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SAFETY OF CANNED TUNA MEAT AFTER OPENING AND STORAGE AT DIFFERENT TEMPERATURES

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Keywords: *food safety, fish, canned tuna, microbial quality, pathogens, histamine*

Abstract
Canned tuna is widely consumed worldwide due to its palatability, nutritional value, and convenience. However, it may pose a health risk to consumers if not properly processed or improperly handled and/or stored by consumers. This study evaluated the microbial safety and histamine content of canned tuna meat and the effect of the storage at different temperatures (4, 28, and 31 °C) for 7 days after opening on the microbial safety and histamine content. Data were analyzed by the SAS program. The aerobic bacteria counts in tuna samples after 48 hours of storage at 4 °C, 28 °C, and 31 °C were 3.2, 2.75, and 5.09 log CFU/g, respectively, with no significant difference observed between 4 °C and 28 °C ($p > 0.01$). Similarly, the anaerobic bacteria counts were 3.3, 2.98, and 5.08 log CFU/g at 4 °C, 28 °C, and 31 °C, respectively, also showing no significant difference between 4 °C and 28 °C ($p > 0.01$). Storage of canned meat at 4 °C showed more significant ($p < 0.01$) microbial inhibition than storage at 28 °C, and 31 °C. No pathogenic bacteria were observed in all samples during storage at different temperatures. For the histamine test, the highest recorded concentrations were 3.53, 9.58, and 28.24 mg/kg in tuna samples stored at 4 °C, 28 °C, and 31 °C, respectively. The storage temperature influenced ($p < 0.01$) histamine formation in tuna meat during storage. Recording histamine concentrations at zero time indicates that histamine was formed before opening the can, which may be due to failure to apply good hygiene practices in handling fish, as histamine does not degrade once formed. However, it did not exceed the maximum permissible limit. Also, the results of the microbial count and histamine content indicate that holding canned tuna meat after opening at 4 °C contributes to maintaining the safety of the tuna during storage.

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Introduction

Canned tuna production is constantly increasing locally and globally [1], due to its high nutritional value and desirable taste. Canning is one of the preserving techniques that makes the food stable at room temperature for a relatively long period ranging from 1 to 5 years [2]. Thus, canned food is distinguished by the possibility of being easily distributed all over the world and needs relatively fewer requirements for storage and distribution [3]. The major steps of the canning tuna process include cleaning and preparing raw materials, precooking, cooling, cleaning, packing with a covering oil or vegetable broth, etc. in sealed cans, the thermal process (retorting), can cooling, labeling, casing, and storage [4].

Fish and fish products are an important nutritional source that is readily digestible and contains biologically highly valuable nutrients, including protein, polyunsaturated fatty acids, minerals, and vitamins [5–7]. Further, fish consumption is known to prevent diseases since fish is a source of omega-3 highly unsaturated fatty acids, which include eicosapentaenoic and docosahexaenoic acids [8]. On the other hand, it is considered a suitable environment for the growth of microorganisms due to the availability of moisture and nutrients, such as non-protein nitrogenous compounds that include free amino acids, peptides, amines, amine oxides, guanidine compounds, quaternary ammonium molecules, nucleotides, and urea [9–11], as well as the low acidity (pH > 6) of the meat [12]. Therefore, fish and fish products could be a cause of foodborne diseases (FBDs).

FBDs represent one of the most widespread public health problems [13,14]. FBDs associated with pathogenic microorganisms, such as bacteria and viruses, parasites, and chemical contaminants in food pose a serious threat to the health of millions of individuals, leading to conditions such as diarrhea, cancer, and even death [15,16]. Fish-borne

pathogenic bacteria include: 1) indigenous bacteria such as *Aeromonas hydrophila*, *Vibrio cholerae*, *Clostridium botulinum*, *Vibrio parahaemolyticus*, *Listeria monocytogenes* and *Vibrio vulnificus*; 2) non-indigenous bacteria present as a result of fecal contamination such as *Yerinia enterocolitica*, *Campylobacter* spp., *Escherichia coli*, *Shigella* spp. and *Salmonella* spp.; and 3) bacteria that are present as a result of contamination during processing such as *Clostridium perfringens*, *S. aureus*, *L. monocytogenes*, and *Bacillus cereus* [17,18]

The incidence of FBDs in some areas of developing countries may be attributed to poor hygiene practices, lack of access to safe adequate food storage facilities, and poorly enforced laws [19]. Food safety awareness, education, and promotion among consumers should be emphasized, as most FBDs outbreaks occur at home, in restaurants, and/or social events [20].

One of the FBDs is histamine-forming bacteria poisoning. Histamine-forming bacteria is one of the compounds named biogenic amines [21]. Histamine-forming bacteria is a toxic metabolite produced by bacteria [22]. It is worth noting that a review of published studies about biogenic amines, suggested indicative levels of histamine-forming bacteria content in fish, pointing out that amounts of 5–20 mg/100 g are possibly toxic [23]. The formation of histamine-forming bacteria depends on the type and amount of free amino acids, the presence of decarboxylase-positive bacteria, the availability of appropriate conditions for the growth of decarboxylase-positive bacteria and production of histamine-forming bacteria, and the extent of application of hygienic practices and food safety standards [24]. Histamine-forming bacteria formation is most often caused by improper temperature control of fish after harvesting and the level of accumulation is influenced by the combination of time and temperature, with accumulation typically occurring rapidly after 12 hours of storage at 25 °C [25]. In addition, the formation of histamine-forming bacteria is affected by the manufacturing process, and the conditions of transport and storage [26]. It is important to mention that histamine-forming bacteria concentration may decrease with storage time because of its decomposition. Once it is produced, the histamine-forming bacteria concentration does not depend only on the histamine-forming bacteria but also on the presence of histamine-decomposing bacteria within the flora [27]. Storage temperature and time can be used as the primary means to monitor and control the quality and safety of canned seafood [28]. Several studies were conducted to evaluate the safety of canned fish related to histamine-forming bacteria [29–32]. In general, the results of these studies show that canned fish is safe for health [24]. However, the histamine-forming bacteria may form after cans are opened due to improper storage practices by consumers, such as temperature abuse. A tuna sandwich is a significant and popular ready-to-eat food made from canned tuna. The histamine-forming bacteria formation in opened canned tuna could rapidly increase if stored at 33 °C for 6 h [33].

It is worth mentioning that food patterns in Libya have changed as in other countries where the Libyans increasingly consume canned fish, especially canned tuna [34]. Consumers may not use all the contents of the can and might store the remainder in various ways, which could alter its characteristics. The various attributes of canned tuna that are important to the consumer, including safety, sensory and nutritional properties, are affected by storage temperature and time; therefore, proper storage of food is essential. Therefore, the main objectives of this study were to: 1) evaluate microbiological safety of canned tuna meat, including aerobic bacteria count (ABC), anaerobic bacteria count (AnBC), coliform bacteria counts, *E. coli*, *Salmonella* spp., *L. monocytogenes* and *S. aureus*; 2) determine the level of histamine-forming bacteria in canned tuna meat; 3) and explore the effect of storage at different temperatures, including 4, 28, and 31 °C, on the microbial quality and histamine-forming bacteria level in canned tuna meat after opening during 7 days of storage.

Objects and methods

Study plan

The study was conducted between September and December, 2024. One carton of Libyan-made tuna cans from the same brand and the same expiration date was purchased from the Al-Krimia market in Tripoli City, Libya. The carton contained 48 cans, and the filling media of tuna was a mixture of olive oil and brine. All cans were free of any leaky conditions or swelling. According to the product label, each tuna can had a net weight of 160 grams and a shelf life of three years from the date of production (July 2024 to July 2027).

Once the tuna cans arrived at the food microbiology laboratory of the Department of Food Science and Technology, Faculty of Agriculture, University of Tripoli, thirty cans were randomly selected and divided into three groups. Each group represented a trial and consisted of ten cans. The tuna cans were kept at room temperature until the opening. After the cans were opened and held in polyethylene bags, the first group was stored in the refrigerator at a temperature of 4 °C. The second and third groups were stored at 28 °C and 31 °C, respectively. The incubator (IN260) used to maintain temperatures of 28 °C and 31 °C was from Memmert GmbH +Co. KG (Germany). The temperature of the refrigerator and incubators was checked using a glass and digital thermometer (HTC2, CNWTC company, China). The storage temperatures tested in this study were chosen as follows: 4 °C is the recommended temperature for the refrigerated storage of fish, which is also available to consumers, while 28 °C and 31 °C were the average temperatures recorded in Tripoli and Sabha during the summer of 2023, respectively.

Sampling

After opening the cans, the samples were taken immediately (zero time) and at different periods during 7 days: after 6 h, 24 h, 48 h, 72 h, 96 h, 120 h, and 168 h. After

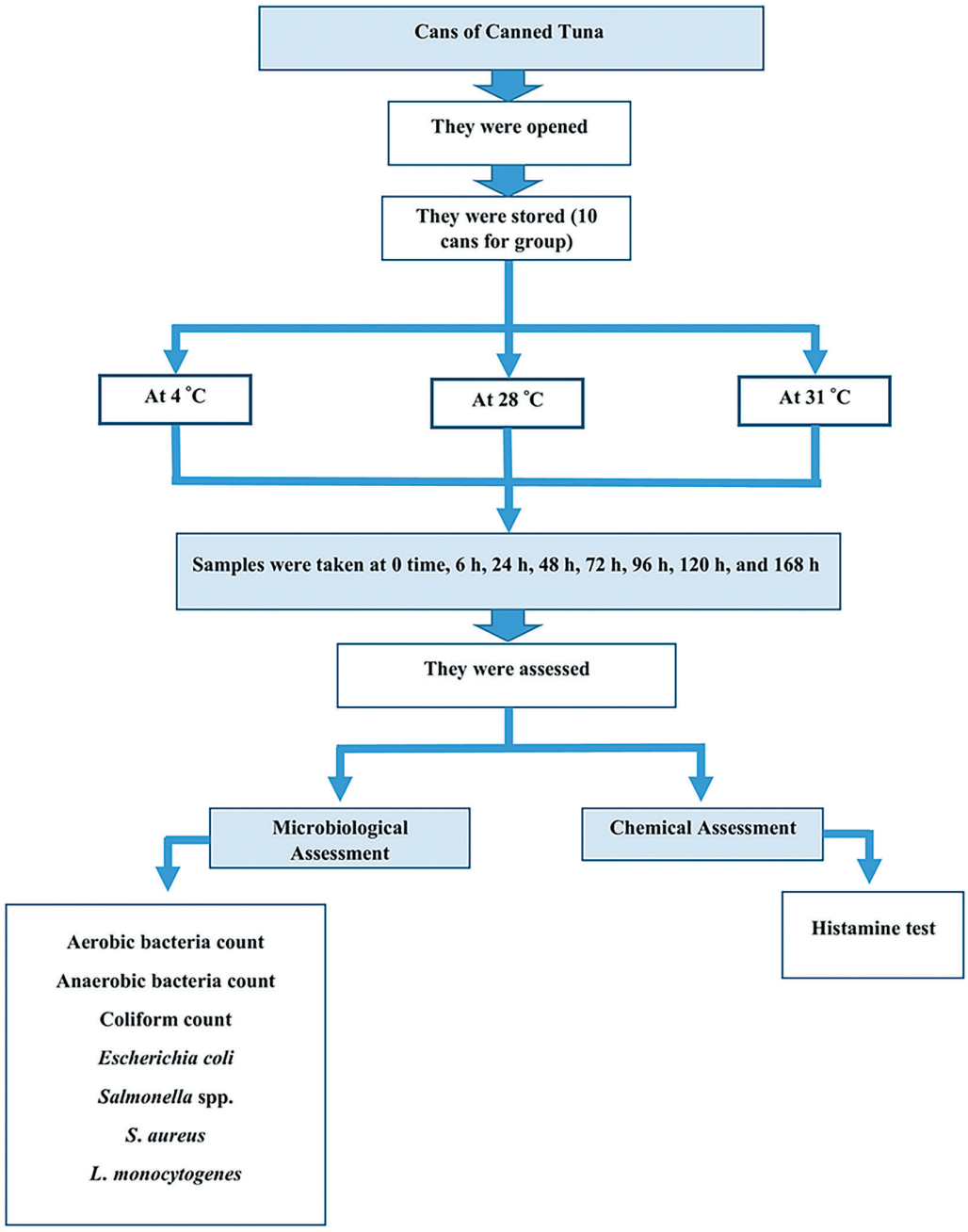

Figure 1. Diagram of the research methodology

that, the samples were held at –18 °C until analyzed in the laboratory belonging to the food and drug control center/ Tripoli branch. The samples were tested for ABC, AnBC, coliform count, *E. coli*, *Salmonella* spp., *S. aureus*, *L. monocytogenes*, and histamine-forming bacteria content. All microbial experiments were carried out in duplicate, and the histamine-forming bacteria experiment was in triplicate, and the mean values were recorded. The study sampling is displayed in Figure 1.

Test methods

Microbiological methods

Aerobic bacteria count was determined using the ISO method (ISO 4833-1:2013)[1]. Using a stomacher (Stomacher 400 Circulator Lab Blender, Seward Ltd., UK), 10 grams of tuna sample was homogenized with 90 ml of sterile buffered peptone water (BPW). A series of decimal dilutions was prepared using test tubes containing 9 ml of sterile BPW. One milliliter was transferred aseptically from the homogenate to the first test tube using a sterile pipette, resulting in a 10^{-1} dilution. This procedure was repeated sequentially to achieve further dilutions up to 10^{-5}. From the 10^{-5} dilution, 1 mL was aseptically transferred into sterile, labeled Petri dishes. Approximately 15 mL of Plate Count Agar (Liofilchem, Italy), previously melted and cooled to 45 °C, was then poured into each dish. The plates were gently swirled to mix the contents. After solidification, the plates were incubated in an inverted position at 30 °C for 72 hours. After the specified incubation period, visible bacterial colonies on the plates were counted, and the results were expressed as colony-forming units per gram (CFU/g).

[1] ISO 4833-1:2013. Microbiology of the food chain — Horizontal method for the enumeration of microorganisms. Part 1: Colony count at 30 °C by the pour plate technique.

Anaerobic bacteria counts were determined according to ISO 15213:2003[2]. The sample and serial dilutions were prepared in the same manner as for aerobic bacteria. From the 10^{-4} dilution, one milliliter was aseptically transferred into sterile labeled Petri dishes. Approximately 15 mL of Iron Sulfite Agar (Liofilchem, Italy) was then added to each dish. Anaerobic conditions were established using an oxygen removal system and carbon dioxide generation. Then, the inoculated plates were placed in an anaerobic jar (IQ2000® GasPak System, BD Company, USA) and incubated (Incubator B50, Memmert GmbH +Co. KG, Germany) for 48 hours at 30 °C. Colonies were subsequently counted using the same principles applied to aerobic bacteria.

Coliform bacteria counts were determined using the ISO method (ISO 4832:2006)[3]. Ten grams of the sample was placed in 90 ml of sterile saline solution to prepare a 1:10 dilution, and the sample was shaken thoroughly to prepare a homogeneous solution. One milliliter of an appropriate dilution was transferred into sterile Petri dishes, and approximately 15 mL of Violet Red Bile Agar (VRBA) (Liofilchem, Italy), cooled to 44–47 °C, was poured into each dish. The contents were gently mixed and allowed to solidify. After solidification, an overlay of VRBA was added to suppress surface spreading of colonies. The plates were incubated (Incubator IN750, Memmert GmbH +Co. KG, Germany) at 37 °C for 18–24 hours. After incubation, red to dark red colonies with a precipitated bile zone were counted as presumptive coliforms.

Escherichia coli were determined using the ISO method (ISO 16649–2:2001)[4]. The sample and dilutions were prepared in the same way as for aerobic bacteria. One milliliter of 10^{-3} dilution was transferred to sterile labeled petri dishes. Approximately 15 ml of Tryptone Bile X-glucuronide (TBX) Agar (Liofilchem, Italy) was then poured. The contents were gently mixed and allowed to solidify. The plates were incubated (Incubator IN750, Memmert GmbH +Co. KG, Germany) at 44 °C for 24 hours. After the incubation period, the plates were examined to identify the characteristic colonies. Positive colonies of *E. coli* produce distinct colonies that appear blue or green on the TBX agar due to β-glucuronidase activity. Non-target bacteria may form colorless or different-colored colonies or may be inhibited entirely.

Salmonella spp. was detected according to the ISO method (ISO 6579: 2002)[5]. A 25 g portion of the sample was aseptically transferred into 225 mL of sterile BPW for non-selective pre-enrichment. The sample was incubated at 37 °C for 16–20 hours. Following pre-enrichment, 0.1 mL of the culture was inoculated into 10 mL of Rappaport-Vassiliadis Soya Peptone (RVS) broth (Liofilchem, Italy) and incubated (Incubator INB200, Memmert GmbH +Co. KG, Germany) at 42 °C for 24 hours for selective enrichment. Presumptive positive colonies of *Salmonella* spp. appear as red colonies with black centers on XLD agar, due to hydrogen sulfide production and the inability to ferment lactose or sucrose.

L. monocytogenes detection was performed according to the ISO method (ISO 11290-1:2004)[6]. Ten grams of tuna were aseptically transferred into sterile incubation bags. Then, 90 ml of Half Fraser Broth (Liofilchem, Italy) supplemented with Listeria Fraser Supplement, was added. The sample was homogenized thoroughly and incubated at 30 °C for 24 hours for primary enrichment. Following this, 0.1 mL of the primary enrichment was transferred into 10 mL of Fraser Broth (Fraser Broth Base supplemented with Listeria Fraser Supplement) and incubated at 37 °C for 48 hours for secondary enrichment. After incubation, aliquots of the secondary enrichment were streaked onto PALCAM Agar (Listeria Agar Base PALCAM, Condalab, Spain) using the spread plate method after solidification of the medium. The plates were incubated (Incubator IN750, Memmert GmbH + Co. KG, Germany) at 37 °C for 24–48 hours. Presumptive *L. monocytogenes* colonies on PALCAM agar appear gray-green with a black center and are often surrounded by a red or dark halo, due to esculin hydrolysis and mannitol fermentation inhibition.

S. aureus enumeration was performed according to the ISO method (ISO 6888-1:2003)[7]. The sample was prepared and the dilution series was prepared in the same way as used for aerobic bacteria. One milliliter of the 10^{-3} dilution was transferred to the labeled petri dish. Then, approximately 15 ml of Baird-Parker Agar Base (Liofilchem, Italy) supplemented with Egg Yolk Tellurite Emulsion, was poured into the dish. After the agar solidified, the plates were incubated (Incubator IN750, Memmert GmbH +Co. KG, Germany) at 37 °C for 48 hours. Positive results for *S. aureus* colonies appear in a shiny black or gray color and are surrounded by a clear zone due to the activity of lipase on the egg yolk. Non-*S. aureus* colonies typically lack this appearance and do not produce a clear zone.

Histamine method

Histamine-forming bacteria content was analyzed using the AOAC-approved method (RIDASCREEN® histamine-forming bacteria (enzymatic) (Art. No. R1605) [35]. It is an enzymatic test in microliter plate format for the quantitative determination of histamine-forming bacteria in fresh fish, canned fish, fish meal. The test kit is sufficient for a maximum of 96 determinations (including standards). Each test kit contains components as displayed in Table 1.

[2] ISO 15213:2003. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions.

[3] ISO 4832:2006. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique.

[4] ISO 16649-2:2001. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli*. Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide.

[5] ISO 6579:2002. Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Salmonella* spp.

[6] ISO 11290-1:2004. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and *Listeria* spp.

[7] ISO 6888-1:2003. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Technique using Baird-Parker agar medium.

Table 1. Reagents and volumes used for histamine-forming bacteria standard curve preparation

Component	Histamine-forming bacteria concentration	volume
Microtiter plate		96 wells
Buffer		15 mL
Standard 1	0 mg/L	1.3 mL
Standard 2	1 mg/L	1.3 mL
Standard 3	5 mg/L	1.3 mL
Standard 4	10 mg/L	1.3 mL
Standard 5	15 mg/L	1.3 mL
Standard 6	20 mg/L	1.3 mL
Enzyme solution		1 mL
Spiking solution	500 mg/L	3 mL
Catalase Catalase to remove ascorbic acid		1 mL

Sample preparation: 5 g of the homogenized sample was placed in a 50 ml polypropylene screw cap vial and 20 ml of distilled water was added. The vial was closed and shaken by using a Vortex device until the sample was evenly suspended. Then, the sample was heated in a boiling water bath at 100 °C for 20 minutes. Every 10 minutes, the vial was removed using protective gloves and shaken for 3 seconds. After that, the vial was placed in an ice bath incubated (Bionics Scientific Technologies (P) Ltd, India) for at least 2 minutes to reach room temperature and was placed in a centrifuge (Hettich Universal 32R Centrifuge, Germany) for 10 minutes at a speed of not less than 2500 × g at a temperature of 4 °C. The lower layer was withdrawn carefully with a pipette and delivered to a new vial. After that, the new vial contents were filtered and centrifuged again and 100 µL of the undiluted clear extract was used per well. The extracted sample was stable at room temperature (20–25 °C) for 2 h. **Analysis steps**: Using a multi-stepper (815, Socorex, Switzerland), 150 µL of buffer was added to the wells and the plate was shaken manually for 3 sec. 100 µL of standards, controls, or samples was added to separate wells in duplicate. Thereafter, the plate was carefully shaken manually for 3 seconds. After 3 min., the absorption (A_1) was measured at 450 nm using a Microtiter plate spectrophotometer (ELx808, BioTek Instruments, USA). Then, 10 µL of the blue-dyed enzyme solution was added to each well using a multi-stepper. The plate was then carefully shaken manually for 3 seconds. After 10 minutes, the absorbance (A_2) was measured at 450 nm. The histamine-forming bacteria concentration was calculated according to the manufacturer's instructions.

Statistical analysis

The experiment was conducted using a Complete Randomized Design (CRD). Analysis of variance and statistical tests were performed to study the effects of storage temperature, storage time, and their interaction, utilizing the Statistical Analysis System (SAS-2002) program. The results obtained were expressed as means with standard deviation (±SD). Duncan's multiple range test was used to determine the significance of differences between the means of different treatments at a probability level of ≤ 0.01. Microsoft Excel 2010 was used to prepare tables and graphs.

Results and discussion

Fish can carry bacteria from the environment naturally or as a result of contamination due to improper handling, processing, storage, distribution, or preparation for consumption. However, under carefully controlled conditions at processing, commercially canned fish is safe [36]. ABC reflects bacterial contamination and give an indicator of the application of hygiene standards in the factory [37]. As shown in Table 2, the results of this study reveal that aerobic bacteria were not detected at zero time in the three trials. On the contrary, the number of microorganisms exceeded the maximum limit in a study carried out by Alhafeth et al. [36], where the average total number of bacteria in 20 samples of canned fish was $23.25 \times 10^7 \pm 3.42 \times 10^7$ CFU/g.

During storage at 4 °C, the highest ABC (3.2 log CFU/g) was found after 48 h, i. e., on the third day of the storage period. Then, ABC decreased to 0.5 log CFU/g after 120 h, i. e., on the sixth day of the storage period, and was not detected after 168 h, i. e., on the seventh day of storage. Low-temperature storage slowed down the growth of ABC. The results of the statistical analysis showed a significant effect ($P \leq 0.01$) of temperature and storage time on the ABC. The ABC in all samples during storage at 4 °C did not exceed the maximum level set by the International Commission on Microbiological Specifications for Foods (ICMSF) of 1.0×10^6 CFU/g [38].

Canned tuna can be contaminated if consumers abuse the storage temperature after opening. The ABC increased gradually during the storage period at 28 °C and 31 °C, reaching the highest values of 6.09 and 6 log CFU/g, respectively, after 120 h and 96 h, i. e., on the sixth and fifth days of the storage period. The highest ABC values at 28 °C and 31 °C reached the maximum level set by the ICMSF [38]. However, the tuna exhibited significant spoilage signs and notable changes in its organoleptic characteristics, including smell and appearance, after 3 and 2 days at 28 °C and 31 °C, respectively.

