide pathways. Both pathways result in the formation of an amide derivative at the N-terminal end of the protein via the α-amidation pathway and the derivative α-keto-acyl at the C-terminal end of the protein via the di-amide pathway [36].

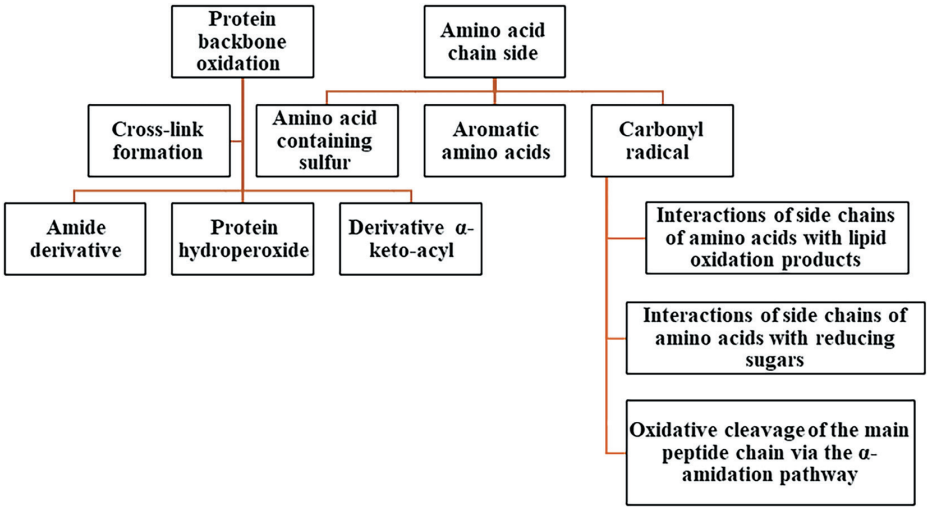

Figure 2. Oxidative modification of protein backbone and amino acid side chains

Oxidation of amino acid side chains

The oxidative processes, such as oxidation of thiol groups in cysteine, hydroxyl in cyclic amino acids, and formation of carbonyl groups, affect the side chains of amino acids. These processes result in the loss of functional properties of proteins, ability of proteins to aggregate and loss of solubility, which affects their ability to carry water. Oxidation of proteins in the presence of ROS can convert histidine to oxohistidine derivatives and imidazolone derivatives, while leucine and valine are transformed into hydroxyderivatives. One of the most significant outcomes of the oxidation of side chains of amino acids is the formation of various products [36,37,38,39].

Loss of thiols

The sensitivity of amino acids to oxidation processes is determined by their chemical properties. Cysteine and methionine are two amino acids that possess sulfur atoms, which make them highly reactive even when the concentration of reactive oxygen species (ROS) is low. When the SH group of these amino acids is oxidized, a hydrogen atom is extracted, leading to the formation of thiyl radicals. These radicals can either react with other thiol groups to create disulfide bonds or interact with oxygen (O_2) to produce thiyl peroxy radicals (RSOO). These thiyl peroxy radicals undergo a series of reactions, as described by [35,40].

Cyclic amino acid oxidation products

Amino acids containing hydroxyl groups, such as histidine, phenylalanine, tryptophan, and tyrosine, are sensitive and prone to oxidation. These amino acid groups are not easily oxidized by metal-catalyzed reactions but are preferred targets for free radicals, according to studies by [41,42]. Tyrosine is particularly vulnerable to oxidation compared to phenylalanine, both within the same protein chain and across different protein chains. When tyrosine is exposed to free radicals, ultraviolet radiation, beta rays, or lipid hydroperoxides, it can be transformed into dityrosine. This conversion occurs when a tyrosyl radical is formed by the loss of one electron from tyrosine, and two single tyrosyl radicals can then combine to form dityrosine, which is a relatively stable molecule that is resistant to enzyme hydrolysis. Dityrosine can form cross-links both between and within proteins [42,43].

The rapid oxidation of the amino acid tryptophan is one of its distinguishing features. This is because tryptophan is among the amino acids that exhibit early oxidation. The oxidation of tryptophan results in several metabolites, such as -hydroxy-tryptophanα when H_2O_2 is present. Additionally, photo-oxidation of tryptophan produces formylkynurenine, kynurenine, and 3-hydroxykynurenine-3. The loss of tryptophan is an essential indicator of meat and meat product oxidation. This information is supported by research conducted by [35,36,41].

Protein carbonyl radical

Proteins are oxidized into carbonyl compounds through various pathways. The oxidation is irreversible and not carried out by enzymes. There are four main pathways for this reaction. The most common pathway involves the direct oxidation of certain amino acid side chains, such as lysine, threonine, arginine, and proline, which results in the formation of a carbonyl radical. Another pathway involves the interaction of amino acid side chains with reducing sugars, which contributes to the formation of carbonyl radicals. The third pathway involves the oxidative cleavage of the main chain of the peptide via the **α-** amidation pathway [34,36]. Finally, certain amino acid side chains can interact with lipid oxidation products, which can also lead to carbonyl compound formation [34,36].

The way, in which certain amino acids are oxidized, directly depends on how metal ions interact with H_2O_2 to create hydroxyl radicals, either •OH or the highly reactive RO· hydroxyl radical. These reactions occur near the metal binding site in the amino acids. In many biological systems, metals such as iron and copper act as either electron donors or acceptors. Metal-catalyzed oxidation (MCO) is thought to be the primary cause of oxidative damage because of the presence of these metals. This idea has been supported by research conducted by [36,44]

H_2O_2 + Fe(II)/Cu(I)→HO· + OH− + Fe(II I)/Cu(I) [1]
ROOH + Fe(II)/Cu(I)→RO· + OH− + Fe(II I)/Cu(I) [2]

When H_2O_2 and metals are present at high concentrations, almost all types of amino acids can be oxidized by resulting HO. However, when the concentrations of iron or copper ions and H_2O_2 are low, protein damage is likely to be limited. Metal binding sites on proteins are particularly sensitive to MCO catalysts, and oxidation occurs through a site-specific mechanism. This involves the binding of Fe (II) or Cu(I) to metal-binding sites on the protein, followed by a reaction with peroxide to form OH· and RO·, which are highly effective in reacting with amino acids. Research conducted by [36,45] has demonstrated this mechanism, as illustrated in Figure 3.

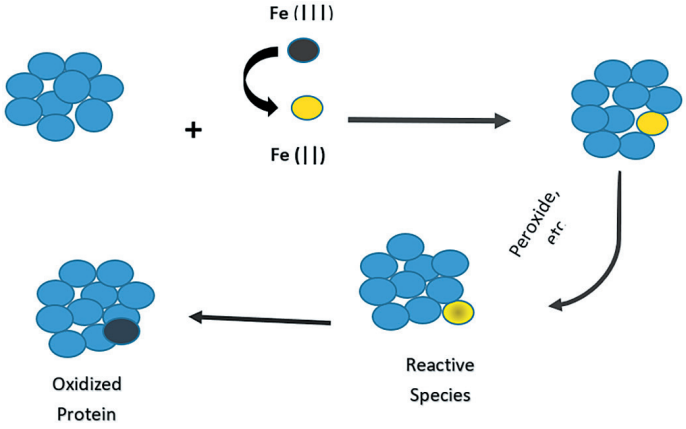

Figure 3. Metal-induced protein oxidation [36]

Formation of crosslinking

ROS are produced in meat when it is exposed to oxygen and light during storage, and they react with the components of meat causing the oxidation of fats and proteins and the creation of radicals. There are various types of cross-links of meat proteins, including direct interaction between two carbon radicals, the formation of dityrosine from the interaction of two tyrosine radicals, and disulfide bonds formed from the reaction of radicals created from the oxidation of cysteine. Cross-links are also formed from the reaction of the carbonyl radical of protein oxidation products with the ε-amino group of lysine in the same or different protein, and the interactions of both aldehyde groups with malonaldehyde with two ε-amino groups in different lysines in the same or different protein. Furthermore, junctions are created between the carbonyl radical of reducing sugars and their oxidation products with lysine or arginine in the same or different protein [36,46].

Impact of frozen storage and production techniques on the oxidation of meat proteins and meat-based food items

Various methods of storage and manufacturing have a significant impact on the physical and chemical properties of protein in food systems, particularly meat, resulting in protein oxidation. As a result, the quality of meat is affected. Numerous research studies have focused on the influence of frozen storage and heat treatment on protein oxidation, and these studies and their respective detection methods are listed in Table 2.

Myoglobin pigment oxidation

The hue of fresh meat and meat products is a crucial quality feature that signals their freshness, and it has a significant impact on how consumers perceive and accept them. Any alteration in color can be a crucial factor that influences whether consumers will buy meat or its products. According to [56,57], there is a strong connection between color preference and purchase decision.

The color of meat is determined by the pigment myoglobin (Mb), and is affected by the myoglobin chemical state and concentration in the muscles [58]. Myoglobin (Mb) consists of a protein part called globin and a non-protein part known as the heme ring. The stability of the color is influenced by both oxidation and reduction states. When oxygen is removed from meat or when it is not exposed to oxygen, deoxymyoglobin is formed, resulting

Table 2. Protein oxidation in various meat types and meat-based products

Product	Method of storage and heat treatment	Indicator	Methods	Sources
Chicken burgers	Frozen	Carbonyl content	DNPH	[47]
Chicken thigh and breast meat	Frozen			[48]
Chicken burgers	Oven cooking and microwaving			[24]
Beef patties	Roasting			[49]
Beef patties	Frozen			[18]
Chicken patties	Boiling, grilling, roasting			[50]
Meatballs	Convection oven precooking, frozen storage and four reheating methods (boiling, pan-roasting, convection oven, microwave oven)	Thiol content	DTNP	[51]
Beef patties	Frozen storage			[18]
Russian sturgeon	Sous-vide cooking			[52]
Fish fillets	Boiling, microwaving, steaming, roasting, deep-frying			[53]
Beef and chicken patties	Frozen	Cross -linking proteins Schiff base	FS	[54]
Beef patties	Roasting			[49]
Chicken patties	Boiling, grilling, roasting	Cross -linking proteins Disulphide bond	4-DPS	[50]
Pork meat	Frozen			[55]

DNPH: 2,4- Dinitrophenylhydrazine, DTNP: 5,5-dithiobis (2-nitrobenzoate), FS: fluorescence intensity, 4-DPS: 4,4-dithiodipyridine.

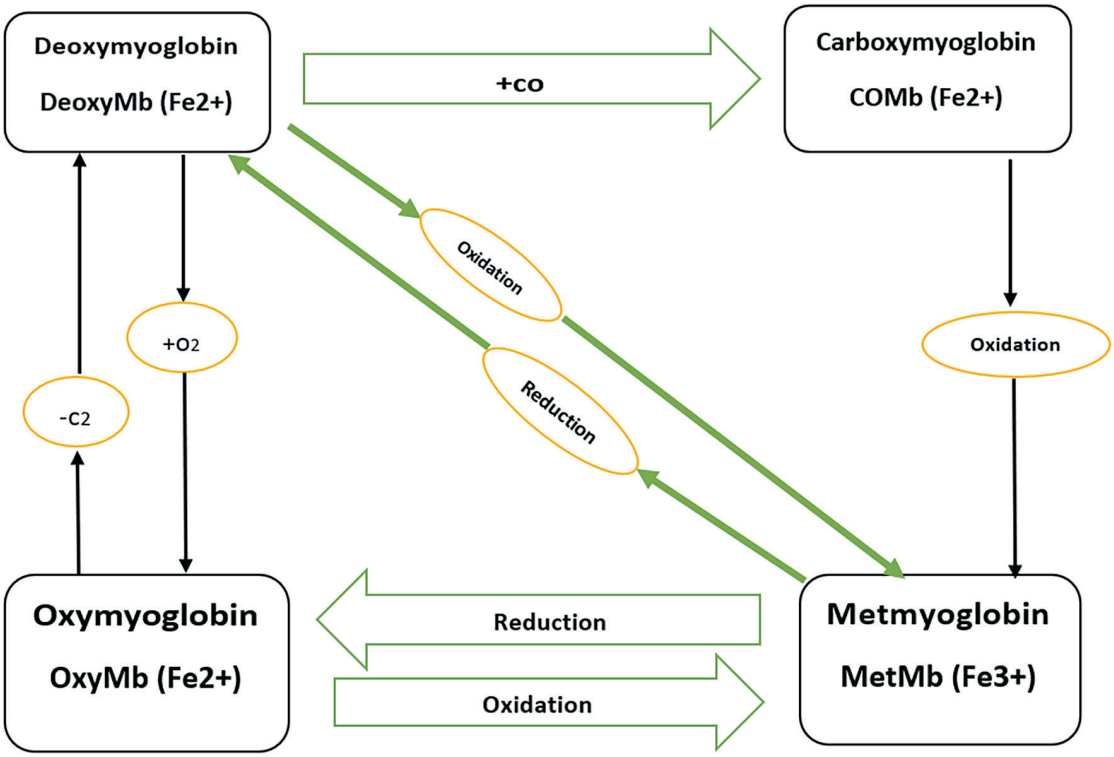

Figure 4. Transformations in the meat pigment

in a purple red color [58] Exposure to oxygen causes the formation of oxymyoglobin, which gives meat a bright red color. However, excessive exposure to oxygen leads to the oxidation of ferrous to ferric state causing the pigment to change from oxymyoglobin to metmyoglobin, resulting in a brown color on the surface of meat (Figure 4). Metmyoglobin values greater than 40% can cause consumer rejection of meat and meat products. This is supported by various studies such as those conducted by [59,60].

The aldehydes that result from the breakdown of lipids are highly active and can easily bond with myoglobin in the sarcoplasm, making it more prone to oxidation. When meat products have a higher fat content, they tend to have a browner color. During meat processing, the redness decreases significantly, and the oxymyoglobin content decreases, while the MetMb content and lipid oxidation increase. Ferric ions can also aid in lipid oxidation. The information in Figure 5 is based on the studies conducted by Wang et al. [61] and Zhu et al. [62].

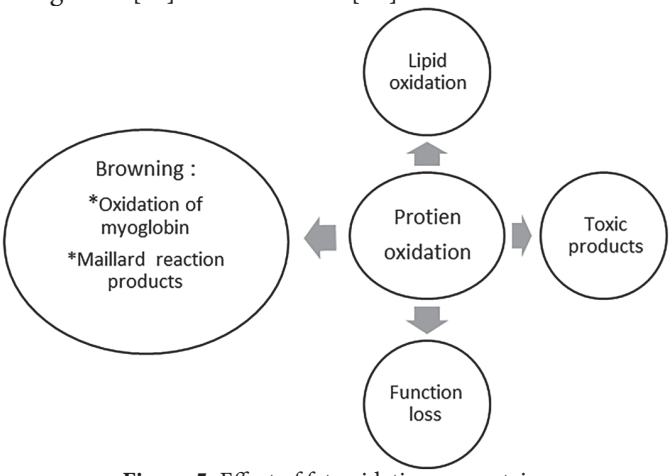

Figure 5. Effect of fat oxidation on proteins

The color of cooked meat at the end is influenced by how the myoglobin pigment reacts to heat treatment. This is determined by the protein's fundamental structure, how it interacts with other biomolecules, and self-oxidation, all of which impact the thermal stability of the pigment and its resistance to denaturation. These findings are depicted in Figure 6 [63].

The final color of cooked meat is influenced by heat treatments, which cause denaturation of myoglobin. It is crucial to consider the early development of the cooked meat color, as pathogenic microorganisms may still be present at this stage, posing a risk to consumers [64]. This early brown color can occur if myoglobin is in a particular chemical state before cooking. The delayed development

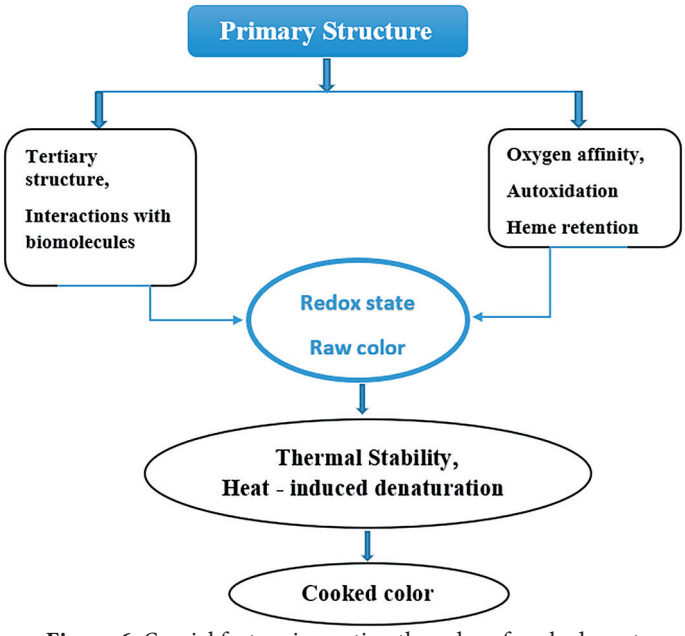

Figure 6. Crucial factors impacting the color of cooked meat

of color requires more time to reach the desired color and also depends on the myoglobin's chemical state in raw meat. Myoglobin's thermal stability varies based on its form, with metmyoglobin being the least stable and most likely to cause early browning. Oxymyoglobin and deoxygenated myoglobin are both more heat-resistant than metmyoglobin. The pH, muscle source and type, product components, packaging, and storage all play a role in the internal color of cooked meat, which is a crucial factor in consumer acceptance and can also serve as an indicator of doneness [65].

Maillard Reaction Products (MRPs)

For centuries, people have used the Maillard reaction to enhance the taste and appearance of foods by heating, frying, roasting, and grilling them. This reaction also produces compounds that give food an appealing flavor and possess the antioxidant activity. However, it can also create harmful compounds in certain food products. The Maillard reaction is influenced by various factors such as a type of reactants, duration and temperature of heat treatment, pH, and water activity. Foods contain oxygen, minerals, and carbonyl compounds from fats that can act as a source of carbonyl for interaction with proteins, leading to the formation of Maillard products and a dark brown color [66, 67]. This reaction is defined as the interaction between the amine group of amino acids, peptides, and proteins with the carbonyl group found in reducing sugars, oxidized lipids, and vitamin C according to studies by [68] and other researchers such as [66,67,69,70].

The Maillard reaction products impact the processes of fat oxidation, with some of these products hindering and others promoting the oxidation processes. They interact with certain intermediate compounds and generate distinct compounds that would not typically form in the absence of fat. For instance, the development of a savory taste in cooked meat relies on the presence of fat. Both pathways involve co-polymerization mechanisms and generate comparable products in high-fat foods like meat and phospholipids. Many Maillard products stem from fat oxidation processes, which include the production of methylglyoxal (MG), glyoxal, and Amadori products [71,72]. The Maillard reactions that occur in meat products can be influenced by a type of heat treatment used depending on a method of heat transfer. The formation of Maillard reaction products (MRPs) is less efficient when using an oven and circulating air for heat (roasting), compared to using a hot surface (barbecue) or frying where heat is transferred through conduction. This information is based on research conducted by [73,74].

The Maillard reaction is a complex chemical reaction that occurs between amino acids and reducing sugars, leading to the formation of a range of intermediate and end products that contribute to the flavor, aroma, and color of cooked and processed foods. The reaction occurs in three main stages:

— Early stage: In the first stage, a reducing sugar and an amino acid form a Schiff base, which undergoes a rearrangement to form an N-substituted glycosylamine. This compound can then undergo further rearrangement to form a variety of intermediate compounds, including 1-amino-1-deoxy-ketoses and 2-amino-2-deoxy-aldoses, which are key intermediates in the reaction.
— Intermediate stage: In the second stage, several key reactions occur, including the formation of diketosamines, which can decompose to form monofructosamines and non-nitrogenous carbonyl compounds. These compounds can undergo enolization, a process that leads to the formation of compounds responsible for flavor and color. Another important reaction in this stage is the Strecker degradation, which leads to the formation of a range of compounds that contribute to flavor.
— Final stage: In the final stage, some of the products formed in the previous stages can condense to form brown pigments and polymers, which are known as melanoidins. These compounds contribute to the brown color of cooked and processed foods and are also responsible for some of the unique flavors associated with these products. Overall, the Maillard reaction is a complex process that involves a series of chemical reactions occurring between reducing sugars and amino acids. The reaction results in the formation of a wide range of intermediate and end products, many of which contribute to the flavor, aroma, and color of cooked and processed foods [66,75]. Figure 7 shows the mechanism of the Maillard reaction.