Table 2. Aerobic bacteria count in canned tuna meat at different storage temperatures

Storage Time	Aerobic Bacteria Counts (log CFU/g ±SD)		
	4 °C	28 °C	31 °C
Zero time	n.d.	n.d.	n.d.
After 6 h.	n.d.	2.86 ± 0.13d	1.59 ± 0.14e
After 24 h.	n.d.	1.54 ± 0.08ef	2.09 ± 0.12e
After 48 h.	3.2 ± 0.00d	2.75 ± 0.00d	5.09 ± 0.00b
After 72 h.	0.5 ± 0.70gh	5.07 ± 0.00b	5.03 ± 0.11b
After 96 h.	1 ± 0.00fg	6.08 ± 0.00a	6.0 ± 0.00a
After 120 h.	0.5 ± 0.70gh	6.09 ± 0.00a	6.0 ± 0.00a
After 168 h.	n.d.	4.47 ± 0.02c	5.13 ± 0.00b

n.d. — not detected.
Means that share one letter within a column are not significantly different ($p \leq 0.01$).

Table 3 presents the results of the AnBC. Anaerobic bacteria were not detected at zero time in the three trials. This finding was not comparable to a study conducted by Alhafeth et al. [36], which reported that the mean AnBC of 20 samples of canned fish was 3.6 x 10^3 CFU/g. In this study, after 48 h of storage at 4 °C, the AnBC recorded the highest count of 3.3 log CFU/g, which decreased to 1 log CFU/g by the end of storage. At 28 °C and 31 °C, the AnBC increased during storage, reaching 6.05 and 5.21 log CFU/g after 120 h and 168 h, respectively. According to statistical analysis, there was a significant difference in the effect of storage temperature at 4 °C on AnBC compared with 28 °C and 31 °C, while there was no significant difference between 28 °C and 31 °C ($p \leq 0.01$). In addition, there was a significant effect ($p \leq 0.01$) of the storage time on the AnBC.

Table 3. Anaerobic bacteria count in canned tuna meat at different storage temperatures

Storage time	Anaerobic Bacteria Count (log CFU/g) ±SD		
	4 °C	28 °C	31 °C
Zero time	n.d.	n.d.	n.d.
After 6 h.	n.d.	2.56± 0.10^{de}	1.82± 0.04^{fg}
After 24 h.	n.d.	2.22± 0.86^{ef}	1.98± 0.18^{ef}
After 48 h.	3.3± 0.04^{d}	2.98± 0.02^{d}	5.08± 0.02^{b}
After 72 h.	0.65± 0.91^{hj}	4.08±0.01^{c}	4.52± 0.08^{bc}
After 96 h.	1.15± 0.21^{gh}	5.09±0.00^{b}	5.14±0.00^{b}
After 120 h.	1.24± 0.33^{gh}	6.05±0.01^{a}	5.19±0.00^{b}
After 168 h.	1± 0.00^{h}	4.33±0.04^{c}	5.21±0.00^{b}

n.d. — not detected.
Means that share one letter within a column are not significantly different ($p \leq 0.01$).

The growth of pathogenic bacteria leads to economic losses, as products are excluded if they are not compliant with regulations, and if these products reach consumers, they can cause FBDs [17]. Proper handling, preparation, and processing steps lead to the safety of canned tuna. Table 4 shows that the pathogenic bacteria considered in this study, including *coliform count*, *E. coli*, *S. aureus*, *L. monocytogenes*, and *Salmonella* spp., were not detected. Thus, the results correspond with the Libyan Standard [39], which states that canned tuna should be free from pathogenic bacteria and/or their toxins. The absence of pathogenic bacteria in the studied canned tuna may be attributed to good manufacturing practices and good hygiene practices, as well as operators' care about the tuna source and contracting with reliable suppliers. Moreover, the absence of pathogenic bacteria in the studied samples may reflect the characteristics of the fish environment [40]. On the contrary, Dhinesh et al. [2] reported the presence of various pathogenic bacteria such as *E. coli*, *S. aureus*, *Salmonella* spp., *Vibrio* spp., and *Listeria* spp. in canned tuna meat of different brands.

The consumption of canned tuna can cause FBDs due to the activity of pathogenic bacteria, including histamine-forming bacteria [41]. Since the histamine-forming bacteria is a thermostable compound, cooking, smoking, or freezing will not eliminate it when forming. Thus, keeping the histamine-forming bacteria at low levels from capture to consumption is an important key to fish safety [24]. Table 5 reports the results of histamine-forming bacteria concentration in canned tuna meat at different storage temperatures. All tuna meat samples in the three trials contained histamine-forming bacteria ranging between 2.69 to 4.46 mg/kg at zero time, which was below the maximum limit of 100 mg/kg established by Libyan standard [39] and also below the safety level of 50 mg/kg established by the Food and Drug Administration [42]. Because low temperatures inhibit the growth of histamine-forming bacteria during fish processing [21], keeping fish cool from the moment of capture until it is eaten serves as an essential step in lowering the incidence of histamine-forming bacteria poisoning. Furthermore, the use of food safety systems such as HAZARD ANALYSIS AND CRITICAL CONTROL POINT (HACCP) SYSTEM in the processing of canned tuna may be the reason for the low histamine-forming bacteria contents in the samples under examination [43]. On the same line, in

Table 4. Pathogenic bacteria in canned tuna meat at different storage temperatures

Pathogenic bacteria	Storage temp.	Storage Time							
		Zero time	After 6 h.	After 24 h.	After 48 h.	After 72 h.	After 96 h.	After 120 h.	After 186 h.
Coliform count	4 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	28 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	31 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Escherichia coli	4 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	28 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	31 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
S. aureus	4 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	28 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	31 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
L. monocytogenes	4 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	28 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	31 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Salmonella spp.	4 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	28 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	31 °C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. — not detected.

Libya, a study of tuna sandwiches being sold to pupils and students determined histamine-forming bacteria content in 19 tuna sandwiches collected from food vendor premises in March and April, 2016. The histamine-forming bacteria concentrations ranged between 0.52 to 4.85 mg/kg and were below the Libyan maximum permitted levels and FDA [44]. The histamine-forming bacteria formation exhibited varying patterns at different temperatures, highlighting the significant impact of storage temperature on histamine-forming bacteria production. After 24 hours, the greatest histamine-forming bacteria concentrations in the present investigation were 3.53 mg/kg for samples kept at 4 °C and 9.58 mg/kg for samples kept at 28 °C. For the samples stored at 31 °C, the histamine-forming bacteria increased with the storage. The results of the statistical analysis showed a significant effect ($P \leq 0.01$) of temperature and storage time on the histamine-forming bacteria. None exceeded the maximum limit established by the Libyan standard of 100 mg/kg [39] and FDA safety level of 50 mg/kg [42]. Although histamine-forming bacteria levels were below the maximum limit, the tuna exhibited significant spoilage signs and notable changes in its organoleptic characteristics including smell and appearance after 3 and 2 days at 28 and 31 °C, respectively. On the same line, Altafini et al. [45] found that no histamine-forming bacteria formation was detected in tuna samples stored at room temperature for six days. However, the tuna showed marked spoilage and changes in organoleptic characteristics after five days. These results agree with findings by Lehane and Olley [46], who observed that decarboxylase-positive bacteria growing at refrigeration temperatures typically produce histamine-forming bacteria in lower quantities than species that grow at warmer temperatures, making it less likely for toxic levels to be reached. In a similar study, Altafini et al. [45] found that storing canned tuna in sunflower oil, to which certain types of vegetables were added after opening, at 4 °C, 12 °C and 20 °C for 8 days, did not result in histamine-forming bacteria formation in the samples collected daily during storage. Also, Kordiovská et al. [47] found that the histamine-forming bacteria was not recorded in carp fish at 3 ± 2 °C during the 7-days storage period. The histamine-forming bacteria concentration not significantly rising during storage may be attributed to the fact that the presence of carboxylase-positive bacteria is necessary for histamine-forming bacteria formation and that temperature alone is not sufficient to stimulate this process [45]. Furthermore, the low histamine-forming bacteria concentrations during storage may be attributed to the brine used as a filling medium in tested tuna that prevents the growth of histamine-forming bacteria [48]. In addition, it was reported that among the factors that affect the histamine-forming bacteria formation is a salt concentration [49,50].

Table 5. Histamine-forming bacteria concentration in canned tuna meat at different storage temperatures

Storage time	Histamine-forming bacteria concentration mg/kg ± SD		
	4 °C	28 °C	31 °C
Zero time	2.69 ± 0.12^{k}	4.46 ± 0.06^{fgh}	4.03 ± 0.26^{fghj}
After 6 hours	2.85 ± 0.37^{k}	4.61 ± 0.49^{fg}	4.24 ± 0.21^{fgh}
After 24 hours	3.53 ± 0.35^{ghjk}	9.58 ± 0.72^{d}	3.58 ± 0.20^{ghjk}
After 48 hours	3.14 ± 0.18^{jk}	6.26 ± 0.05^{e}	4.24 ± 0.06^{fghj}
After 72 hours	2.80 ± 0.18^{k}	4.9 ± 0.05^{f}	7.31 ± 0.05^{e}
After 96 hours	2.39 ± 0.22^{k}	4.62 ± 0.20^{fg}	11.53 ± 0.75^{c}
After 120 hours	2.89 ± 0.67^{k}	4.40 ± 0.13^{fgh}	21.35 ± 1.67^{b}
After 168 hours	3.38 ± 0.34^{hjk}	4.44 ± 0.13^{fgh}	28.24 ± 1.22^{a}

Means that share one letter within a column are not significantly different ($p \leq 0.01$).

Conclusion

Prioritizing proper storage is the key to ensuring the safety of the canned tuna meat. Storage at a refrigeration temperature of 4 °C effectively suppressed microbial growth and histamine-forming bacteria formation. The storage of canned tuna meat at 28 °C and 31 °C provided a suitable environment for microbial growth and accelerated the process of histamine-forming bacteria formation, resulting in higher histamine-forming bacteria levels, although the concentration did not exceed the maximum level established in the Libyan standard. Thus, fish should be stored at temperatures of 4 °C or below to maintain the safety of the tuna. Consumers should be aware of the proper storage of canned tuna after the opening. Depending on the results of this study, it is recommended to conduct broader studies on the safety of canned tuna comparing locally manufactured brands and on the extent, to which the filling medium affects the safety of canned tuna.

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ANTHRAX IN INDONESIA: A ONE HEALTH APPROACH TO ZOONOTIC THREATS AND INTEGRATED PREVENTION STRATEGIES

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Keywords: *anthrax, Bacillus anthracis, Indonesia, One Health, zoonotic disease*

Abstract

Anthrax is a zoonotic disease caused by Bacillus anthracis and remains a significant threat in Indonesia. The disease has a substantial impact on public health, livestock productivity, and economic stability, particularly in areas with traditional farming practices and limited animal health surveillance systems. This study aims to analyze the epidemiology of anthrax in Indonesia using the One Health approach, which integrates human, animal, and environmental health aspects. Data were collected from official government documents, such as the Decree of the Minister of Agriculture of the Republic of Indonesia, as well as scientific literature obtained from reputable databases. The results indicated that approximately 76 % of Indonesia is categorized as suspect areas for anthrax, while 14 % are infected areas, with the highest prevalence in Java, Nusa Tenggara, and Sulawesi. The primary risk factors include the consumption of non-veterinary inspected animal meat, unhygienic handling of carcasses, and the persistence of B. anthracis spores in the environment. Recommended control strategies include periodic livestock vaccination, conducting active surveillance, increasing public awareness, and enhancing the diagnostic laboratory's capacity. The One Health approach has proven effective in reducing the risk of cross-species transmission and improving responses to outbreaks. This study advocates for strengthening the integrated surveillance system, increasing synergy between sectors, and further research on the impact of climate change on zoonotic disease dynamics. With an integrated and collaborative strategy, Indonesia has a significant opportunity to control anthrax sustainably and enhance the resilience of public and animal health.

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Introduction

Anthrax is a zoonotic disease that mainly affects herbivorous animals and can be transmitted to humans [1,2]. The causative agent of the disease is *Bacillus anthracis*, an aerobic Gram-positive bacterium of the genus *Bacillus* [3]. *B. anthracis* is non-motile, spore-forming, and grows optimally at 37 °C in blood or nutrient agar media. This bacterium exhibits two morphological forms during its life cycle: metabolically active vegetative and dormant spore forms. Infections in herbivorous animals typically occur through direct and indirect contact. Meanwhile, transmission of the disease generally occurs in humans through contact with infected animals, animal carcasses, or contaminated animal products [4,5]. *B. anthracis* spores are

formed when vegetative cells are exposed to the external environment, mainly through body fluids from infected animal carcasses. These spores are highly resistant to a variety of extreme environmental conditions, including high and low temperatures, chemical disinfectants, desiccation, salting of skin, pH extremes, and irradiation [6,7].

Anthrax is widely recognized for its potential as a biological weapons agent [8]. Historically, the disease has been a significant threat to human health for centuries. Although countries in Asia and Africa are often considered endemic regions, anthrax cases have also been reported in the United States, Australia, Sweden, Italy, and various countries in Europe [3,9,10]. The largest anthrax outbreak was recorded in Zimbabwe, with approximately 10,000 human cases during the period 1978–1980 [8]. Anthrax fatality rates can reach up to 30 %, especially if the skin infection progresses to a systemic form [7]. Globally, an estimated 1.83 billion people (95 % confidence interval: 0.59–4.16 billion) live in areas at high risk of anthrax exposure [11]. In China, a total of 1,244 human anthrax cases were reported between 2018 and 2022 [12]. Overall, anthrax is estimated to affect between 20,000 and 100,000 people per year worldwide [13,14]. More than 1.8 billion people, including more than 60 million farmers and 1.1 billion animals, are also at high risk of the disease [11].

Anthrax is a zoonotic disease that has long been recorded in Indonesia with a history of recurrent endemicity in various regions [15,16]. In Indonesia, human anthrax deaths have been reported in several provinces, especially in endemic areas [17,18]. The first case was reported in 1832 in Kolaka, Southeast Sulawesi, and spread to Lampung (1884) and East Java (1885). Outbreaks returned in 1975–1977 and 1981–1986 in several provinces, including Java, Sulawesi, Nusa Tenggara, and Sumatra. The disease became more widespread in 1988–1994, and the incidence continued to recur until 2020 [19,20]. Data from the Indonesian Ministry of Health recorded fluctuations in cases, with a peak of 77 cases in 2017 in five provinces [21,22]. Major outbreaks occurred again in 2022 in Central Java and Yogyakarta, and recurred in July 2023 and February 2025 in Gunungkidul District [23–26].

Anthrax disease is globally distributed, with the highest incidence reported from endemic regions such as Asia and Africa [26,27]. Vaccination of susceptible animals has proven effective in reducing disease incidence, but implementation remains uneven in many developing countries [28,29]. In the context of global zoonotic threats and the potential use of *Bacillus anthracis* as a biological weapon agent, a comprehensive and cross-sectoral understanding of the dynamics of this disease is required. Therefore, this article aims to take an in-depth look at anthrax disease through a One Health approach, highlighting the interactions between environmental factors, animal, and human health. This review is expected to provide a scientific basis for developing integrated and sustainable prevention and control strategies to minimize the risk of zoonoses in the future.

Objects and methods

This research is a literature review that aims to describe essential aspects of anthrax disease in animals and humans, and how they relate to the One Health approach. Data on the status of anthrax in animals in Indonesia was obtained from official sources, including the Decree of the Minister of Agriculture of the Republic of Indonesia Number 311/KPTS/PK.320/M/06/2023 [30]. In addition, relevant scientific articles were accessed online through trusted databases such as PubMed NCBI, DOI, ScienceDirect, and Scopus. Inclusion criteria in this review included articles that discussed the etiology, pathogenesis, epidemiology, diagnosis, clinical manifestations, prevention, and control of anthrax in animals and humans. The collected data were analyzed descriptively to evaluate six main aspects, namely: (1) anthrax etiology and epidemiology, (2) clinical manifestations and diagnosis, (3) control and prevention, (4) One Health approach in anthrax management, (5) socio-economic and environmental implications, and (6) recommendations and future research directions.

Etiology and epidemiology of anthrax

Anthrax is a zoonotic disease caused by *Bacillus anthracis*. This gram-positive, encapsulated bacterium is facultatively anaerobic and can survive in aerobic and anaerobic conditions. The organism can form spores when exposed to the external environment, particularly through the bodily fluids of dead animals, which allows its survival over very long periods [16,31]. The term "*anthrax*" comes from the Greek anthrakites, meaning "like coal", referring to the black eschar that characterizes the skin manifestations of the disease [32]. *B. anthracis* spores survive in soil for up to 40 years [33]. These endospores resist extreme environmental conditions, including desiccation, high and low temperatures, ultraviolet (UV) radiation, gamma rays, and disinfectants [34]. The virulence of *B. anthracis* is mainly determined by two main factors, namely the tripartite toxin and the anti-phagocytosis polypeptide capsule [7]. An overview of the life cycle of *B. anthracis* can be seen in Figure 1.

The genes responsible for the virulence factors of *Bacillus anthracis* are located on two plasmids, pXO1 (182 kb) and pXO2 (95 kb) [35]. The pathogenicity of this organism decreases significantly if one of these plasmids is missing. The tripartite toxin produced consists of three main components: protective antigen (PA), lethal factor (LF), and edema factor (EF). PA's primary function is mediating the entry of LF and EF into target cells, allowing them to interact with critical cellular pathways [36,37]. These toxins are secreted during the vegetative proliferation phase of *B. anthracis* and are responsible for the characteristic symptoms of anthrax [7,38]. Anthrax is recognized as a toxin-mediated disease, with two main toxins, lethal toxin (LT) and edema toxin (ET), acting as virulence factors. LT inhibits immune responses and causes vasomotor instability, while ET induces edema at the cellular and tissue levels.

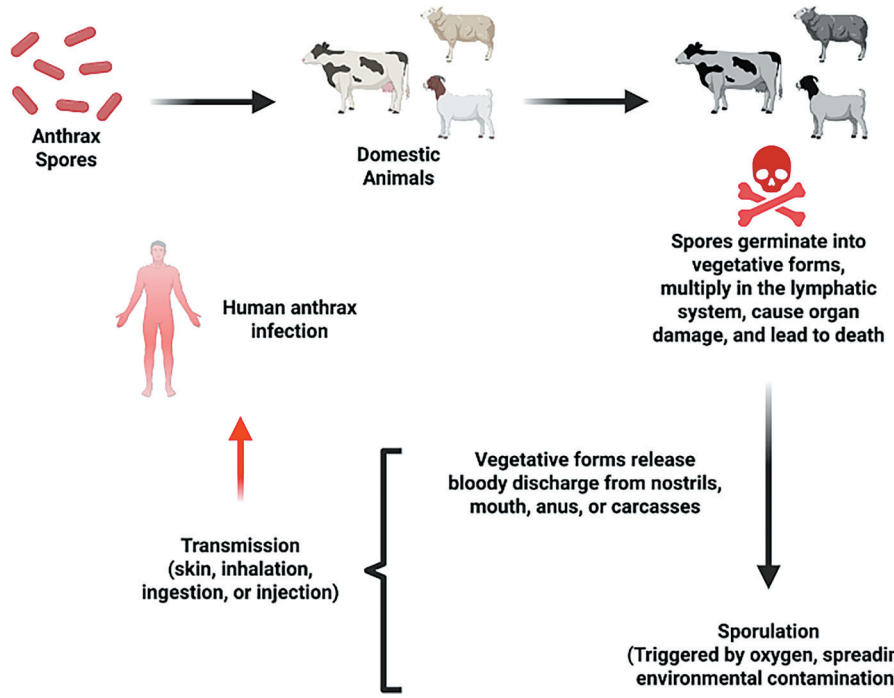

Figure 1. *Bacillus anthracis* transmission cycle in the context of One Health. *B. anthracis* spores infect herbivorous animals through environmental exposure. Infected animals may die suddenly and release spores back into the environment. Humans are infected through direct contact with contaminated animals or animal products. This illustration emphasizes the importance of the One Health approach to anthrax control. The figure was created using BioRender (https://BioRender.com) under a licensed agreement

LT is formed through the interaction between PA and LF, while ET is formed through the interaction between PA and EF [39]. Both plasmids play a crucial role in the virulence of *B. anthracis*. Plasmid pXO1 encodes the anthrax toxin components (PA, LF, and EF), while plasmid pXO2 encodes a polypeptide capsule that protects the bacteria from phagocytosis by the host immune system [40,41]. These genetic products are key to the offensive (toxin) and defensive (capsule) mechanisms of *B. anthracis* and determine the pathogenicity of the bacteria [42]. Thus, losing one of the plasmids, pXO1 or pXO2, will significantly reduce the organism's virulence.

Global warming has the potential to significantly impact the infection dynamics of several pathogenic agents, including anthrax [43]. Increased temperatures have led to the thawing of permafrost, which can release previously trapped *Bacillus anthracis* spores, increasing the risk of human and animal exposure. In addition, global warming also affects the spectrum of disease vectors, altering the development and behavior of various insect and arthropod species, which, although not the primary vectors of anthrax, can still indirectly influence the ecological dynamics of the disease [44]. Rising temperatures and the resulting environmental stress can potentially weaken the immune systems of livestock and wildlife, thereby increasing susceptibility to infections, including anthrax. Anthrax outbreaks tend to occur more frequently and with greater severity in animals stressed by climate change. Global warming also alters the relationship between host and pathogen, creating conditions favoring the persistence and spread of *B. anthracis*. For example, changes in animal population density, migration patterns, and behavior due to climate change may increase the likelihood of previously unexposed animal populations coming into contact with anthrax spores, triggering outbreaks [35,45].

Decree of the Minister of Agriculture of the Republic of Indonesia Number 311/KPTS/PK.320/M/06/2023 on the Determination of Animal Disease Situation Status is based on the recommendation of the National Veterinary Authority through epidemiological studies as stated in document Number B-305/HK.100/F4/05/2023 [30]. This decree determines animal disease status in areas classified into: a) Free Area, b) Suspected Area, c) Infected Area, and d) Outbreak Area. The determination of the situation status is based on several parameters, namely: a) disease incidence, b) disease level, c) surveillance system, d) pathogenic nature of the disease, e) disease epidemiology, f) susceptible animal population, and g) geographical location. Figure 2

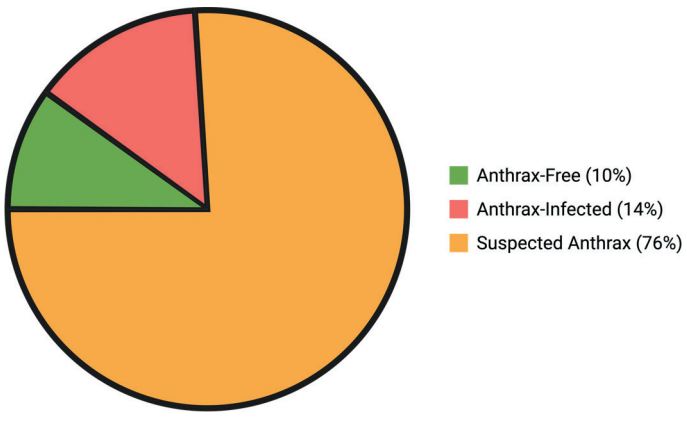

Figure 2. Epidemiological analysis of anthrax disease in Indonesia. The figure was created using BioRender (https://biorender.com) under a licensed agreement, based on the Decree of the Minister of Agriculture of the Republic of Indonesia No. 311/KPTS/PK.320/M/06/2023 [30]

illustrates the epidemiological analysis of anthrax disease in Indonesia. The data shows that approximately 10 % of Indonesia is recorded as anthrax-free, reflecting successful disease control and prevention efforts in some regions. On the other hand, around 76 % of Indonesia is classified as anthrax suspect areas, indicating a potential risk of disease spread that requires close monitoring and ongoing preventive measures. Meanwhile, around 14 % of other regions have been declared as anthrax-infected areas, indicating active infection and the need for further intervention to control the spread of the disease. These findings provide an important insight into the geographical distribution of anthrax disease in Indonesia and emphasize the urgency of strengthening surveillance, biosecurity, vaccination, and rapid response programs to prevent the expansion of cases in suspected and infected areas (Figure 3).

Table 1 shows areas in Indonesia that are free from anthrax. A free area is an area or region where no infectious animal disease agent has ever been found, historically based, or where there was originally a case of a contagious animal disease agent. After observation, it turns out that no more cases of infectious animal disease agents were found [46]. Based on the Decree of the Minister of Agriculture of the Republic of Indonesia No. 311/KPTS/PK.320/ M/06/2023, several regions in Indonesia have been designated as anthrax-free areas [30]. This status is an essential indicator in zoonotic disease control efforts at the national level through an area-based approach. This designation reflects the successful implementation of strategic infectious animal disease surveillance and prevention programs, including applying One Health principles in these areas. Geographically, anthrax-free regions are primarily in eastern Indonesia. East Nusa Tenggara Province is one of the provinces declared anthrax-free, covering districts/cities such as Kupang, South Central Timor, North Central Timor, Belu, Alor, Lembata, Rote Ndao, Malacca, and Kupang City. Districts/municipalities recorded as anthrax-free in Papua include Jayapura, Yapen Islands, Biak Numfor, Sarmi, Keerom, Waropen, Supiori, Mamberamo Raya, and Jayapura City. West Papua Province also has a wide coverage of anthrax-free areas, including Fakfak, Kaimana, Teluk Wondama, Teluk Bintuni, Manokwari, South Sorong, Sorong, Raja Ampat, Tambrauw, Maybrat, South Manokwari, Arfak Mountains, and Sorong City. Meanwhile, new administrative regions such as Papua Mountains (Tolikara, Nduga, Lanny Jaya, Central Mamberamo, Yalimo), South Papua (Merauke, Boven Digoel, Mappi, Asmat), and Central Papua (Nabire, Paniai, Puncak Jaya, Mimika, Puncak, Dogiyai, Deiyai, Intan Jaya) have also been designated as anthrax-free areas.

A suspect area is an area or region with a free status of infectious animal disease directly adjacent to an outbreak or infected area, or where a free status or infected status cannot be determined [46]. Table 2 illustrates the situation

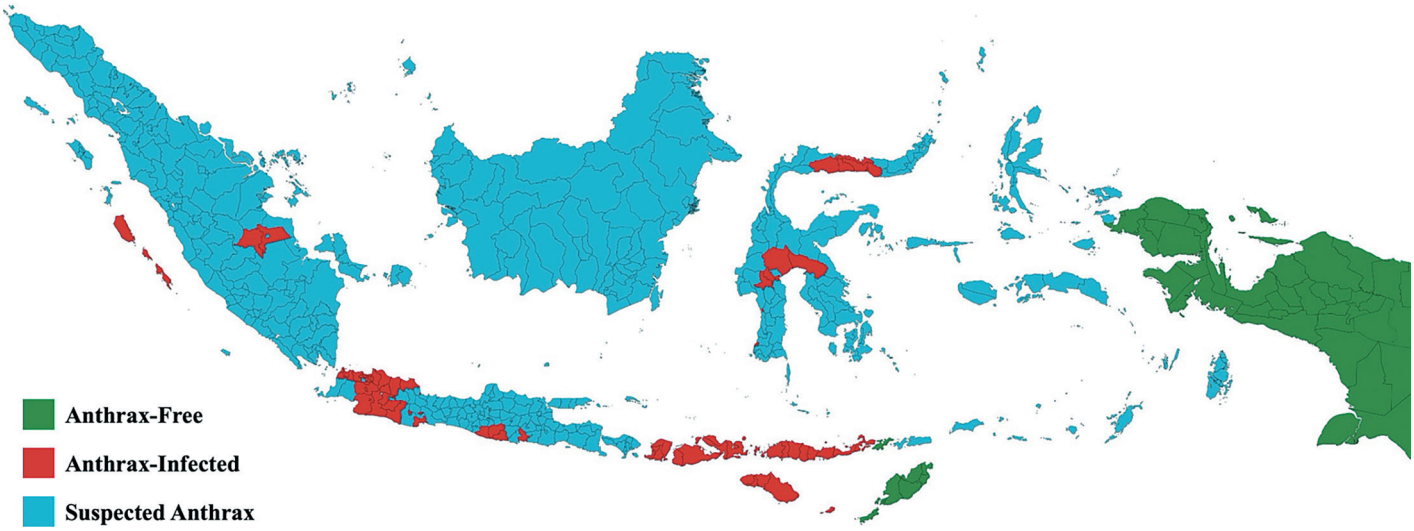

Figure 3. Geographical distribution of anthrax in Indonesia. This figure was created using MapChart.net, based on the Decree of the Minister of Agriculture of the Republic of Indonesia No. 311/KPTS/PK.320/M/06/2023 [30]

Table 1. Situation analysis of anthrax-free areas in Indonesia (Decree of the Minister of Agriculture of the Republic of Indonesia Number 311/KPTS/PK.320/M/06/2023 concerning the Determination of Animal Disease Status [30]

Province	District/City	Disease Situation
Nusa Tenggara Timur	Kupang, South Central Timor, North Central Timor, Belu, Alor, Lembata, Rote Ndao, Malacca, Kupang City	Free
Papua	Jayapura, Yapen Islands, Biak Numfor, Sarmi, Keerom, Waropen, Supiori, Greater Mamberaino, Jayapura City	Free
West Papua	Fakfak, Kaimana, Teluk Wondama, Teluk Bintuni, Manokwari, Sorong Selatan, Sorong, Raya Ampat, Tambrauw, Maybrat, South Manokwari, Arfak Mountains, Sorong City	Free
Papua Mountains	Tolikara, Nduga, Lanny Jaya, Central Mamberamo, Yalimo	Free
South Papua	Merauke, Boven Digoel, Mappi, Asmat	Free
Central Papua	Nabire, Paniai, Puncak Jaya, Mimika, Puncak, Dogiyai, Deiyai, Intan Jaya	Free

Table 2. Situation analysis of anthrax suspect areas in Indonesia (Decree of the Minister of Agriculture of the Republic of Indonesia Number 311/KPTS/PK.320/M/06/2023 concerning the Determination of Animal Disease Status [30])

Province	District/City	Disease Situation
Aceh	Simeulue, Aceh Singkil, South Aceh, Southeast Aceh, East Aceh, Central Aceh, West Aceh, Aceh Besar, Pidie, Bireuen, North Aceh, Southwest Aceh, Gayo Lues, Aceh Tamiang, Nagan Raya, Aceh Jaya, Bener Meriah, Pidie Jaya, Banda Aceh, Sabang, Langsa, Lhokseumawe, Subulussalam	Suspected
Bali	Jembrana, Tabanan, Badung, Gianyar, Klungkung, Bangli, Karang Asem, Buleleng, Denpasar	Suspected
Banten	Pandeglang, Lebak, South Tangerang	Suspected
Bengkulu	South Bengkulu, Rejang Lebong, North Bengkulu, Kaur, Seluma, Mukomuko, Lebong Kepahiang, Bengkulu Tengah, Bengkulu	Suspected
Daerah Istimewa Yogyakarta	Kulon Progo, Bantul, Sleman, Yogyakarta City	Suspected
DKI Jakarta	Seribu Islands	Suspected
Jambi	Kerinci, Merangin, Sarolangun, East Tanjung Jabung, West Tanjung Jabung, Tebo Bungo, Jambi City, Sungai Penuh	Suspected
West Java	Tasikmalaya, Ciamis, Kuningan, Cirebon, Majalengka, Sumedang, Bandung City, Cimahi City	Suspected
Central Java	Cilacap, Banyumas, Purbalingga, Banjarnegara, Kebumen, Purworejo, Wonosobo, Magelang, Boyolali, Klaten, Sukoharjo, Karanganyar, Sragen, Grobogan, Blora, Rembang, Pati, Kudus, Jepara, Demak, Semarang, Temanggung, Kendal, Batang, Pekalongan, Pemalang, Tegal, Brebes, Magelang City, Surakarta, Salatiga, Semarang City, Pekalongan City, Tegal City	Suspected
East Java	Ponorogo, Trenggalek, Blitar, Kediri, Malang, Lumajang, Jember, Banyuwangi, Bondowoso, Situbondo, Probolinggo, Pasuruan, Sidoarjo, Mojokerto, Jombang, Nganjuk, Madiun, Magetan, Ngawi, Bojonegoro, Tuban, Lamongan, Gresik, Bangkalan, Sampang, Pamekasan, Sumenep, Kediri Cty, Blitar Cty, Malang City, Probolinggo City, Pasuruan City, Mojokerto City, Madiun City, Surabaya, Batu.	Suspected
West Kalimantan	Sambas, Bengkayang, Landak, Mempawah, Sanggau, Ketapang, Sintang, Kapuas Hulu, Sekadau, Melawi, North Kayong, Kubu Raya, Pontianak City, Singkawang	Suspected
South Kalimantan	Tanah Laut, Kotabaru, Banjar, Barito Kuala, Tapin, Hulu Sungai Selatan, Hulu Sungai Tengah, Hulu Sungai Utara, Tabalong, Tanah Bumbu, Balangan, Kota Banjarmasin, Banjar Baru City	Suspected
Central Kalimantan	West Kotawaringin, East Kotawaringin, Kapuas, South Barito, North Barito, Sukamara, Lamandau, Seruyan, Katingan, Pulang Pisau, Gunung Mas, East Barito, Murung Raya, Palangka Raya	Suspected
East Kalimantan	Paser, Kutai Barat, Kutai Kertanegara, Kutai Timur, Berau, Penajam Paser Utara, Mahakam Hulu, Balikpapan, Samarinda, Bontang	Suspected
North Kalimantan	Malinau, Bulungan, Tana Tidung, Nunukan, Tarakan City	Suspected
Bangka Belitung Islands	Bangka, Belitung, Bangka Barat, Central Bangka, South Bangka, East Belitung, Pangkal Pinang	Suspected
Riau Islands	Karimun, Bintan, Natuna, Lingga, Anambas Island, Batam, Tanjung Pinang	Suspected
Lampung	West Lampung, Tanggamus, South Lampung, East Lampung, Central Lampung, North Lampung, Way Kanan, Tulang Bawang, Pesawaran, Pringsewu, Mesuji, West Tulang Bawang, West Pesisir, Bandar Lampung, Metro	Suspected
Maluku	Maluku Southeast West, Southeast Maluku, Central Maluku, Buru, Aru Islands, West Seram, East Seram, Southwest Maluku, South Buru, Ambon City, Tual City	Suspected
North Maluku	West Halmahera, Central Halmahera, Sula Islands, South Halmahera, North Halmahera, East Halmahera, Morotai Island, Taliabu Island, Ternate City, Tidore Islands City	Suspected
Riau	Kuantan Singingi, Indragiri Hulu, Indragiri Hilir, Pelalawari, Siak, Kampar, Rokan Hulu, Bengkalis, Rokan Hilir, Meranti Island, Kota Pekanbaru, Dumai	Suspected
West Sulawesi	Majene, Polewali Mandar, Mamasa, Mamuju, Pasangkayu, Central Mamuju	Suspected
South Sulawesi	Selayar Islands, Bulukumba, Bantaeng, Jeneponto, Takalar, Gowa, Sinjai, Maros, Pangkajene Islands, Barru, Bone, Soppeng, Wajo, Sidenreng Rappang, Pinrang, Enrekang, Luwu	Suspected
Central Sulawesi	Banggai Islands, Banggai, Morowali, Poso, Donggala, Toli Toli, Buol, Parigi Moutong, Tojo Una Una, Sigi, Banggai Laut, North Morowali, Palu City	Suspected
Southeast Sulawesi	Buton, Muna, Konawe, Kolaka, South Konawe, Bombana, Wakatobi, North Kolaka, North Buton, North Konawe, East Kolaka, Konawe Islands, West Muna, Central Buton, South Buton, Kendari City, Baubau City	Suspected
North Sulawesi	Bolaang Mongondow, Minahasa, Sangihe Islands, Talaud Islands, South Minahasa, North Minahasa, North Bolaang Mongondow, Siau Tagulandang Biaro, Southeast Minahasa, South Bolaang Mongondow, East Bolaang Mongondow, Manado City, Bitung City, Tomohon City, Kotamobagu City	Suspected
West Sumatra	Pesisir Selatan, Solok, Sijunjung, Tanah Datar, Padang Pariaman, Agam, Lima Puluh Kota, Pasaran, South Solok, Dharmasraya, Pasaman Barat, Padang, Solok City, Sawah Lunto, Padang Panjang, Bukit Tinggi City, Payakumbuh, Pariaman	Suspected
South Sumatra	Ogan Komering Ulu, Ogan Komering Ilir, Muara Enim, Lahat, Musi Rawas, Musi Banyuasin, Banyu Asin, South Ogan Komering Ulu, East Ogan Komering Ulu, Ogan Ilir, Empat Lawang, Penukal Abab Lematang Ilir, North Musi Rawas, Palembang, Prabumulih City, Pagar Alam, Lubuklinggau	Suspected
North Sumatra	Nias, Mandailing Natal, South Tapanuli, Central Tapanuli, North Tapanuli, Toba Samosir, Labuhan Batu, Asahan, Simalungun, Dairi, Karo, Deli Serdang, Langkat, South Nias, Humbang Hasundutan, Pakpak Bharat, Samosir, Serdang Bedagai, Batu Bara, North Padang Lawas, Padang Lawas, South Labuhan Batu, North Labuhan Batu, North Nias, West Nias, Sibolga City, Tanjung Balai, Pematang Siantar, Tebing Tinggi, Medan, Binjai, Padang Sidempuan, Gunungsitoli City	Suspected

of anthrax suspect areas in Indonesia, based on Decree of the Minister of Agriculture of the Republic of Indonesia No. 311/KPTS/PK.320/M/06/2023 [30]. Several areas in Indonesia have been identified as suspect areas. Based on Decree of the Minister of Agriculture of the Republic of Indonesia Number 311/KPTS/PK.320/M/06/2023, many districts/cities in various provinces of Indonesia have been classified as anthrax suspect areas [30]. This status indicates the potential presence or risk of spreading *Bacillus anthracis*, either through a history of previous cases, environmental factors that support spore persistence, or limitations in the monitoring and reporting system in the area [30]. The distribution of suspect areas covers almost all provinces in Indonesia, including Sumatra, Java, Kalimantan, Sulawesi, Maluku, and parts of Papua. Densely populated provinces such as East Java, Central Java, and West Java include dozens of districts/cities in the suspect category, raising concerns about the potential impact of zoonoses on public health, the livestock industry, and trade in animals and animal products.

In addition to the central agricultural regions of Java and Sumatra, suspect areas include border areas and islands such as Riau Islands, Bangka Belitung, and North Maluku, which can complicate the response due to limited animal health infrastructure and geographical access. Urban areas such as DKI Jakarta (Thousand Islands) and other major cities are also included in the suspect list, reflecting the possible risk of transmission through the distribution of animals or animal products from different areas. This "suspect" classification does not necessarily indicate an active outbreak, but rather shows the need for increased vigilance, early detection, and strengthening of surveillance systems under the One Health approach. In this context, synergy between the animal health, human health, and environmental sectors is crucial to prevent risk transformation into an outbreak. This status determination provides an essential basis for local and central governments to design strategic policies, including implementing routine vaccination programs, capacity building of veterinary laboratories, and educating communities and farmers on zoonotic risk management. Responsive and collaborative response in these suspected areas will determine Indonesia's success in achieving a national anthrax-free status in the long term.

An infected area is an area or region where cases of certain infectious animal diseases are found in vulnerable animal populations, based on observations [46]. Outbreak areas are areas or regions where cases of certain infectious animal diseases are found in vulnerable animal populations, based on observations [46]. Based on the Decree of the Minister of Agriculture of the Republic of Indonesia No. 311/KPTS/PK.320/M/06/2023, several districts/cities in Indonesia are categorized as anthrax-infected areas, which are areas that have experienced or are currently experiencing confirmed cases of anthrax, both in animals and humans [30]. The distribution of infected areas covers 13 provinces in Indonesia and is spread across major islands such as Java, Sumatra, Sulawesi, and Nusa Tenggara (Figure 3). West Java and East Nusa Tenggara provinces have the highest number of infected districts/cities. West Java includes densely populated areas with high livestock intensity, such as Bogor, Garut, and Bandung, indicating a significant risk of cross-species transmission and unsafe distribution of animal products. On the other hand, East Nusa Tenggara recorded almost the entire mainland of Sumba and Flores as infected areas, indicating that anthrax has become an endemic disease in the region.

Other provinces, such as South Sulawesi (especially Tana Toraja and North Toraja) and DKI Jakarta, are also categorized as infected. Even the entire administrative area of DKI Jakarta is recorded as an infected area, reflecting that even urban areas are not free from anthrax risk, most likely due to the distribution of animals or animal products from infected areas. Areas such as the Mentawai Islands in

Table 3. Situation analysis of anthrax-infected areas in Indonesia (Decree of the Minister of Agriculture of the Republic of Indonesia Number 311/KPTS/PK.320/M/06/2023 concerning the Determination of Animal Disease Status [30])

Province	District/City	Disease Situation
Banten	Tangerang, Serang, Tangerang City, Cilegon City, Serang City	Contracted
Daerah Istimewa yogyakarta	Gunung Kidul	Contracted
DKI Jakarta	South Jakarta, East Jakarta, Central Jakarta, West Jakarta, North Jakarta	Contracted
Gorontalo	Boalemo, Gorontalo, Pohuwato, Bone Bolango, Gorontalo Utara, Gorontalo City	Contracted
Jambi	Batanghari, Muaro Jambi	Contracted
West Java	Bogor, Sukabumi, Cianjur, Bandung, Garut, Indramayu, Subang, Purwakarta, Karawang, Bekasi, Bandung Barat, Pangandaran, Kota Bogor, Kota Sukabumi, Kota Cirebon, Kota Bekasi, Kota Depok, Kota Tasikmalaya, Kota Banjar	Contracted
Central Java	Wonogiri	Contracted
East Java	Pacitan, Tulungagung	Contracted
West Nusa Tenggara	West Lombok, Central Lombok, East Lombok, Sumbawa, Dompu, Bima, West Sumbawa, North Lombok, Mataram, Bima City	Contracted
East Nusa Tenggara	West Sumba, East Sumba, East Flores, Sikka, Ende, Ngada, Manggarai, West Manggarai, Central Sumba, Southwest Sumba, Nagekeo, East Manggarai, Sabu Raijua	Contracted
South Sulawesi	Tana Toraja, North Luwu, East Luwu, North Toraja, Makassar, Pare Pare, Palopo City	Contracted
West Sumatra	Mentawai Islands	Contracted

West Sumatra and Wonogiri in Central Java, although only one district, remain important to monitor as they have the potential to be the starting point for further spread. This geographical distribution emphasizes the urgency of implementing an integrated One Health approach, which combines animal health, public health, and environmental management. This approach should be realized through strengthening active and passive surveillance systems, vaccinating vulnerable animals, closely monitoring the movement of animals and animal products, improving farmer and community education, and cross-sector coordination between health services, livestock services, and local governments. As an archipelago with high inter-regional connectivity, Indonesia's success in controlling anthrax will depend on the ability to implement a collaborative and sustainable risk-based control system.

Anthrax is a severe zoonotic disease that can infect various domestic and wild animal species and, under certain conditions, humans. Its potential to cause large-scale outbreaks, including at a global level, has made it one of the main focuses of veterinary public health and zoonotic disease surveillance systems [3]. Herbivorous animals, especially ruminants, are known to be the main reservoirs of *Bacillus anthracis*, with sheep having higher susceptibility than goats, cattle, and horses. Conversely, some species, such as dwarf pigs and Algerian sheep, show relatively higher levels of resistance to infection [31,47]. As a spore-forming pathogen naturally distributed in the environment, *B. anthracis* is classified as a high-priority threat agent due to its widespread availability in nature, ease of dispersal, and potential to cause significant morbidity and mortality in humans and animals [48]. Anthrax transmission in animals and humans generally occurs through direct contact with infected animals or contaminated animal products, such as meat, blood, skin, or internal organs [12]. Environmental transmission can also occur through inhalation of spores from contaminated soil, while in animals, spores enter mainly through the gastrointestinal tract. In the body, the anthrax bacillus produces a lethal toxin that can cause death, even after treatment with antibiotics [16].

Anthrax can manifest in several clinical forms depending on the route of infection, namely cutaneous, gastrointestinal, and inhalation anthrax [12]. Cutaneous anthrax is the most common form, occurring through direct contact with spores through skin wounds, and is characterized by characteristic lesions of black eschar; the mortality rate is relatively low (~5 %) if appropriately treated [7,49]. Gastrointestinal anthrax results from ingestion of spore-contaminated food and can cause severe symptoms such as abdominal pain, bloody diarrhea, and shock, with a mortality of around 50 % [7,50]. Inhalational anthrax, caused by spore inhalation, is the most fatal form, causing hemorrhagic mediastinitis and pulmonary edema; without immediate treatment, the mortality rate approaches 100 % [49]. Other variants, such as injectional anthrax and welder's anthrax, have been reported in certain at-risk groups, expanding the clinical spectrum and routes of transmission of the disease [32,51].

Human infection is generally a consequence of animal outbreaks and is often associated with the slaughter or consumption of sick animals, especially in regions with low food security, weak animal health surveillance systems or inadequate vaccination coverage, and can occur through inhalation of anthrax spores [11,52,53]. Of these three forms, cutaneous anthrax is the most common and accounts for more than 95 % of human cases [12]. Clinical manifestations are localized skin lesions on areas of the body that have frequent direct contact with animals or animal materials, such as the face, neck, hands, and arms [54]. Studies in Bangladesh have consistently shown that the risk of anthrax transmission to humans is highly correlated with slaughtering practices of infected animals and consumption of unhygienically processed animal products, including raw meat, blood, skin and internal organs [55–57]. Although most cases of natural anthrax are limited to the non-systemic cutaneous form, the infection can progress to systemic if not treated appropriately, especially when entered through an open wound on the skin [28,29].

Clinical manifestations and diagnosis

Diagnosis of anthrax in animals generally begins with observation of typical clinical symptoms, such as elevated body temperature, depression, respiratory distress, bloody discharge from the body orifices, tremors, and sudden death within a few hours of the onset of initial symptoms. To confirm anthrax cases, field disease investigation laboratories (FDILs) and veterinary hospitals in endemic areas usually use the polychrome methylene blue (PMB) staining technique, known as the McFadyean reaction, as the basic diagnostic method [58,59]. In addition to these conventional methods, more sophisticated molecular-based diagnostic techniques have been applied, such as polymerase chain reaction (PCR), which allows for rapid and accurate detection of target DNA [60]. In some cases, more sophisticated molecular approaches such as multilocus variable number tandem repeat analysis (MLVA) are also used for epidemiological analysis and genetic characterization of bacterial isolates from suspected infected animal samples [61].

In humans, clinical symptoms of anthrax generally begin with the appearance of painless skin lesions, which may take the form of papules or vesicles, and progress to solid black colored eschar. This manifestation is often considered an early indication or tentative case of cutaneous anthrax. To confirm the diagnosis, swab samples are taken from the exudate of the skin lesions and then analyzed using various diagnostic methods. The techniques used range from conventional methods such as Gram stain and Loeffler's methylene blue stain to advanced molecular approaches such as polymerase chain reaction (PCR) and multilocus variable-number tandem repeat analysis (MLVA) [55,57]. Each diagnostic method has varying sensitivity levels (Se) and specificity (Sp), requiring different laboratory

infrastructure and technology. In resource-limited settings, diagnosis generally relies on clinical manifestations and basic microbiological techniques, including bacterial culture. However, in countries with more advanced laboratory facilities, competence has been developed to perform molecular-based detection and more specific microbial cultures to identify and confirm the presence of *Bacillus anthracis* in human clinical samples [61,62,63].