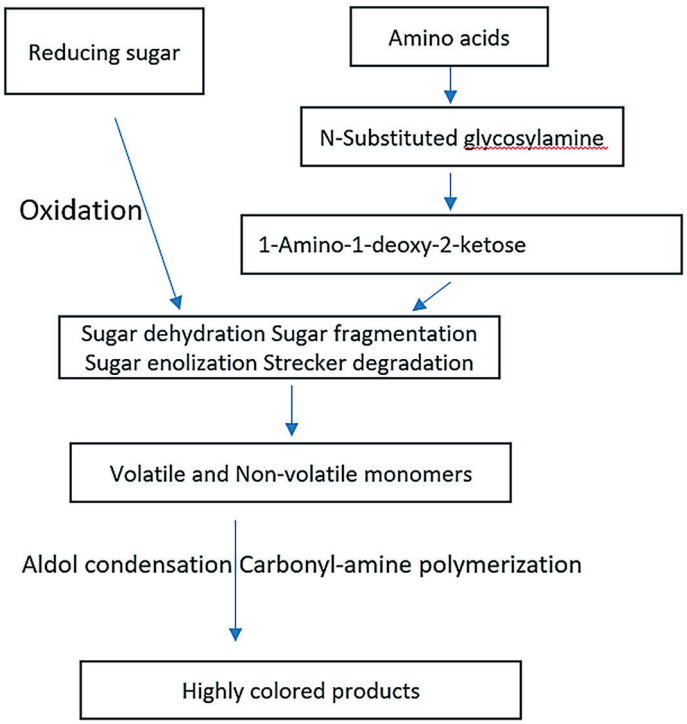

Figure 7. The mechanism of Maillard interactions

The analytical methods to determine lipid and protein oxidation in meat and meat products

Analyzing lipid and protein oxidation in meat and meat products requires various analytical methods to assess the extent and progression of oxidation. Here are some common analytical methods used for this purpose:

— Peroxide value (PV) for lipid oxidation: PV measures the primary oxidation products in fats and oils, including meat lipids. It quantifies the concentration of peroxides, which are formed during the initial stages of lipid oxidation. Iodometric titration is a widely used method for determining PV [76].
— Thiobarbituric acid reactive substances (TBARS) assay for lipid oxidation: TBARS measures secondary lipid oxidation products such as malondialdehyde (MDA), a marker for oxidative rancidity. It involves reacting MDA with thiobarbituric acid to form a colored complex, which is measured spectrophotometrically [77].
— Anisidine value (AV) for lipid oxidation: AV assesses the quality of meat lipids by measuring the concentration of secondary oxidation products such as aldehydes and ketones. It is particularly useful for evaluating fish products. AV is determined by reacting the sample with anisidine reagent and measuring the absorbance at specific wavelengths [78].
— Free fatty acid (FFA) content for lipid oxidation: FFA content is indicative of hydrolytic rancidity and can be measured through acid or enzymatic hydrolysis of lipids followed by titration or colorimetric methods [79].
— Total oxidation (TOTOX) value for lipid oxidation: The TOTOX value combines PV and AV measurements to provide a more comprehensive assessment of lipid oxidation [78].
— Carbonyl content for protein oxidation: Carbonyl content in proteins is determined using the 2,4-dinitrophenylhydrazine (DNPH) assay. It quantifies the oxidative damage to amino acid side chains, particularly to lysine and arginine residues [80].
— Sulfhydryl (–SH) group assay for protein oxidation: Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid)) can be used to measure the -SH groups in proteins, which are susceptible to oxidation [81].
— Electrophoresis for protein oxidation: Gel electrophoresis techniques, such as sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), can reveal changes in protein profiles due to oxidation [82].
— Mass spectrometry for lipid and protein oxidation products: Mass spectrometry techniques, including liquid chromatography-mass spectrometry (LC–MS), are used to identify specific lipid and protein oxidation products and assess their abundance [83].
— Fourier transform infrared spectroscopy (FTIR): FTIR can analyze changes in the chemical structure of lipids and proteins in meat samples due to oxidation, providing information on functional groups and molecular changes [84].
— Electronic nose and electronic tongue: These instrumental methods can detect volatile compounds and taste profiles associated with oxidation, helping to assess meat quality [85].
— Sensory evaluation: Trained sensory panels or consumer panels can provide valuable information on the flavor, odor, and overall quality of meat products affected by lipid and protein oxidation [86].

The choice of method(s) depends on the specific research or quality control objectives, as well as a type of a meat product being analyzed. Often, a combination of these methods is used to obtain a comprehensive understanding of lipid and protein oxidation in meat and meat products [78].

Conclusion

The evaluation of meat and meat product oxidation is an important consideration for ensuring product quality and safety. The oxidation of lipids and proteins can result in the formation of off-flavors and undesirable odors, which can reduce consumer acceptance and ultimately result in economic losses for producers. However, the management of oxidative changes can be achieved through the use of various strategies, including the addition of antioxidants, the use of modified atmosphere packaging, and the control of processing and storage conditions. These approaches can help to minimize the formation of off-flavors and extend the shelf-life of meat and meat products. Overall, managing oxidative changes is crucial for maintaining the quality and safety of meat and meat products and ensuring their acceptability to consumers.

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CYBER-PHYSICAL SYSTEMS IN FOOD PRODUCTION CHAIN

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Keywords: *cyber-physical systems, Big Data, smart production, Industry 4.0, food systems, meat products*

Abstract
The article reviews the state-of-the-science in the field of cyber-physical systems (CPSs). CPSs are intelligent systems that include physical, biological and computational components using engineering networks. CPSs are able to integrate into production processes, improve the exchange of information between industrial equipment, qualitatively transform production chains, and effectively manage business and customers. This is possible due to the ability of CPSs to manage ongoing processes through automatic monitoring and controlling the entire production process and adjusting the production to meet customer preferences. A comprehensive review identified key technology trends underlying CPSs. These are artificial intelligence, machine learning, big data analytics, augmented reality, Internet of things, quantum computing, fog computing, 3D printing, modeling and simulators, automatic object identifiers (RFID tags). CPSs will help to improve the control and traceability of production operations: they can collect information about raw materials, temperature and technological conditions, the degree of food product readiness, thereby increasing the quality of food products. Based on the results, terms and definitions, and potential application of cyber-physical systems in general and their application in food systems in particular were identified and discussed with an emphasis on food production (including meat products).

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Introduction

The Strategy for Scientific and Technological Development of the Russian Federation, approved by Decree of the President of the Russian Federation dated December 1, 2016, No. 642, indicates the need for a transition to advanced digital intelligent production technologies, robotic systems, new materials and design methods, and the creation of systems for processing big data, machine learning systems and artificial intelligence.

The concepts of Industry 4.0 and Society 5.0 were introduced in 2011 and 2016 respectively, but digitalization of food systems began less than 5 years ago.

According to the Industry 4.0 concept proposed by physicist Henning Kagermann, the "fourth industrial revolution" is a means of increasing the competitiveness of the German processing industry through the increased integration of "cyber-physical systems" into factory processes.

As a result, one of the current trends is the development of cyber-physical systems for digital transformation, robotization of processes in the field of storage and processing of agricultural raw materials and food products. Digital transformation as the process of introducing modern digital technologies into business processes of production (processing) systems at all levels in practice will lead to the creation of a system of end-to-end IT/agro-biotechnological processes.

The purpose of this study is to give a comprehensive review of cyber-physical systems: purpose, history of creation, directions and prospects for use in the food industry.

Objects and methods

The authors conducted a search and a comprehensive analysis of publications using key phrases: "cyber-physical systems", "Industry 4.0", "smart industry", "smart production" in the Scopus, PubMed, MEDLINE, Web of Knowledge, Google Scholar, IEEE Xplore, Science Direct, eLibrary (RSCI) databases for the period of 2000 to August 2023.

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) standard was chosen to ensure reproducibility of the selected information (http://www.prisma-statement.org/).

The identified publications were preliminarily analyzed by abstract. The inclusion criteria were as follows:

1. Scientific research on cyber-physical systems, their principles, architectures, implementation tools;
2. Conditions of use in industry;
3. Limitations of use;
4. Mainly publications in Russian and English.

The exclusion criteria were as follows:

1. Scientific articles, monographs published before 2000;
2. Publications related to Industry 4.0 tools (Big Data, IoT, Digital Twin, etc.) without the use of CPSs.

Terms and definitions

The term "Cyber-Physical Systems (CPSs)" was first introduced in 2006 by the Director of Embedded and Hybrid Systems of the US National Science Foundation (NSF), Dr. Helen Gill, at the "NSF Workshop on Cyber-Physical Systems" conference (October 16–17, 2006, Austin, Texas) to denote complexes consisting of natural objects, artificial subsystems and controllers.

Currently, the following definitions of the cyber-physical systems exist.

The book "Introduction to Embedded Systems — A Cyber-Physical Systems Approach" [1] states that CPSs represent the integration of computing with physical processes. The need to understand the interaction of the computational and physical process is noted. It is not enough to understand the computational and physical process separately. In other words, CPSs are monolithic connection of physical objects or phenomena and calculations combined into a network.

In [2], CPSs are systems that combine physical and computer-based or cyber components. The physical components are biological objects, as well as systems developed by man (for example, transport or energy systems). A physical component exists, functions, and interacts with its environment in a continuous or routine manner. A computational component includes systems and objects involved in the processing, transmission and controlling the information by computing means. These are algorithms implemented in software and digital systems interfaced with physical components through analog-to-digital converters (ADCs), digital-to-analog converters (DACs) and digital communication networks. Computational components are artificial systems that operate in discrete time or based on events. Sanfelice [2] notes that the complexity of integrating components in cyber-physical systems is due to the fact that the computational component is distributed throughout the system and is closely related to the physical component. Thus, CPSs are highly complex systems that combine continuous and discrete dynamics.

The following definition is given in [3,4,5]: CPSs are the junction of cyber (electrical/electronic) systems with physical object. CPS helps mechanical systems to sense the physical world, process that sensing as data on computers, make calculations, and inform the systems of actions to change the outcome of the process.

Trappey et.al. define in their work that "CPSs are a set of transformative technologies for managing interconnected physical and computational capabilities" [6].

According to Baheti et al. [7], cyber-physical systems refer to transformative technologies for managing interconnected systems between physical parameters and computational capabilities.

Shafiq et al. [8] agree that CPSs are "the convergence of the physical and digital worlds by creating global networks for businesses that include their equipment, warehouse systems and manufacturing facilities."

A number of studies suggest that CPS, as an emerging technology, has the potential to offer promising solutions to transform the operation and role of many existing industrial systems [9–14].

Gürdür et al. [10] note that the development of CPSs requires tool support for tasks associated with various engineering tasks at different stages of the product life cycle. These tools must evaluate product data based on internal and external dependencies. The study examines a method for visualizing the node-link diagram (NLD) in the CPS development chain. An assessment of the current compatibility status and various solutions for integration scenarios are provided.

Mao et al. [11] state that in the context of the Internet of Things (IoT) of an industrial enterprise, all objects, such as tools, materials, machines and persons, are networked by radio channels that have not only the capabilities of measurement, processing, communication and control, but also location information. To meet these requirements, the authors believe that future RFID systems will provide both reliable identification and high-precision positioning. In the paper, the authors propose an integrated asymmetric UHF/UWB reader transceiver for industrial IoT applications.

Yan et al. [13] proposed a new wearable wireless sensor network (WWSN) for health anomaly detection, discussed the network architecture, established a detection model, and developed a set of algorithms to support the operation of WWSN. Thus, changes in medicine will be due to the personalization of human data, and the system will select treatment individually.

Zhai et al. [14] presented a multi-frequency time division multiple access (MF–TDMA) protocol for a radio frequency identification (RFID) monitoring system in the industrial Internet of Things (IoT).

CPSs are industrial automation systems that integrate innovative functions via a network. Therefore, the operations of physical reality are connected with computing and communication infrastructures [5,8,15,16,17,18,19].

In [15], a unified framework for integrating CPSs into production is presented. The method of adaptive clustering is described as an advanced analytical method for interconnected systems, and a practical example of self-aware machines through CPS integration is shown.

Harrison et al. [16] consider the industrial context for the development of CPSs. Examples of engineering methods, approaches and tools that are currently available are provided. The study focuses on a set of tools for designing CPSs. An example is shown to explain how a component-based design toolkit may support an integrated approach to the virtual and physical design of automation systems throughout the life cycle. The method allows for the

efficient integration of equipment from different suppliers and provides support for the specification, verification and use of such systems throughout the supply chain.

Jazdi [17] describes the significance of the Internet of Things and Services (IoT), its important role in professional and everyday life. The author notes that Industry 4.0 has already begun and directly affects our lives and business models, demonstrates software for an industrial coffee machine at the Institute of Industrial Automation. Future work is expected to focus on implementing a distributed remote application based on software agents.

Lee et al. [18] propose a unified five-layer architecture to implement CPSs. The article provides a practical guide for the processing industry to implement CPSs in order to improve product quality and system reliability using intelligent and failsafe equipment facilities.

The development of CPSs is associated with a new paradigm of technical systems. For implementation, the following is necessary: 1) online configuration for a set of systems; 2) coordinated functioning of interacting systems; 3) provision of appropriate infrastructure. Mosterman and Zander [19] focus on the second aspect, the collaboration function. In their study, they present a number of specific examples of CPSs, one of which is illustrated using a pick-and-place machine solving a distributed version of the Towers of Hanoi puzzle. The system includes a physical environment, a wireless network, parallel computing resources, and computing functions (service arbitration, various forms of control, and streaming video processing). The entire research is conducted at the computational model level aiming at contributing to the research agenda to develop next-generation systems.

The study by Putnik et al "What is a Cyber-Physical System: Definitions and models spectrum" [20] analyzed 44 scientific publications with different definitions of CPSs. Since there are many definitions, models, and structures of CPSs, this study focused on identifying their characteristics and classifying them by approaches or applications. An overview of definitions from the literature and their position in the presented synchronous spectrum of CPSs is presented. The classification is based on the basic characteristics, behavior and supporting technologies of CPSs.

Potential applications

The vast majority of authors in the studied publications agree that CPSs are related to the fourth industrial revolution. It is stated that it is the introduction of cyber-physical systems into industry that will contribute to the early transition from Industry 3.0 to Industry 4.0.

The fourth industrial revolution is closely related to the Internet of Things (IoT), cyber-physical systems (CPSs), information and communications technologies (ICTs), enterprise architecture (EA) and enterprise integration (EI).

A systemic analysis of researches [18,21,22,23] showed that the interoperability architecture of Industry 4.0 includes four levels: operational (organization), systematical (applicable), technical and semantic interoperability.

These four levels make Industry 4.0 and CPSs more productive and cost-effective. The interaction diagram between Industry 4.0 and CPSs is shown in Figure 1.

Interoperability indicates the common structures of concepts, standards, languages and relationships within CPSs and Industry 4.0. Systematical interoperability defines the guidelines and principles of methodologies, standards, domains and models. Technical interoperability brings together tools and platforms for technical development, IT systems, ICT environment and related software. Semantic interoperability enables the exchange of information between different groups of people, malicious application packages, and institutions at different levels.

Industry 4.0 interoperability requires specific principles to ensure the accuracy and efficiency of the entire process, i. e. accessibility, multilingualism, security, use of open-source software and multilateral solutions. Accessibility means that Industry 4.0 must offer equal opportunities for public access by participants without their discrimination. Multilingualism means that Industry 4.0 must support multiple languages to effectively deliver information and knowledge to CPSs. A security policy means that appropriate risk assessments and security measures are required. Multilateral solutions achieve Industry 4.0 interoperability by meeting the different requirements of different partners [24].

CPSs include microcontrollers that control sensors and actuators. Data and information are exchanged between embedded computer terminals, wireless applications,

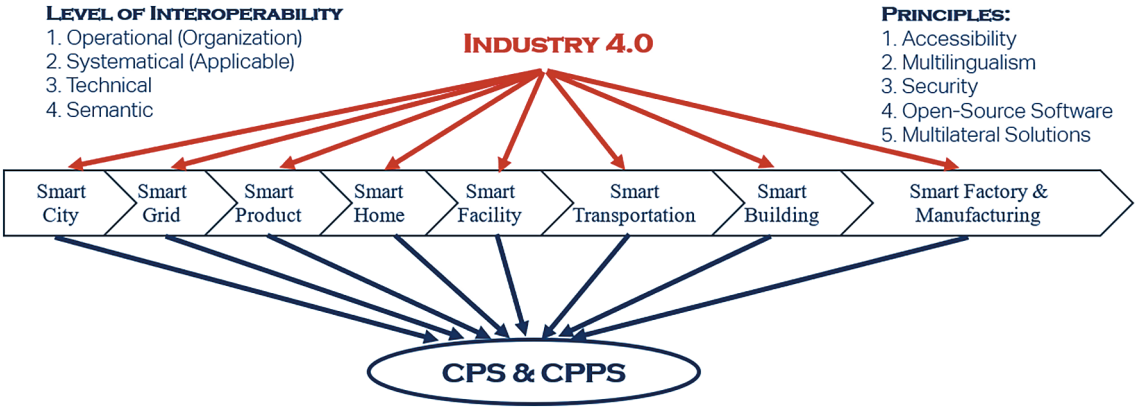

Figure 1. The interaction diagram between Industry 4.0, CPSs and CPPSs (cyber-physical production systems) [18,21,22,23,24]

homes, and even cloud services. A complex, dynamic and integrated CPS will support planning, analysis, modeling, design, implementation and maintenance in the manufacturing process [17,25]. CPSs are capable of increasing productivity, stimulating growth, changing labor productivity, and producing higher quality products at lower costs due to the collection and analysis of malicious data [26].

Since CPSs integrate information and materials, decentralization and autonomy play an important role in improving the overall performance of industrial production [27].

Ivanov et al. [28] state that to coordinate actions in production procedures and to achieve production optimization, dynamic models are needed in CPSs that describe changes in a system or process over time, take into account the influence of external factors, interactions with other elements of the system, and predict course of events and consequences. Based on the dynamic structure control mechanism, the authors develop a service-oriented dynamic model for dynamic scheduling and collaboration of CPS networks in Industry 4.0.

CPSs have characteristics such as timeliness, reliability, failure tolerance, security, scalability and autonomous operation [29,30,31].

Systematic reviews of CPS technologies [24,32,33] highlight the following areas of knowledge: real-time embedded systems, distributed computing systems, automated control systems for technical processes and objects, wireless sensor networks, Internet of Things (IoT), industrial Internet, machine-to-machine (M2M) interaction, fog and cloud computing, complex adaptive systems, holon (agent) production systems.

Figure 2 illustrates the product's impact and potential applications. Information technology and low-cost sensors offer new capabilities to improve the services [34].

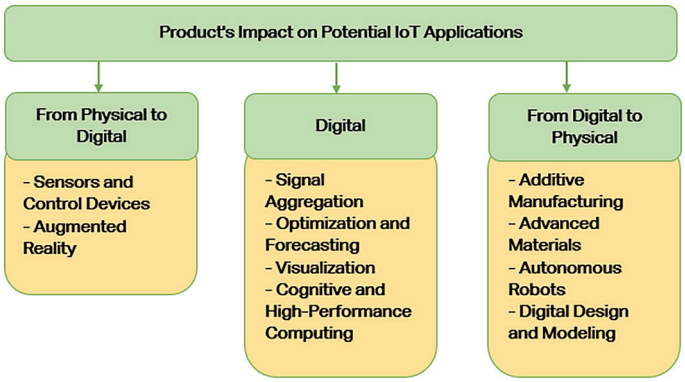

Figure 2. Product's impact and potential applications

In food production, CPSs consist primarily of three modules: 1) field device process; 2) production equipment process; 3) production management process using a service-oriented architecture for management [36].

Structure of CPSs

As noted in [24,36,37], CPS processes at the logical level are described in a formalized language and implemented using standard technologies for collecting, converting and storing information in information and communication systems. The physical level considers the implementation of CPS designed or adapted to interact with the expected operating environment to achieve one or more intended goals while respecting the limitations of the system. Communication between the logical and physical levels is carried out using converters: various sensing device, sensors that collect data about the physical state of the cyber-physical environment, the interpretation of which may be used to change the logical state of the system, as well as actuators that can influence the physical state of the environment. It is the converters that play a central role in CPSs, ensuring the interaction between physical and logical components.

The main categories of components in CPSs are logical components, physical components, users, and converters, which include sensors and actuators.

Vatamaniuk and Iakovlev [38] presented a generalized set-theoretical model of CPS in their paper:

$$CPS = \langle Ph, Lg, Sens, Act, Hum \rangle \quad (1)$$

where *Ph* is a set of physical components;
Lg is a set of logical components;
Sens is a set of sensors;
Act is a set of actuators;
Hum is a set of persons involved in the processes of CPS functioning or located within the cyber-physical environment and being the end users of the system.

According to the researchers, each functional component of CPS should have the following capabilities [39]:

- availability of computing power and software necessary to implement its own functions;
- availability of sufficient memory to store all the data necessary to carry out its own activities;
- ability to establish network connections with other system components and ensure targeted data transfer;
- ability to obtain and collect the necessary information about the state of the environment and other components of the system;
- ability to perform self-diagnosis in the context of identifying its own malfunction, as well as to inform related components in the event of such a malfunction.