Anthrax control and prevention

Anthrax control requires a multidisciplinary approach that includes medical interventions, animal health policies, environmental surveillance, and active community involvement. Various strategies have proven effective in suppressing the spread of the disease, including timely reporting and monitoring of cases, rapid response to extraordinary events, restricting the movement of animals and animal products from affected areas, and managing animal carcasses through safe burning or burial methods. Routine disinfection of livestock facilities and vaccination of at-risk animals are also key components of control strategies [12,51].

Live attenuated anthrax vaccines have been widely used and shown to provide adequate protection. However, they still have limitations, including residual toxicity, relatively short duration of protection, and reports of post-vaccination mortality [64,65]. Therefore, developing a new generation of safer vaccines that provide long-term immunity is urgently needed, utilizing advances in recombinant vaccine technology and adjuvants. In addition, the high mortality rate in gastrointestinal and inhalation anthrax is due to toxin production by *Bacillus anthracis* and the limited effectiveness of conventional therapies. The development of therapies targeting the toxin is a top priority in improving survival rates [51]. Currently, antimicrobial therapy remains the mainstay of anthrax treatment. However, adjuvant therapies in the form of antitoxins have been developed and approved by the Food and Drug Administration (FDA), including Anthrax Immune Globulin Intravenous (AIGIV/Anthrasil), raxibacumab (Abthrax), and obiltoxaximab (Anthim). These three agents work by binding to protective antigen (PA) and inhibiting the formation of lethal toxin (LT) and edema toxin (ET) [48]. In addition to therapy, raxibacumab and obiltoxaximab can be used as post-exposure prophylaxis (PEP). The Centers for Disease Control and Prevention (CDC) recommends the use of antitoxins as adjunctive therapy for systemic anthrax cases, with no restrictions on age or risk group [66–68].

Non-medical aspects also play an important role in anthrax prevention and control. Continuous education of the public, especially farmers and field workers, is crucial in increasing risk awareness and preventing panic during an outbreak. Public education can be done with a comprehensive campaign about anthrax, its transmission symptoms, and preventive measures [6,10]. The media, farmer communities, animal trader communities, government, and other relevant institutions are key actors in the efficiency and effectiveness of anthrax mitigation in Indonesia [69,70]. This is important so that anthrax does not become a prolonged public threat [71,72].

Community education is becoming increasingly important as collective behavior and culture change [73,74]. Such efforts require a consistent and coordinated cross-sectoral approach, manifested by implementing Behavior Change Programs based on the Information, Education, and Communication/Behavior Change Communication (IEC/BCC) approach. However, some of these efforts are insufficient if not accompanied by direct intervention against the transmission source. Unfortunately, the current vaccination coverage of livestock against anthrax is still not optimal. Therefore, we recommend maximum annual vaccination of livestock, especially cattle. Routine vaccination significantly mitigates and reduces anthrax incidence [10,62].

To achieve effective herd immunity, at least 80 % of the cattle population in an area should receive annual vaccination [24]. To enable rapid response, cross-sector coordination between health, livestock, and forestry services must be strengthened, especially in reporting suspicious animal deaths. An active surveillance system and daily reporting by veterinary services should be implemented to detect disease early and prevent its spread. In addition, human resource capacity building through regular training for health workers and veterinarians is important in preparedness for zoonoses such as anthrax. Furthermore, anthrax is also categorized as a potential threat in bioterrorism, given that the pathogen is highly lethal and *B. anthracis* spores can survive in the environment for long periods. Therefore, national preparedness should be enhanced through the provision of isolation facilities, development of reliable diagnostic reagents, and stockpiling of vaccines and antibiotics as part of an emergency response to a possible bioterror attack [51,75].

One Health: an integrated approach to anthrax management

Prevention and control of zoonotic diseases, including anthrax, requires close coordination between the human health, animal health, and environmental management sectors. The One Health approach is a strategic framework that emphasizes the close linkages between these three components in global health [76]. The basic principles of One Health include cross-sector collaboration, integration of surveillance systems, and synergy in implementing public and animal health programs. One Health implementation enables early detection and rapid response to potential outbreaks through efficient and effective data-driven information exchange [77]. Furthermore, One Health encourages the development of integrated policies that consider shared risks such as socio-economic and ecological factors that influence zoonotic disease spread at local, national, and global levels [6].

Implementing the One Health approach includes important activities such as two-way communication between sectors, exchange of epidemiological surveillance data, use

of shared diagnostic tools, and adoption of best practices in zoonosis control. These efforts have supported accurately depicting the endemic situation, early detection of extraordinary events, rapid response to outbreaks, and implementation of vaccination programs in high-risk areas [78,79]. Furthermore, strengthening local capacity, improving access to treatment in endemic areas, and interdisciplinary research initiatives play an important role in strengthening the effectiveness of anthrax control. Outbreaks and pandemics in recent decades have exposed the weaknesses of the global health system, especially in dealing with diseases that involve complex interactions between humans, animals, plants, and the environment. This emphasizes the importance of One Health as a collaborative, cross-sectoral, integrative, and holistic framework [80].

The One Health approach also addresses the limitations of conventional sectoral and fragmented approaches. By emphasizing the importance of integrated management of zoonotic risk factors, it supports capacity building of cross-species surveillance systems. It identifies critical points of human-animal-environment interaction within a syndemic framework. The approach allows for more efficient and risk-based intervention strategies. Case studies from several countries show the successful application of the One Health approach in anthrax control, especially in areas with endemic status [4,6,27]. Strategies include inter-agency collaboration, mass vaccination of animals and humans, development of an integrated surveillance system, improvement of health infrastructure, and evaluation of public knowledge and perception. In addition, developing technical guidelines and operational protocols is an important component in supporting sustainable disease management [81–83].

In Indonesia, One Health implementation faces various challenges, including public knowledge, stakeholder knowledge, governance and policy, social and cultural factors, limited cross-sector coordination, low livestock vaccination coverage, and limited diagnostic laboratory capacity in the regions [54,84,85]. However, opportunities for integrating these approaches remain wide open, particularly through strengthening national policies, developing integrated information systems, and interprofessional training. With adequate regulatory support and strong political commitment, the One Health approach has great potential to be adopted as the primary strategy in managing priority zoonoses, including anthrax, in Indonesia.

Socio-economic and environmental implications

Anthrax not only impacts public health, but also has far-reaching consequences on social, economic, and environmental aspects. In the livestock sector, anthrax outbreaks often lead to mass livestock deaths that have a direct impact on farmers' income, food security, and economic stability, especially in rural areas that rely heavily on the agricultural sector. In addition to losses due to livestock deaths or due to livestock reduction, restrictions on the mobility of animals and animal products during outbreaks also disrupt local and regional trade [18]. Farmers must incur additional costs for outbreak control, such as emergency vaccination, environmental disinfection, and compensation for losses. Long-term anthrax disruption can weaken the country's agricultural supply chain [3].

From a social perspective, the emergence of an anthrax case can create fear in the community and stigmatize affected individuals or communities [69,77]. This could exacerbate the marginalization of vulnerable groups and hinder the effectiveness of public health interventions. In some cases, excessive fear has led to the rejection of health workers or volunteers, creating barriers to vaccination and open reporting of cases. Socially, anthrax cases also have the potential to undermine social cohesion, which should be an asset in building community wellbeing [86].

Anthrax also impacts resource use efficiency and livestock productivity [87]. In most developing countries, vaccination programs in susceptible animals in enzootic areas have reduced disease prevalence to relatively low levels nationwide. However, significant losses can still occur in specific population groups [3]. These losses include post-vaccination animal mortality, reduced livestock production, destruction of infected carcasses and by-products, and temporary closure of abattoirs [88]. Anthrax fatality rates vary between animal species [89]. Pigs generally have a high cure rate, whereas clinical infections in ruminants and horses tend to result in death [90]. Despite relatively low mortality rates in carnivores, information on infection rates in wildlife is limited [91].

The environment is a crucial element in the epidemiological dynamics of anthrax, especially given the ability of *Bacillus anthracis* to form highly resistant spores that can persist in soil for many years. Global climate change, land degradation, and the increasing frequency of extreme climate events such as floods and droughts have accelerated the distribution of infectious diseases, including anthrax, through their effects on microbial ecosystems and wildlife habitats [92]. Complex interactions between climatic factors, environmental management practices, and human activities such as wildlife consumption and trade increase the risk of zoonotic pathogens emerging and spreading across regions [93].

Studies show that soil characteristics play an important role in the persistence of anthrax spores. Endemic areas with clay or loam soils containing high calcium and organic carbon are known to be highly conducive to spore survival. High temperatures, extreme rainfall, and acidic soil pH also increase the potential for environmental contamination [57]. Research by Vieira et al. [62] found that 77.08 % of clay and 22.92 % of loam samples from endemic areas contained anthrax spores, with an average pH of 6.38. Clay soils were noted to be more than three times more likely to be contaminated with spores than non-clay soils. Inadequate carcass management practices, such as burial without disinfection or spore removal, exacerbate this persistence. Such practices create hotspots of infection for wild animals and livestock grazing in contaminated

areas [63]. Therefore, waste and carcass management strategies, including sterilization of contaminated materials and decontamination of outbreak sites, are crucial steps in breaking the transmission cycle.

Socio-cultural aspects also have a significant influence on the dynamics of anthrax transmission. In some remote communities, the practice of consuming the meat or blood of animals that die suddenly persists and is a significant route of infection [83,94]. Low community knowledge of the risk of anthrax, limited access to health services and veterinarians, and reluctance to accept modern medicine further exacerbate the situation [10,95,96]. In the context of global environmental change, future One Health approaches should be able to integrate climate projections and ecological risks in health system planning. Cross-disciplinary collaboration between climatologists, ecologists, and health experts is needed to design adaptive strategies to mitigate the emergence of new pathogens and re-emerging diseases. Integrating climate data, animal habitat maps, and zoonotic surveillance information is important in realizing a resilient and sustainable early warning system.

Recommendations and future research directions

Effective anthrax control requires a multisectoral strategy integrating human, animal, and environmental dimensions within the One Health framework. Addressing zoonoses' complex and dynamic risk factors requires a holistic approach that includes improving surveillance, diagnostic innovation, strengthening human resource capacity, and empowering local communities. One of the top priorities is developing an integrated surveillance system that combines data from the human, animal, and environmental health sectors. This surveillance should be participatory and community-based, especially in endemic areas with limited infrastructure. Early reporting by farmers and local communities can be an effective detection tool, but this requires support in the form of technical training and reporting incentives. Significant barriers remain in diagnosis, especially in rural areas without basic laboratory facilities. Therefore, providing simple diagnostic tools such as Gram stain and polychrome methylene blue (PMB) tests and training field laboratory technicians should be an integral part of surveillance strengthening programs [57].

Diagnostic innovations and community-based interventions are also urgent. Molecular-based rapid diagnostics and cheap and easy-to-use point-of-care testing methods must be developed to detect anthrax cases accurately at the point of source. On the other hand, digital technology, such as mobile app-based reporting systems, can also be utilized to accelerate case tracking and risk area mapping. Community involvement in disease education and control through social and behavior change communication (SBCC) campaigns will increase risk awareness and strengthen compliance with veterinary health protocols [57].

Capacity building of health workers across sectors is the primary foundation for implementing the One Health approach. Medical personnel, veterinary paramedics, and environmental officers must receive continuous training on anthrax detection, handling, and mitigation, including safe waste and carcass management. Authorized veterinarians must handle infected animals according to protocols. Meanwhile, the community needs to be equipped with practical information on safe management of livestock and foodstuffs and instill a responsive and responsible attitude. The community needs to receive guidance regarding the direct application of various practices in daily life.

Empowering animal health cadres and extension workers is crucial in bridging the information between authorities and grassroots communities. Strategically, policy and funding support from the government is needed, especially in providing door-to-door vaccination services, surveillance of meat and animal products during the outbreak season, and compensation for affected farmers. Strict inspection of animal products entering rural markets will help reduce human cases of cutaneous anthrax [59]. Future research directions need to focus on the development of climate-based and geospatial prediction models to identify new anthrax hotspots; evaluation of the effectiveness of One Health approaches in local and multicultural contexts; innovation of vaccines that are more durable and easier to distribute, especially for remote areas; and socio-cultural research related to risk perception, trust in health services, and local practices in livestock and carcass management. With an evidence-based approach and consistent cross-sector collaboration, anthrax control can be implemented more effectively and sustainably, while strengthening the resilience of public health systems at the local and national levels.

Conclusion

Anthrax is a serious zoonotic threat in Indonesia that requires an integrated and cross-sectoral approach. The study results show that around 76 % of Indonesia is classified as suspected infected areas, while 14 % have been confirmed infected, mainly in Java, Nusa Tenggara, and Sulawesi. Key risk factors include unhygienic farming practices, consumption of contaminated animal products, and the ability of *Bacillus anthracis* spores to persist in the environment. The One Health approach, which integrates human, animal, and environmental health aspects, has proven effective in controlling this disease. The study recommends strengthening integrated surveillance systems, increasing livestock vaccination coverage, and evidence-based public education. In addition, further research is needed to develop adaptive strategies to deal with disease dynamics due to climate change. The One Health approach is seen as increasingly relevant in dealing with zoonotic threats in the era of globalization, with great potential to strengthen the resilience of public health systems and livestock sustainably.

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POST-MORTEM IDENTIFICATION OF MEAT WITH ABNORMAL AUTOLYSIS BY NON-INVASIVE METHODS

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Keywords: *meat autolysis, quality group, electrical conductivity, optical methods, fluorescence spectroscopy, physical methods*

Abstract
The theme under consideration is of great interest for researchers and practical specialists engaged in the development of methods for identification of meat with different courses of autolysis. In this review, modern non-invasive methods for meat quality assessment are presented. The authors describe methods developed for identification of meat with nontraditional course of autolysis, including determination of electric properties of meat (electrical conductivity, electrical resistance, electrical impedance), optical methods (light scattering, reflection, absorption, Raman spectroscopy, fluorescence spectroscopy, visible/near/mid-infrared spectroscopy), investigation of physical parameters of meat (determination of meat color coordinates using spectrocolorimeters, nuclear magnetic resonance, ultrasound spectroscopy and others). The results of studies carried out by various researchers on the use of the proposed methods for meat sorting into quality groups and certainty of the obtained data are presented. It is shown that meat quality can be predicted using the obtained values of electrical parameters and optical spectra. Analysis of published materials shows that up to now there is no definite answer to the question about a choice of a method for identification of meat quality group. This problem requires further research and discussion.

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Introduction

Modern conditions of intensive raising of farm animals as well as target action of genetic transformations lead to formation of specific features of meat quality. For more than fifty years, the efforts of scientists and specialists in the field of meat production and processing are aimed at establishing all factors that cause predisposition of animals to stress, assessing a degree of their negative effect on the development of deviations in meat quality. Along with the problem of assessment of causes that induce quality peculiarities of meat raw materials, there is a problem of identification of meat with abnormal course of autolysis with the purpose of its rational use [1]. The objective determination of meat quality indicators requires labor-consuming laboratory investigations and the results of analyses can be obtained after several days. Joo et al. [2] stated that a method for effective grading of carcasses should be economical, easy to use and provide information as soon as possible after animal slaughter.

According to the opinion of many researchers [3–6], the most accessible and reliable method of meat grading into quality groups is measuring pH of muscle tissue of hot carcasses (pH_{45}) and then 24 hours after animal slaughter (pH_{24}). Comparison of the pH_{45} and pH_{24} values enables detecting meat with abnormal development of autolysis and identifying NOR, PSE and DFD quality groups. The rate of pH decline over a day of autolysis allows relatively accurate sorting of meat into quality groups. However, this method is quite labor-consuming as it requires certain expenditures on organization of pH measurement in carcasses after a day of holding. In addition, this method cannot be applied for assessing quality of meat in cuts.

Over the last years, scientists have focused their attention on the development of methods that make it possible to assess quality characteristics of meat already at the moment of measurements. As a result, many non-invasive methods have been developed. These methods are based on measuring electrical properties, optical characteristics and physical properties of meat.

It is possible to assess quality characteristics of muscle tissue by measuring its electrical conductivity [7], electrical resistance and electrical impedance [8]. Possibilities of meat quality identification by measuring its optical parameters (light scattering, reflectance and absorption) were demonstrated in [9,10]. Brøndum et al. [11], Hoving-Bolink et al. [12], Barbin et al. [13], Andersen et al. [14] have developed spectrophotometric methods for meat quality assessment. Methods of spectrometric determination of color coordinates, nuclear magnetic resonance, ultrasound spectroscopy are considered quite acceptable from the point of view of their use in practice [15–17]. During the last years, the interest of researchers to the development of the sensory technology for meat quality determination has been growing [18]. These methods are preferable as they are non-invasive.

The aim of this paper is to examine and compare non-invasive methods for identification of meat with abnormal course of autolysis and reveal advantages and disadvantages of each of them, which will allow seeing trends to understand the development of this theme.

Objects and methods

This review includes publications that present results of experimental investigations and review papers of national and foreign authors published from 1928 to 2022. The search included databases of search systems, such as eLibrary, CyberLeninka, Google Scholar, ScienceDirect, Springer open, PubMed, using keywords such as meat autolysis, quality group, electrical conductivity, optical methods, fluorescence spectroscopy, physical methods. In addition, leading Russian and foreign journals on the direction "Meat science" were used, publications in the Proceedings of the International conferences and bibliography from references lists were studied. One hundred fifteen sources were chosen. Criteria for selection of publications were relevance, practical significance, indexing in Russian and international scientometric databases.

Electrical conductivity methods

The main electrical properties of muscle tissue include its electrical conductivity, electrical resistance, and electrical impedance. Electrical properties of meat have been studied for a long time for many reasons, including the possible use for meat quality assessment [6,19–21]. As a result of investigations, it has been established that measuring electrical characteristics of meat is expedient for its quality identification. Thus, Antosik et al. [22] obtained relatively high coefficients of correlation between electrical conductivity and quality traits of meat from different animal groups. According to their data, measurement of this parameter in pork allows revealing PSE and DFD carcasses.

To predict pork quality, Oliver et al. [23] и Guerrero et al. [24] used electrical impedance spectroscopy (EIS). Impedance spectroscopy is based on the measurement of electrical conductivity of a sample under study when weak alternating currents with various frequencies are passed through it [25]. It is known that a live muscle is a poor conductor as intracellular and extracellular fluids are separated from each other by membranes [26]. Intact muscle tissue has low values of conductivity. As a result of disturbance of the state of cell membranes, redistribution of tissue fluid takes place, which directly influences a value of its electrical conductivity. The authors established that with an increase in the time of meat autolysis, cell membranes lose their semipermeability, and electrical conductivity tends to the maximum value.

Pliquett et al. [27] proposed using the parameter of electrical impedance P(y) for assessment of integrity of cell membranes and meat quality. The results obtained correlate well with a pH value and drip losses during autolysis, which enables differentiating NOR and PSE meat. The researchers found that the P(y) indicator gives the most significant results within a period of 4 to 24 hours of autolysis.

Egelandsdal et al. [28] also state that measurement of impedance directly reflects damages of the cellular structure of meat and allows judging about drip losses.

According to data of Suliga et al. [29], it is possible to assess a wide spectrum of morphological and physiological parameters of pork carcasses and reveal quality defects using bioimpedance analysis. The authors established the interrelation between physico-chemical indicators of meat quality (ultimate pH, color characteristics in the CIE L*a*b* system, drip losses) and results of the bioimpedance analysis. To compare defects of meat quality revealed using bioimpedance and physico-chemical indicators, the authors carried out proteomic analysis, which showed that differences in meat quality are reflected by the condition of eleven proteins that characterize meat quality.

Recently, EIS has found wide application for assessment of quality and safety of food products, including meat products.

It is known that electrical conductivity of biological tissues depends on the presence of various ions in cellular fluid. During autolysis, an increase in meat electrical conductivity is facilitated not only by the disruption of cell membranes and redistribution of tissue fluid, but also by protein destruction under the effect of tissue proteases as a result of rupture and fragmentation of myofibrils and degradation of cytoskeleton. With that, as Kristensen and Purslow [30] believe, ions migrate into intracellular space increasing conductivity of tissues.

According to data of Antosik et al. [22], to assess meat quality, electrical conductivity of muscle tissue can be measured both 1–3 hours and 24 hours after slaughter, when the rate of autolytic changes is slowed down and pork quality is forming.

Borzuta et al. [31] and Czyżak-Runowska et al. [32] suppose that measurement of electrical conductivity of meat 24 hours after animal slaughter correlates to a higher degree with meat parameters under study compared to measurements carried out after 90 min. Łyczyński et al. [33] hold the same viewpoint and state that measurement of electrical conductivity of pork 24 hours after slaughter can be used in practice to diagnose its quality.

To identify pork quality group (PSE, DFD, RFN) in the post-mortem period, Castro-Giráldez et al. [34] suggest determining dielectric spectra of muscle tissue from 100 Hz to 0.4 MHz in parallel and in perpendicular to the direction of muscle fibers. Notable differences in the permittivity between groups were observed 24 and 48 hours after slaughter. The authors assume that determination of dielectric properties of muscle tissue can be carried out 24 hours after slaughter to reveal meat quality group.

According to data of other researchers [35], determination of electrical conductivity of pork enables dividing semi-carcasses into PSE and DFD groups when measuring the parameter in the first 45–50 min. after slaughter.

Dielectric properties of meat with different character of autolysis were studied by Zakharov and Sus [36,37]. The authors found that, one hour after slaughter, electrical conductivity of m. *longissimus dorsi* of PSE pork was 6.83 mS/cm, while this parameter was equal to 4.73 and 3.39 mS/cm in NOR and DFD pork, respectively. The values of electrical conductivity of the studied muscle were 7.41, 5.29 and 3.73 mS/cm in PSE, NOR and DFD meat, respectively, after 24 hours of autolysis. Based on the results obtained, the authors proposed a method for meat sorting into quality groups according to the value of electrical conductivity.

According to Richer et al. [38], a rapid and convenient method for detection of DFD meat is a method of measuring rectangular electric pulse (h parameter), which allows making conclusion about structural changes in muscle tissue. The authors believe that it is possible to characterize the state of meat structure with high confidence using indicators pH, R (ratio of ATP to IMP concentrations) and h with data of electronic microscopy. It is shown that the specific features of autolysis of PSE meat are manifested in a rapid decrease in the value of pH and R and a reduction in the h parameter. The dependence between the rate of lactic acid accumulation and changes in tissue structure was established by optical methods.

Optical methods

The ability of muscle tissue to scatter the light flux is based on differences in the muscle fiber packing density in meat structure. Unlike NOR muscle tissue, PSE meat is characterized by looser fiber packing, which absorbs light better, and is perceived by the eye as lighter meat. On the contrary, DFD meat is characterized by tighter myofibrillar packing, reflects light less and is visually assessed as darker. Thus, assessment of color characteristics of meat enables determining its quality group quite confidently.

Raman spectroscopy can be considered one of the available spectroscopic methods for meat quality assessment. It is based on inelastic scattering of monochromatic light usually from a laser in visible, infrared or near ultraviolet spectra. It is also possible to use X-ray irradiation [39–41].

Raman light scattering was discovered in 1928 [42] and named after one of the authors (effect of Ch. V. Raman). The researchers described light scattering in liquids. Independently of them, in the same year in the Soviet Union, physicists G. S. Landsberg and L. I. Mandelshtamm recorded the similar light scattering in quartz independently of each other [43]. Preferability of this method consists in its ability to give information about concentration, structure and interaction of biomolecules inside intact cells and tissues (*in situ*) by the non-invasive method.

As regards meat quality assessment, this method enables determining its characteristics indirectly using correlations between one or several biophysical indicators and sensory properties. Herrero [44] established that the results of Raman spectroscopy correlate with data obtained by the traditional methods of meat quality assessment, such as protein solubility, viscosity, water holding capacity, structural-mechanical characteristics, peroxide value, fatty acid composition. The author believes that Raman spectroscopy can be applied for prediction of functional properties of proteins in situ and sensory indicators of meat products.

Results obtained by Scheier et al. [45] indicate that conversion of glycogen into lactic acid and degradation of ATP to IMP can be revealed using Raman spectra of pork obtained immediately after animal slaughter. The authors are of the opinion that it is possible to identify meat raw materials with abnormal course of autolysis at the early stages of autolysis with the help of Raman spectra.