Thus, each functional component is associated with a specific set of sensors, actuators and persons.

There are many architectures for CPSs. Highly detailed 5-level CPS configuration is presented in [34]. CPS consists of two main functional components: 1) advanced connectivity providing real-time data collection from the physical world and feedback from cyberspace; 2) intelligent data management, analytics and computing capabilities that form cyberspace. Figure 3 presents a generalized architecture of CPSs.

"Communication/Connection" is the first level towards achieving integration using such elements as sensors, actuators and protocols. Connections are necessary to create complex systems such as enterprise resource planning

(ERP), customer relationship management (CRM), and supply chain management (SCM).

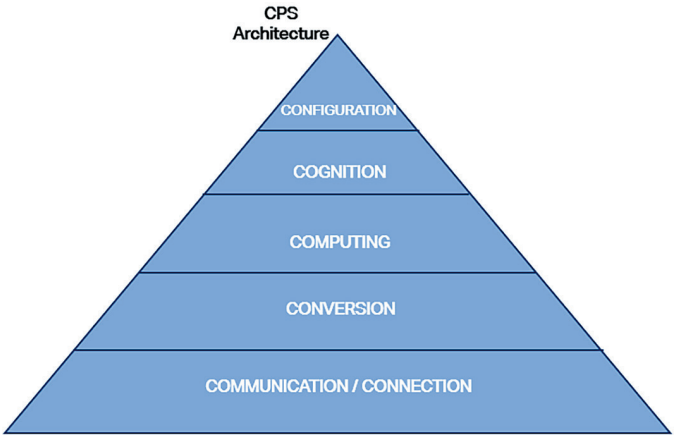

Figure 3. Generalized architecture of CPSs

"Conversion" is the second level. Interferences are filtered out based on information obtained from such sources as big data analytics and cloud computing.

At the third level, algorithms, software and computer infrastructures are used to analyze current data and predict the future behavior of the system or process.

"Cognition" is the fourth level that represents the knowledge collected at the above stages for decision making.

"Configuration" is the final, fifth level. There is a transformation of intelligence into action (moving from cyberspace to physical world).

The very first and simplest structure is proposed by Zachman [40] and shows that information should affect not only internal resources, processes and personnel, but also external resources of the organization. Along with this, Wolfert et al. [41] proposed a general information integration method based on service-oriented architecture (SOA) in agrifood supply chains. Närman et al. [42] proposed an analytical modeling method using a hybrid probabilistic relational model. Le and Wegmann [43] proposed hierarchical-oriented modeling with all stakeholders. Săsă and Krisper [44] proposed information-based analysis of business process support based on a systematic review of important aspects. Mamaghani et al. presented in their work [45] a conceptual model of enterprise IT architecture using Shannon entropy. Wang et al. [46] recommend using a hybrid multi-agent negotiation protocol in the implementation of virtual enterprises.

Food system applications

Digital engineering technologies are increasingly adopted by the food and processing industries and agriculture. This is not only due to the use of sophisticated robotic technology in carrying out technological operations and processes, but also due to the use of engineering concepts in relation to the food product.

It should be noted that food production based on CPSs is a very complex heterogeneous system including several types of physical systems and many computing and communication models.

Cyber-physical system of the food system life cycle should integrate smart technologies at each stage using various tools.

Smart technologies include distributed CPSs, open API (application programming interface) and fog computing network.

From a hardware point of view, a distributed system is a set of interconnected autonomous computers or processors; from a software point of view, it is a set of independent processes (executable software components of a distributed system) interacting by messages to exchange data and coordinate their actions.

CPSs are complex distributed systems driven or controlled by computer algorithms tightly integrated with the Internet and its users. The technological basis of which is the Internet of things (IoT) or "thin Internet" technologies. Thin Internet is a general term for the growing number of physical devices around the world that are connected to the Internet and, ultimately, to each other; a networked world of interconnected devices, objects and persons.

Open API is a new web technology such as Simple Object Access Protocol (SOAP). It is necessary for interoperability in the food supply chain, where CPS-managed service system may also adapt to any tracking in a managed services environment with another CPS through centralized system integration.

Fog computing, also known as fogging, is a new paradigm operating directly at the edge of the network that extends the capabilities of cloud computing running on machine-to-machine communications based on a large-scale and geospatially distributed programming model to efficiently operate a network of smart physical objects for the future Internet-applications without human intervention [47,48].

There are a lot of studies and scientific publications related to tracking food products throughout the supply chain. Aung and Chang [49] provided in their study extensive information on safety and quality tracking in the food supply chain.

Pizzuti et al. [50] specify the description by presenting an ontology of forward (track) and backward (trace) traceability of food products (Food Track and Trace Ontology, FTTO). The main goal of the proposed FTTO is to integrate the most representative food concepts involved in the entire supply chain (SC) into a single ordered hierarchy capable of integrating and linking the main functions of food traceability. FTTO consists of four modules: food, services, processes and supply chain participants.

Kang and Lee [51] proposed and developed a new set of services called tracking services (TS) using the EPCglobal certified EPCIS system. EPCglobal Architecture Framework is a standard for connecting distributed RFID systems in the supply chain. The system allows tracing the entire chain of product movement and integrates with other systems including external ones.

Overall, it should be noted that companies gain a sustainable competitive advantage through the implementation of innovative food traceability systems [52].

In a review study, Suprem et al. [53] discuss the application of technology systems in agriculture and food processing, such as embedded computing, robotics, wireless technology, GPS/GIS (Geographic Positioning System/Geographic Information System) software and DBMS (Database Management System). The article describes: 1) soil sampling methods and their application; 2) mapping fields and yields using GPS and GIS; 3) harvesters and future research in robotic harvesters; 4) food processing and packaging technologies, such as traceability and RFID tags; 5) application of a sensor network; 6) data management and execution systems; 7) automation and control standards.

A review article by Bosona and Gebresenbet [54] on the modern food supply chain concludes that future research should be focused on: 1) integrating food traceability with logistics; 2) technological aspects of FTSs (Food Traceability Systems); 3) connections between the traceability system and food enterprises; 4) standardization of data collection and information exchange; 5) awareness raising strategies; 6) continuity of information flow and effective communication of traceability information to consumers and other stakeholders; 7) connections between different FTS drivers; 8) strategies for improving FTSs; 9) development of systems for assessing the effectiveness of FTSs.

Recalling food products, especially perishable or gourmet foods, is extremely expensive for a company. It is associated both with direct financial and reputational losses. A study by Piramuthu et al. [55] illustrated the importance of more details in both forward and backward traceability. The appropriate levels of responsibility between participants in the production and supply chain are determined depending on the identification speed of contamination and its source. The recall of contaminated products is tracked using radio frequency tags.

RFID tags have been successfully applied and described by Parreño-Marchante et al. [56] to trace the movement of aquaculture. RFID tags are combined with environmental data collected through wireless sensor network (WSN) infrastructure. By reducing the time spent on monitoring, the company's performance (in a pilot project) increased by 89% to 95%.

Olsen and Aschan [57] describe a method of process mapping (a graphical description of the material and information flow of value stream creation in the form of flow charts using special symbols and a description of data obtained from measurements or statistics) in a processing plant throughout the entire traceability chain. This method helps to standardize company reporting and supply chain reporting, and also allows for comparisons and benchmarking. The study focuses on identifiers and conversions. Once the process has been mapped and systematic information loss have been identified and bottlenecked, new or improved software should be installed to improve the food traceability system.

Using a vegetable supply chain traceability system as an example, Hu et al. [58] use the Unified Modeling Language along with a set of suitable templates, i. e. a series of Unified Modeling Language class diagrams.

Lack of process automation is the main reason why special high-resolution tracking tools are difficult to implement. Lavelli [59] describes a promising algorithm for implementing traceability in enterprises with a low level of automation.

Based on the above, in a food traceability system based on CPSs, it is necessary to coordinate the actions of internal and external parties, i. e. both network and distribution systems must be used.

Example of a food supply chain monitoring and traceability system

Food production based on CPSs includes all elements that have industrial automation capabilities. This includes an intelligent system model, intelligent programmable logic controllers (PLCs), sensors, actuators, cameras, subsystem control units, etc.

A three-layer food production system based on CPSs includes: 1) physical level with cyber capabilities deeply embedded in physical processes; 2) network level to strengthen cybersecurity; 3) service level for distributed operational services.

Chen [35] proposed intelligent CPS for food production based on the value stream, the diagram of which is presented in Figure 4.

The food traceability system in the supply chain is divided into 5 levels: 1) operational level; 2) level of traced points; 3) level of details; 4) level of stakeholders; 5) technological level (process level).

The first level (operational) consists of transport, packaging, production or processing of the food product with a traceability process in place. From raw materials to the sale of a product to the consumer, more and more detailed traceability information needs to be collected at the stakeholder level.

The final, fifth level (receipt of raw materials) describes: sowing/irrigation for the farmer, growing agricultural crops, animals and poultry for the manufacturer, slaughter/meat processing for the processer, harvesting/packaging for the distributor, processing/repackaging for the retailer.

In Russia, the life cycle of a food product differs from that presented in Chen's work [35].

Thus, a value stream management (VSM) model is presented, i. e. a cyber-physical systematic approach integrated into processes at the corporate and global levels using a fog computing network for traceability and improving the efficiency of the company. At the same time, a feature of VSM is the formation of a product based on the customer's request, the business capabilities of all participants in the chain and the customer's degree of satisfaction with the final product.

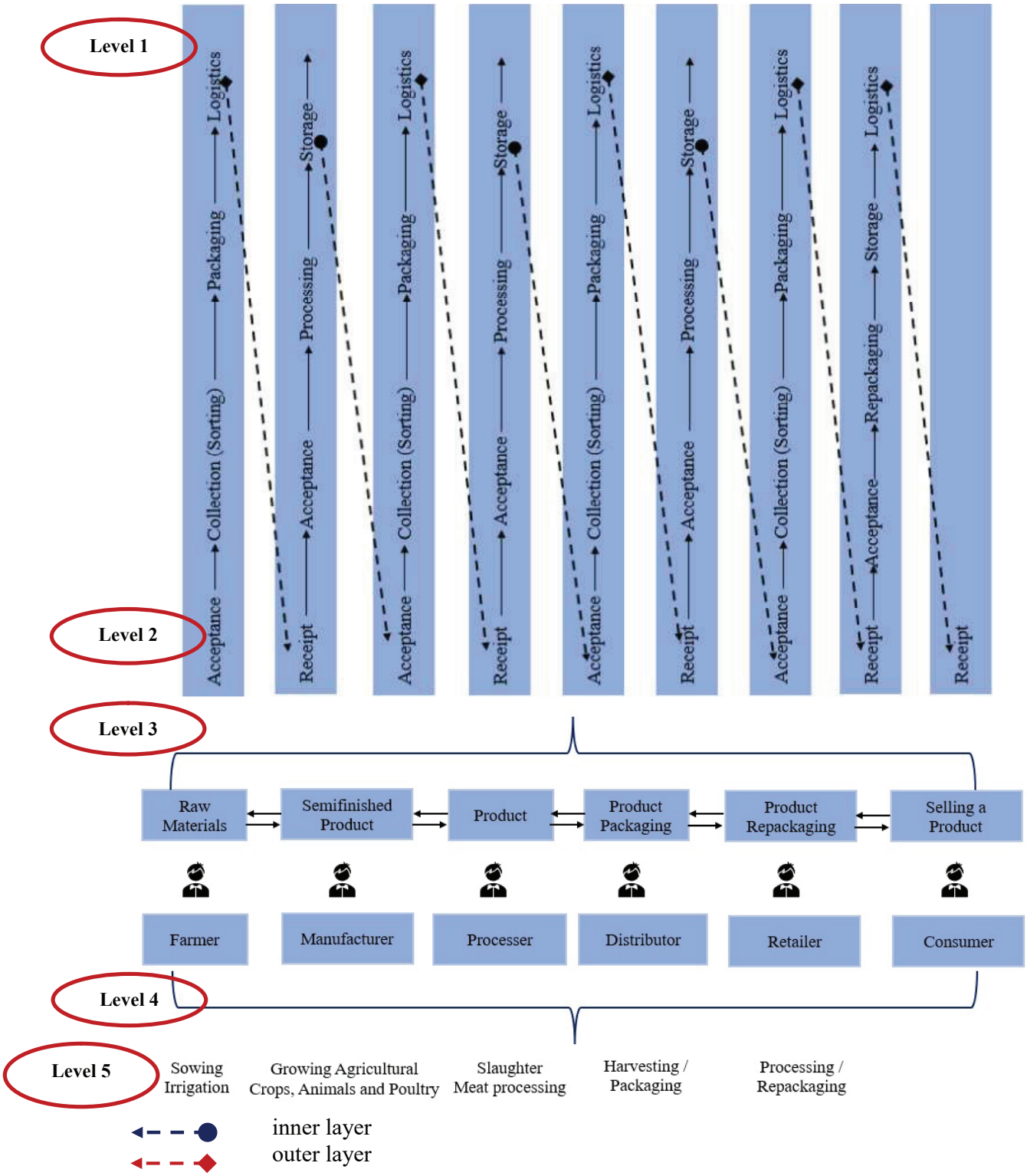

Figure 4. Hierarchical food traceability diagram

Conclusion

Digital transformation, or implementation of digital technologies into industrial production in general, and into the life cycle of food systems in particular, is expected to contribute to the sustainable development of industry. It is noteworthy that the use of cyber-physical systems in food production will help to increase the share of qualified personnel capable of creating and managing CPSs, opposite to mechanization and even automation of technological processes aimed at facilitating the work of lower-skilled personnel.

It is expected that at each stage of the food product life cycle (including meat products) specific tools will be used, from predictive analytics and big data analysis (Big Data, Data Mining), neural networks, fogging and artificial intelligence to the development of digital twins for food products (meat products), technological operations and the technological process as a whole. Due to differences in the stages of a food product life cycle (including meat products) and technological approaches at enterprises in Russia, the hierarchical traceability using CPSs must be adapted and adjusted for specific conditions.

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STRATEGIES FOR REPLACING SATURATED FAT IN MEAT PRODUCTS: A REVIEW

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Keywords: *healthy meat, saturated fat, fat replacer, vegetable oil, emulsion gel*

Abstract
This paper aims to provide a better understanding of how to replace saturated fat in meat products due to concerns about its high amounts as health consciousness improves and consumers look for changes. In particular, we focused on various approaches and technologies to replace saturated fat in meat products. A systematic literature review was conducted using Web of Science, Google Scholar, and Scopus based on existing papers. The use of vegetable oils in meat products, oleogel and emulsion gel technologies, as well as application of protein substitutes were reviewed. The results show that the mentioned methods are potentially effective techniques for reducing the saturated fat content of meat products. As research on new approaches to fat substitutes continues to attract interest, we would like to highlight the research needs for the development of healthy meat products in the long term.

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Introduction

Meat and its derivatives constitute crucial elements of the human diet due to their high-quality protein content and the presence of essential nutrients such as vitamins, indispensable amino acids, fatty acids, and water-soluble minerals, all of which are vital for human biochemistry and physiology [1]. Despite their nutritional value, concerns have arisen regarding the high salt and fat content in these products. To address the issue of excessive salt, an alternative method of processing meat raw materials involves high-pressure processing. This technique offers several advantages, including the creation of stable emulsions, enhanced water retention, prolonged shelf life, and intensified salty flavors [2]. The prevailing perception of meat fat as being high in saturated fatty acids (SFAs) has instilled caution, especially concerning red meat consumption, with concerns linked to adverse health effects such as obesity, type 2 diabetes, hyperlipidemia, elevated cholesterol levels, and cardiovascular diseases [3,4].

Consequently, a key focus of ongoing academic research is the development of healthier meat products. Central to this pursuit is the partial or complete replacement of saturated fats with unsaturated fats, aiming to create products that are more appealing to consumers. However, achieving this objective while preserving the desired sensory qualities inherent in full-fat meat products poses a formidable challenge [4,5]. This challenge is particularly daunting because fats play a pivotal role in enhancing the functional and sensory attributes of meat products, encompassing critical aspects such as texture, tenderness, flavor, and juiciness [6].

Given the rising demand for processed meat items among consumers, scientists specializing in meat science are actively investigating the potential and advantages of employing structured emulsions, such as oleogels and emulsion hydrogels, as viable fat substitutes in an array of meat products. These products include bologna, frankfurters, pâtés, fermented sausages, hamburgers, patties, and meat batters [7]. Meat emulsions consist of a delicate balance of fat, muscle proteins, water, salt, and other ingredients. The interplay between fat and proteins, acting as natural emulsifiers, alongside their chemical interactions, profoundly shapes the final product quality. Significant reductions in fat concentration, alterations in fat particle size, or imbalanced fat-to-protein ratios lead to compromised emulsification capabilities, directly affecting critical aspects such as flavor, aroma, and texture. Thus, a major challenge in making meat emulsion products, for example, frankfurters and bologna, lies in developing stable products that endure cooking processes without experiencing fat and water separation [8]. Concurrently, in the pursuit of healthier meat options, researchers are investigating a blend of olive and chia oil as a substitute for pork fat. Research has demonstrated that substituting a portion of pork back fat with oleogels and emulsion gel systems results in an enhanced lipid profile, marked by reduced saturated fatty acids and increased polyunsaturated fatty acids, ensuring lipid stability during storage [9].

The market for emulsion-based meat products is poised for rapid growth, as companies seize the opportunity to reformulate existing products for health benefits and innovate new, healthier alternatives [10]. Modern consumer

lifestyles demand that the meat industry meet evolving expectations, emphasizing the need to deliver increasingly healthier and cleaner meat products. This review aims to provide an academic overview of novel meat processing methods and technologies that reduce saturated fat and offers strategic implications for the industry.

Objects and methods

This review aims to provide an update on the state of research analyzing saturated fat in meat and meat products, with a focus on how new processing methods and technologies can reduce saturated fat in meat products. Below we describe in detail our search strategy, article selection methods, and data synthesis procedures.

Search strategy

For this review, we searched six databases in the areas of food science, food and nutrition, social sciences, and practical sciences, following PRISMA flow guidelines: PubMed, Scopus, ResearchGate, and Google Scholar. The following keyword sets were used as search terms: (a) meat products and saturated fat, (b) meat saturated fat replacement technologies, and (c) meat saturated fat substitutes. Figure 1 is a flowchart showing the process of selecting studies for inclusion in this review.

Eligibility criteria

Articles used in this review had to meet the eligibility criteria, which included selecting studies on saturated fat in meat products, nutritional properties of saturated fat in meat, meat saturated fat replacement technologies, and meat saturated fat substitutes.

Screening and data extraction

Articles were included in the corpus if they (1) investigated the characteristics of meat saturated fat, (2) included meat saturated fat substitution technologies, (3) included consumer perceptions of meat saturated fat, (4) included all types of meat saturated fat substitutes, (5) were peer-reviewed, and (6) were journal articles or conference presentations. We excluded articles that (1) did not target animal fats, (2) did not investigate saturated fat substitutes, or (3) did not introduce a new technology or substance as a means of replacing meat saturated fat. We considered a range of article types, including original articles, full-text articles, Internet articles, summary reports, and series, and did not place restrictions on publication date or language. Exclusion criteria included inaccessible full text, full text without raw data, inappropriate topics, and doctoral dissertations, and we searched through the ProQuest Dissertations and Theses global database.

Study selection and data extraction

A literature review approach was used to select a total of 402 references from the major journal search sites PubMed, Google Scholar, ResearchGate, Medline, and Scopus using the PRISMA flowchart, which resulted in the final selection of 52 articles from a total of 402 articles. The PRISMA flowchart is shown in Figure 1.

Results

Consumer demand for healthy food is steadily increasing around the world. Processed foods high in sodium or fat are criticized for their health effects as consumers' lifestyles and nutritional structures are changing due to a better understanding of the relationship between nutrition and health, and knowledge of food processing is expanding [11].

Vegetable oil as a fat substitute

Beef and pork fat are rich in saturated fatty acids, making them a concern due to their association with coronary artery disease resulting from elevated triglyceride levels in the bloodstream. To create healthier meat products, successful strategies involve substituting or reducing beef or pork fat while enhancing the fatty acid profile [12]. However, from a technological perspective, reducing fat levels in meat emulsions poses challenges, as significant reformulation can detrimentally affect attributes such as flavor intensity and tenderness [13].

One promising strategy involves the integration of vegetable oils, such as olive, soy, sunflower, flax, rapeseed, or marine oils, as fat substitutes. Olive oil, which is widely consumed in the Mediterranean area, has associations with a reduced risk of specific diseases [16]. Approaches involving the incorporation of olive oil, either partially or completely replacing traditional fats in meat emulsions, provide a pathway to align with recommended fatty acid guidelines, yielding meat products that offer enhanced health benefits to consumers [17,18].