Scheier et al. [45] и Bauer et al. [46] demonstrated that using Raman spectroscopy it is possible to classify meat into five quality groups (PSE, PFN, RFN, RSE, and DFD) both at the early stages of autolysis (from 0.5 to 10 h) and 24 hours after animal slaughter. It is also possible to reveal meat with signs of oxidative spoilage. The authors established that several indicators of quality, such as pH24, lightness L^*, drip losses and meat hardness (shear force), can be predicted using this method 24 hours after animal slaughter.

Nache et al. [40] showed that values of pH_{45} и pH_{24} can be predicted with the help of Raman spectra measured 1–2 h and 24 h after animal slaughter. This is of great interest for the meat industry for the rapid assessment of meat quality by the non-destructive method.

By now, it has been established that Raman spectroscopy provides insights into changes in the protein structure [47,48], water holding capacity of meat [44], shear stress [49,50], changes in lipids [51]. Scheier et al. [45] demonstrated that it is possible to detect degradation of glycogen to lactic acid and ATP to IMP by the use of Raman spectra.

Schmidt et al. [52], Scheier et al. [53], and Andersen et.al. [54] stated that the development of a portable device made it possible to assess pork quality, which correlated with the ultimate pH and drip losses, using Raman spectra obtained between 30 and 120 min. after animal slaughter. The possibility of prediction of pH_{45} and pH_{24} values, as well as drip losses, in pork during post-mortem storage employing a portable Raman system was confirmed by [53,55]. Raman spectra of muscles obtained from the signals of glycogen, lactic acid, creatinine, ATP, ADP and phosphate group correlated well with the indicators mentioned above. According to the opinion of Osborne [56], PSE meat can be revealed using this method, which is promising for introduction into slaughterhouses.

Methods of infrared spectroscopy

To predict quality characteristics of meat, several authors [16,56–58] suggest using electromagnetic radiation in near-, mid- and far-infrared regions, which interacts with an object under study and the infrared spectra (IR-spectra) obtained are processed to obtain necessary information about meat quality.

IR-spectroscopy in the near-infrared region of radiation (NIR — near-infrared spectroscopy) is based on the absorption of infrared radiation with wavelengths of 700 to 2500 nm, or from 12 800 to 4000 cm^{-1} by a sample under study. According to data of Sahar et al. [59], spectra can be registered by means of an optical sensor on the open surface of a carcass in the area of chuck or round 1 h and 2 h after animal slaughter.

Multiple studies [60–65] demonstrated the possibility of using near-infrared spectroscopy to identify pork properties and differentiate it into quality groups.

Neyrinck et al. [64] and Kennedy et al. [65] suppose that the use of NIR-spectroscopy for pork sorting into NOR and PSE groups is possible at wavelengths of 4500 to 9500 cm^{-1}. The authors established that NIR-spectroscopy ensures high confidence of results of detecting PSE pork 24 hours after slaughter (90–93.3 %).

Pankratova et al. [66] and Monroy et al. [67] showed that the non-destructive spectral method of the control in the visible and near-infrared regions in a wave range from 350 to 2500 nm with the use of discriminate analysis enables identification of five pork quality groups (NOR, RFN, PSE, RSE and DFD) with the accuracy of 79 %.

According to the data of Prieto et al. [68], spectroscopy in the near-infrared range of 350–2500 nm (Vis-NIR) can be used to classify beef carcasses into DFD and NOR. The method makes it possible to reliably classify up to 95 % of carcasses.

Cafferky et al. [69] made a conclusion that the method of infra-red spectroscopy can be applied to predict quality indicators in beef both at the early and later (24–48 h) stages of autolysis.

To detect meat with quality defects (PSE *и* DFD), the authors [70,71] recommend employing infrared thermography of the porcine skin surface. Temperature measurement was carried out with a portable radiometer "Raytek11" at a distance of about 60 cm above the surface of porcine back immediately before their stunning. Based on the results of the investigations, the authors established that when a temperature of the surface of animal skin rose from 21.1 to 35 °C, the probability of occurrence of PSE and DFD meat increased. Thus, at a temperature of more than 32.2 °C, about 73 % of studied animals gave PSE and DFD pork after cutting with approximately the same proportion. With that, 6 % of pork showed moderate PSE properties, 39 % was PSE pork, 22 % was meat with moderate DFD properties and 33 % was DFD pork. At indications of the device lower than 26.5 °C, most animals gave NOR meat. When a skin surface temperature was 29.4 % meat tended to PSE.

According to the opinion of other researchers [68,72–74], this method has limited possibilities for assessment of meat properties, which is conditioned by meat heterogeneity. However, this method allows direct or indirect qualitative and quantitative assessment of chemical, physical and physico-chemical characteristics of an analyzed object, including pH (Prieto et al. [73]), color (Cecchinato et al. [75]), water holding capacity (Ripoll et al. [76]) and tenderness of meat (Leroy et al. [77]).

Čandek-Potokar et al. [78] demonstrated a possibility of application of near-infrared spectroscopy to determine pH, color parameters (L*, a*and b*), drip losses in pork during autolysis 24 h and 48 h after animal slaughter. Meat samples were scanned in a wavelength range of 400–2500 nm using a NIR Systems spectrophotometer model 6500. For the development of models and calibration, the method of partial least squares was used. The best calibrations were obtained for the meat samples in a spectral range of 400–1100 nm. According to authors' opinion, it is necessary to improve methods, which are used to calibrate data obtained by spectroscopy, and/ or their accuracy to improve the method of near-infrared spectroscopy.

Brøndum et al. [79] compared data on prediction of drip losses in pork in visible and near-infrared regions of reflectivity with low-voltage nuclear magnetic resonance spectrometry (NMR). The best results were obtained with the use of NMR.

However, there are not so many studies devoted to application of near-infrared spectroscopy to assess technological properties of meat. They suggest that first of all the results of different studies differ significantly from each other and, secondly, prediction of quality characteristics of meat with the application infrared spectroscopy is less accurate compared with the prediction of the chemical composition of meat.

Spectrocolorimetric methods

It is known that the color of an opaque body is conditioned by the spectral composition of light flux reflected from it. At present, the spectrocolorimetric method of small color differences in the uniform color space system CIE L*a*b* is used to assess meat color. The CIE L*a*b* color space is an international standard for color measuring adopted by the International Commission on Illumination (CIE) in 1976. L* is the lightness coordinate, which varies from 0 to 100 (from black to white) and coordinates a* (from green if negative to red if positive) and b* (from blue if negative to yellow if positive) are chromatic components, which vary from –120 to +120 [80].

In 1931, the International Commission on Illumination (CIE) published color spaces CIE XYZ 1931, which determine interrelation between the visual spectrum and visual sensation of particular colors with the help of the human color vision.

All know colors are developed from various ratios of three spectral colors: red (700 nm), green (546.1 nm) and blue (435.8 nm). According to data of McDarrah et al. [81], each color is characterized by hue, saturation and lightness but is not a physical quantity and therefore does not have a unit of measurement. As Klettner et al. [82] showed, hue is a characteristic, which distinguishes achromatic (black, white and gray) color from chromatic. Saturation characterizes a ratio of colorfulness to brightness that is it

is the highest for spectral colors. Brightness is determined by the content of black and white colors.

Analyzing various methods for meat quality assessment, Chizzolini et al. [83] и Kudryashov et al. [84] proposed to identify PSE и DFD meat by the spectrophotometric method, determining values of color coordinates: L* — lightness, a* — redness, b*- yellowness and H — hue. The quantitative assessment of color intensity can be used as a basis for meat classification into quality groups depending on the character of autolysis [85,86]. Results obtained when determining color in the L*a*b* system allowed collecting a large databank of color characteristics of different meat products [86,87].

McKee and Sams [88] established the correlation between a pH value of poultry meat and the lightness value (L*). A pH decrease by 0.2–0.3 units in poultry meat is accompanied by an increase in the lightness (L*) value by 3–4 units.

Owens et al. [89] believe that color evaluation is the most rapid and available method of meat quality assessment for manufacturers of meat products and it is easy to perform both visually and instrumentally using optical scanning.

Kudryashov et al. [90] developed a method for identification of NOR, PSE and DFD meat using measurement of a ratio between the reflection intensity of the light flux of a sample under test and that of the reference (T) using a color comparator KTsSh (KTsZ). Comparison of their values allows determining to which quality group meat belongs. Thus, when the T value is lower than 1.100 and higher than 1.050 meat corresponds to NOR, when T is less than 1.250 and more than 1,200 meat has DFD properties, and when T is less than 0.950 and more than 0.900 meat is classified as PSE.

Computer technologies

To identify PSE and DFD pork and beef, Tomasevic [91] proposed to use computer technologies, in particular, methods of digital processing of images based on registering changes in color characteristics of meat, meat extract and broth. It has been established that images of PSE and DFD meat differ significantly from NOR meat by color characteristics. Results can be used to develop a method for identification of different meat quality groups.

Sun et al. [92] developed a computer vision system (CVS) for objective color assessment in pork loin to determine its correspondence to the requirements of the industry. Based on the artificial intelligence, a model for prediction of pork quality groups by color has been created. The results show that the accuracy of prediction of pork quality using CVS achieves 92.5 %.

Girolami A. et al. [93] showed a possibility of applying the computer vision system (CVS) to assess quality of beef, pork and chicken meat. The authors found that the digital values of samples displayed on the monitor were more similar to the color generated by the computer vision system than to the color obtained using the Minolta CR-400 colorimeter. The authors believe that the CVS method gives more reliable data of meat color measurement than a colorimeter. Therefore, this method can be recommended for revealing quality of meat with abnormal autolysis.

Chmiel et al. [94] applied the computer vision system (CVS) to identify DFD beef. L*, a* и b* coordinates were determined using the CIELab and CVS systems. Also, the total content of heme pigments was determined. The results obtained using CIELab and CVS characterized DFD meat as darker than normal beef. Beef with lower content of heme pigments was lighter compared to beef with the high level of heme pigments. The authors suppose that the CVS method can be employed to determine DFD meat.

The computer vision system (CVS) was applied to reveal PSE pork using color parameters [95]. The authors found differences between PSE and RFN meat in terms of lightness (L*). According to the data of the authors, however, the coefficients of correlation and determination between color parameters characterized by lightness had low values of 0.44 and 19.4 %, respectively. A significant spread of results of color parameters obtained for certain meat quality groups indicate a limited possibilities of using the CVS method for classification of pork carcasses using the *Semimembranosus* muscle.

Comparative data on the color of pork loin obtained by Sun et al. [96] with the use of the computer vision system and colorimeter Minolta indicate a significant correlation ($P < 0.0001$) of lightness L* (0.91), redness a* (0.80) and yellowness b* (0.66) values recorded using these methods. To assess results of predicting coordinates of pork color based on the artificial intelligence, two regression models were developed. These models enable on-line identification of meat quality group by color. The authors showed that the highest accuracy of predicting pork color characteristics is achieved using the CVS method, which corroborates data obtained by Girolami et al. [93].

The use of the computer vision system (CVS) makes it possible to acquire information both about external characteristics and the internal structure of an object under investigation. To confirm meat quality characteristics measured by CVS, the authors [97,98] carried out meat classification by pH_{45} and pH_{24}, electrical conductivity, drip losses, water binding capacity and color in the CIEL*a*b* system. The most accurate classification was recorded when determining color parameters (hue, saturation, lightness). It has been established that using the CVS method it is possible to identify PSE and DFD meat but it is not possible to differentiate RSE from RNF. In conclusion, the authors believe that the computer vision system can be used for rapid analysis of pork (m. *longissimus lumborum*) quality under industrial conditions.

Physical methods

The specific features of structure and condition of proteins, amino acids, nucleotides, lipids, organic acids, water and other substances in the composition of meat of different quality groups can be assessed by the nuclear magnetic resonance (NMR) method [99,100]. The method is based

on emission and absorption by these substances of energy in the radio frequency range of the electromagnetic spectrum [101]. Molecules of each substance in the composition of a sample under study are determined as specific peaks, including those that characterize conformational changes in proteins, water mobility and other changes.

Magnetic resonance tomography (MRT) makes it possible to obtain spatial resolution of the composition of a sample and measure quantitatively parameters closely associated with meat properties, such as pH and water holding capacity, in each point of a NMR image [101].

Renou et al. [102] analyzed pork of different genotypes (halothane-positive and halothane-negative) applying the nuclear magnetic resonance method. The authors registered spin–spin relaxation time of water protons. The study demonstrated that the spin-spin interaction is closely related to a rate of a post-mortem decrease in pH and denaturation changes in proteins. Data obtained by Decanniere et al. [103] showed that using NMR imaging of porcine muscles it is possible to reveal animals that carry the halothane-positive gene, which allows differentiation of stress-sensitive pigs from normal animals.

Borowiak [104] identified NOR and PSE pork by the non-destructive and rapid method of pulsed nuclear magnetic resonance. According to the data of the author, quality of meat can be assessed by excitation of proton spin, which makes it possible to determine its properties, including technological characteristics and establish the character of autolysis. Investigations were carried out on the *longissimus dorsi* muscle of different genotypes of pigs. The spin-lattice and spin-spin relaxation times of water protons were determined. The authors established that the two-component relaxation behavior of spin lattice is closely linked with the rate of post-mortem drop in pH and protein denaturation. Based on the results obtained, it was shown that the differences in the pH value of muscle tissue were the largest two hours after animal slaughter. According to the results of the study, the authors classified meat with pH 6.3 as normal, with 6.0 <pH> 6.3 as meat with intermediate properties and meat with pH < 6.0 as PSE.

Taking into consideration that structural changes in animal muscles in the process of rigor mortis can be determined by the method of proton NMR relaxation, Miri et al. [105] and Bogner et al. [106] used this method to study meat from pigs that were sensitive and resistant to stress. As experiments showed, this method allows identification of pork with the normal course of autolysis with confidence of up to 85 %. It was established that animals that were susceptible to the malignant hyperthermia syndrome give PSE meat.

Bogner et al. [106] investigated nuclear magnetic resonance (NMR) relaxation parameters of muscle tissue of normal pigs and pigs that were susceptible to malignant hyperthermia. It is known that the electronic envelope of the molecular responses to the external magnetic field and strives to shield it. The results of the relaxation measurement and multiexponential analysis of T2 curves showed a shift between different levels of water molecule protection upon action of NMR, which can predict an increased loss of water in muscle tissue after slaughter. The authors believe that using NMR it is possible to differentiate meat into one of three groups: normal, pale-soft-exudative, and dark-firm-dry.

Li et al. [107] in their study reported that it is possible to identify pork with different quality using NMR with low strength of magnetic field.

High performance in meat quality analysis was shown by the method of fluorescence spectroscopy, which is a variation of electromagnetic spectroscopy and analyzes fluorescence from a sample. This method is based on using a beam of light, as a rule ultraviolet light, which excites electrons in molecules of an analyzed sample and makes them emit light of lower energy. Amino acid tryptophan is a basis for the formation of fluorescent proteins with chromophores being in the anion state upon the physiological conditions. Interaction of tryptophan with the molecular environment of the cell is the most important factor influencing changes in spectra [14].

Fluorescence spectroscopy was proposed for the first time for quality analysis of meat and fish products based on their own fluorescence by Jensen et al. in 1986 [108]. According to the patent, a meat product, which quality is to be controlled, is subjected to an impact of electromagnetic radiation in a range of about 325–360 nm with the subsequent analysis of fluorescence that is typical for product biological components determining its quality. This analysis makes it possible to determine fluorescence that is characteristic of bones, cartilages, connective tissue and/or fat, as well as changes in pH and meat structure [14,109].

Despite availability and wide possibilities, the NMR method has not found application in meat science, apparently, due to the high cost of NMR spectrometers.

For rapid assessment of meat quality traits, a non-destructive method of hyperspectral imaging was proposed. This method combines a possibility of digital visualization and spectroscopy in a single system and is part of methods which are called spectral imaging or spectral analysis. The term “hyperspectral imaging” comes from the development of aerial photos by NASA (AIS) and AVIRIS in the middle of the 1980s. A hyperspectral image is a three-dimensional data array, which includes spatial information (2D) about an object supplemented with spectral information (1D) for each spatial coordinate. In other words, each point of an image corresponds to the spectrum obtained in this point [110].

The main advantage of the method consists in the fact that hyperspectral imaging collects and processes information from the entire electromagnetic spectrum emitted by an object [111]. Disadvantages include cost and complexity. Fast computers, sensitive detectors and big capacities for data storage are necessary for analysis of hyperspectral data.

The use of this method for meat quality assessment gained momentum at the beginning of the 21st century. Huang et al. [112] and Barbin et al. [113] obtained hyper-

spectral images of porcine m. *longissimus dorsi* of three quality groups (PSE, RFN and DFD) in near infrared range (from 900 to 1700 nm). Later Barbin et al. [113] observed reflectance differences between samples of three quality groups at wavelengths of 960, 1074, 1124, 1147, 1207 and 1341 nm. The data obtained indicate that pork groups can be reliably distinguished with the accuracy of up to 96 %. The researchers used the results of spectral information for prediction of color characteristics (L*a*b*), pH and drip losses. To predict these traits, certain wavelengths that were linked with each of them were chosen. It was established that values of lightness (L*), pH and drip losses can be predicted with the coefficients of correlation of 0.93, 0.87 and 0.83, respectively.

According to earlier results obtained by Qiao et al. [114], drip losses, pH and pork color can be predicted using hyperspectral imaging with coefficients of correlation of 0.77, 0.55 and 0.86, respectively. The authors suppose that this method can classify pork quality groups with regard to its exudative characteristics and color.

Therefore, it can be assumed that the hyperspectral imaging method is a potential tool for rapid identification of pork quality.

There have been attempts to use ultrasound for meat quality assessment. El Karam et al. [115] applied ultrasound to classify bovine muscles by scanning their acoustic parameters (velocity, attenuation and backscattering intensity). As investigations showed, the positive results of identification that correlated with the chemical composition of the samples were obtained in more than 80 % of analyses.

Conclusion

It is known from national and foreign publications that most often meat with non-traditional course of autolysis is revealed after animal slaughter by measuring, as a rule, pH, water binding capacity and drip losses, which requires preparation of samples (mincing) and certain time for analysis. The review presents non-invasive methods for identification of meat quality groups, which preferability consists in the fact that they allow objective, rapid and quite reliable qualitative and quantitative assessment of chemical, physical and physico-chemical characteristics of meat in real time without destruction of a sample. Taking into consideration the heterogeneity of the meat composition and structure, the specialists face the task of modernization of non-invasive methods and their adaptation to samples under study.

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DEVELOPMENT OF A SPECTROPHOTOMETRIC APPROACH FOR ASSESSING PORK QUALITY DURING STORAGE

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Abstract
The annual growth of meat production, accompanied by significant quality deterioration at all stages of the production chain, drives the development of fast and highly accurate control methods. The work is devoted to the adaptation of the spectrophotometric method for assessing pork quality based on the analysis of muscle tissue extracts. The purpose of the work is to generalize and systematize knowledge about spectrophotometric analysis and the application of this method for pork quality control during storage. The work provides a comparative spectrophotometric assessment of various methods for extracting protein and non-protein components of pork muscle tissue. Aqueous, buffer, NaCl and KCl extracts of muscle tissue were studied, their absorption spectra in the wavelength range of 315–1000 nm were analyzed. It was found that KCl and NaCl extraction ensured the maximum degree of myofibrillar and sarcoplasmic protein extraction, and also formed the most pronounced and stable spectral peaks. Particular attention was paid to the analysis of KCl extracts demonstrating the best resolution and clarity of spectral curves, which is important for a detailed study of changes in muscle tissue properties during storage. During meat storage, statistically significant changes in the intensity and geometry of key spectral peaks ($\lambda_{325–335}$, λ_{355}, $\lambda_{410–415}$, λ_{545}, λ_{580}, $\lambda_{610–620}$, $\lambda_{635–650}$) were revealed, which were simultaneous with histostructural transformations of muscle tissue. A high correlation was established between the change in the area of minor peaks and the dynamics of muscle fiber diameter, which allows using spectral characteristics as objective indicators for the degree of changes in muscle tissue at the cellular and molecular levels during storage. The results obtained confirm the feasibility of using spectrophotometric analysis of KCl extracts for an objective assessment of meat quality and monitoring its changes at various stages of storage.

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Introduction

Global meat production has more than tripled over the past 50 years and currently amounts to approximately 370 million tons per year [1,2]. With increasing production, meat quality remains a key factor in consumer choice. However, significant quality deterioration and losses occur at all stages, from production to consumption of food products. The share of meat losses worldwide varies from 14 % to 20 % [3,4]. Significant losses occur at various stages before and during slaughter, and quality deterioration occurs throughout the whole food distribution chain [3]. One of the main reasons for this is the insufficient development of a quality control strategy, as well as the limited use of modern analytical methods due to the requirements for the use of traditional approaches. This is due to the lack of adaptation and metrological evaluation of new methods [5].

As noted by the authors [6], traditional methods of meat quality assessment based on physical and chemical analytical approaches retain the status of standard ones, however, they have significant limitations, such as invasiveness, labor intensity and duration [7], which complicates their use in express control systems in production environment [8].

In a critical review by Chen et al. [9], it is shown that in order to overcome the limitations of traditional methods and ensure effective quality control of meat at all stages of production and distribution chain, it is necessary to develop and implement appropriate innovative analytical systems. Such systems should be high-sensitive, compact, integratable into production processes, non-invasive and economically feasible.

An analysis of scientific papers published from 2015 to 2025 on the topic of meat quality and its detection methods showed a shift in emphasis towards non-invasive and high-precision technologies, such as hyperspectral imaging (HSI) [10], Raman spectroscopy [11], ultraviolet (UV) spectrophotometry [12], visible and near infrared (VIS-NIR) spectroscopy [13]. The methods described by the authors allow for multiparameter analysis (water, fat, protein content, freshness) with minimal data processing duration and the ability to integrate into production lines.

According to the study by Ayaz et al. [14], the use of hyperspectral imaging (HSI) to detect meat adulteration demonstrates a fairly high accuracy (94 %), which is significantly higher than most traditional methods. By integrating spectroscopy and visualization, this technology allows for simultaneous acquisition of spectral and spatial data, which significantly expands the capabilities of meat quality and safety assessment. But despite the progress of HSI technology, it has a number of significant disadvantages: a large volume of data, which complicates processing and application in real time; high cost of equipment, which limits widespread implementation; labor-intensive calibration and updating of models, which require significant resources [15].

As shown in a review by Pchelkina et al. [16], Raman spectroscopy allows predicting the quality indicators of meat raw materials with a high degree of reliability and obtaining a large amount of information about the object without its destruction. The method allows to evaluate the sensory indicators, autolytic changes, spoilage, authenticity of raw materials, technological properties, protein structure, fatty acid composition, as well as the differentiation of muscles and tissues [17]. An important advantage of the method is the comparability of the obtained results with the data of traditional analytical methods. However, despite the advantages of this method, there are also some disadvantages, including the complexity of the equipment and data interpretation, as well as limited availability.

The advantages of using visible (VIS) and near infrared (NIR) spectroscopy in the meat industry are thoroughly described in the works by Nechiporenko et al. [18], Wu et al. [19], Tang et al. [20]. The method is successfully used to analyze the composition of muscle tissue, describe the destructive processes occurring during aging, cooling, freezing and storage, as well as for the simultaneous determination of several meat quality parameters, identifying the fact of adulteration, determining the moisture, protein, and fat content, pH, freshness of beef, lamb, pork, chicken and seafood.

Despite the fact that VIS-NIR spectroscopy is a promising tool for monitoring the quality and safety of meat, it requires taking into account the specifics of the samples, numerous physical and chemical experiments before modeling, optimization of models, and has some limitations [21]. Wang et al. noted [22] that for a comprehensive assessment of meat quality, NIRS should be combined with other non-destructive methods to increase its efficiency. The works by Zheng et al. [23] and Cheng et al. [24] emphasize the need for efficient extraction of spectral information, increasing the signal-to-noise ratio, and proper processing of the obtained spectral data to eliminate errors in their interpretation.