This approach provides a compelling avenue for the development of meat products that align with both the technical demands of production and the health-conscious preferences of consumers. In experiments involving a beef model system, the incorporation of olive oil yielded positive outcomes by altering the lipid composition, increasing levels of monounsaturated and polyunsaturated fatty acids, and decreasing the saturated fatty acid content. This not only reduced the overall fat content but also resulted in a meat system that exhibited enhanced oxidative and technological stability. Samples containing olive oil showed lower fat and jelly separation, along with higher water retention compared to the control samples [19]. Likewise, in a research endeavor focused on improving product quality, scientists opted to replace beef fat with an inverted emulsion system comprising olive oil and carrot powder. This replacement led to an increase in unsaturated fatty acids and a decrease in both monounsaturated fatty acids and total fat. The incorporation of carrot powder played a crucial role in maintaining the appropriate technological and oxidative characteristics of the resulting emulsion samples [17].

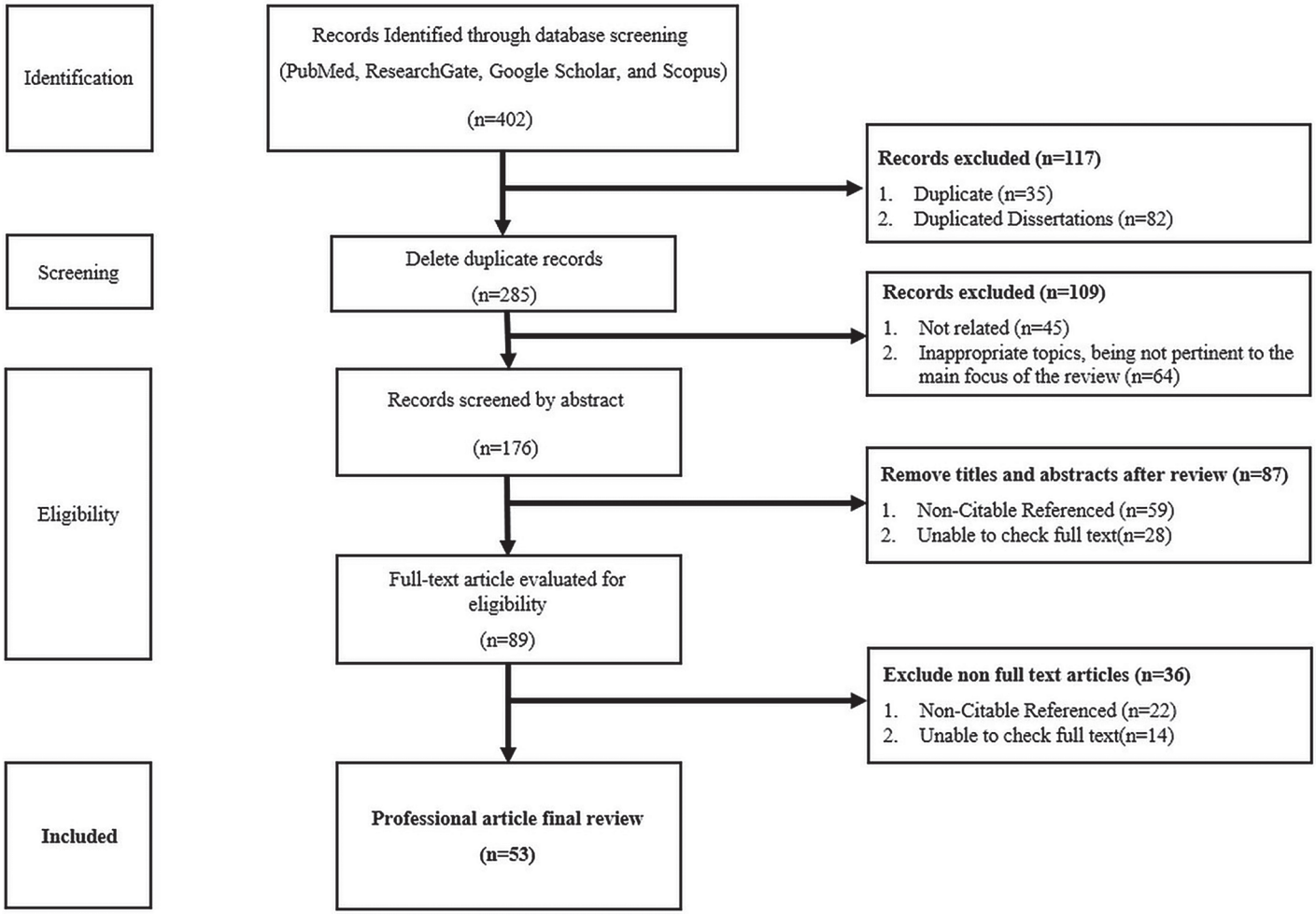

Figure 1. PRISMA flow chart for literature review search results

The increasing awareness among consumers regarding the relationship between food choices and health outcomes has spurred the rapid evolution of functional foods and generated heightened interest in "superfood" products [20]. Acai berry oil, despite its abundance in polyunsaturated fatty acids [21], possesses noteworthy antiproliferative and anti-inflammatory properties without causing genotoxic effects [22]. Additionally, the oil is rich in polyphenolic compounds such as vanillic acid, ferulic acid, catechins, and syringic acid [23,26].

Regarding the analysis of lipid oxidation in foods with acai oil as a fat substitute at 0% (CO), 25% (S-25%), 50% (S-50%), 75% (S-75%), and 100% (S-100%) substitution, the findings (S-100%) revealed that structuring the supplementary fat in the shape of a lyophilized hydrogel emulsion encapsulating acai oil efficiently slowed down the oxidation process [26]. Lipid oxidation, a crucial factor affecting the specificity and sensory quality of meat products, leads to an expanded range of flavors, including undesirable rancidity, while diminishing color parameters and altering texture. These changes ultimately impact consumer acceptance of the product [24,25]. However, in the case of foods with acai oil as a fat substitute, the lipid oxidation values recorded after 7 days of storage (ranging from 0.279 to 0.429 mg MDA/kg sample) were significantly below established thresholds. Moreover, this fat replacement strategy positively influenced the nutritional profile of food by reducing saturated fatty acids (SFAs) and increasing polyunsaturated fatty acids (PUFAs). Notably, the fat replacement approach significantly decreased all analyzed texture parameters, including hardness, springiness, and cohesiveness. These findings highlight that freeze-dried hydrogels encapsulating acai oil can effectively substitute up to 50% of animal fats, thereby enhancing the health benefits of the resulting food product [26].

Protein-based hydrogel emulsions

Alternative substitutes for saturated fats can be effectively incorporated into hydrogels or oleogels [27]. Although oleogels have been thoroughly studied as substitutes for saturated fats, their broad utilization in the food industry faces challenges due to their expensive production processes and the negative impact of organic gelling agents on fatty acid profiles, primarily due to the high polymerization temperatures required. In contrast, emulsion hydrogels offer a more cost-effective solution compared to oleogels and do not necessitate high temperatures for production. Hence, hydrogel emulsions are utilized to immobilize or encapsulate compounds sensitive to heat. Moreover, the oil content in these emulsions does not exceed 50%. The application of such fat substitutes has the prospect of improving the fatty acid composition

while simultaneously decreasing the total fat content in food products [27,28].

Emulsions can be structured in the aqueous phase, oil phase, or at the interface. However, the majority of research has concentrated on aqueous-phase continuous emulsions, neglecting oil-phase continuous emulsions. Emulsions formed with oleogels have substantial potential as replacements for trans-fats and for reducing saturated fats in fat-based food products. These emulsions are generated through mechanical forces that encourage the formation of small droplets, complemented by the use of compounds that act on interfaces, thereby reducing interfacial tension [29].

Numerous strategies have been developed to decrease the fat content and enhance the lipid profile of meat products. Among these approaches, the production of fat substitutes using hydrocolloids, emulsification, encapsulation, or gelation of oils has emerged as a prominent method. Utilizing gels enables the creation of fat replacements that exhibit properties similar to those of animal fats, such as comparable rheological, physical, and appearance characteristics. However, these substitutes have a significantly healthier lipid profile due to the incorporation of polyunsaturated oils [30].

Protein-based emulsion gels employed in meat products frequently contain soy protein and sodium caseinate (SC) due to their high nutritional value and their capacity to emulsify, thicken, and form gels [31,34]. Soy protein acts as a surfactant, lowering the interfacial tension between oil and water, thus improving the stability of emulsion gels [32]. Research indicates that soy protein isolate (SPI) can be employed to create emulsion gels with robust freeze-thaw stability and desirable rheological properties when combined with NaCl, underscoring its potential as a fat substitute. However, achieving consistent hardness and gel strength in emulsion gels using proteins proves to be more challenging compared to polysaccharide or protein-polysaccharide complex-based gels [33]. Marie-Christin Baune et al. [35] assumed that increasing the internal phase (oil) content beyond 50% and elevating protein concentration could enhance the rigidity and viscoelasticity of emulsion gels. They investigated commercially available proteins isolated from soy, pea, or potato to create pH-neutral (6.5) and heat-resistant (72 °C) emulsion gels as substitutes for solid animal fats. Their experiments revealed that legume proteins, involving both interfacial and protein-protein interactions, improved structural integrity. The firmness correlated with the cysteine content and interactions were of electrostatic, hydrophobic and hydrophilic nature. Potato protein formed the least stable emulsion, but a stable gel was formed. In general, legume proteins seem more promising for making stable solid animal fat substitutes with a neutral pH that can be shaped and stored for a long time. However, further analysis is needed to provide more accurate information, and research into the effects of salt, which can fuse into emulsions when added to foods, should also be watched carefully [34,35]. Figure 2 provides an overview of the primary gel types used to replace saturated fat in meat products.

Oleogel systems

Oleogel systems are emerging as a viable alternative to traditional fats, which are rich in trans and saturated fatty acids. These systems involve structuring lipids, primarily unsaturated triacylglycerols found in liquid vegetable oils or semi-solids, into gels [36]. Oleogels essentially comprise a liquid organic phase structured by a gelling agent, forming a three-dimensional network capable of capturing oil. Remarkably, oleogels exhibit thermal reversibility and behave akin to solid fats, even in the presence of high levels of unsaturated fatty acids. Importantly, the process of oleogel formation does not necessitate any chemical or structural

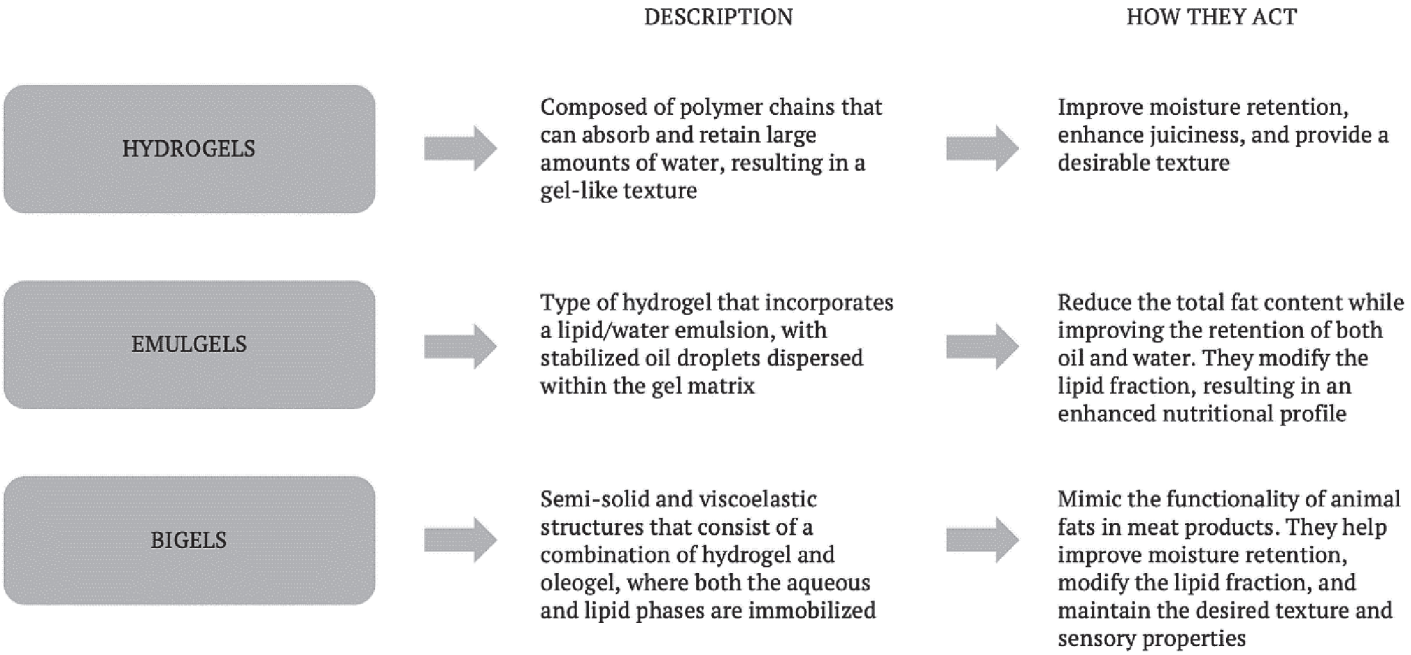

Figure 2. Examples of foods with oleogel [30]

alterations in the triacylglycerol molecule. Consequently, the nutritional properties, unsaturated fatty acid content, and natural regional distribution of the oil used can be preserved [29].

Oleogels are commonly produced by dissolving gelling agents, such as waxes, fatty acids, fatty alcohols, monoacylglycerols, and phytosterols, in vegetable oils at low concentrations. This mixture is then heated above the melting point of the gelling agent and subsequently cooled to form a gel structure [37]. Soybean oil, high-oleic sunflower oil, olive oil, and palm oil are considered viable options for oleogel production because of their advantageous composition, widespread availability, and cost-effectiveness [36]. The amount of a gelling agent needed for gelation decreases with the higher saturation level of the oil. An ideal gelling agent is one that can structure liquid oils at low concentrations, is considered safe for consumption, and exhibits thermally reversible properties. Studying these properties of gelling agents at different concentrations and different vegetable oils is essential for developing diverse oleogels tailored for specific technical applications [29].

Oleogels offer several advantages, including simplifying existing technological processes, producing trans-fat-free and low-saturated products, enabling the creation of low-fat products, and allowing for the enrichment of products with polyunsaturated fatty acids. Additionally, they contribute to cost reduction in manufacturing facilities. Nevertheless, these benefits are accompanied by challenges, including the necessity to adapt existing processes and facilities, preserve sensory attributes, achieve efficient structuring with minimal agents, navigate regulatory hurdles regarding the use of recognized safe structuring agents, maintain thermal and mechanical stability, manage oxidation concerns, and account for the seasonality of specific agents [38,39]. Research on W/O emulsions containing oleogels with sunflower seed wax in rice bran oil and esterified fats demonstrated intricate crystallization dynamics arising from variations in melting properties and chemical composition between the wax esters and fats. Consequently, sausages formulated with these rice bran wax oleogels exhibited properties akin to their fat-laden counterparts, showcasing similar firmness, chewiness, and elasticity while retaining their taste and aroma profiles [40]. Although a variety of food products are being produced from oleogels, further research is needed at various levels to fulfil the potential uses of oleogels. This includes validating the application of oleogels in food products to replace hard fats and understanding the overall impact of oleogels on digestion and metabolism. In addition, flavor development, texture, and physical properties of oleogels and oleogel-formulated foods need to be evaluated; these evaluations may play an important role in consumer acceptance of oleogels in foods. If the mentioned points are addressed, consumer acceptance of oleogel foods can be increased [39]. An overview of oleogel technology applications across various food products, extending beyond meat, is presented in Table 1.

Features of MP112 emulsion

MP112 emulsion, derived from mannoproteins and yeast polysaccharides, serves multiple purposes in the food industry, acting as a dietary fiber, emulsifier, and fat substitute. Created via enzymatic hydrolysis of microbial β-1,6-glucanase glueM, MP112 showcases impressive emulsifying capabilities when used in emulsion formulations. When incorporated into emulsified sausages, a meat product, MP112 emulsion significantly reduced the fat content while elevating the moisture and protein content without compromising organoleptic quality. Particularly noteworthy was its impact on texture that led to enhanced hardness, chewiness, and cohesiveness of sausages, especially at replacement ratios between 50 and 75%. Substituting animal fat with MP112 emulsion resulted in sausages exhibiting enhanced nutritional

Table 1. Applications of oleogels in food as fat replacements [39]

Food products	Liquid oil type	Organogelator type
MEAT PRODUCTS		
Frankfurter	Soybean; canola; sunflower	Rice bran wax; ethylcellulose; y-oryzanol and phytosterol
Meat patties	Linseed; sesame	Oryzanol and β-sitosterol; beeswax
DAIRY PRODUCTS		
Cream cheese and processed cheese products	High oleic soybean; soybean	Rice bran wax; ethycellulose and sunflower wax
Ice cream	High oleic sunflower; sunflower	Rice bran wax; y-oryzano and phytosterols
SPREADS		
Margarine	Soybean	Sunflower wax, rice bran wax, and candelilla wax
Spread	Sunflower; virgin olive and hazelnet	Shellac wax; beeswax and sunflower wax
CONFECTIONARIES		
Chocolate paste	Sunflower; pomegranate seed and palm	Shellac wax; monoglyceride, beeswax, and propolis wax
Chocolate Filling	Sunflower; hydrogenated palm kernel Rice bran and palm; canola	y-oryzano and β-sitosterol; ethycellulose Beeswax; hydroxypropyl methylcelluose and methylcelluose
OTHER APPLICATIONS		
Oleogels as carriers of bioactive compounds	High oleic sunflower; canola	Beeswax with β-carotene; ethycellulose with β-carotene

characteristics, marked by a higher ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) and a reduced n-6/n-3 ratio [41].

To further confirm the effectiveness of the MP112-based emulsion, composite gels of porcine myofibrillar protein and MP112 emulsion were prepared and their gel strength and water holding capacity (WHC) were measured. The results showed that both gel strength (penetration) and WHC improved exponentially with increasing amounts of MP112 emulsion added (10–20%, v/v). An increase in myofibrillar protein gel formation was observed after the addition of lipids and vegetable oils, suggesting that animal fat was partially replaced by mannoprotein MP112 with similar gelling properties [42]. Utilizing diverse emulsions as substitutes for animal fat emerges as an effective strategy to reduce the total fat content in various meat products by replacing fat with water [41].

On the other hand, ultra — high pressure treatment can be used to affect the MP gel, which has the advantage of improving the rheological properties of the MP gel and significantly improving the properties of the gel, creating a more digestible particle size and improving its nutritional value [43]. This idea finds validation in a study carried out by Kavuşan et al. [44], who made fresh chicken sausages using a gelled emulsion infused with black cumin and flaxseed oil. In this study, the chemical composition of uncooked fresh sausages displayed a clear linear relationship with the level of the gelled emulsion, emphasizing the prospect of fine-tuning nutritional attributes through strategic emulsion usage.

Discussion

Exploring diverse methods to substitute animal fats in meat products with non-animal fat ingredients reveals a range of physical forms during processing. These include powders, pastes, vegetable oils, combinations involving dietary fiber, and oleogels and emulsion gels. It is necessary to carry out a thorough analysis delving into ingredients, characteristics, techniques, mechanisms, merits, and drawbacks of these fat replacements. This comprehensive exploration is imperative due to shifting consumer perceptions regarding saturated fat in meat products. Among these approaches, oleogels and emulsion gels have emerged as particularly practical methods. They offer a healthier fatty acid profile compared to other techniques while preserving solid-like properties. Nevertheless, the research findings highlight the challenge of the high expense associated with the oleogel method.

Despite this limitation, oleogels excel in emulating animal fats by dissolving both hydrophobic and hydrophilic elements, while also exhibiting remarkable thermodynamic stability. Furthermore, they enable preserving the nutritional, physicochemical, and sensory attributes of meat products as well as economical manufacturing. Addressing the economic aspects of the oleogel approach is vital for its widespread adoption in the industry [45].

The challenge of replacing saturated fat in meat products

The ongoing trend in food innovation revolves around creating products tailored for vegetarians. However, developing viable vegetarian alternatives presents substantial challenges, both technical and societal. Meat products have unique characteristics related to the amino acid structure, peptide sequences, and intermolecular connections. It is exceptionally difficult to replicate these features in plant-based substitutes, especially concerning sensory aspects. The texture heavily relies on the minute particles that bind water. To preserve these properties, plant proteins must undergo various structuring processes, such as thermodynamic extrusion or shearing. Despite the attempts to alter the structures of plant proteins or improve their ability to retain moisture, significant obstacles remain. A notable example is juiciness, a distinctive characteristic of meat, originating from water absorption and the unique bonding of water with proteins and fibers [46].

Although plant proteins are frequently used as substitutes for meat proteins, they exhibit specific flavors that are absent in meat. For instance, the aftertaste associated with soy in legume protein products is believed to stem from secondary lipid oxidation products. Additionally, replicating the natural reddish or pink color of meat products without resorting to artificial coloring agents presents a significant challenge. This challenge is further complicated by preferences of consumers interested in vegetarian options, who often seek additive-free alternatives, which increase the technical difficulties for food developers [46].

What further unsettles consumers regarding meat protein substitutes is the absence of a clean label: vegetarian products often contain elevated levels of preservatives, stabilizers, colorants, or thickeners, which contradicts the preferences of individuals seeking natural and minimally processed alternatives [47].

Protein substitutes must also serve as nutritional replacements, providing sufficient nutrient density. However, these substitutes, derived from highly processed protein sources, often lack the same nutritional value as meat products sourced directly from the animal. This discrepancy arises because the proteins used in alternative products have already undergone significant thermal and other forms of processing. It remains uncertain whether replacing meat protein with plant-based alternatives might adversely affect human health by reducing the intake of essential components such as heme protein, zinc, or selenium, which are inherent to meat-derived products [48].

Codifying the nomenclature of animal alternatives

With the significant growth of meat alternatives, there is a lack of clarity on whether or not names similar or reminiscent of 'meat' should be allowed when referring to these foods, and how they should be recognized [49]. Recent

scholarly reviews, including investigations by Knappila et al. [50], discuss the categorization of protein-rich foods sourced from non-animal origins that were engineered to replicate meat and act as substitutes. These items are often referred to as meat analogs, meat substitutes, or meat alternatives. In line with Fiorentini et al. [51], products made from plant-based ingredients that replicate the sensory properties of meat are commonly termed meat analogs, vegetable meats, or imitation meats. Elzerman et al. [52], in their definition, categorize meat substitutes as products specifically formulated as direct replacements for meat, while they classify meat alternatives as other protein sources typically consumed in vegetarian diets, such as legumes or nuts. On the contrary, as outlined by Choudhury et al. [53], plant-based meat alternatives are viewed as sustainable protein sources capable of imitating the taste, texture, color, and nutritional composition of specific meat types.

Conclusion

Due to health concerns and changing consumer perceptions regarding meat foods, several techniques have been developed to reduce saturated fat in meat foods that are discussed in this paper. Among the ways to replace or reduce fat in meat is the application of emulsion technology using vegetable oils and fat replacer materials. Recent studies have shown their potential for reducing the saturated fat content and producing healthier meat products. However, there is a need for more extensive research into different saturated fat replacement methods and materials to ensure quality and achieve the texture desired by consumers. Future research should go beyond the development of different types of emulsions for saturated fat replacement in meat to evaluate blending methods and formulations that reflect consumer needs in real-world conditions. There is also a need to unify terms that name them for consumers as there are many different meat substitutes and methods.

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MOLECULAR GENETIC METHODS FOR IDENTIFYING RAW MATERIALS IN MEAT PRODUCTS: DIVERSITY, OPPORTUNITIES AND PROSPECTS

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Abstract
In the current economic situation, after easing the Covid pandemic restrictions, almost all laboratories, which are focused on evaluation of the conformity of food products, have faced issues in supplying for their laboratories. In this regard, in the last years many laboratories have been forced to validate new approaches and introduce new methods for assessing conformity of the food products. Very often it is not possible to use only one method to resolve the issue of the food product ingredients, especially for the purpose of traceability of their names and the used raw materials, listed on the label. Survey of the raw food materials to determine whether they correspond to the type name is a simpler task, in contrast to survey of the multicomponent food product. Many researchers have to estimate the opportunities and feasibility of application of various methodologies in their workplaces. Therefore, this review is relevant for the researchers in this field, as it focuses on aspects and special features of similar methodologies. The prospect of molecular genetic methods for identification of the raw materials used for manufacturing of meat products is presented below. This review also represents characteristics of methods for identification of the sources of raw materials used for the manufacturing of the meat products, based on the recognition of species-specific sections within the nucleic acids structures. The variety of methods (hybridization methods, polymerase chain reaction, different types of isothermal amplifications, methods using CRISPR/Cas systems), the principles of their implementation, and achieved analytical characteristics are considered. The capacities and competitive potential of various methods are discussed, as well as approaches being developed to overcome the existing limitations.

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Introduction

In recent years the intensification of interregional and interstate trade flows and the development of tracking information about manufactured and distributed commodities and products necessitate the methodical re-equipment of the tracking and control system to ensure compliance of food products with their declared composition. Unfortunately, the unfair producers remain interested in violating the declared composition of food products, including meat products, and using cheaper raw materials [1,2]. The significance of this issue goes beyond the incorrect informing of the consumers and unjustified increases in their expenses. Eating counterfeit meat products can be dangerous for health and also violate religious food restrictions [3–6].

In this regard there is necessity to expand the opportunities of obtaining data on the composition of meat products at all stages of their production and trade chains. It is extremely important that, along with the use of chromatographic, microscopic, electrophoretic and other analytical methods implemented in specialized laboratories and successfully solving the issues of confirmatory and arbitration control [7], the availability of simple and fast testing methods focused on widespread general laboratory equipment that does not require and special conditions for its implementation and professional training of the tests performers.

The observed dynamics of development of analytical methods proves the growing potential of molecular genetic assay as the methods of mass testing [8]. When solving issues of species identification, receptor molecules — oligonucleotides — are selectively bound due to complementary interactions of the nucleic acids contained in the tested samples, which are peculiar for the given organism. After this binding the following stages lead to the formation of intermolecular complexes, which include an enzymatic,

Table 1. Key review publications of the recent years on molecular genetic identification of the raw materials in meat food products

Article	Thematic specialization	Link
Authentication of meat and meat products using molecular assays: A review	General principles of molecular genetic methods, examples of their application	[9]
Market drivers and discovering technologies in meat species identification	Place of molecular genetic methods among other methods, diversity, integration with technological processes	[7]
A systematic review of DNA-based methods in authentication of game and less common meat species	Comparative evaluation of the development of different approaches based on bibliometric data	[10]
Current analytical methods for porcine identification in meat and meat products	Evaluation of molecular genetic methods in solving the problem of pork detection	[11]
Species identification and animal authentication in meat products: a review	Variety of molecular genetic methods, their comparison with alternatives	[12]
Authentication issues in foods of animal origin and advanced molecular techniques for identification and vulnerability assessment	Features of various practical problems, new methods	[2]

fluorescent, colloidal or the other type of the tags. The signal recorded due to this tag allows drawing a conclusion on presence in the sample of a biomaterial of the corresponding origin. In some cases the test result also includes a quantitative assessment of the content of the peculiar type of raw material [1,2].

Several reviews have been published describing the variety of already implemented developments and giving an idea of the principles of implementation and key differences between the various options of analysis — refer to the Table 1, summarizing the list of references and features of the material given in various reviews. However, against the background of the well-known polymerase chain reaction (PCR), as the historically first of the amplification methods for the selective detection of nucleic acids, the variety of methods still remains pretty poorly characterized, and the comparative evaluation of their advantages and disadvantages is not sufficient.

The purpose of this review is a unified comparative evaluation of the main groups of methods, description of their differences in their applicability for solving various issues, factors that limit the expansion of these methods, and the most promising directions for their prospective development. The properties of the main considered approaches and the results of applying the new methodological solutions are illustrated in the article with examples from the publications of the recent years, including the works of the authors.

Objects and methods

The object of the study was the developments of domestic and foreign scientists on the issues of molecular genetic control of the composition of meat products, presented in the articles and patents. The area of research included modern developments of analytical methods, features of their application in the identification of the species used as raw materials for the meat products. The search was run within the databases ScienceDirect, PubMed, Google Scholar, eLibrary, catalogs of patents of the Russian Federation, USA and EU, and the other publicly open electronic sources. Combinations of the keywords like control of the composition / ingredients, identification, detection, molecular genetic analysis, amplification analysis, hybridization methods, polymerase chain reaction, isothermal amplification, and non-amplification analysis were used. Keywords were used in English and Russian versions. In addition, thematically similar articles were searched also with the help of citation chains. Non-peer-reviewed, uninformative and duplicate sources were excluded from the results of the search; the same was done to the sources included in the search samples that were not related or just indirectly related to the topic of the research.

General issues in the development of molecular genetic analytical methods

The demand for nucleic acids as detectable targets is determined by the combination of conservative and varying (including species-specific) sectors within their structure, as well as the possibility to implement high-affinity interactions with complementary oligonucleotides, thereby ensuring high specificity of analytical methods [13,14]. DNA is present in all animal tissues and features very high stability when exposed to high temperatures during the production of meat food products [11]. These factors determine the using of DNA as a detectable target in the identification of raw meat materials.

When selecting target genes and DNA fragments as markers for evaluation the composition of products and identifying falsification, the conservation of the gene, its copy number, and the possibility of rapid extraction are taken into account. The listed requirements are well met by mitochondrial DNA (mtDNA) (regions of the mtDNA D-loop, cytochrome b genes (CytB), genes of subunits I, II and III of cytochrome c oxidase (COI, COII and COIII), genes of subunits 6 and 8 of adenosine triphosphatase (ATPase6 and ATPase8), genes encoding 12S and 16S ribosomal RNAs [15]. It is worth noting that mtDNA has a number of advantages compared to genomic DNA — more numerous copies, better accessibility for its isolation and the presence of conserved genome elements [16]. However, to detect counterfeits in the meat products, the markers related to genomic DNA are also used, for example, the genes for replication protein A1 (RPA1) [17], melanocyte-stimulating hormone receptor (Mc1r) [18].

Further in the review, the most significant approaches to DNA identification for the food industry are presented, reviewed and compared.

Hybridization methods

Hybridization plays a key role in detecting any type of DNA-DNA or DNA-RNA interactions [19]. The concept of nucleic acid hybridization was proposed in the 1950s, and in 1987 hybridization was used for the first time to identify the cooked meat [20,21]. Note that hybridization interactions occur in the course of all DNA identification methods, being necessary for recognition of target DNA. The hybridization methods discussed in this section differ from PCR and isothermal amplifications in that they are not accompanied by an increase in the number of target DNA copies. Therefore, these methods either use multicopy genes, or can detect rather high threshold levels of contamination, or should use special instrumental methods to ensure high sensitivity.

The mandatory stage of hybridization analysis is denaturation of the double-stranded DNA (dsDNA) target; usually it is thermal denaturation that takes place within the temperature range from 70 to 95 °C. After denaturation, specific recognition of the target by a complementary single-stranded DNA (ssDNA) probe occurs, carried out either in a heterogeneous or homogeneous format [22]. The formation of a complementary complex leads to the generation of a signal — electrochemical, fluorescent, colorimetric one (including visually detectable signal), etc. The scheme of the typical hybridization analysis is presented below in Figure 1.

In many developments nanoparticles with attached ssDNA probes are used to detect hybridization [23]. As far as identification of meat products is concerned, such methods demonstrate detection limits that are quite acceptable for practice: 6 μg/ml (pork) [24], 0.23 μg/ml (pork) [25], 4 μg/ml (pork) [26], 28 μg/ml (chicken) [27], 6 μg/ml (pork) [28], 12.3 ng/ml (horse meat) [29].

The principle of operation of this approach is well illustrated by the biosensor proposed by Ali et al. [25]. ssDNA was immobilized on the surface of gold nanoparticles (GNPs) to recognize pork gene fragment *CytB*pig mtDNA; The 3′-end was modified with a sulfhydryl group for immobilization on GNPs, the 5′-end was modified with tetramethylrhodamine (fluorescent label). The analyzed sample was heated to ensure denaturation of dsDNA, then incubated with the GNP-ssDNA hybrid structure. In the absence of the target fragment, the fluorescent label was located at the surface of the GNP and it showed no fluorescence. In the presence of a target DNA, the ssDNA probe attached to the surface of the GNP formed dsDNA with a complementary ssDNA target, which ensured distancing of the label and provided for fluorescence. The proposed biosensor made it possible to detect up to 1% pork in raw and heat-treated meat products. Due to using of short DNA fragment (27 bp) as a target, the analysis is possible even for samples that contain highly damaged DNA.

Hybridization methods serve as the basis of DNA microarrays, which are the clusters of dots on silicon or glass substrates with an ordered arrangement of ssDNA probes that differ in nucleotide sequence for target DNA recognition [30]. The detection limits of hybridization DNA microarrays range from 0.1% to 0.01% [31].

For the hybridization approach, the influence of processing of meat products on the identification of raw materials (chicken, pork, beef and horse meat) was evaluated [25,32]. It was shown that thawing and freezing did not lead to a significant decrease in hybridization. When exposed to high temperatures — 100–120 °C — signals fading was observed due to degradation of DNA, but the raw material remained identifiable. The type of analyzed tissue and pretreatment at high temperatures provided the great-

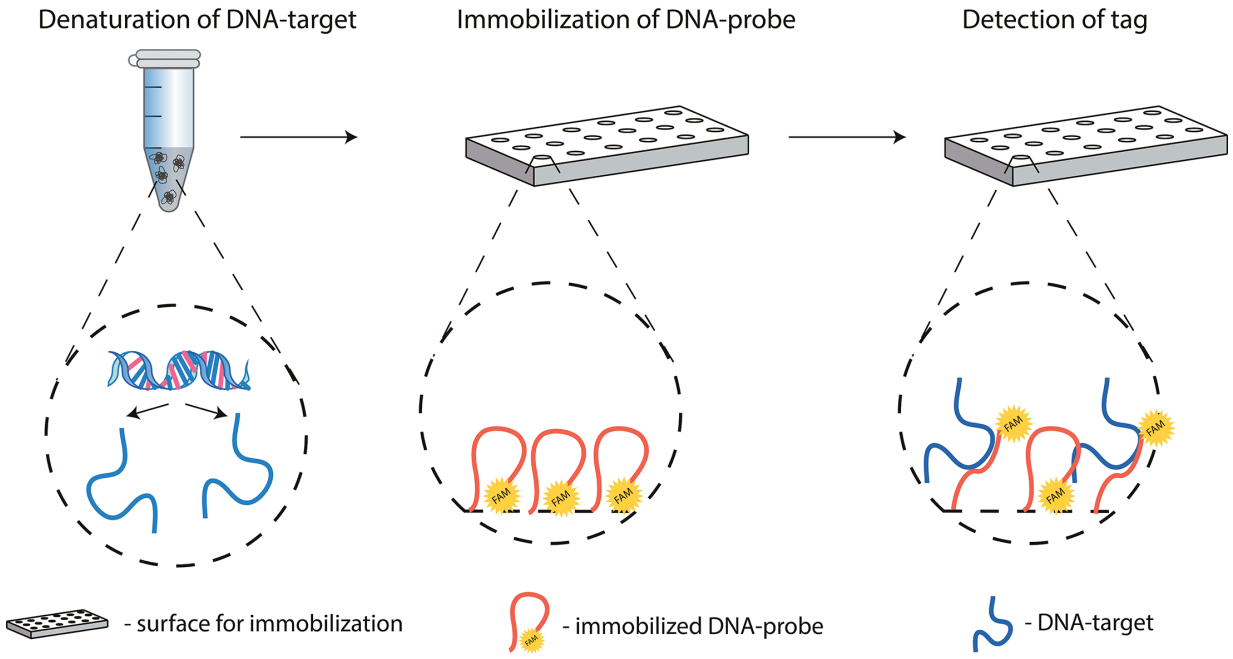

Figure 1. Hybridization analysis scheme (Source: Compiled by the authors)

est influence on the efficiency of hybridization analysis. Changing storage conditions had a limited effect, with the exception of storing meat at room temperature. The authors concluded that DNA hybridization provides a reliable basis for detecting animal species used in most meat products when the meat share exceeds 5%.

Summarizing the discussed results, we can conclude that DNA hybridization is effective for identifying the meat products. The use of nanomaterials opens up the new opportunities for more convenient, effective and low-cost application of this approach.

Methods based on polymerase chain reaction (PCR)

Today PCR and its variants are the most often used methods to detect meat and meat products counterfeits. It is explained by the sensitivity, simplicity and reliability of this method. PCR is based on increasing the number of copies of the target DNA through repeated cycles: high-temperature denaturation of the target DNA (94–98 °C), annealing of the primers on complementary single-stranded fragments of the target DNA (DNA-DNA hybridization) (50–64 °C), elongation — the polymerase synthesis of the DNA chain following the primer (72–80 °C). This canonical pipeline makes possible to produce multiple copies of the target DNA (amplicons) due to precise regulation of temperature. To visualize amplicons, it is possible to use electrophoresis in agarose gel (an approach that requires additional time and labor), fluorescent staining, or immunochromatographic tests (ICT). The latter option is a simple and promising approach that requires the use of a pair of primers with tags that shall be recognized by ICT with high specificity and sensitivity [33]. Figure 2 shows a scheme of the ICT for amplicon detection. Thus, visualization of PCR products using ICT made it possible to increase sensitivity by 10 times in comparison with gel electrophoresis and significantly reduce duration of the analysis [34–36].

Among DNA-based meat identification methods, the most popular and widespread are the following ones:

- PCR with endpoint analysis of the results. The results are recorded at the end of the PCR. Intercalating dyes or hybridization probes with a fluorescent label can be used as fluorophores [38]. However, the significant drawback is the inability to estimate the increment of

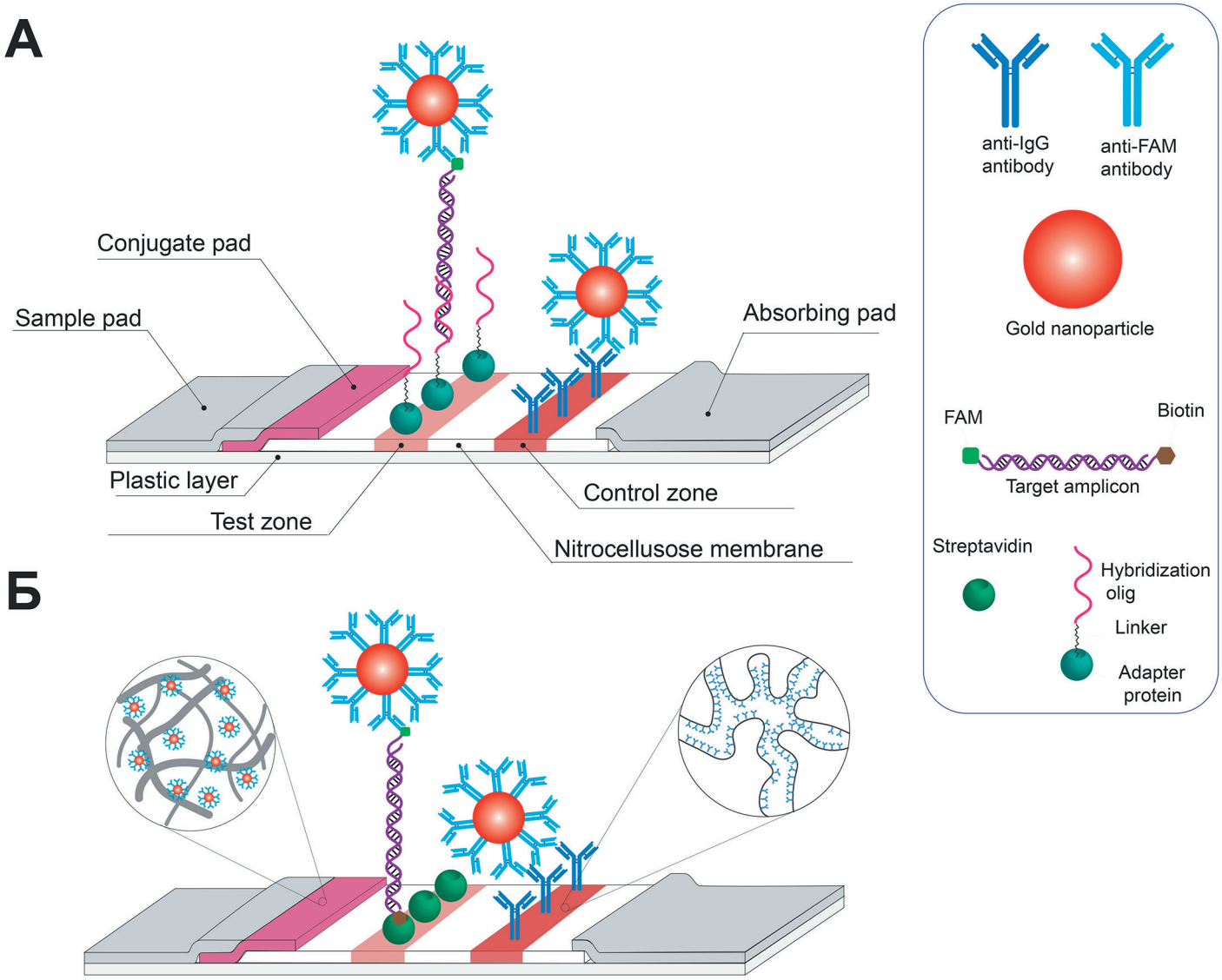

Figure 2. Scheme of an immunochromatographic test for the detection of amplicons (based on [37], with changes). FAM — fluorescein label

the fluorescent signal per unit of time at different stages of amplification, which can cause false positive results;

- Real-time PCR using SYBR Green. The fluorescent signal is detected during the amplification process [39–43]. SYBR Green is the most common intercalating dye. The drawbacks are the ability of SYBR Green to get bound to any dsDNA, and its inhibitory effect on the polymerase;
- Real-time PCR with TaqMan probe. The fluorescent signal is detected during the amplification process. In addition to the primers, the reaction mixture contains a hybridization DNA probe (TaqMan), complementary to the target DNA with fluorophore and fluorescence quencher at the opposite ends. DNA polymerase features exonuclease activity, due to which the annealed TaqMan probe is cleaved during the elongation stage, and fluorescence is recorded [44];
- multiplex PCR is the simultaneous amplification of two or more DNA targets in one tube. It is implemented by several pairs of primers, each of which is specific per one target [41,45–47]. Its efficiency depends on the specificity, copy number of each DNA target, and selection of the annealing temperature that is optimal for all primers' pairs;
- digital PCR in droplets. The PCR reaction mixture is sprayed into tens of thousands of tiny droplets. One microdroplet hits either one target or none. PCR takes place in the droplets (Figure 3 illustrates the principle of the analysis) [48–50]. The method allows determining the absolute concentrations of nucleic acids without the application of calibration curves. To implement the method, an emulsion generator and an amplifier are required.