Pre-processing of spectral data to improve predictive performance requires the correct choice of appropriate mathematical methods, depending on the objectives [25]. The main goal of pre-processing in spectroscopy is to reduce the influence of scattering and noise in order to isolate the part of the spectrum associated with chemical, physical or biological properties of the studied object. For example, the Savitzky Golay Smoothing Filter (SGSF) method is widely used to eliminate high-frequency noise interference and preserve the peak shape [26]. Cubic spline fitting (CSS) is used to smooth spectra and reduce random noise. The use of first and second derivatives may eliminate the influence of systemic background, such as baseline drift, and increase the resolution of overlapping absorption bands, which allows revealing hidden peaks and improving the identification of chromophores [27]. Moreover, averaging and centering are also common methods of spectral pre-processing.

Modern advances in spectrophotometry, including the use of visible (VIS), near infrared (NIR) and ultraviolet (UV) ranges, as well as integration with chemometric methods, bring new opportunities for analyzing the structure and composition of muscle tissue. However, as the analysis of publications in this area has shown, today there is no common spectral database for meat raw materials, and there are no standardized methods for conducting research and processing the results. Thus, the use of spectroscopic and spectrophotometric methods is a new, promising and relevant area of research in the field of meat quality.

The purpose of this study is to adapt the spectrophotometric method for assessing autolytic changes and monitoring the quality of meat during storage. To achieve this goal, it was proposed to use muscle tissue extracts, since spectroscopy of a piece of meat only evaluates its surface properties, which leads to multiple errors and unreliability of this approach. Therefore, one of the objectives of this work was to determine the optimal extractant for the isolation of protein and non-protein components, suitable for spectrophotometric analysis of meat quality during storage, because sarcoplasmic, myofibrillar and matrix proteins of muscle tissue have different solubility depending on their structure and environmental conditions [28,29].

Objects and methods

The objects of the study were chilled samples of pork longissimus muscle (*Sus scrofa M. longissimus dorsi*) 24 hours after slaughter with a pH value of 5.82 ± 0.20. The meat samples were obtained from various representative half-carcasses of crossbred pork (Yorkshire × Landrace × Duroc) aged 170–180 days, weighing 77–95 kg at Slutsk Meat Processing Plant JSC, Republic of Belarus, and delivered to the laboratory in an isothermal bag within one hour after sampling. The meat was packed in polyethylene bags and stored at a temperature of 2 ± 2 °C for 6 days. Sampling and sample preparation for testing were carried out in accordance with GOST 7269–2015[1] and GOST R 51447–99

[1] GOST 7269–2015 "Meat. Methods of sampling and organoleptic methods of freshness test". Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200133105 Accessed August 20, 2025 (In Russian)

(ISO 3100–1–91)[2]. Longissimus muscle samples were tested according to the scheme below every day during 6 days of storage.

Preparing aqueous meat extracts

The connective tissue and fat were trimmed, then meat raw material was minced twice in Vitek VT-3624 meat grinder (Star Plus Limited, China) pre-cooled to 4 ± 2 °C with a grate outlet diameter of 2–3 mm. To obtain an aqueous extract, a sample of the minced meat (20.00 ± 0.02 g) was placed in a 250 cm^3 titration flask and extracted with distilled water with a temperature of no higher than 4 ± 2 °C (minced meat to water ratio of 1:5) on SHR-1D laboratory shaker for 30 min. The resulting extract was filtered first through cheesecloth folded in four, and then through a "white ribbon" paper filter with a pore size of 8–12 μm at an ambient temperature of 20 °C.

Preparing NaCl meat extracts

A sample of minced meat (20.00 ± 0.02 g), prepared according to the scheme described above, was placed in a 250 cm^3 titration flask and extracted with a cold (4 ± 2 °C) isotonic sodium chloride solution with NaCl concentration of 0.9 % (minced meat to extractant ratio of 1:5) on SHR-1D laboratory shaker for 30 min. The resulting extract was filtered similarly to aqueous extracts.

Preparing borate buffer meat extracts

A sample of minced meat (20.00 ± 0.02 g) was placed in a 250 cm^3 titration flask and extracted with a borate buffer (sodium tetraborate decahydrate $Na_2B_4O_7 \cdot 10H_2O$) with a concentration of 0.01 mol/l, pH 9.18 and a temperature of no higher than 4 ± 2 °C (minced meat to buffer ratio of 1:5) on SHR-1D laboratory shaker for 30 min. The resulting extract was filtered similarly to aqueous extracts.

Preparing KCl meat extracts

A sample of minced meat (20.00 ± 0.02 g) was placed in a 250 cm^3 titration flask and extracted with a 5 % KCl solution with a temperature no higher than 4 ± 2 °C (minced meat to extractant ratio of 1:5) with constant stirring on SHR-1D laboratory shaker for 30 minutes. The resulting extract was filtered similarly to aqueous extracts.

Registration of absorption spectra

Registration of the absorption spectra of the extracts was carried out on research-grade spectrophotometers, SF-2000 (Zagorsk Optical and Mechanical Plant, Russia) and PE5400VI (Ekokhim, Russia). Extractant solutions were used as comparison solutions (blank solutions). Spectrophotometer operation and preliminary data processing were performed using the included software. Scanning of the absorption spectra of the extracts was carried out in quartz cuvettes with an optical path length of 1 cm and wavelength range of 315–1000 nm, with a scanning step of 5 nm in the optical density measurement mode.

[2] GOST R51447–99 "Meat and meat products. Methods of primary sampling". Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/1200028183 Accessed August 19, 2025 (In Russian)

Processing of data obtained

The data obtained as a result of spectrophotometry were averaged using Excel 2019 software (Microsoft, USA) for each sample and presented graphically as a linear diagram or spline.

When processing the spectra in OriginPro 2024 software (OriginLab Corporation, USA), the baseline was calculated using asymmetric least-squares smoothing. The asymmetry factor was taken equal to 0.001. The threshold determining which peaks are considered significant, was taken equal to 0.05. The number of iterations that the method performs to refine the baseline was set equal to 100.

An increase in the absorption spectra resolution was achieved by calculating the second derivative. The smoothing window size was set to 0. Noise filtration during the second derivative analysis was performed using Savitzky Golay Smoothing Filters (SGSF). The points of window used in SGSF method was set to 5. Peak filtration was not applied, and all peaks found were taken into account for further analysis.

The following output data was obtained as a result of the calculations described above:

1) Peak Area;
2) Percent Area;
3) Curve Area;
4) Beginning X;
5) Ending X;
6) Peak Center;
7) Peak Height;
8) FWHM (Full Width at Half Maximum);
9) Peak Centroid;
10) Total area under curve at baseline $Y = 0$.

Histological study

To study the microstructure, 3 × 3 × 3 cm samples were taken from each object of study and fixed in 10 % neutral buffered formalin solution for at least 72 hours at room temperature. For further study, two 1.5 × 1.5 × 0.5 cm pieces with longitudinal and transverse orientation of muscle fibers were taken from each fixed sample. The pieces were washed with cold running water for 4 hours, then compacted in gelatin (AppliChem GMBH, Germany) in ascending concentration (12.5 %, 25 %) using TS-1/20 SPU thermostat (Smolensk SKTB-SPU, Russia) at a temperature of 37 °C for 8 hours in each. Sections 14 μm thick were prepared on Kedee KD-3000 cryostat (Kedee, China) at a temperature of minus 35 °C. Three sections were made from each piece. The obtained sections were mounted on glass (Minimed LLC, Russia), stained with Ehrlich hematoxylin and 1 % aqueous-alcoholic eosin solution (BioVitrum, Russia), and then embedded in glycerin-gelatin.

Histological preparations were studied and photographed with AxioImaiger A1 light microscope (Carl Zeiss, Germany) using AxioCam MRc 5 video camera (Carl Zeiss, Germany) and AxioVision 4.7.1.0 image analysis software (Carl Zeiss, Germany).

Statistical analysis

Statistical analysis of the results was performed using Excel 2019 software (Microsoft, USA). Multiple comparisons between sample groups were performed using OriginPro 2024 software (OriginLab Corporation, USA). The results obtained were considered significant at $p < 0.05$.

Literature data analysis

To select and analyze literature data, taking into account the degree of their relevance, scientific articles, monographs and dissertation abstracts published between 2015 and 2025, available in scientific databases such as Elsevier, PubMed, ResearchGate, Web of Science, Scopus, eLIBRARY.RU, cyberleninka.ru, and CNKI (China National Knowledge Infrastructure) were analyzed.

The search was carried out using the keywords: "meat extract absorption spectra", "myoglobin spectroscopy", "muscle tissue optical properties", "spectrophotometric analysis of meat", "肉类提取物吸收光谱" (absorption spectra of meat extracts), "肉类蛋白质光谱分析" (spectroscopy of meat proteins). The obtained information was processed using systematic approach and logical generalization.

Results and discussion

Spectrophotometric evaluation of extraction methods

As a result of comparative spectrophotometric analysis (Figure 1), it was found that when extracting with different extractants (distilled water, borate buffer, 0.9 % NaCl solution, 5 % KCl solution), the shape and nature of the spectral curves were almost the same in all cases. In the UV–VIS region of the spectrum in the wavelength range of $\lambda_{315–600}$, several of the most intense (major) peaks were observed. In the infrared region (over 700 nm), absorption was significantly reduced, and the spectral curves were flatter.

The main difference between the obtained absorption spectra was the different intensity due to the concentration, chemical nature and physicochemical properties of the extracted substances and extractants [30,31]. The spectra of aqueous extracts were characterized by the lowest integral value of optical density, since distilled water extracts only water-soluble (sarcoplasmic) proteins [32], which are comparatively less numerous than myofibrillar proteins [33,34]. Despite the fact that aqueous extraction is simple and cost-effective, it is limited by the extreme instability of solutions [35] and the isolation of only water-soluble proteins, which may affect the sensitivity of spectrophotometric analysis.

The absorption spectra of buffer extracts had a comparatively higher integrated optical density compared to the spectral profiles of aqueous extracts. This is due to the ability of the borate buffer to extract both sarcoplasmic and myofibrillar proteins, but the completeness of extraction was noticeably lower than that of the KCl and NaCl extracts due to the probable formation of aggregates, which is consistent with the results by Perry et al. [36].

KCl and NaCl extracts demonstrated the highest integrated optical density values compared to aqueous and buffer extracts, providing stable and reproducible results even with small pH fluctuations, since solutions of these salts effectively extract both myofibrillar and sarcoplasmic proteins without pronounced side effects [37], which is consistent with the results by Vasilevskaya et al. [38], Munasinghe et al. [39].

When comparing the shape of the spectra, it was found that aqueous and NaCl extracts had smoother spectral curves with less pronounced (diffuse) peaks, while buffer and KCl extracts were distinguished by clearer and more resolved peaks. The observed differences in the spectra are due to the ability of the extractants used to extract specific substances of muscle tissue (proteins, nucleic acids) and affect their optical properties, which is consistent with the results in [32,36–39] and the Beer-Lambert law.

For a more detailed study of the obtained spectra and an objective choice of the optimal extractant suitable for spectrophotometric analysis of meat quality during storage, a comprehensive processing of the experimental data was carried out. In particular, preliminary processing of the spectra, integration, as well as the search and analysis

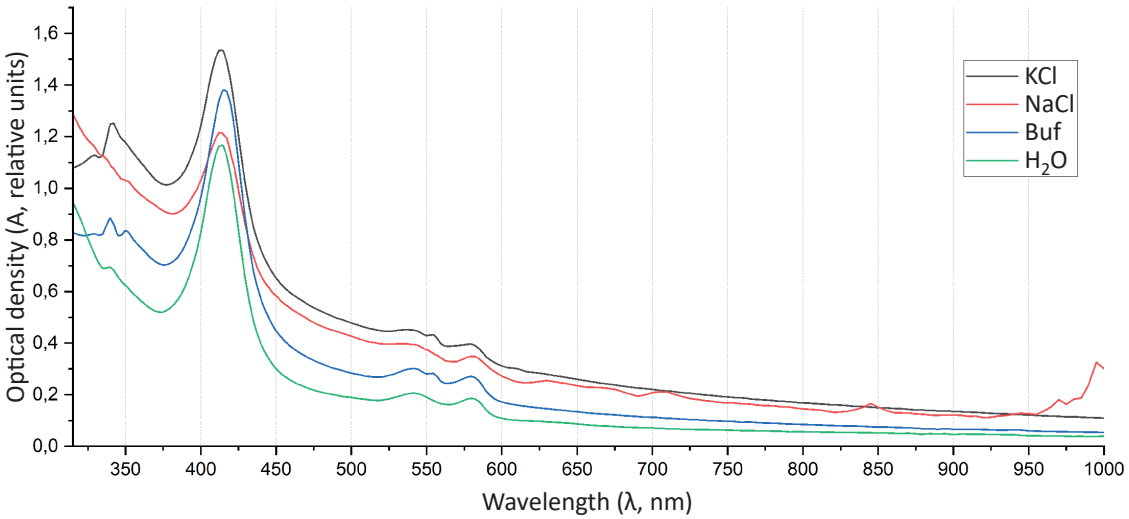


Figure 1. Absorption spectra of pork muscle tissue: (from top to bottom) KCl extract; NaCl extract; borate buffer extract; aqueous extract

of the maxima and minima of absorption in the spectral curves were carried out in accordance with the methods described in [40,41].

Analysis of absorption spectra

When analyzing the absorption spectra, it was taken into account that muscle tissue extracts are multicomponent colloidal systems containing proteins, lipids, and other biologically active compounds. It is known [42] that the main contribution to the absorption spectra in the visible region is made by chromoproteins, such as myoglobin, which amounts 90–95 % of all meat pigments, and hemoglobin. Myoglobin, which contains a heme group with an iron ion (Fe^{2+} or Fe^{3+}) and a porphyrin ring, exhibits typical absorption bands that depend on its oxidation-reduction state.

Deoxymyoglobin demonstrates an absorption maximum at 557 nm, while metmyoglobin demonstrates it at 503 nm. Oxymyoglobin and carboxymyoglobin are characterized by double peaks (doublets): 542/582 nm and 543/581 nm, respectively. The intersection of the spectra of all myoglobin forms at 525 nm (isobestic point) allows to estimate its total concentration in solutions and extracts of fresh meat [43].

An additional contribution to the spectra is made by lipopigments (lipofuscin) [44] and heme breakdown products (bilirubin, biliverdin), which are active in the UV and visible regions [45–47].

A comparative analysis of the spectral profiles of the extracts revealed key differences due to the type of extractant. The most pronounced peaks were observed in KCl and NaCl extracts, which is associated with the high efficiency of extraction of myofibrillar and sarcoplasmic proteins.

In all spectra, regardless of the extraction method, a major peak was observed in the region of 410–415 nm (γ-band), which is characteristic of heme-containing proteins (hemoglobin, myoglobin, cytochromes) and mucopolysaccharides. This peak is associated with $\pi \rightarrow \pi^*$ transitions in the porphyrin ring of heme [48,49] and serves as an indicator of the presence of these compounds in tissues.

In the Q-range of the obtained spectra, α-band (λ_{580}) and β-band (λ_{545}), corresponding to myoglobin [50] were distinguished, as well as some minor bands: $\lambda_{610–615}$, $\lambda_{625–635}$, $\lambda_{640–645}$, $\lambda_{665–670}$.

In the near UV region (330–365 nm), peaks due to $\pi \rightarrow \pi^*$ transitions in unsaturated fatty acids of lipids were identified [51]. The highest intensity and resolution of these peaks in KCl extracts confirmed the high efficiency of this type of extraction (Figure 1).

The spectra of aqueous and buffer extracts demonstrated lower intensity of bands in the Q-range and γ-band compared to KCl and NaCl extracts, which is associated with limited solubilization of proteins [37]. Despite the better resolution of peaks in aqueous and buffer extracts (Table 1), KCl and NaCl extracts provided an optimal ratio of intensity and stability of spectral characteristics.

Based on the data obtained, it was concluded that KCl and NaCl extractions are superior to other methods for spectrophotometric analysis. Absorption maxima in the range from 0.2 to 1.0, minimal distortions of peak geometry, and high reproducibility confirm the suitability of these extraction methods. In turn, KCl extracts demonstrated more pronounced peaks, which makes them preferable for monitoring changes in meat quality during storage and establishing a correlation between spectral characteristics and tissue histostructure.

Spectrophotometric analysis of meat quality during storage

Spectrophotometric analysis of pork KCl extracts during six-day storage revealed consistent changes in key spectral parameters reflecting autolytic processes in muscle tissue. Figure 2 shows averaged absorption spectra demonstrating the preservation of the general shape of the curves with changes in peak intensity and position depending on the storage period.

Analysis of the second derivative of the spectra allowed to isolate and identify 125 unique peaks, including key

Table 1. Results of absorption spectra integration of different extracts

Extracts	Peak center, nm	Peak centroid, nm	FWHM	Peak height	Peak area	Percent area, %	Curve area
KCl	415 ± 1.0	411.3 ± 1.0	30.02 ± 1.05	0.78 ± 0.03	27.05 ± 0.95	61.49 ± 2.15	43.99 ± 1.54
	545 ± 1.0	537.3 ± 1.0	19.15 ± 0.67	0.07 ± 0	1.58 ± 0.06	3.58 ± 0.13	
	555 ± 1.0	556.8 ± 1.0	10.00 ± 0.35	0.07 ± 0	0.87 ± 0.03	1.98 ± 0.07	
	580 ± 1.0	579.1 ± 1.0	25.00 ± 0.88	0.08 ± 0	1.7 ± 0.06	3.87 ± 0.14	
NaCl	415 ± 1.0	411.9 ± 1.0	28.96 ± 1.01	0.50 ± 0.02	16.47 ± 0.58	45.24 ± 1.58	36.41 ± 1.27
	545 ± 1.0	538.5 ± 1.0	38.02 ± 1.33	0.07 ± 0	2.88 ± 0.1	7.92 ± 0.28	
	580 ± 1.0	584.0 ± 1.0	30.15 ± 1.06	0.08 ± 0	2.25 ± 0.08	6.17 ± 0.22	
$Na_2B_4O_7 \cdot 10H_2O$	415 ± 1.0	413.7 ± 1.0	28.10 ± 0.98	0.86 ± 0.03	28.13 ± 0.98	66.59 ± 2.33	42.25 ± 1.48
	545 ± 1.0	536.3 ± 1.0	18.89 ± 0.66	0.1 ± 0.01	2.28 ± 0.08	5.40 ± 0.19	
	555 ± 1.0	554.8 ± 1.0	5.00 ± 0.18	0.09 ± 0.01	0.85 ± 0.03	2.01 ± 0.07	
	580 ± 1.0	578.2 ± 1.0	30.85 ± 1.08	0.11 ± 0.01	2.96 ± 0.1	7.00 ± 0.24	
H_2O	415 ± 1.0	413.0 ± 1.0	27.92 ± 0.98	0.78 ± 0.03	24.15 ± 0.85	61.83 ± 2.16	39.06 ± 1.37
	545 ± 1.0	539.0 ± 1.0	31.52 ± 1.1	0.09 ± 0.01	3.11 ± 0.11	7.95 ± 0.28	
	580 ± 1.0	581.1 ± 1.0	32.16 ± 1.13	0.1 ± 0.01	3.08 ± 0.11	7.89 ± 0.28	

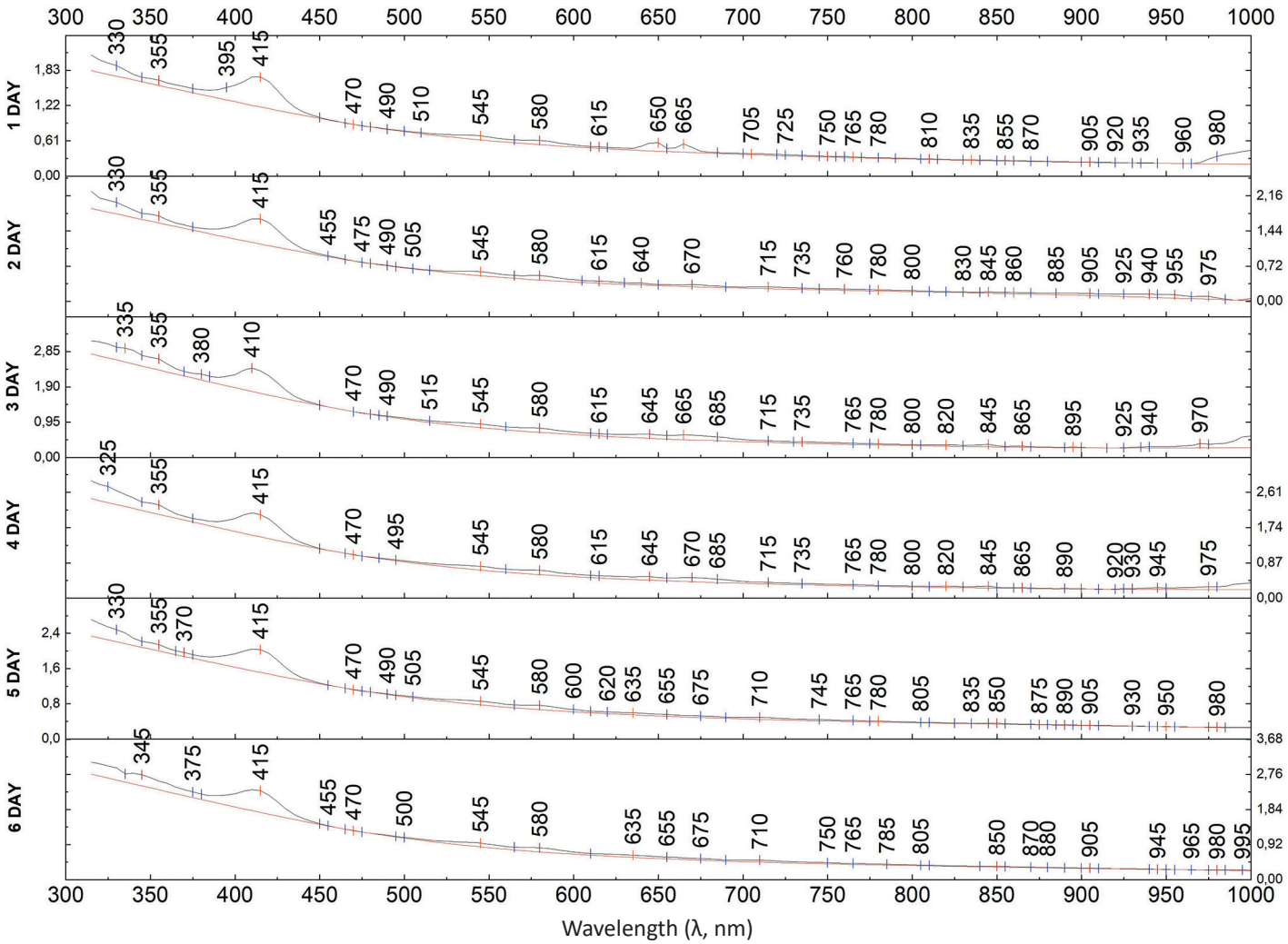


Figure 2. Average absorption spectra of pork muscle tissue KCl extracts at different storage periods

maxima: $\lambda_{325–335}$, λ_{355}, $\lambda_{410–415}$, λ_{545}, λ_{580}, $\lambda_{610–620}$, $\lambda_{635–650}$ (Table 2). The use of the Blackman-Tukey correlogram method in combination with the Tukey HSD test for multiple comparisons between sample groups confirmed the statistical significance ($p < 0.05$) of the differences in the intensity of these peaks between sample groups formed by storage periods.

Minor peaks at $\lambda_{325–335}$ and λ_{355} showed simultaneous dynamics with histological changes in muscle tissue (Figure 3). In the first four days, their height, area and percent area increased, correlating with muscle fiber relaxation and restoration of transverse striation. By the sixth day, the peaks completely disappeared, which corresponded to the development of the aging stage and the onset of destructive processes.

FWHM of peak at $\lambda_{325–335}$ remained stable, while for peak at λ_{355}, narrowing was observed. The hypsochromic shift of peak centroid at λ_{355} by the fifth day (with an unchanged position of the peak center) indicated structural changes in the extracted components associated with lipid oxidation [51,52] and a decrease in NADH/NADPH level [53,54].

According to [54], NADH level decreases statistically significantly ($p < 0.05$) with increasing of meat storage period. This is due to the depletion of metabolites involved in the restoration of NADH, such as fumaric acid, creatinine and fructose, which also decrease over time. For NADPH, there is little direct data on post-slaughter changes in meat, however, given its role in biochemical reactions, it may be assumed that its amount also decreases, since metabolic processes in muscle tissue cease after slaughter. The study [55] notes that during meat storage, there is a change in the oxidation-reduction balance, which may lead to a decrease in the NADPH level, but specific measurements are not presented.

A strong correlation ($r = 0.91$) between the change in peak area at λ_{355} (including its percent area) and the dynamics of muscle fiber diameter indicated the influence of autolytic processes in muscle tissue on the spectral properties of the extracts.

Thus, changes in the indicated values of minor peaks at $\lambda_{325–335}$ and λ_{355} reflect biochemical transformations of extracted components of muscle tissue.

The peak of the γ-band at $\lambda_{410–415}$, associated with heme-containing proteins and mucopolysaccharides [48,49], demonstrated an increase in area up to the third day (19.17 ± 0.64 arbitrary unit) with subsequent stabilization. The hypsochromic shift of peak centroid (4.13 ± 1.24 nm) and the change in FWHM (up to $31.95 \pm .57$ on the sixth day) reflected the degradation of glycosaminoglycans and proteins.

Table 2. Results of absorption spectra integration of chilled pork KCl extracts at different storage periods

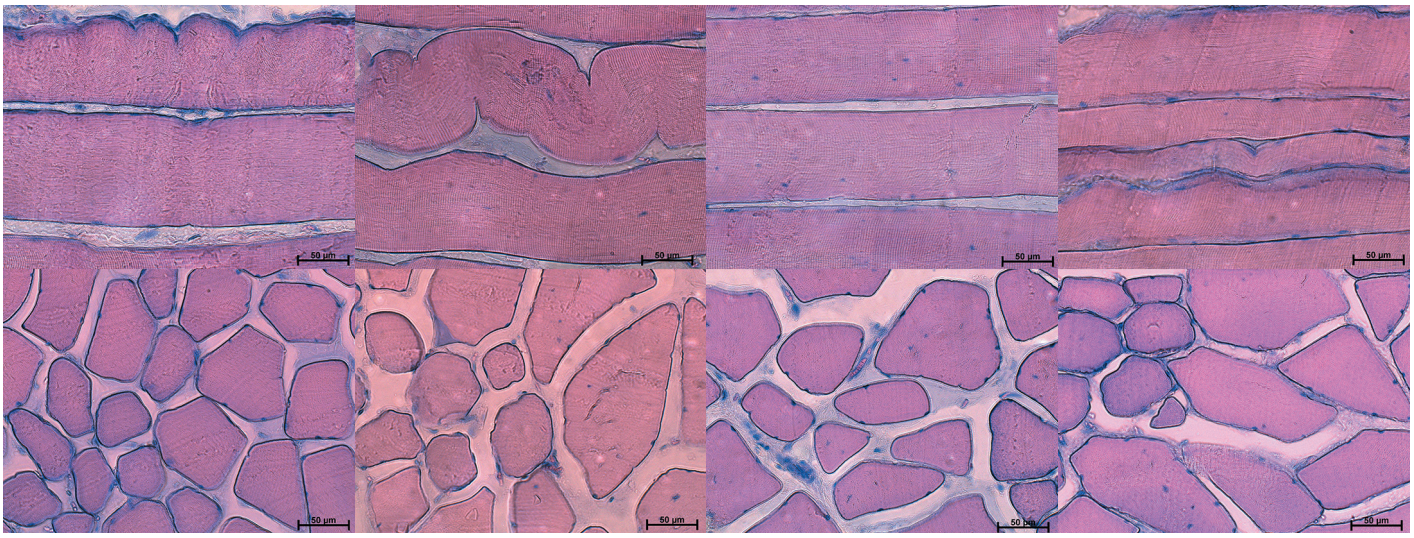
Peak (λ, nm)	Days	Peak area	Percent area, %	Curve area	FWHM	Peak height	Peak center, nm	Peak centroid, nm
$\lambda_{325–335}$	1	1,81 ± 0,05	4,26 ± 0,13	42,46 ± 1,27	10 ± 0,31	0,18 ± 0,01	330 ± 1,00	336,32 ± 1,00
	2	2,63 ± 0,08	5,7 ± 0,17	46,24 ± 1,39	10 ± 0,30	0,23 ± 0,01	330 ± 1,00	336,66 ± 1,00
	3	5,27 ± 0,16	7,65 ± 0,23	68,92 ± 2,07	10 ± 0,31	0,37 ± 0,01	335 ± 1,00	337,33 ± 1,00
	4	6,03 ± 0,18	10,24 ± 0,31	58,83 ± 1,76	15 ± 0,45	0,4 ± 0,01	325 ± 1,00	334,02 ± 1,00
	5	3,06 ± 0,09	6,86 ± 0,21	44,63 ± 1,34	10 ± 0,33	0,28 ± 0,01	330 ± 1,00	336,52 ± 1,00
	6	—	—	—	—	—	—	—
λ_{355}	1	2,4 ± 0,07	5,65 ± 0,17	42,46 ± 1,27	25 ± 0,75	0,09 ± 0,01	355 ± 1,00	359,55 ± 1,00
	2	3,16 ± 0,09	6,83 ± 0,2	46,24 ± 1,39	23,94 ± 0,72	0,14 ± 0,01	355 ± 1,00	357,72 ± 1,00
	3	5,75 ± 0,17	8,35 ± 0,25	68,92 ± 2,07	19,53 ± 0,59	0,3 ± 0,01	355 ± 1,00	355,57 ± 1,00
	4	5,02 ± 0,15	8,53 ± 0,26	58,83 ± 1,76	19,63 ± 0,59	0,24 ± 0,01	355 ± 1,00	357,28 ± 1,00
	5	2,56 ± 0,08	5,73 ± 0,17	44,63 ± 1,34	15 ± 0,45	0,15 ± 0,01	355 ± 1,00	354,24 ± 1,00
	6	—	—	—	—	—	—	—
$\lambda_{410–415}$	1	15,14 ± 0,45	35,66 ± 1,07	42,46 ± 1,27	30,23 ± 0,91	0,52 ± 0,02	415 ± 1,00	415,59 ± 1,00
	2	17,52 ± 0,53	37,88 ± 1,14	46,24 ± 1,39	29,56 ± 0,89	0,52 ± 0,02	415 ± 1,00	412,61 ± 1,00
	3	19,71 ± 0,59	28,59 ± 0,86	68,92 ± 2,07	29,12 ± 0,87	0,63 ± 0,02	410 ± 1,00	412,5 ± 1,00
	4	18,89 ± 0,57	32,11 ± 0,96	58,83 ± 1,76	30,35 ± 0,91	0,55 ± 0,02	415 ± 1,00	410,92 ± 1,00
	5	18,25 ± 0,55	40,9 ± 1,23	44,63 ± 1,34	31,69 ± 0,95	0,52 ± 0,02	415 ± 1,00	411,47 ± 1,00
	6	19,81 ± 0,59	37,03 ± 1,11	53,51 ± 1,61	31,95 ± 0,96	0,57 ± 0,02	415 ± 1,00	411,46 ± 1,00
λ_{545}	1	2,69 ± 0,08	6,33 ± 0,19	42,46 ± 1,27	35,47 ± 1,06	0,07 ± 0,01	545 ± 1,00	541,39 ± 1,00
	2	2,94 ± 0,09	6,35 ± 0,19	46,24 ± 1,39	32,84 ± 0,99	0,08 ± 0,01	545 ± 1,00	543,56 ± 1,00
	3	3,55 ± 0,11	5,15 ± 0,15	68,92 ± 2,07	35,5 ± 1,06	0,1 ± 0,01	545 ± 1,00	539,77 ± 1,00
	4	3,85 ± 0,12	6,55 ± 0,2	58,83 ± 1,76	34,91 ± 1,05	0,09 ± 0,01	545 ± 1,00	534,6 ± 1,00
	5	3,54 ± 0,11	7,93 ± 0,24	44,63 ± 1,34	37,79 ± 1,13	0,09 ± 0,01	545 ± 1,00	540,04 ± 1,00
	6	4,08 ± 0,12	7,62 ± 0,23	53,51 ± 1,61	37,72 ± 1,13	0,1 ± 0,01	545 ± 1,00	538,78 ± 1,00
λ_{580}	1	2,26 ± 0,07	5,33 ± 0,16	42,46 ± 1,27	32,11 ± 0,96	0,07 ± 0,01	580 ± 1,00	584,14 ± 1,00
	2	2,74 ± 0,08	5,93 ± 0,18	46,24 ± 1,39	35 ± 1,05	0,09 ± 0,01	580 ± 1,00	583,38 ± 1,00
	3	4,32 ± 0,13	6,27 ± 0,19	68,92 ± 2,07	45 ± 1,35	0,11 ± 0,01	580 ± 1,00	583,55 ± 1,00
	4	4,01 ± 0,12	6,81 ± 0,2	58,83 ± 1,76	45 ± 1,35	0,11 ± 0,01	580 ± 1,00	583,37 ± 1,00
	5	2,68 ± 0,08	6,01 ± 0,18	44,63 ± 1,34	30 ± 0,9	0,1 ± 0,01	580 ± 1,00	582 ± 1,00
	6	3,52 ± 0,11	6,57 ± 0,2	53,51 ± 1,61	36,19 ± 1,09	0,11 ± 0,01	580 ± 1,00	585,19 ± 1,00
$\lambda_{610–620}$	1	0,24 ± 0,01	0,57 ± 0,02	42,46 ± 1,27	5 ± 0,15	0,02 ± 0	615 ± 1,00	615,05 ± 1,00
	2	1,05 ± 0,03	2,27 ± 0,07	46,24 ± 1,39	20 ± 0,6	0,04 ± 0	615 ± 1,00	617,24 ± 1,00
	3	0,61 ± 0,02	0,88 ± 0,03	68,92 ± 2,07	5 ± 0,15	0,06 ± 0	615 ± 1,00	615 ± 1,00
	4	0,29 ± 0,01	0,49 ± 0,01	58,83 ± 1,76	610 ± 18,3	0,06 ± 0	615 ± 1,00	612,51 ± 1,00
	5	0,39 ± 0,01	0,87 ± 0,03	44,63 ± 1,34	610 ± 18,3	0,04 ± 0	620 ± 1,00	615,07 ± 1,00
	6	—	—	—	—	—	—	—
$\lambda_{635–650}$	1	2,37 ± 0,07	5,59 ± 0,17	42,46 ± 1,27	10,04 ± 0,3	0,15 ± 0	650 ± 1,00	643,19 ± 1,00
	2	0,67 ± 0,02	1,44 ± 0,04	46,24 ± 1,39	15 ± 0,45	0,04 ± 0	640 ± 1,00	639,31 ± 1,00
	3	3,05 ± 0,09	4,42 ± 0,13	68,92 ± 2,07	30 ± 0,9	0,11 ± 0	645 ± 1,00	639,25 ± 1,00
	4	2,74 ± 0,08	4,66 ± 0,14	58,83 ± 1,76	35 ± 1,05	0,09 ± 0	645 ± 1,00	636,41 ± 1,00
	5	1,51 ± 0,05	3,39 ± 0,1	44,63 ± 1,34	30 ± 0,9	0,05 ± 0	635 ± 1,00	637,44 ± 1,00
	6	2,35 ± 0,07	4,4 ± 0,13	53,51 ± 1,61	40 ± 1,2	0,06 ± 0	635 ± 1,00	632,97 ± 1,00

In the first three days, β-band (λ_{545}) and α-band (λ_{580}) in the Q-range showed an increase in area, with the latter being characterized by a significant ($p < 0.05$) change in geometry (increase in FWHM and height).

It was found that the indicated processes on the α-, β- and γ-bands occurred simultaneously with histostructural changes in muscle tissue and the onset of various autolysis stages.

As is the case of the minor peak at λ_{355}, similar dynamics were observed in $\lambda_{610–620}$ region, where an increase in peak area occurred on the first day, followed by a decrease and complete disappearance by day 6. In turn, a bathochromic shift of peak center by 5 nm was detected in the indicated wavelength range while maintaining the position of peak centroid.

A fourfold increase in FWHM and a hypochromic shift of peak centroid (10.22 ± 1.92 nm) in $\lambda_{635–650}$ region indicated the accumulation of lipid oxidation products, which is consistent with [52].



(a) (b) (c) (d)

Figure 3. Histostructure of *Sus scrofa M. longissimus dorsi* muscle tissue on the first (a), third (b), fifth (c) and sixth (d) days of storage (40× magnification)

A change in the integral parameters of the peaks indicates an increase or decrease in the concentration of certain substances in meat during storage. In turn, a change in the geometry (FWHM, peak center and peak centroid) of the peaks indicates structural and chemical changes in these substances. The results of the study prove that, depending on the degree of autolytic changes in muscle tissue, characteristic changes in the intensity and geometry of the above peaks occur. The use of KCl extraction in combination with data preprocessing made it possible to establish clear patterns consistent with histological studies (Figure 3).

The dynamics of minor peaks at $\lambda_{325–335}$, λ_{355} and γ-band at $\lambda_{410–415}$ reflected the stages of rigor mortis and meat aging simultaneously with histological transformations (restoration of transverse striation, relaxation and change in the diameter of muscle fibers). Changes in the Q-range (λ_{545}, λ_{580}) and $\lambda_{635–650}$ region confirmed the accumulation of oxidation products, which allows using these parameters as markers of oxidative spoilage. This confirms the potential of the method for objective assessment of meat quality, predicting shelf life and monitoring structural and chemical changes in muscle tissue.

Conclusion

The study confirmed the effectiveness of spectrophotometric analysis of pork KCl extracts for monitoring meat quality during storage. It was found that KCl extraction provides high solubilization of myofibrillar and sarcoplasmic proteins, forming stable spectral profiles with clearly defined peaks. Key spectral parameters (peak area, FWHM, peak centroid) demonstrated statistically significant correlation ($p < 0.05$) with autolytic processes in muscle tissue, including physicochemical and histostructural changes.

The obtained data confirm that the spectral characteristics of muscle tissue KCl extracts, reflecting the dynamics of pigments (myoglobin, NADH), structural proteins and other compounds interaction, serve as reliable markers of autolytic transformations. Quantitative analysis of the integral parameters of key peaks at $\lambda_{325–335}$, λ_{355}, $\lambda_{410–415}$, λ_{545}, λ_{580}, $\lambda_{610–620}$, $\lambda_{635–650}$ allows objectifying the assessment of the autolysis degree. Integral analysis of spectral characteristics allows not only to assess the autolysis degree, but also to identify specific patterns characteristic of meat at different storage periods.

The ability to monitor changes occurring in pork muscle tissue during storage using spectrophotometry emphasizes its value as a tool for assessing meat quality and brings opportunities for the development of fast and reliable methods for quality control of meat products. However, to fully realize the potential of the method, further research is needed to standardize the analysis conditions and expand the spectral characteristics databases.

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MEAT CONSUMPTION TRENDS: HEALTH IMPACTS, ALTERNATIVES, AND SUSTAINABILITY PERSPECTIVES

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Abstract

Meat is a valuable source of energy since it contains protein and fat. It is also a source of key vitamins and minerals, such as vitamin B12, iron and zinc. However, high meat consumption can have adverse health and environmental effects. The objective of this paper is to discuss the global trends in consumption of meat and meat substitutes and understand their impacts on human health and the environment. It is accepted that the growing emphasis on sustainability underscores the importance of switching to alternatives, as the traditional meat production system faces substantial environmental and resource limits. Reducing meat consumption is vital in decreasing health and environmental impacts caused by meat production and consumption. Nevertheless, veganism may not be the best solution for all people because nutritious plant-based foods are not readily available particularly in low-income nations. Furthermore, livestock farming provides a significant source of earnings for many low-income households. Further research is required to encourage technical and behavioral improvements, while balancing the environment. Considering the above information, this study provides valuable insights into the consumption trend for meat and meat alternatives, encompassing their strengths, weaknesses, opportunities, and threats.

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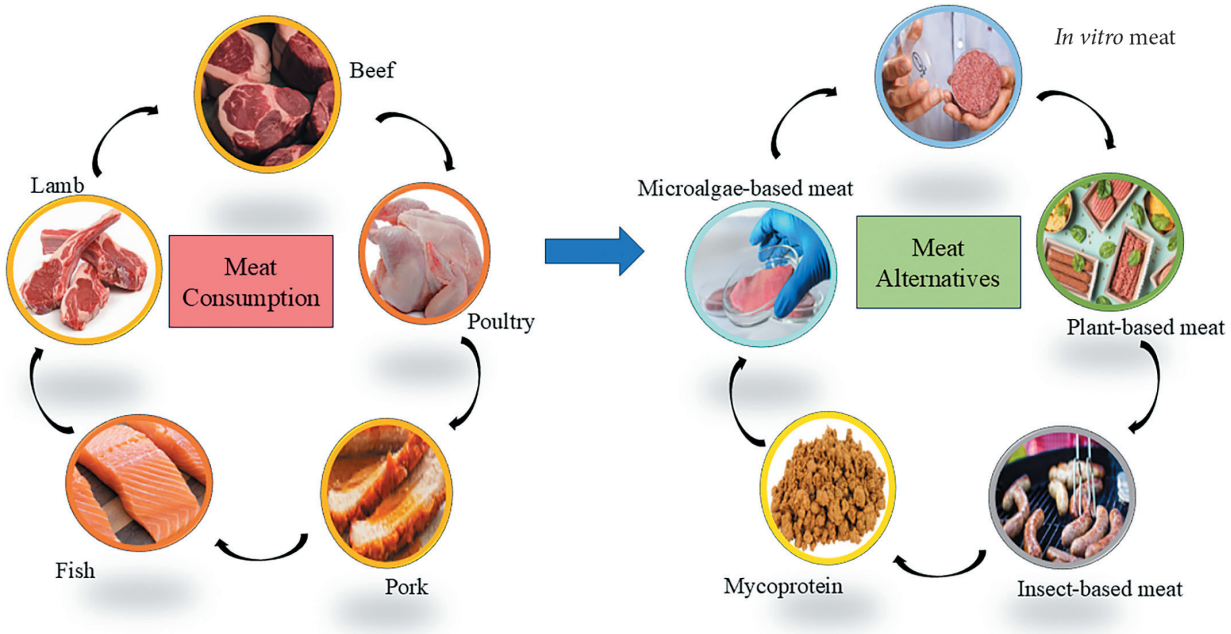
Highlights

- Meat protein is a nutrient-rich source but contributes significantly to environmental pollutants.
- Reliance on conventional meat production should be reduced.
- Demand for meat alternatives is rising; the aim is to meet nutritional needs with lower environmental impact.
- Meat alternatives provide more sustainable option to global food security.
- Food technological innovative production should be revolutionized in the coming decades.

Introduction

Meat is an esteemed protein source that contains all essential amino acids for human health. Fat in meat is a



Graphical abstract

significant energy source and imparts flavor, deliciousness, and delicacy, with its composition varying with the species, quality, and cuts. Other edible parts, such as the liver, kidneys, and offals, are also known for their significant composition of nutrients and minerals that are effectively absorbed by the human body [1]. However, meat is an exceptionally "inefficient" food source. Meat production requires more energy, water, and land than production of other foods. It is also an enormous wellspring of ozone-depleting substance emanations and in this way assumes a significant part in environmental change. According to the European legislation, the term meat is defined as the edible portion obtained from domestic bovine, caprine, ovine, and porcine animals, domestic solipeds, poultry, rabbits, wild and farmed game, as well as some other animals. Meat is identified as the chief source of protein availability with 28 g of protein/per capita/day, trailed by wheat products and dairy products [2]. Over the past 50 years, there has been a notable growth in the meat consumption, which has led to expenses and potential health problems. An estimated 350 million tons of meat are consumed annually worldwide, which results in the annual slaughter of 72 billion animals for human consumption. Also, meat production has doubled since 1988 and tripled since the mid-1960s.

The foods that humans obtain from animal sources are a major provider of essential nutrients. Even though the foods from plant sources can convey the daily nutrients needed by the human body, there should be a sizable number of plants that have to be included in the diet. Some nutrients cannot be digested in the human body whereas they can be digested within the animal body and thus humans can intake nutrients that are in a digestible form from animal products by consuming them. For example, cellulose cannot be digested in the human body but cellulose can be digested by ruminant animals and it turns into products that are thus useful to humans [3]. Society is now having a shift towards diets with more fat, sugar, processed foods, etc., and thus witnessing a situation with a more diseased community. Meat consumption is increasing with increased economic development and thus affecting human health and the environment. This growth in meat consumption has negative impacts on human health and the environment [4]. Meat creates more emission per unit of energy compared to that of plant-based food sources since energy is lost at each trophic level. Within the types of meat, ruminant animals generally prompt a bigger quantity of emissions than non-ruminant warm-blooded creatures, and poultry production ordinarily prompts fewer outflows than warm-blooded animals.

Meat production harms global biodiversity and causes pollution. The resources used in meat production, such as land, energy, and water, could be better employed to grow plant-based foods for humans. The food sector, notably animal-based diets, accounts for 30 % of global greenhouse gas emissions. Livestock husbandry reduces biodiversity, depletes water sources, alters nutrient cycles, and emits greenhouse gasses, all of which have significant consequences for human health and the environment. With a growing worldwide population, demand for meat and animal products is predicted to double by 2050, compounding these difficulties [5,6]. Researchers are looking into the factors that drive people to limit their meat intake and the role that meat substitutes play in reaching this goal. Global meat consumption is linked to chronic diseases such as diabetes, cancer, and cardiovascular problems [7]. Reduced cattle production can efficiently cut greenhouse gas emissions and address concerns including interrupted nitrogen cycles, biodiversity loss, climate change, and pollution [8]. With limited land and water resources, growing meat production is unsustainable. As a result, the development of meat alternatives, such as plant-based, cultured, and insect-based choices, is gaining traction as a feasible solution to fulfill future protein demands [9].

A shift is required from meat and animal-based product consumption to meat alternatives. Recently many plant-based meat alternatives (PBMA), as well as insect-based proteins, are becoming an emerging trend in the market as a suitable choice for meat alternatives. Consumer acceptance of meat alternatives is one of the main challenges [5]. It is considered necessary to reduce meat consumption and thus to identify more sustainable methods for protein intake. Accepting the innovation would permit us to compete with the issues presented by the conventional production of meat. Meat alternatives, especially lab-grown meat, can act as a continuous meat source in the future for space missions. As compared with plant-based foods meat production efficiency is unfavorable. Studies indicated that meat production should be reduced in the future to conserve the environment and human health and also to reduce animal suffering. People consume meat for pleasure, to express their economic and social status, and also for a personal identity beyond its nutritional value. By identifying the meat consumption trend, it is important to propose its impacts on the environment and human health [10], and introducing meat analogs will help in ensuring the health and safety of consumers by thus reducing the climate change caused by commercial meat production [11]. Given the complex nutritional, environmental, and ethical implications associated with rising global meat consumption, there is an urgent need to explore sustainable, health-conscious alternatives. While meat remains a valuable source of high-quality protein and essential nutrients, its production comes at significant ecological and public health costs. In light of growing consumer awareness, environmental pressures, and evolving dietary trends, it becomes crucial to assess the feasibility and acceptance of meat substitutes that can fulfill nutritional needs while minimizing environmental burdens.

Therefore, this research aims to critically examine the health and environmental impacts of conventional meat consumption and to explore the potential of alternative protein sources including plant-based, cultured, and

insect-derived options as sustainable substitutes. This study also aims to investigate consumer perceptions and barriers to the adoption of meat alternatives, providing insights into how future dietary transitions can be guided to strike a balance between human health, environmental sustainability, and food security.