These and other types of PCR are used to detect counterfeits in meat and meat products, thus showing high specificity, sensitivity and speed.

Isothermal amplification methods

For the recent years several alternatives to PCR have emerged that also increase the number of copies of the original target DNA, but capable to run at the same temperature. All methods that fit this definition are called isothermal amplification methods [37,51,52]. Isothermal methods do not only make amplification easier, but also allow detecting the product using simple tools and instruments, for example, membrane test strips — ICT (the scheme of a typical analysis that combines isothermal amplification and ICT is shown in the Figure 4).

More than ten types of isothermal amplification are known, the main ones are listed in the Table 2. The most common and promising types are recombinase polymerase amplification (RPA) [53], loop-mediated isothermal amplification (LAMP) [54], and rolling circle amplification (RCA) [55]. These methods can be implemented at a single temperature (LAMP ~60 °C, RPA and RCA ~37 °C) within 10–60 minutes depending on the sequence being recognized and the type of amplification. LAMP, RPA and RCA have been described with the very high score of sensitivities that are not worse than PCR and allow detecting single copies of nucleic acids. At the same time, each of these methods has advantages and disadvantages.

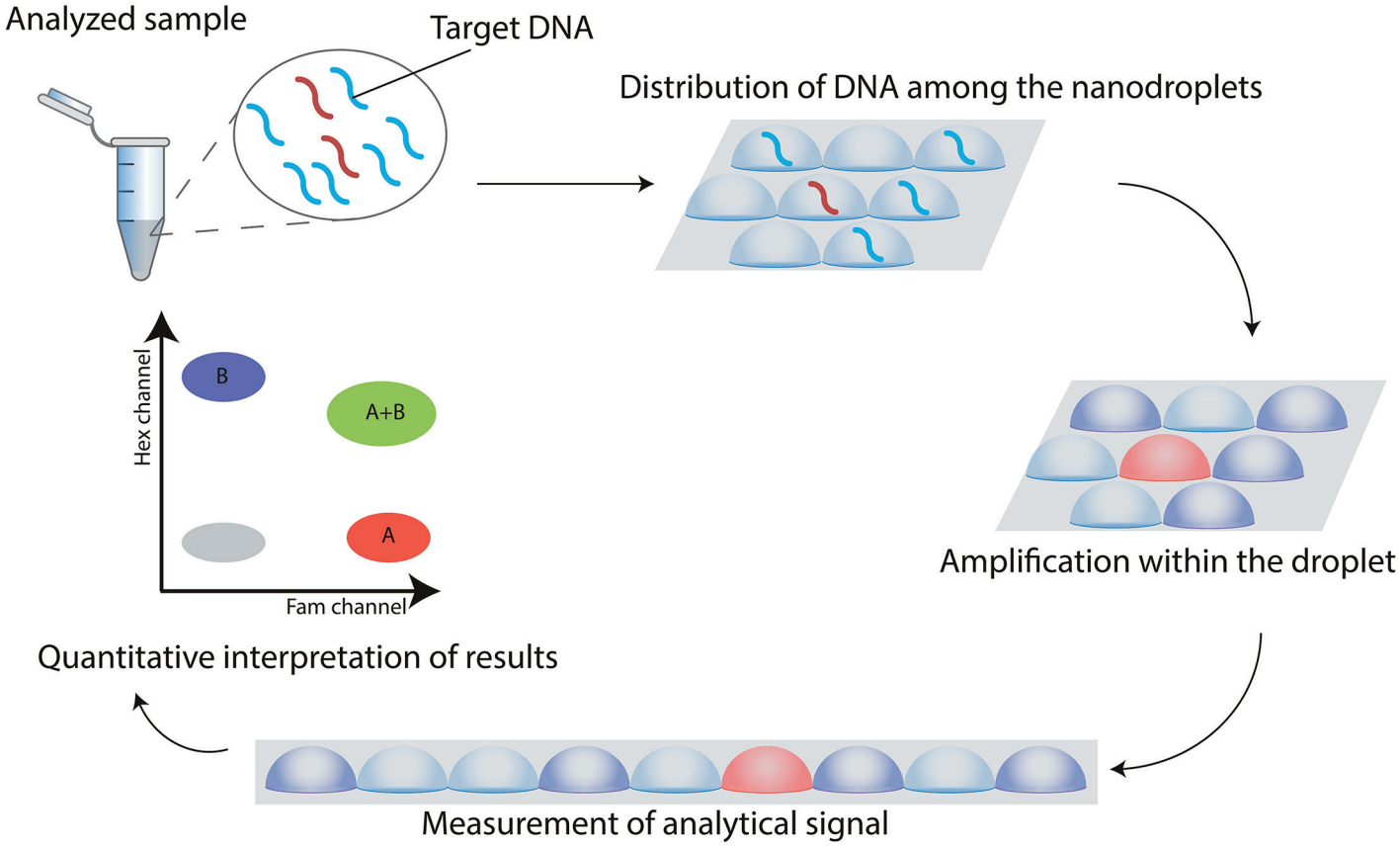

Figure 3. The principle of digital PCR in the droplets (Source: Compiled by the authors)

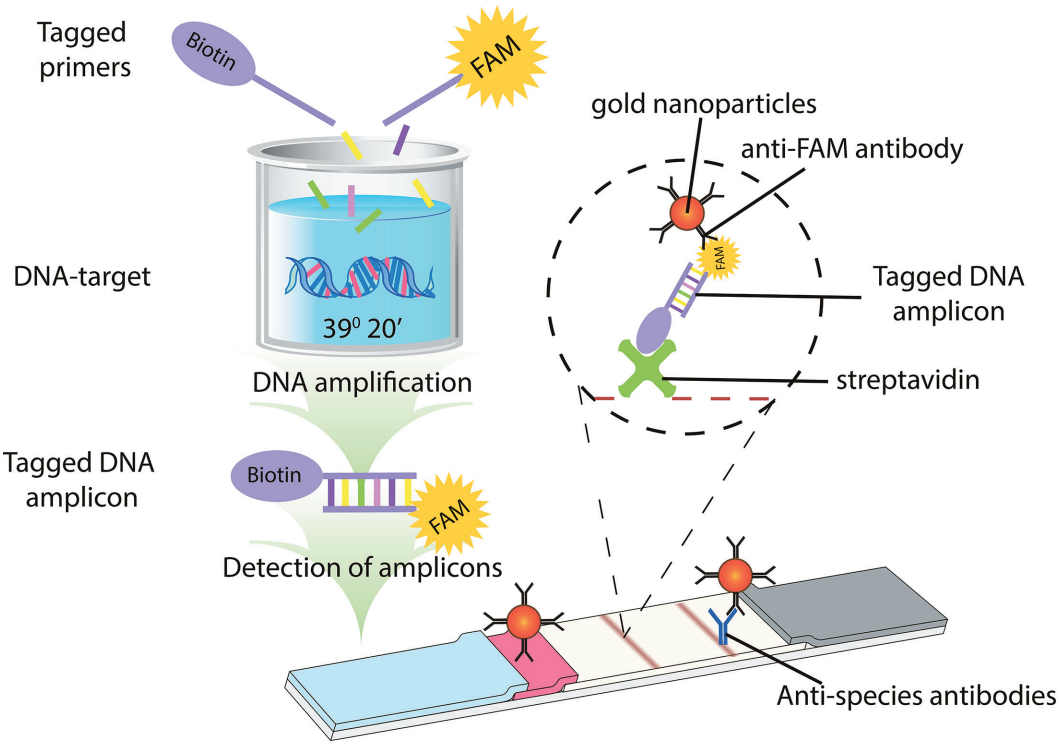

Figure 4. Scheme of an assay combining isothermal amplification and ICT fluorescent label (Source: Compiled by the authors)

Table 2. Comparison of isothermal amplification methods performances (based on [37])

Amplification type*	LAMP	RPA	RCA	NASBA	HDA	CRISPR/Cas
Parameter/property						
Temperature, °C	60	37	30	42	37	37
Duration, min	30–60	10–30	200–240	60–120	60–120	20–30
Specificity	+++	+	++	++	+++	+++
Sensitivity	+++	++	++	++	++	+
Non-laboratory analysis	+++	+++	+	+	+++	+++
Multiplex analysis	+	+++	++	++	+++	++
Resistance to inhibition	+++	+++	+	+	+++	++

* LAMP — loop-mediated isothermal amplification, RPA — recombinase polymerase amplification, RCA — rolling circle amplification, NASBA — nucleic acid sequence-based amplification, HDA — helicase-dependent amplification.

Thus, RPA is the simplest to implement, requiring only one pair of primers and a commercially available reaction mixture (manufacturer TwistDX, UK). As a result of RPA, homogeneous dsDNA product of a given length is obtained. The successful application of RPA in combination with fluorescent and colorimetric detection [56,57] or with ICT [58–65] for detecting the impurities in meat (chicken, duck, pig, etc.) has been described.

RCA reproduces circular DNA as multiple linear copies. The process starts with the presence of one primer and Phi29 DNA polymerase. RCA has great potential due to the variety of options for obtaining circular DNA by ligation [66]. This approach, combined with SYBR Green I fluorescent staining, was used to detect horse meat in beef food products (cytB gene) (limit of detection (LOD) = 63 ng/ml, 0.01% of horse meat in beef food) [67].

LAMP requires two or three pairs of primers and leads to the formation of fragments of various lengths [68,69]. LAMP is isothermal amplification, which is most widely used for identification of meat products. LAMP products can be determined by various methods, including recording the turbidity in the reaction mixture [70], running gel electrophoresis [71], measuring the fluorescence of a dye incorporated into DNA (intercalating) [72], indicating via metals ions [73,74], and monitoring changes in pH [75], detection based on the formation of pyrophosphate, visual detection using test strips [76], etc. Detection of LAMP products based on gel electrophoresis, turbidity measurements and intercalator fluorescence, despite examples of successful use for detecting the counterfeited food products [77], have limited prospects due to its duration or subjectivity in evaluation of the results. The use of primers with fluorescent labels significantly speeds up the detection of LAMP amplification products. So, Qin et al. [78], using LAMP in combination with fluorescence polarization to detect undeclared admixture of pork in beef food products showed high specificity and sensitivity of the 30-minute analysis.

pH-sensitive indicators such as phenol red, cresol red, neutral red and m-cresol violet can also be used to monitor the formation of DNA amplicons in LAMP. LAMP processes are accompanied by the accumulation of H^+ and, accordingly, decrease of pH in the reaction solution [79]. Thangsunan et al. [75] used neutral red as a pH indicator in LAMP with colorimetric detection in order to detect counterfeit in raw and processed meat products, which allowed achieving limit of detection (LOD) of 0.01% in poultry meat.

DNA targets amplified by LAMP can be visually detected with the help of fluorescent dyes or colorimetric tests. However, operator's interpretation of may lead to errors due to the turbidity of the tested sample or ambiguity in color changes. ICTs that detect amplification products with tags introduced through primers (see Figure 2 and Figure 4) minimize the subjectivity of evaluation of the results due to clear visualization of the test zone and control zone [80]. For example, Jawla et al. [81] developed a method combining ICT and LAMP for detecting the counterfeited admixtures in beef food products. Registration of its results via ICT provided the high sensitivity of this method [81].

Isothermal amplification methods deny the need for an amplifier, which allows for simple and fast out-of-laboratory analysis [51,72,77]. The preference of LAMP for identifying the authenticity of meat products in comparison with the other isothermal methods is explained by its low cost and the availability of enzymes. The main disadvantage of LAMP is the interactions between the 4–6 primers used, which can cause the formation of a nonspecific DNA product.

CRISPR/Cas system methods

Another promising tool for specific identification of meat products is Cas endonuclease-based recognition, the clustered regularly interspaced short palindromic repeats (CRISPR). CRISPR/Cas is a bacterial adaptive immune system but it can also be used for *in vitro* diagnostics. The discovery of collateral DNase activity (trans-cleavage) for Cas12a endonucleases has become the pivot point for diagnostics [82]. The principle of this approach is based on the acquired nuclease activity of Cas12a — when the Cas endonuclease is included into the complex with guide RNA (CRISPR RNA, gRNA), then if there is a dsDNA target (cis-target) complementary to the gRNA region in the sample, its recognition and cleavage occurs (cis-cleavage). Moreover, Cas this way acquires the ability to perform off-target collateral cleavage of any ssDNA (trans-targets). Over the past years, Cas endonucleases of various families (Cas9, Cas12, Cas13 and Cas14), that differ both in the structure of the protein and the structure of the required gRNA, and in the types of recognized cis-targets and trans-targets, have proven their efficiency for identifying DNA/RNA targets with high selectivity, being capable to recognize even the sequences with single nucleotide substitutions [83].

The CRISPR/Cas approach has also proven its efficiency in detecting adulterated meat products. Wu et al. used the CRISPR-Cas12a system to rapidly (30 min) detect pork DNA in mixture samples at 37 °C without prior amplification [84]. However, CRISPR/Cas systems, being used on its own only, often fail to achieve the required sensitivity. Therefore, to detect nucleic acids, they are supplemented with the other approaches, in most cases preliminary isothermal amplification is performed (Figure 5). Liu et al. combined Cas12a with RPA to detect adulterated beef, pork

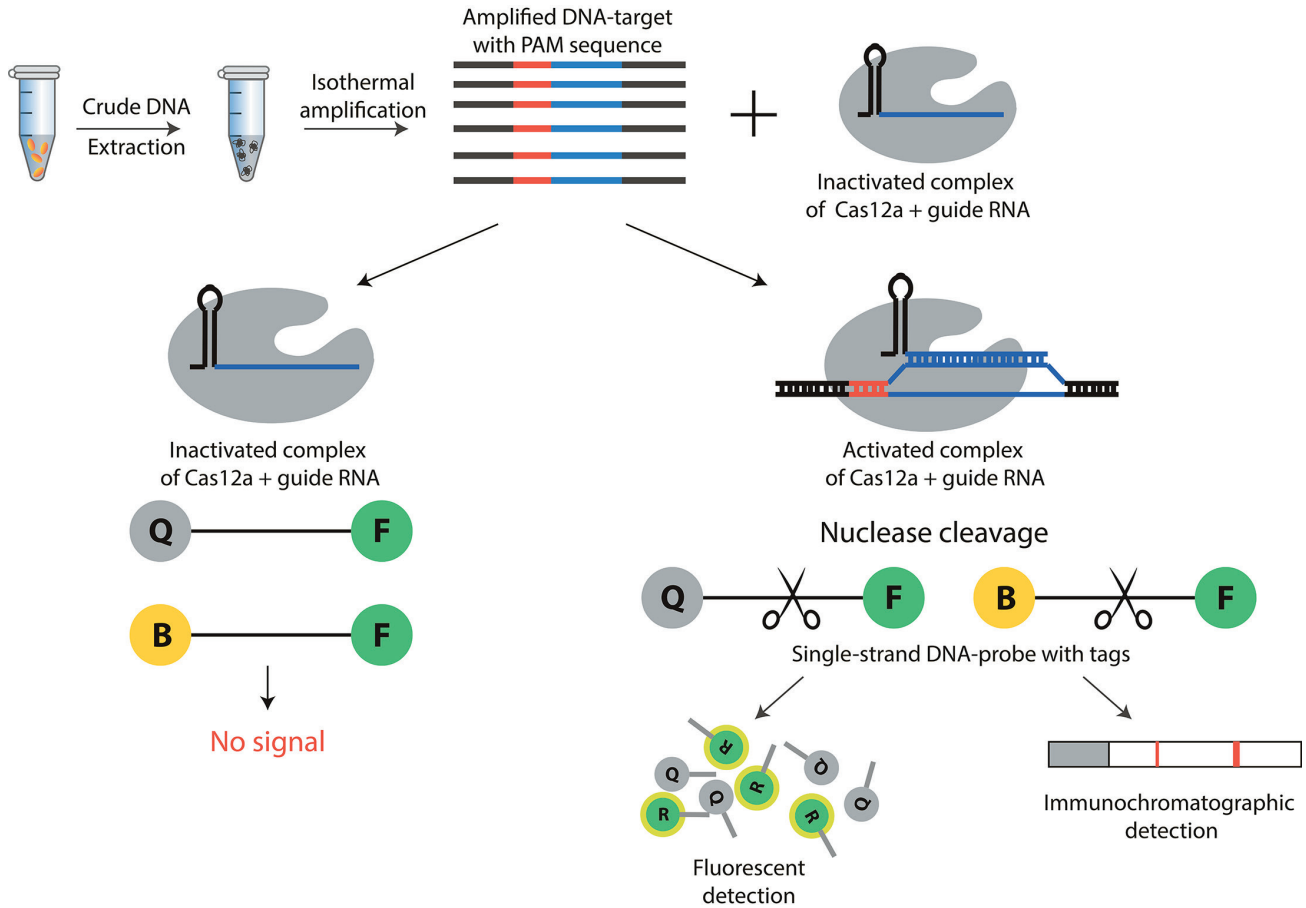

Figure 5. Scheme of analysis, including isothermal amplification, recognition of amplicons by the system CRISPR-Cas12a, fluorescent or immunochromatographic detection of single-stranded DNA probes cleaved by activated Cas12a
(Source: Compiled by the authors)

and duck meat (RPA-Cas12a-FS), achieving the limit of detection per 10 copies in 45 minutes [85]. In the research of Zhao et al. [86] the combination of RPA and CRISPR/Cas12a allowed detecting pork in the food composition, with a detection limit of up to 10^{-3} ng within 30 minutes, and also detect up to 0.1–0.001% share of pork in the meat products that were subjected to freezing, boiling and autoclaving.

In comparison with the other technologies the application of CRISPR/Cas systems requires minimal laboratory equipment, which significantly increases the efficiency of nucleic acid detection and the practical applicability of this approach. Disadvantages of CRISPR/Cas systems include the necessity for preliminary amplification to achieve high sensitivity. This necessity increases the risk of contamination and increases the duration of the analysis. Further progress, of this approach is associated with the development of a single-stage "closed" system that provides the necessary sensitivity. Nowadays only Cas12a has been used to detect adulterated meat products, while the capabilities of other Cas family proteins still remain undefined.

Molecular genetic methods are actively used to test and control the composition of meat food products. The fundamental feature of these methods is the absence of necessity for complicated instrumentation, as the testing does not involve fractionation and identification of sample components, but is limited to the registration of a tagged specific complex. To date, a number of commercialized analytical kits and methods is available and is included into the official recommendations of state and international regulatory authorities. However, the capabilities of molecular genetic testing are not fully implemented in practice. Actively progressing new developments, primarily related to isothermal amplification, move towards the autonomous analytical systems that can be used beyond the specialized laboratories. That significantly reduces labor intensity of testing and strives to autonomously functioning systems [87,88].

Conclusion

The aspects of molecular genetic research methods considered in the review are not the new direction in the field of test and control in the global laboratory practice. But they are practically not used in the Russian Federation, of course, with the exception of the PCR-based method. In connection with this, in our country all the considered methodologies have not yet been standardized. As a result of review of the recent publications, the characteristics and performance of the major considered approaches and the results of applying new methodological solutions were systematized. The analysis presented above will undoubtedly be useful for orientation in the current state of development of molecular genetic methods aimed at the species-specific identification of components in the meat products.

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COMPARATIVE ANALYSIS OF LOCAL PIG BREEDS IN CHINA AND RUSSIA

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Abstract
Pork is a favorite type of meat with a large share in the structure of consumption worlwide, including China and Russia. Pork production in China and Russia has been constantly growing over the last years. This type of meat remains to be in high demand due to its sensory properties despite consumer attitude to pork fat content. This review presents the short history of pig farming in China and Russia, as well as the modern trends in the development of this industry. The data on the pork production and consumption in two countries over the last years are compared. Characteristics that consumers consider important when buying pork and negative factors influencing consumer choice are described. Consumer properties of meat products from pork depend on keeping conditions and feeding of animals. Meat composition and functional properties depend greatly on pig breed. Information about pig breeds that are raised in China (depending on a region) and Russia, as well as indicators of productivity of pigs of certain breeds, are presented.

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Introduction

History of pig breeding

Domestication of pigs began 4–9 thousand years ago, taming 7–10 thousand years ago. The development of breeds dates back thousands of years [1]. It is believed that the progenitors of domestic pigs are European wild boar, which lives in the significant part of Europe, Northern Africa and certain regions of Asia, and Asian wild boar (often called Indian wild boar), which is found in the South and East Asia (China, Japan and others). The first group is known under the name of *Sus scrofa* and the second one under the names of *Sus orientalis*, *Sus cristatus*, *Sus vittatus* [2].

China

The history of pig breeding in China can be traced back to 9,000 years ago [3]. A variety of local pig breeds had gradually formed under the complex and diverse ecological environments and socio-economic conditions in China [4]. Before the 20th century, the local breeds accounted for the vast majority of pigs in China. The breeding of pigs was focused on environmental adaptation, disease resistance, meat quality, and local cultural aesthetic preferences and consumption requirement. As a result, many pig breeds with local characteristics were formed, such as the cold-resistant Northeastern native pig, the Jinhua pig, which is suitable for making cured ham, and the prolific Taihu pig [5].

From the 1950s to the early 1970s, China began to adopt intensive pig farming, and pig breeds were gradually optimized mainly through specialized breeding of boars [6]. In particular, a number of foreign breeds were imported, such as the Large White, Duroc and Pietrain pigs. At the same time, breed improvement was also carried out domestically, such as selecting and breeding on the basis of the Taihu pig, resulting in new breeds such as Sutai and Dianlu [7]. From the 1980s to the early 1990s, the pig farming in China gradually moved towards modernization, large-scale, and scientific management, and pig breeds were continuously crossbred, with the high-quality foreign breeds as the mainstay [8]. During this period, based on the comprehensive evaluation system, multiple high-quality pig

breeds were established in China, such the Sanjiang white pig, Hubei white pig, and Duhu [9]. Since the beginning of the 21st century, the pig breeding industry in China has paid more attention to bio-security and sustainable development, as well as the pig breeds with high quality and disease resistance [10].

During this process, the pig farming industry in China confronted many challenges and opportunities. In particular, China experienced an outbreak of swine fever in 2004, and then suffered the African swine fever in 2013 and 2018, which brought the great threat to the pig farming industry [11]. As Chinese economy grows, the demand for pork increases. In 2007, China signed a pork import agreement with the United States of America, and the price of pork constantly increased to a historical peak in 2011. Therefore, to stabilize pork prices in the market and prevent social instability caused by price fluctuations, the Chinese government announced the launch of the pig reserve system.

Russia

Based on the data of ancient literature sources, Russian scientists came to a conclusion that animal husbandry was more significant than hunting on the middle part of the territory of the future Russia and then the USSR as far back as the 1st millennium BCE. After studying ancient settlements and bone remains, researchers presented the following data on the main species of raised livestock: cattle 50.5%, horses 41.5%, pigs 4.05%, sheep and goats 4.0%. In the 2nd millennium CE the ratio between the livestock species changed: cattle from 20 to 60% depending on the location of settlements; pigs from 21 to 58%, sheep and goats from 12 to 35%; horses from 6 to 22% [12].

According to the classification of the German zoologist Hermann von Nathusius, stud breeds are characterized by the following traits: animals that are more productive with regard to the weight of the consumed part of the body (that is, meat and fat) have a shape of the body close to a parallelogram, their head and limbs are smaller, and they are more valuable for a household. Pigs of common breeds have a length of the head (from the eyes to the end of the face) that relates to the length of the whole body as 1:6, this ratio in stud breeds of pigs is 1:9 and sometimes 1:11 [13].

To promote pig farming in Russia, the government organized the state-owned farms, for which foreign breeds were brought into the country at the end of the 19th century. The most common were English stud breeds: Yorkshire and Berkshire. In the 1880s, over 6,000 pigs of the Hungarian breed, Tamworth breed (England) and several heads of the Spanish breed were brought into the country.

According to the census of 1898, the total pig population in the world was 100 million. The highest number was in the United States (about 40 million), the second place was occupied by Germany (14 million), the third place by Russia (11 million), followed by Austria-Hungary (10.5 million), France (6 million), Great Britain (2.5 million), Italy (2 million), Spain (2 million), Romania and the Netherlands (1 million each), Serbia (900 thousand), Denmark (800 thousand), Sweden and Norway (900 thousand) and others [12].

In Russia, there were 15,670,000 pigs in 1912 and 21,438,000 pigs in 1916 according to the data of the Veterinary Directorate. The First World War, revolutions of 1905 and 1917, and the Second World War led to problems with food, including a sharp decline in agricultural production. The period of animal husbandry recovery lasted up to 1953 [12].

The number of pigs was 28,341.3 thousand heads in March 2023, which was 5.7% higher than in March 2022. The vast majority of pigs (almost 93%) are raised in agricultural organizations, 6.5% in the households and 0.8% on farms [14].

Materials and methods

Sources. Articles were searched both on the Chinese, Russian and international information and analytical portals and databases like CNKI (China National Knowledge Infrastructure, http://www.cnki.net), Wanfang database (https://www.wanfangdata.com.cn), elibrary.ru, Scopus and Web of Science. The following keywords were used: pig, breed, productivity, carcass quality, pork.

The review includes data from published articles, reviews, thesis, the Russian Encyclopedia, the State Register of Breeding Achievements in Animal Breeding in the Russian Federation, the Federal State Statistics Service, the National Bureau Statistics of China, from 2010 to 2023. Monographs of 1952 and 1985 were used to describe the history of pig breeding.

Inclusion and exclusion criteria were used to define the following indicators: pork production, meat and pork consumption, pig breed composition, pig breed productive popularity profile in Russia and China. The data obtained were summarized in tables. During the selection, attention was paid to sources where information about consumer preferences and factors influencing changes in pork consumption were presented. When identifying publications with the same type of results, later editions were selected. At the next stage, data were combined into tables for comparison and analysis.

The aim of the study was to introduce pig husbandry, consumer attitude to pork and pork consumption in China and Russia, reveal and describe local pig breeds.

The current situation and trends of pork industry development in both countries

Pig farming is one of the most dynamically developing and profitable agricultural branches worldwide, including Russia and China.

According to USDA and EC statistics, the global pork production was 122.4 million tons in 2021. China is the largest pork producer in the world followed by the United States and the European Union, which produced 40 (32.68%), 23 (18.79%), and 12 (9.80%) million tons of

pork per year, respectively [15]. In addition, China is the largest pork food supplier in the world. It has been shown that pork food supply in China reached 385 million tons in 2021, while it was 210 and 124 million tons in the EU and the United States, respectively [16]. In 2021, for the first time, the scale rate of pig farming reached 60% in China.

Table 1. Pork production in China and Russia

Years	Pork production (million tons)		Growth rate of pork production (%)	
	China [17,18]	Russia [19]	China	Russia
2010	51.38	2.34	4.16	7.27
2011	51.32	2.43	–0.12	2.88
2012	54.44	2.56	6.08	5.08
2013	56.19	2.82	3.21	9.22
2014	58.21	2.96	3.59	4.73
2015	56.45	3.08	–3.02	3.9
2016	54.25	3.36	–3.90	8.33
2017	54.52	3.52	0.50	4.76
2018	54.04	3.74	–0.88	5.88
2019	42.55	3.94	–21.26	5.08
2020	41.13	4.28	–3.34	7.94
2021	52.96	4.30	28.76	0.47
2022	55.41	4.53	4.63	5.08

The volume of pork production in China reached 51.38 million tons in 2010, followed by a minor decline in 2011. From 2011 to 2014, pork production was increasing year by year, and reached 58.21 million tons in 2014. Then, a decrement and stagnation of pork production was observed in the following 4 years. In 2019, pork production fell by 21.26% and then gradually recovered to 55.41 million tons in 2022 (Table 1).

In Russia, the volume of pork production of 2.8 million tons was achieved in 1992 with the proportion of industrial pork production accounting for 60% of total (1.684 thousand tons in slaughter weight). Then stagnation came, which lasted more than 10 years. By the beginning of the 2000s, pork production reduced almost by half to 1.5 million tons in slaughter weight with the four-fold decrease in the industrial sector [20].

In 2005, transformations began within the framework of the "National projects on the agro-industrial complex", which was further developed in the "The state program for the development of agriculture in 2008–2012". The main task of the national project "Pig farming" was the modernization of the industry, including construction of new and reconstruction of the existing pig raising complexes based on the modern achievements in the practice of keeping and feeding; the use of modern achievements of genetics in formation of the parent herd of commercial complexes that determine the best quality indicators of productivity; specialization of enterprises in breeding and commercial production, including within agroholdings and their cooperation with personal and farm enterprises.

As a result, the volume of industrial pork production increased by almost three times (by 2.6 million tons) in 2010–2021 compared to 2000, while production increased by more than 8 times [21].

The main strategic challenge of pig farming for the next 10 years is entering the top five world pork exporters [22].

Pork is a favorite product both for the Russians and the Chinese. Pork is the most common animal protein for the Chinese [23]. The muscle fibers of pig are fine and soft, with less connective tissue and intermuscular fat. Pork accounts for close to or more than 60% of domestic meat production and consumption in China. From 2013 to 2022 the meat consumption in China was more than 24 kg/capita/year. The proportion of pork consumption was basically maintained at more than 75%, with a minimum of 73.39% in 2020 and a maximum of 78.13% in 2014 (Table 2).

Table 2. The meat and pork consumption in China and Russia [17,21,24]

Year	Meat consumption without regard for poultry meat (kg/capita/year)		Pork consumption (kg/capita/year)		Ratio of pork to overall meat consumption (%)	
	China	Russia	China	Russia	China	Russia
2013	25.6	44.9	19.8	26.6	77.34	35.42
2014	25.6	41.4	20.0	23.5	78.13	32.10
2015	26.2	39.4	20.1	23.3	76.72	32.50
2016	26.1	40.0	19.6	24.5	75.10	33.84
2017	26.7	40.8	20.1	25.6	75.28	34.18
2018	29.5	40.8	22.8	25.5	77.29	34.00
2019	26.9	41.7	20.3	26.7	75.46	35.13
2020	24.8	42.7	18.2	27.9	73.39	36.52
2021	32.9	42.6	25.2	28.3	76.60	36.80
2022	/	47.8	/	29.8	/	37.72

In Russia, the proportion of pork in the meat balance was 52% in 1940 [2]. Nowadays, the proportion of pork in the overall consumption is slightly more than 30% (Table 2) and exceeds the corresponding indicator in China (by more than 1.5 times in 2013, and by 1.3 times in 2021). Pork consumption per capita in Russia is higher than in China, but the difference between Russia and China in terms of this indicator is gradually decreasing. For example, it was 6.8 kg in 2013 and 3.1 kg in 2021. In 2022, pork consumption in Russia was 37.72%.

The survey of 2018 showed that more than 70% of the Russians over the age of 14 eat pork several times a week [25].

Curiously, the share of beef, goat meat, lamb and meat from other animal species is slightly more than 6 kg. Thirty five years ago, however, the ratio between pork, beef and poultry meat was 30:30:30%.

Pork consumption specificity in China and Russia

Pork is the major meat commodity consumed by Chinese people, while quality and price are the two most important factors that consumers consider when purchasing pork [26]. In addition, the nutritional content of meat, such as the

content of fat, protein, minerals and vitamins, is also important for consumers [27]. Fresh pork is bright red in color, tender, with white and elastic fat and gray tendon on the surface [28]. Most consumers choose fresh pork, and surveys showed that the vast majority of people (90.77%) accepted fresh pork priced below 40 RMB/kg [29].

Apart from quality and price, consumers also take into account pork cuts. It has been found that consumers have a wide range of choices regarding the cut, with fatty pork and tenderloin being the most popular, followed by front leg meat [30,31].

Russian consumers also consider positively consumption of fatty pork [32].

Pork is widely used in manufacture of meat products in enterprises in Russia. The first and second dishes, sausages, hams, rolls, "buzhenina" (roasted pork), loin, belly, semi-finished products, dried meat and other meat products that are in high demand among the population are produced from pork. Products from speck, especially, "salo" (backfat), are very popular among the Russians.

It is worth noting that the opinion of Russian consumers about pork has changed noticeably. Aggressive pressure of mass media, publications and TV shows have linked the upsurge in cardiovascular diseases and excessive body weight to the presence of cholesterol in animal fat. Physicians and dietarians supported this company. Pork was especially targeted. As a result, consumers began to reject pork explaining rejection by the presence of harmful speck in it. In response to consumer demand, bacon pigs (Landrace) were bought from abroad, and then several local breeds were developed. It is remarkable that in 1935–1940 there were also discussions among pig breeders about the expediency of lean-meat pig development ("baconization"). The opponents of baconization argued that "hound-gutted, flat animals with the weak back" were obtained as a result [2].

Surveys note several groups among consumers: (1) those who eat pork without paying attention to negative publications about it in mass media; (2) those who choose only lean pork and consider pork speck harmful to health and (3) a small group of consumers who understand both positive and negative nutritional characteristics of pork and eat it according to this knowledge.

A survey of consumers carried out in 2022 along with netnographic studies showed that from 39% (for minced pork) to 49% (for chilled meat) of consumers continue to buy pork despite the negative information appearing in mass media. Curiously, the proportion of respondents who answered that they did not buy frozen meat was 59%. With that, respondents prefer to buy meat in specialized farm stores [33].

The optimal ratio of ω-6 to ω-3 PUFA (10.76) in lipids of muscle tissue allows assigning lean pork and food products from it to healthy nutrition products [34].

Pig breeds with marble meat are considered to be very valuable nowadays. It is worth noting that the consumer characteristics of marble meat were already mentioned by A. P. Redkin back in 1952 [2]!

Over the last years, the growth in consumption has been recorded for chilled and frozen semi-finished products, including those from pork. This trend is clearly seen in large and medium-sized cities and is conditioned by the dynamic lifestyle of the Russians [35].

Pork palatability, composition, tenderness and fatness to a large extend depend on a breed, feeding and raising conditions.

Breed composition and productivity of local pig breeds

Local pig breeds in China

Pig industry has a long history in China, and owns rich pig breeds and genetic resources. There were as many as 76 local pig breeds in China in 2011 [36]. Based on their characteristics, such as size, appearance, production performance, distribution location, and ecological and natural conditions, Chinese local pig breeds are roughly divided into North China, South China, Central China, Jianghai, Southwest China and Plateau types [37]. These local pig breeds show diversity in terms of body shape, reproductive performance, intramuscular fat content, and flavor substance content. However, since the 1980s, a large number of foreign pigs have been imported because of their good growth performance and high leanness. Since then, the number of local pig breeds in China has gradually declined.

Local pig breeds in north China

North China refers to the north of the Qinling Mountains-Huaihe River line, including North China, Northeast China and Mengxin District [38]. The main farm crops in these areas are winter wheat and summer corn. This region is one of the important grain production bases in China. The major local pig breeds in north China include Laiwu pig, Min pig, Huabei pig, and Dingyuan pig, and their growth performance is shown in Table 3. Among them, Laiwu pig is the representative pig breed, which is mainly found in Laiwu District, Jinan City, Shandong Province, China. The head bones of modern Laiwu pigs and those of ancient Laiwu pigs (lived 5,300–6,500 years ago) are almost identical, providing archaeological evidence of the long history of Laiwu pig, which is more than 5,000 years [39]. The Laiwu pig is famous for its high reproductive and nursing ability, excellent meat quality, and rich flavor substances [40]. In particular, it is characterized by a high intramuscular fat content (with an average of 11.6%), which is 9 times and 2–3 times higher than that of Yorkshire pig and other local pig breeds, respectively [41]. The intramuscular fat can improve the muscle texture by increasing the water-holding capacity and juiciness without affecting tenderness. Therefore, meat of the Laiwu pig has a rich and mellow flavor (Table 3).

Local pig breeds in south China

Local pig breeds in south China are mainly found in the south of Nanling and Pearl River basins in China, including the southwestern and southern border areas of

Table 3. Indicators of productivity of local Chinese pig breeds [36–53]

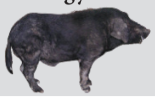
Characteristic \ Breed	North China type				South China type			
	Laiwu pig	Min pig	Huaibei pig	Dingyuan	Tunchang pig	Luchuan pig	Bama Miniature pig	Diannan Xiaoer pig
								
Body length, cm	94–104	147	129–147	119–128	93–105	124–125	50–75	103–109
Backfat thickness, mm	37–45	37	35	27–37	25–42	36–60	22–24	32
Age to 90 kg body weight, days	180–210	240	240	300	315 (90 kg)	240 (70 kg) 360 (90 kg)	180 (20 kg)	300 (70 kg)
Piglets per litter, number	10–13	13–14	6–12	7–12	8–13	8–10	8–12	7–10
Average weight gain, gram	700–1,000	326	434	533–547	300–450	321	100	155–360
Feed conversion ratio, units	2.6–3.0	4.2	3.5–3.6	3.3–3.4	3.5	4.13	4.7	4.3–5.0
Average carcass yield, %	69–73	72–74	70–73	72–76	69–77	63–68	60–61	68–74
Average lean meat yield, %	41–51	47–58	44–52	46–48	45	36–39	49	35–40
Longissimus dorsi area, cm²	25–36	20	21	26–30	27–30	21	12–19	13

Table 3. (continued) [54–64]

Characteristic \ Breed	Central China type				Jianghai type				
	Jinhua pig	Ningxiang pig	Xiangxihei pig	Fuzhouhei pig	Erhualian pig	Meishan pig	Jiaxinghei pig	Shawutou pig	Shawutou pig
									
Body length, cm	122–127	119–122	127–136	150–164	123–142	143–161	124–139	143–150	136–142
Backfat thickness, mm	38	46	33	33–35	36	36–40	38	37–40	39–42
Age to different body weight, days	300 (75 kg)	240 (70 kg)	240 (70 kg)	240 (70 kg)	210 (70 kg)	300 (100 kg)	240	240	240 (80 kg)
Piglets per litter, number	13	8–11	7–11	10–12	12–16	15–16	11–15	11–13	12
Average weight gain, gram	456–472	368–400	300–354	400–500	350–380	430–480	425	425–451	434–453
Feed conversion ratio, units	3.0–3.6	4.5–4.7	3.5–3.7	3.5–3.9	3.7	3.5–4.0	3.0–4.0	3.7	3.7
Average carcass yield, %	68–72	68–72	73–74	72	61–65	64–68	68	65	66–69
Average lean meat yield, %	43	35–40	41–46	47	43–45	42–45	42–56	46	35–47
Longissimus dorsi area, cm²	19–20	18–22	21–23	21–25	25–29	17	16	18–19	15–19

Table 3. (continued) [65–84]

Characteristic \ Breed	Southwestern type local pig breeds					Tibetan pigs	
	Rongchang pig	Chenghua pig	Yanan pig	Wujin pig	Neijiang pig	Diqing Tibetan pig	Zangxiang pig
							
Body length, cm	139–157	135–138	139–141	120–125	130–152	82–88	68
Backfat thickness, mm	55	25–29	25–33	45–52	33–40	2,1–5,4	2,1
Age to different body weight, days	240 (80 kg)	240 (90 kg)	270 (90 kg)	240 (80 kg)	190 (90 kg)	360 (50 kg)	540 (40 kg)
Piglets per litter, number	6–11	9–12	8–10	8–9	8–12	6–8	5–7
Average weight gain, gram	513–571	358–464	350–400	120–320	373–544	235–342	173
Feed conversion ratio, units	3.2–3.7	4.1–4.6	4.2–4.5	4.5	4.0–4.2	4.3–4.8	—
Average carcass yield, %	69–76	68–70	70–73	72–78	68–71	60–65	65–66
Average lean meat yield, %	40–46	41–46	43–44	45–56	40–44	52	52–55
Longissimus dorsi area, cm²	18–21	17–19	18–23	18–24	18–23	—	10–17

Yunnan, Guangdong, Guangxi, Fujian, Hainan and Taiwan Provinces. Representative pig breeds are the Guangdong-Guangzhou floret pig, Xiang pig, South Yunnan small-eared pig, Hainan pig, Guangdong black pig, Huai pig, Taiwan pig and so on. This type of pig breeds is characterized by the small size, short mouth, concave face, small and erect ears, and mostly black and white coat color. Specifically, the local pig breeds in south China are generally short, broad and round, with a large, pendulous belly, plump and round legs and hips, thin and hairless skin, short bristles, and loose bodies. Local pig breeds in south China are sexually mature at early age with estrus at 3–4 months and bred at 6 months with 8–10 piglets per litter. In addition, the slaughter rate is higher and meat is tender than meat from other breeds.

Tunchang pigs, a type of Hainan pig, are native to Tunchang County, Hainan Province, China. They have the advantages of early puberty, high fertility, resistance to rough feeding, strong disease resistance, and good meat quality. However, Tunchang pigs grow slowly and have low birth weights. Therefore, Duroc boars are usually used for cross-breeding with Tunchang sows. The pig is also named "Tunchang black pig" because of the all-black fur [46,47]. The typical rearing regime of Tunchang pigs is mainly a combination of indoor and outdoor rearing. The feed formula for housed Tunchang pigs mainly consists of corn, cassava, sweet potato, rice bran, peanut cake and so on. At the same time, fresh stems and leaves of cassava or sweet potato, sweet elephant grass, and king grass are used as green fodder. At the medium pig stage (body weight 30–65 kg), green fodder may account for more than 15% of the diet; at the large pig stage (body weight 66–100 kg), grazing may be practiced [46] (Table 3).

Local pig breeds in central China

Local pig breeds in central China include Ningxiang pigs, Jinhua pigs, Jianli pigs, and Dahuabai pigs, which are mainly found in the vast area between the Yangtze River and the Pearl River. The growth performance of local pig breeds in central China is between the North China and South China types, while the reproductive performance is above average. Meat is tender and of good quality.

The Jinhua pig, also known as the Jinhua two-headed black pig, has a long history and is one of the four famous local pig breeds in China. Jinhua pigs have excellent characteristics such as early sexual maturity, good meat quality, and high reproductive rate. The cured "Jinhua ham" is of excellent quality and famous at home and abroad.

The marbling score of meat from Jinhua pigs at 35, 80, and 125 days of age was 50.48%, 50.00%, and 28.92%, respectively, which was higher than that of meat from their Landrace pig counterparts. The drip loss in meat of Jinhua pigs at the same ages was 34.43%, 34.00%, and 33.08%, respectively, which was lower than that in meat of their Landrace pig counterparts [56]. In addition, the intramuscular fat content of meat from Jinhua pigs reached 4.45%, which was 3.11 times higher than that of meat from Landrace pigs at 180 days of age [57]. However, Jinhua pork pigs grow slowly in the late period, and the feed utilization rate is low (Table 3).

Jianghai type local pig breeds

The Taihu pig, also known as the Taihu Black pig, is a Jianghai type pig breed. Native to the Taihu Lake Basin in Jiangsu and Zhejiang, the Taihu pig is one of the most productive breeds in the world and enjoys the reputation of "national treasure". Taihu pigs have many excellent characteristics, such as ideal meat quality, high disease resistance and good adaptability to the local environment [53]. Taihu pig includes six varieties: Erhualian (EH), Meishan (MS), Fengjing (FJ), Jiaxing Black (JX), Mizhu (MI), Shawutou (SW), and Hengjing (a currently extinct variety), which have been collectively referred to as "Taihu pig" since 1974. The MS breed is further divided into two strains, called Zhongmeishan (MMS) and Xiaomeishan (SMS) [53,62].

Erhualian pigs are known for their high fertility and hold the highest farrowing record worldwide. They are one of the representative populations of high-yielding pig breeds in the Taihu Lake basin of China. The average litter size of multiparous sows is about 16, of which 14 piglet survive. Erhualian pigs are native to Jiangyin, Wujin, Xishan, Changshu and other cities in Jiangsu province, China. At the same time, Erhualian pigs also have excellent characteristics such as long reproductive life, early sexual maturity, large number of teats, good meat quality, docile temperament, tolerance to rough feeding, and good hybridization effect [63] (Table 3).

Local pig breeds in southwestern China

Local pig breeds in southwestern China are mainly found in Yunnan-Guizhou plateau, most of the Sichuan Basin, and western Hunan and Hubei province. The coat color of southwestern pig breeds is mostly black, with a considerable number of black and white, and a small number of red-haired pigs. Southwestern type pigs have large heads, thick and short legs, and the litter size is usually 8 to 10. The slaughter rate of southwestern type pigs, such as Neijiang pigs, Rongchang pigs, and Wujin pigs, is low and the fat content is high.

Rongchang pig is a high-quality local pig breed in China. It is mainly produced in Chongqing Rongchang and Sichuan Longchang [69]. It is one of the eight excellent pig breeds in the world. Rongchang pigs are typical fat-type pig breeds, which have the advantages of early sexual maturity, fast growth, resistance to rough feeding, strong adaptability, docile temperament, stable genetic performance and excellent meat quality [69,70]. Studies have shown that compared with Duroc pork, Rongchang pork has significantly higher intramuscular fat content, lower shear force, and richer monounsaturated fatty acids [71] (Table 3).

Tibetan pig

Tibetan pigs mainly live in the Qinghai-Tibet plateau, including Diqing Tibetan pigs in Yunnan, Aba and Ganzi Tibetan pigs in Sichuan, Juema pigs in Gansu, and Tibetan pigs in Shannan, Linzhi, and Qamdo in the Tibet Autonomous Region. Tibetan pig is the rarest plateau pig breed in the world, and it is also a precious local breeding resource in China. Tibetan pigs have been living for a long time in pollution-free and pure natural alpine mountainous areas. They have the characteristics of adapting to the harsh climatic environment at high altitudes, disease resistance and ruggedness, but the fertility is low.

The Diqing Tibetan pig is a unique plateau pig breed in China. It has advantages of strong stress resistance and good meat quality, including the pH value of meat from commercial fattening pigs (≥ 80 kg) of 5.8–6.8, the ash content of less than 1.0%, the thawing water loss rate of less than 5%, and the total amino acid content of more than 15%. However, it has disadvantages of slow growth and few litters [80]. At present, reports on Diqing Tibetan pigs mainly focus on hypoxia adaptation, growth and succulence [81–84] (Table 3).

Pig breed profile in Russia

Soviet zootechnicians developed several pig breeds such as Ukrainian Steppe White, North Siberian, Mirgorod, Breitovo, Livny, Kemerovo, Prudishchenskaya, Kalikin, Urzhum and some others. By 1952, pigs of the following breeds were raised in the Soviet Union: Large White, Ukrainian Steppe White, Mirgorod, North Siberian, Breitovo, Livny, Berkshire, White Short-Eared, White Long-Eared, Large Black, Mangalitsa [2]. By 1980, there were 32 pig breeds in the USSR, including 22 breeds that were developed and registered during 1917–1980 (Figure 1) [85]. The number of pigs of 22 breeds at that time was 29 million heads out of the total number of livestock in the USSR of 79 million.

At present, the breeding base of pig farming in Russia is presented by a small number of breeds. Pigs of four main breeds are raised in industrial pig farming, namely, Large White, Yorkshire, Landrace and Duroc, which account for 98% of total stock. The structure of the stock of the main pig breeds raised in the enterprises of the Russian Federation is as follows: Large White 56.9%, Yorkshire 18.52%, Landrace 18.18% and Duroc 5.83%. According to various data, other breeds (Pietrain, Livny) account for 0.56 to 2% [86]. Three breeds are raised in pig farming on the homestead: Altai Meat-type, Short-Eared White and Tsivislk [87].

Pigs are raised in 53 pedigree pig farms and 40 reproducing farms in 38 regions of the RF. The "State Register of Breeding Achievements Approved for Use. Volume 2. Breeds of animals" in edition of 2022 contains 20 breeds, 23 types and one line [86,88].

Pigs of fat-type and meat-and-fat type breeds have not been in high demand during the last years. The exclusion is the population living in cold regions that prefer fatter meat with high amounts of saturated fatty acids and cholesterol and at the same time, has a healthy lifestyle. In Russia, the preference is given to breeding universal pigs because these

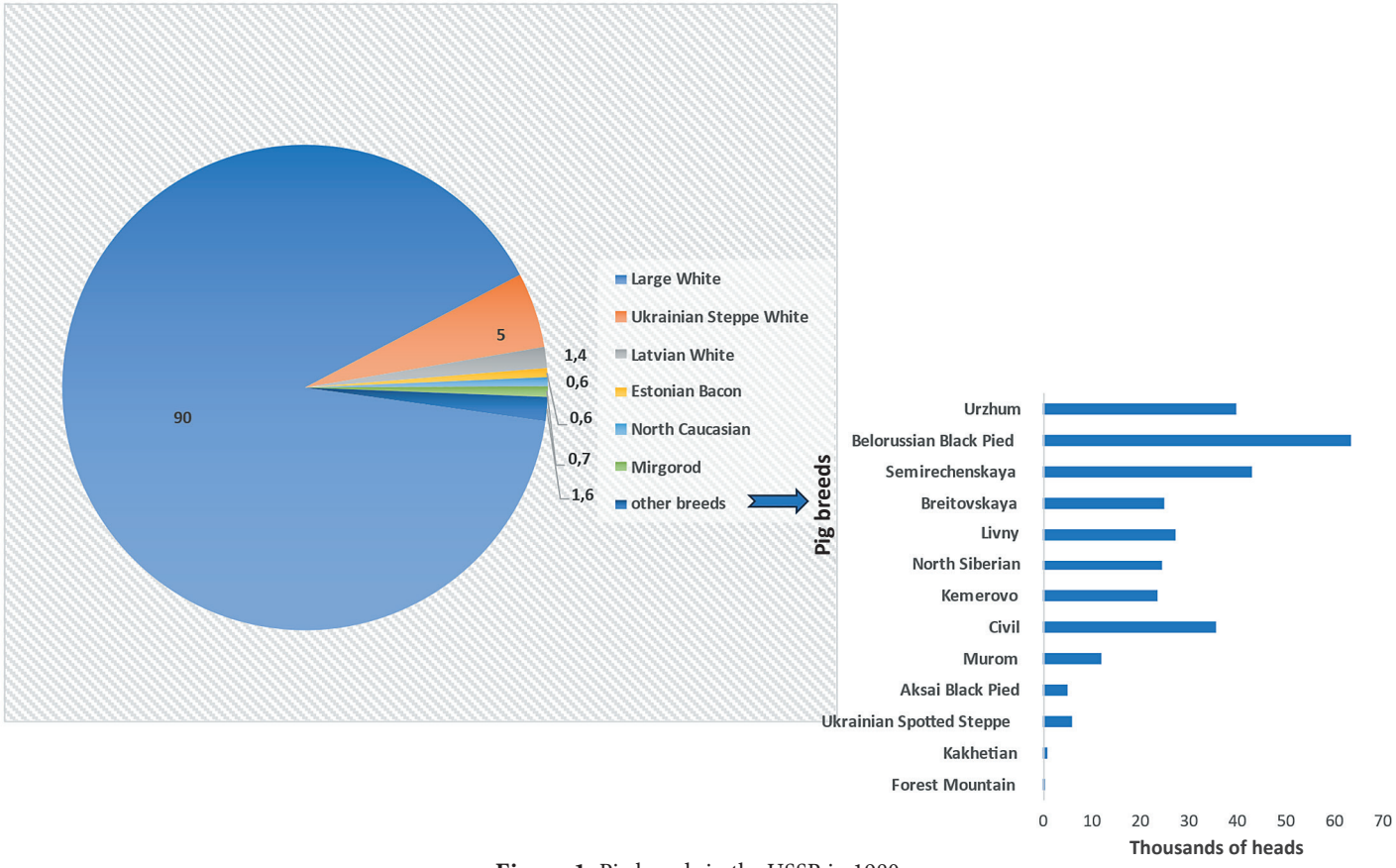


Figure 1. Pig breeds in the USSR in 1980

animals are grown both for meat and for backfat depending on the type of feeding (meat, bacon, fat types).

Recently, the meat-type direction of pig farming has been in demand; therefore, pig stock of the Landrace, Duroc, Pietrain and other breeds has been growing. Meat-type or bacon-type pigs are distinguished by the elongated body (some breeds, for example Landrace, has an increased number of vertebrae), their front part has a smaller size than the back part, the head is small, rump is large, hams are meaty, sides are roundish and resilient. Some meat-type pigs are distinguished by the genetic predisposition to accumulation of muscle mass rather than fat (the fat interlayer is thin, 18–20 mm and is located under the skin).

The majority of breeds have been developed by crossing several breeds on the basis of targeted choice of the best animals, selection for a desired type and targeted raising of new generations. The following breeds have played an important role in the breed development process: Large White, Berkshire, Landrace and Duroc. Nowadays, these breeds are widely used and are specialized in the meat direction.

Currently, the lean meat content is one of the main traits for pig selection on local basis [86,89].

Unlike China, there is now no pronounced regional distribution of breeds in Russia. The livestock' breed profile is about the same everywhere: crosses based on the Large White, Landrace and Durok. Several breeds still exist with strict regional localization.

Large White

A. P. Redkin (1952) states that the exact time of the development of the Large White breed is unknown [2]. He writes that the breed was obtained by long-term crossing, which involved English breeds (Long-Eared, Leicester and Yorkshire from Sus ferus), pigs of the Romance group and even Chinese pig breeds — Siamese and small Sus vittatus. In 1885, the National Pig Breeders' Association developed the standard for the Large White breed.

The Large White breed was brought into Russia for the first time in 1887–1888; that is, only two years after the creation of the standard for the breed in England.

Pigs that are raised in our country are assigned to three directions of productivity (production types): meat, meat-and-fat (or universal) and fat. It is quite possible that this classification was formed in the process of improvement of the Large White breed in Russia in the 1930s. In the process of crossing, productive and economic traits of the breed in general changed. Only the type (fat or bacon) did not change. Then, the third (meat-and-fat) type of Large White pigs was developed.

The Large White breed improved in the USSR became an important tool for developing many domestic pig breeds. For example, the following breeds were produced based on Large White pigs: Ukrainian White Steppe (in Askania Nova, the breed was approved in 1932); North Siberian (1942, Novosibirsk); Urzhum (1957, Kirov region); Kemerovo (1961, Kemerovo region), Breitovo (1948, Yaroslavl region), Latvian White and Lithuanian White (both approved in 1967), Semirechensk (firstly approved in 1968 as Kazakh hybrid, approved in 1978 as Semirechensk/Semirechenskyaya), Mirgorod (1940), Tsivilsk (a cross of native Chuvash pigs with Large White boars, the breed is not registered), Mangalitsa, Altai (registered in 2017), and others that were created by multiple crossbreeding procedures.

Brief description of several local pig breeds that are raised nowadays in the RF is given below.

Livny (Livenskaya)

The Livny breed was officially recognized in 1949 and was produced by crossing of the long-eared pigs with boars of the Yorkshire, Large White, Berkshire breeds [90,91]. The breed is assigned to the meat-fat type and was produced for pasture raising. Pigs are sturdy and undemanding to feed.

Tsivilsk (Tsivilskaya, Civilian, Civil'skaya)

The breed was developed in Chuvashiya by crossing pigs of local breeds and Large White. It was registered in 1951 as the Tsivilsk breed group. It is undemanding to feed and resistant to climatic variations. The undeniable advantage of this breed is the capacity of maximum transfer of the genetic potential of the breed in terms of production and economic traits. According to the expert opinion, meat from pigs of this breed is distinguished by good taste and aroma [92]. The breed is assigned to the meat-and-fat type.

Altai (Altaiskaya)

The Altai meat-type breed was registered in 2017. The White Large and Landrace breeds as well as boars of the MAXGRO™ Terminal Line were used in its development. The breed is adaptive to environmental conditions, stress resistant, has good zootechnical and technological characteristics [93,94]. The breed was developed as the meat type. Nevertheless, speck from pigs of the Altai breed is interesting in terms of its composition as it has the high content of unsaturated fatty acids, in particular omega 3 and omega 6 PUFA [32].

Kemerovo (Kemerovskaya)

The breed was produced by the complex reproductive crossing of sows of local breeds and boars of the Large White breed, and then by using boars of the Berkshire and Large Black, North Siberian and Siberian Black Pied breeds. The breed was approved in 1960/1961. Presumably, individual animals are raised on the territory of the Kemerovo Oblast and the Far East, as well as in Kazakhstan [95].

The breed was produced with consideration for Siberian climate. It is adapted to it and is distinguished by cold resistance. Pigs are undemanding to feed. According to the opinion of V. A. Bekenev et al. [96,97], meat and speck from pigs of the Kemerovo breed differ from that of other breeds, in particular commercial breeds, by a ratio of unsaturated to saturated fatty acids. Speck is distinguished for

Table 4. Indicators of productivity of Russian pig breeds [2,90–97,99]

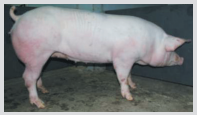
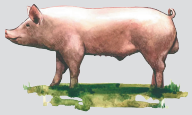
Characteristic \ Breed	Large White	Landrace	Duroc	Yorkshire	Livny	Altai	Kemerovo	Tsivilsk
								
Body length, cm	167–182	140–180	160–180	169–180	162–180	110–112	160–180	160–177
Backfat thickness, mm	28	20–27	17–21	9.5–13. 3	34	20–23	23–35	24–28
Age to 100 kg body weight, days	165–189	167–194	160–180	153–168	150–196	160	175–180	178–185
Slaughter age, days	185	160–170	159	160–168	160	152–155	180–182	160
Piglets per litter, number	11–12	11–12	9–11,5	10–13 (up to 14)	10–11	14–15	10–11	11–12
Milk, kg	58	55–64	43	54.6–65	51–70	—	58–65	47
Average weight gain, gram	720	700–730	715–820	750–850	700–800	750–800	730–780	700–800
Feed conversion ratio, units	3.4–3.85	2.97–4.05	2.7–3.75	2.6–3.1	3.8–4.11	2.6–2.8	3.7–3.9	3.5–4.5
Average carcass yield, %	82	75	86	82	75	75–82	72–75	70
Average lean meat yield, %	57–63	65–68	60–69	64	54–55	58–59	53–60	50–55

Table 5. Breeds mentioned in the text [2,90,98–100]

Characteristic \ Breed	White Short-Eared	White Long-Eared	Pietrain	Mirgorod	Breitovo	Berkshire	Mangalitsa	Ukrainian Steppe White
								
Body length, cm	162–180	165–175	—	—	155–180	—	141	
Backfat thickness, mm	31–40	25–35	7–10	30–37	31–38	33–36	65–90	25–36
Age to 100 kg body weight, days	195–210	179	160–210	154–182	191–230	180	160–180	192–209
Piglets per litter, number	9–11	10–12	7–9	10–11	10–12	6–9	5–9	10–12
Milk, kg	51	70–80	60	48–50	52–70	48–55	—	55–60
Average weight gain, gram	650	765–770	500–550	700	680–770	670–750	700–800	
Feed conversion ratio, units	4.11	3.91	2.5–3.4	4.2–4.5	3.8–4.5	2.5–4.0	3.9–4.0	
Average carcass yield, %	80	—	78–80	80–85	74–77,9	84–88	up to 70	77–84
Average lean meat yield, %	60	47–53	62	53–55	54–58	—	54	54–55

its high-melting properties and at the same time for good sensory characteristics.

Many breeds have not stood the test of time as they did not correspond to the requirements for industrial raising. For example, the Chukhonskaya breed gradually disappeared. The main reason for the lack of demand for it was the fact that pigs of this breed grow very slowly and parameters suitable for the manufacturing process are formed only after two years [13].

Changes in exterior, productivity, biological, morphological and physiological features of animals have occurred under an effect of long-term selective breeding [1]. Tables 4 and 5 present parameters of productivity for breeds that are prevalent in Russia and some other breeds that are mentioned in this paper.

Conclusion

The majority of breeds comprising the stock of pigs in the RF are commercial breeds (Landrace, Duroc, Yorkshire and Large White), which proportion prevails in the overall breed profile (more than 60%). These breeds became a basis for the development of practically all pig breeds that exist today in the RF.

Selective breeding has been carried out in our country from the beginning of the 20th century and was intensified in the period from 1935 to 1975. The result was a successful development and registration of 22 pig breeds. Unfortunately, only less than 30% of local breeds remained in the pig stock in Russia in the first quarter of the 21st century. This is determined, primarily, by the insufficient productive characteristics of pigs: insignificant fertility, low milking capacity of sows, and low yield of muscle tissue.

Consumers required more lean meat and less fat. In response to their demands many crosses of pigs, which carcasses corresponded to these requirements, were produced.

At the same time, animals of the meat type especially those with a thickness of backfat less than 10 mm turned to be quite sensitive to feeds, water, stress and temperature fluctuations, contrary to pig breeds developed in Russia.

In addition, meat of such pigs is characterized by the low water binding capacity and low color stability; their speck is low-melting and, as a result, its technological processing is more difficult.

As the experience of Chinese zootechnicians show, preservation of local breeds and their diversity allows varying both zootechnical and technological characteristics to develop new breeds and increase manufacturability of meat raw materials.

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