Global trends in meat consumption

From the study conducted by the Food and Agricultural Organization, it is estimated that there has been a critical expansion in overall consumption of meat over time and the consumption rate is accelerated by the growing population [5]. At the global level, meat consumption trends increase mainly due to two factors: rising population and rising income in countries [12]. In the analysis conducted from 2000 to 2022, meat consumption patterns have increased noticeably in Indonesia and China with 89 % and 54 % growth, respectively. The growth was much slower in Australia and the United States with 13 % and 8 % respectively, whereas, the meat consumption trends decreased in Japan by 3 %. Figure 1 represents the meat consumption trends in Indonesia, China, Japan, Australia, India and the United States over 22 years to 2022 [5,13,14].

In middle and low-income countries there was an increasing trend for consumption of fat, processed, animal foods. Some factors which are related to an increase in meat consumption include the growing incomes in developing countries. In developed countries, the meat consumption growth has not shown a significant change because it has been high for a long time. Studies evidence that consumers have moved toward white meat in the last two decades. Fish and poultry were marked as the most consumed meat across the world [13]. The trend in meat consumption patterns varies with meat categories and it was found that people have moved to higher consumption of white meat compared to red meat due to its low price [15]. Decreases in price, trade liberalization, extension in the food system, and urbanization are other factors that influence the trends in global meat consumption.

A number of studies suggest that meat intake will increase in the coming years in developing countries with the rise in income and population [13]. There is a need for an approach to create food that represents ecological externalities, while assuring that the worldwide population has **a** sufficient healthy food supply. From the environmental point of view, the future patterns for the food system should be adaptable, sustainable and more efficient [12].

Meat consumption impacts on human health

Daily intake of 50 grams of processed meat has been linked to an 18 % increase in colorectal cancer (CRC) risk, according to the International Agency for Research on Cancer (IARC), which classifies processed meat as carcinogenic and red meat as probably carcinogenic to humans [16]. Processed meats are high in saturated fats and cholesterol, contributing to coronary heart disease, diabetes, and obesity [9]. The WHO recommends limiting red and processed meat intake, especially in high-income countries, and shifting toward more plant-based diets for better health outcomes and sustainability [2]. The American Cancer Society suggests that replacing red meat with poultry or fish may reduce cancer risk [17].

Red and processed meat contains heme iron, which plays an important role in formation of N-nitroso compounds (NOCs) in the gut. It has been postulated that NOCs can damage intestinal lining and potentially lead to CRC [18]. Furthermore, processed meat may contain such compounds as nitrates (which convert to nitrosamines), polycyclic aromatic hydrocarbons (PAHs), and dioxin-like PCBs [18–20]. These compounds have been associated with an increased risk of gastrointestinal, liver, and bladder cancers [18]. Some studies also note that vegetarian and pescatarian diets may lower CRC risk compared to non-vegetarian diets (Table 1) [21,22].

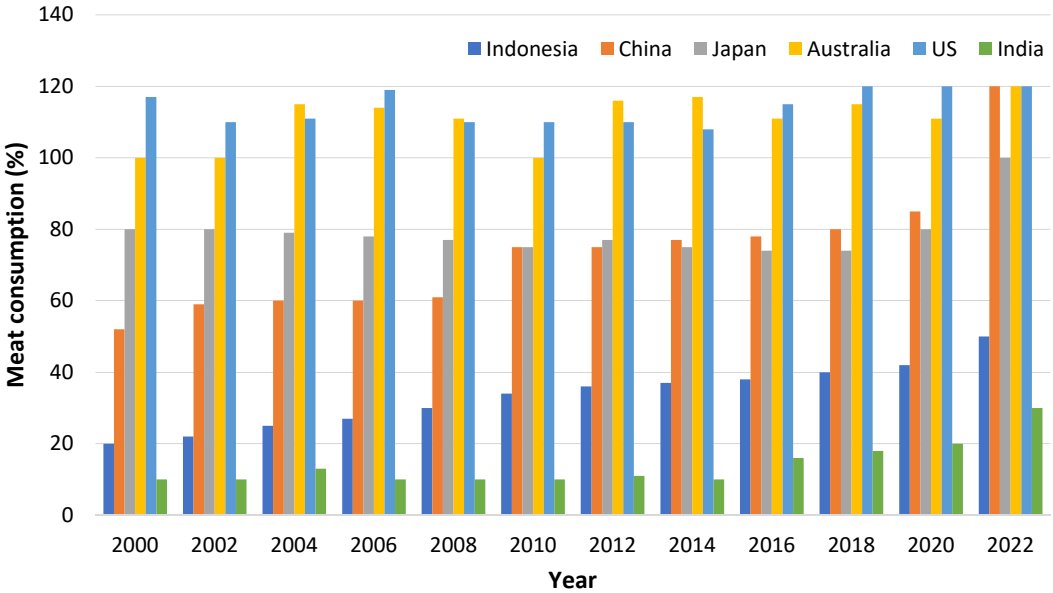


Figure 1. Meat consumption per person from 2000 to 2022

Table 1. Impact of meat consumption on human health

Health impacts	Inference	Reference
Colorectal cancer (CRC)	CRC risk is higher with red meat, such as beef and lamb than with processed meat	[25,26]
Esophageal cancer	Processed meat intake is associated with an incremental chance of esophageal cancer	[29]
Gastric cancer	Processed meat consumption has a greater risk of occurrence of gastric cancer than unprocessed meat	[30]
Bladder cancer	Processed meat was found to be associated with bladder cancer	[31]
Cardiovascular disease	Meat consumption was found to be related to an elevated risk for stroke	[32]
Diabetes type 2	Red meat consumption was found to have a link with the occurrence of diabetes type 2	[33]

In addition to cancer, high consumption of red and processed meats has been linked to an increased risk of type 2 diabetes and cardiovascular diseases. Consuming 50 grams of processed meat daily can raise diabetes risk by up to 51%, likely due to preservatives and fat content impairing insulin sensitivity [18,23]. Moreover, diets high in saturated fat and cholesterol common in processed meat are known to promote arterial plaque buildup, elevating cardiovascular disease risk, particularly more so with processed than unprocessed meats [24].

In essence, health outcomes related to meat consumption depend largely on meat type, processing level, portion size, and overall dietary context. While plant-based diets have shown benefits in reducing chronic disease risks, well-balanced omnivorous diets that include lean meat and fish can also support optimal health, particularly when integrated with healthy plant based diets, as depicted in Figure 2. Increased intake of meat can lead to a relative risk of developing heart disease. The diets high in red and processed meats show the greatest risk up to 160% due to their high content of saturated fats, cholesterol, and pro-inflammatory compounds. According to the findings (Figure 2), red meat consumption is associated with a higher relative risk increase (140%) for heart disease compared to processed meat (125%). This difference may be attributed to higher levels of heme iron, saturated fats, or other bioactive compounds in unprocessed red meat that influence cardiovascular risk factors [25,26]. In contrast, shifting toward plant-based diets significantly reduces the risk. A general plant-based diet lowers the risk to around 60%, while a healthy plant-based diet rich in fruits, vegetables, whole grains, legumes, and nuts can cut the risk even further, down to 25% [27,28].

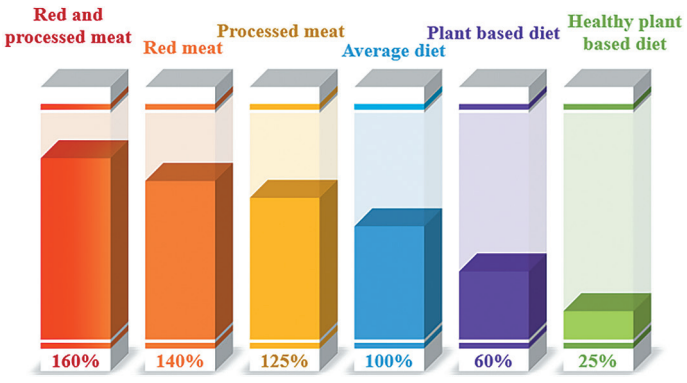


Figure 2. Probability of heart disease based on diets

Impacts on the environment

The environmental implications of meat production, particularly from ruminant livestock such as cattle and sheep, are a growing concern [2]. These animals contribute significantly to greenhouse gas (GHG) emissions, mainly methane, which has a high global warming potential. Studies estimate that by 2050, ruminant meat production could account for over two-thirds of greenhouse gas (GHG) emissions from global agriculture [34]. In addition to GHG emissions, meat production impacts land and water use, biodiversity, and air quality [35]. Intensive livestock farming practices contribute to deforestation, overgrazing, and pollution from animal waste and fertilizers. The majority of pollutants from the environment often detected in meats including polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polychlorinated naphthalenes (PCNs), are easily absorbed in fats. Beef production, for instance, requires considerably more land and water compared to plant-based alternatives, such as grains and legumes [36,37]. The GHG contribution to a variety of diets is shown in Figure 3.

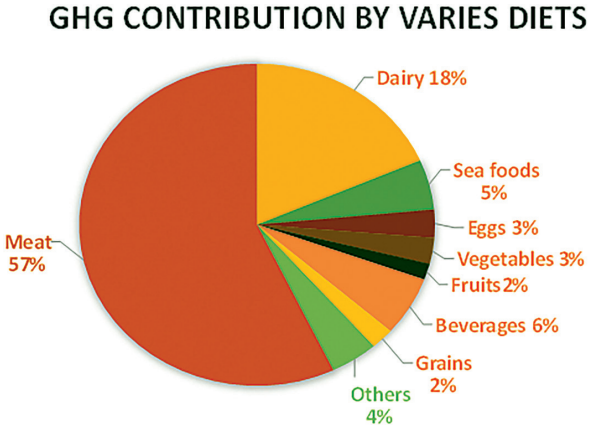


Figure 3. GHG contribution by various products

Benefits of meat consumption

Nevertheless, it is important to recognize that meat, when consumed in moderation and sourced sustainably, can play a beneficial role in both nutrition and the environment. Rather than advocating for complete elimination, a balanced consumption and responsible production are emphasized as more realistic and effective approaches for achieving public health and environmental goals. Nutritionally, meat, especially lean and unprocessed varieties,

remains a rich and efficient source of essential nutrients. It offers high-quality, complete protein along with bioavailable iron, zinc, selenium, and vitamin B_{12}, all of which are vital for maintaining muscle mass, immune health, cognitive function, and preventing nutrient deficiencies. These benefits are particularly important for vulnerable populations such as children, pregnant women, and the elderly, where deficiencies in these nutrients can lead to long-term health consequences [38,39].

In fact, including moderate amounts of lean red meat or poultry in a balanced diet has been shown to improve satiety and aid in weight management without a negative effect on cardiovascular health. Clinical studies, such as those examining the DASH (Dietary Approaches to Stop Hypertension) diet, have demonstrated that lean meats can be part of a heart-healthy eating pattern, helping to control blood pressure and lipid levels when consumed alongside plenty of fruits, vegetables, and whole grains [40].

From an environmental standpoint, not all livestock systems are equal in their impact. When managed sustainably, livestock can contribute positively to ecosystems. Practices such as rotational grazing and agroecological animal husbandry can help restore soil health, sequester carbon, and enhance biodiversity [41]. In many parts of the world, especially in low- and middle-income countries, livestock are not just a food source; they play vital roles in livelihoods, nutrient recycling, and farm resilience [42].

Furthermore, innovations in animal nutrition, such as low-emission feed additives, and the adoption of waste-to-energy systems are helping reduce the carbon footprint of meat production. These sustainable strategies show that with thoughtful policy and technological advancement, meat can be produced in a way that supports both food security and environmental stewardship [43].

Ultimately, the conversation around meat should move beyond extremes. Rather than eliminating meat entirely, encouraging mindful consumption focused on quality, source, and quantity can help strike a balance between nutritional needs and environmental priorities. Choosing lean, unprocessed meats, reducing portion sizes, and supporting ethical farming practices are practical steps individuals can take toward better health and a more sustainable food system.

Meat alternative sources

Many studies revealed that 30 % of GHG emissions occur from the food sector, mainly the animal-based food production system, causing all kinds of biodiversity losses. FAO has proposed that livestock handling is the major contributor to climate change. Therefore, reducing animal meat consumption is considered important. To this end, a shift is required from conventional meat-based products to meat analogs. Research from the past reflected that consumers do not know about the enormous effect that the consumption of meat has on the environment. A large part of customers does not consider eating meat substitutes even though a minority of them have consideration for it [44].

Meat alternatives are also known as meat analogs, faux meat, fake meat, mock meat, meat substitutes, imitation meat, and meat surrogates. Technologically a transition from meat-to-meat alternatives can be obtained by "protein transition" [45]. Studies evaluated that soy protein has high health benefits practically identical to animal protein and represents a great base for meat substitutes. In the 1960s, soy protein was first presented in the US market as a significant meat analog in the form of tofu and fermented soy cake. As an alternative to meat, consumers consider mainly plant-based meat alternatives (PBMA). In addition to plant-based meat alternatives, other meat analogs include *in vitro* meat, insect-based protein sources, microalgae-based meat, and mycoprotein-based food products [46].

***In vitro* meat**

In the current scenario, livestock meat production is increasing day by day due to its nutritional importance and increasing consumption of meat by the population. A new technology to develop meat in the laboratory called *in vitro* meat, cultured meat or lab meat evolved as a substitute for conventional meat. This technology operates on the principles of tissue engineering by isolating stem cells from livestock (e. g., cattle, pigs, or sheep) and culturing them in a bioreactor. The bioreactor provides a growth medium enriched with nutrients and growth factors, enabling the cells to proliferate and differentiate into mature muscle tissue, ultimately forming in vitro meat [36]. Thus, it includes a new method of developing meat from animal muscles to avoid slaughtering process [47].

The proposal behind *in vitro* meat came from the idea put forward by Winston Churchill. He once suggested that "We shall escape the absurdity of growing a whole chicken in order to eat the breast or wing, by growing these parts separately under a suitable medium". Later Frederick Edwin Smith foresaw a future where raising entire cattle for meat would become unnecessary. He suggested that from a single starter steak, it would be possible to cultivate vast quantities of equally tender and flavorful meat [36].

The world's first *in vitro* meat was cooked and tested by a sensory panel in London in 2013. The *in vitro* meat was more like white meat, and thus some amount of beetroot juice and saffron were added to give the meat its particular color. The panelists found that the cultured meat tasted quite similar to animal meat.

Many kinds of research have been conducted by NASA on *in vitro* meat to make it a "long-term food" available for astronauts [48]. The production of *in vitro* meat has many advantages, including health, financial, environmental, and animal welfare advantages, over traditional meat. Studies revealed that *in vitro* technology is used for the production of steak, sausages, nuggets, etc., and researchers are still working on developing meat for commercial use [36].

Techniques for production of in vitro meat

The development of meat from muscle cells with the technology of tissue engineering without rearing animals is called *in vitro* meat production. Two technical methods are used for developing *in vitro* meat in laboratories such as self-organizing technique and scaffold-based technology [48].

Self-organizing technique

The method arose with the application of tissue engineering by Benjaminson, Gilchriest, and Lorenz to develop meat in the 21st century. This method is used for the production of structured muscle tissue. They first developed this technique experimentally by isolating the explants from a golden fish and culturing them in a proper nutrient medium and identified the expansion in the growth of muscle explants. These isolated muscle fibers contain all components in correct proportion thus mimicking an *in vivo* meat structure. However, the explants lack blood circulation, which exert a negative impact on substantial growth and thus this method is unable to produce large amounts of meat without vascularization [36]. Nevertheless, meat developed with this process will have a well-organized 3-D structure [47].

Scaffold-based technology

This technique consists of culturing stem cells isolated from farm animals in appropriate bioreactors [47]. The embryonic myoblasts are multiplied, then attached to a scaffold and perfused with a nutrient-rich culture medium. These culturing results in myofibers formation and these myofibers may then be processed and utilized as *in vitro* meat. Figure 4 represents the production process of *in vitro* meat by scaffold-based technique. Scaffold-based techniques are applied for the production of boneless meats with soft consistency, but structured meats such as steaks cannot be produced using this technique [36].

Benefits of in vitro meat

In vitro meat was developed to reduce animal suffering and to satisfy the need of meat-eaters with an alternative to conventional meat. In light of the sizable adverse consequences of current meat production for climate and human well-being, a reasonable solution lies with *in vitro* meat production [36]. The production of *in vitro* meat offers health and environmental benefits by lessening ecological contamination, as well as water and land utilization related to current meat production methods [49,50]. The nutritional composition of cultured meat can be redesigned and developed with suitable needs.

In vitro meat manufacturing reduces the GHG emission and carbon footprint of meat by reducing the rearing of animals for food. Food scarcity, increased risk of cancer, and many other diseases that occur due to the consumption of conventional meat can be eliminated by utilization of *in vitro* meat. The fat and other nutrient content in the meat can be controlled in cultured meat and unhealthy saturated fats can be replaced in it. The *in vitro* meat

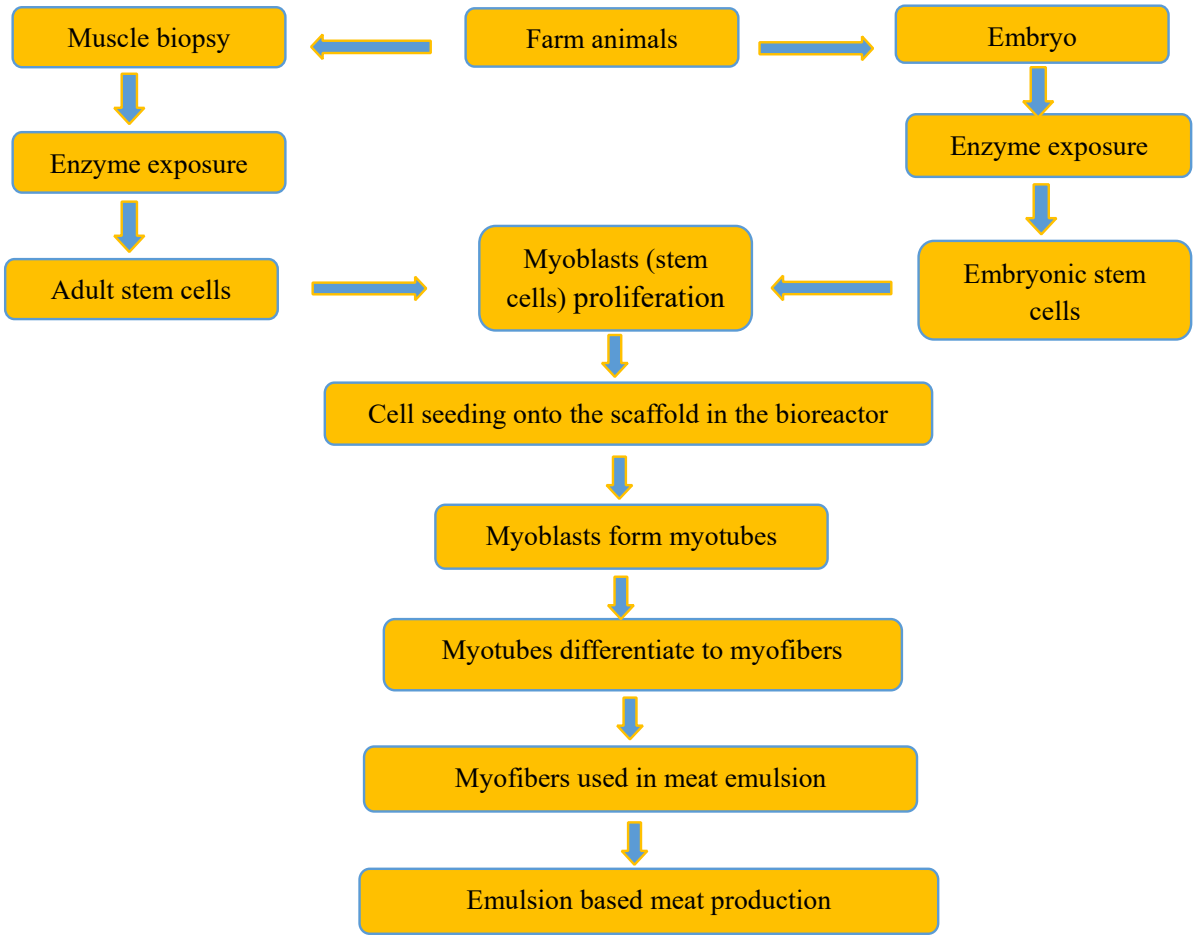


Figure 4. The production process of *in vitro* meat by scaffold-based technique [36]

production system is time-efficient and can produce meat within weeks when compared with conventional meat production from animals, which takes months to years. Many traditional animal slaughtering methods that are used to meet the increasing demand for meat consumption require high amounts of resources. Around 70 % of fresh water and 20 % of energy utilization is straightforwardly or in a roundabout way used for food manufacturing, of which an impressive extent is utilized for the rendering of meat. *In vitro* meat production reduces land use and biodiversity losses because it is built up vertically by utilizing less land area when compared with land used for grazing animals Researchers have found that 80 % of the land and water resources used are reduced by utilizing *in vitro* meat. This also lowers GHG emissions to 80–95 % [36].

Another important issue is food safety. The condition of food poisoning occurs through the consumption of meat slaughtered and handled in an unhygienic environment. Many food-borne diseases, including zoonotic diseases, that spread through commercial meat products can be prevented by the consumption of lab meat [51]. In addition, the risk of the presence of other hazards, for example hormones, can be reduced through this advanced technology [36,39,40]). *In vitro* meat is a good replacer for palates, steaks, etc. in the sense of flavor, taste, and tenderness. This technology can generate meat of consumer preferences by utilizing very low energy and is a time-efficient process. Theoretically, the *in vitro* meat developed using a single farm animal may create the world's meat supply [36].

Challenges in developing in vitro meat

The major challenge with *in vitro* meat is its acceptability as a meat substitute. A survey conducted globally on the acceptability of *in vitro* meat revealed that 80 % of the US population was not willing to consume *in vitro* meat, but in the UK 68 % of the respondents said that they would eat *in vitro* meat. The major disadvantage of *in vitro* meat is the absence of the natural pigment myoglobin, which gives the red color to meat, and the lack of minerals that are abundant in red meat [47].

Another challenge in developing *in vitro* meat is the mimicking of natural meat flavor. The technical challenge faced in the manufacturing of *in vitro* meat is the isolation of correct stem cells from animals [52,53].

Cultured meat is an unpreventable fate of humankind, but the high cost of manufacturing is a significant barrier to development. The cost of manufacturing cultured meat is high and thus this process is deliberate in society [36]. The growth media for meat should be less costly and of plant origin because it is more realistic than growth media made from animal sources. However, *in vitro* meat developed in this plant-based growth medium may cause an allergic reaction in some consumers [51].

Maintenance of the growth medium for stem cell culture was found to be a great challenge. Finally, the most important challenge is the so-called "yuck factor". This is the reluctance of consumers to take up with the idea of eating unnatural meat that is developed in the lab [47,54].

Plant-based meat alternatives (PBMA)

Protein-rich plants such as soybean, oilseeds, legumes, wheat, and fungi are well-known meat analogs [55]. The technologies used for the manufacturing of PBMA include shear cell technology and extrusion technology. Some of the common plants with their proteins that can be used as a meat substitute are illustrated in Table 2. Currently, the market for plant-based meat analogs is expanding with expanding social requests, and consistent endeavors are being taken to work on enhancing the sensorial characteristics of PBMA. PBMA has been regarded as the best meat analogs in the market recently [56,57]. Beyond Burger (BB) and Impossible Burger (IB) are the major two companies that have developed plant-based meats and they use soy protein, and wheat protein as the major alternatives [56].

Table 2. Plants with their proteins that can be used as meat substitutes

Plant	Protein
Soybeans	ß-conglycinin
Oilseeds	Legumin, albumin, globulin, glutenin
Wheat rye, barley	Gluten (Glutenin, gliadins)
Legumes	Glycinin, vicilin
Filamentous fungi	Mycoproteins

Benefits of PBMA

According to the nutritional sources, soy protein and wheat gluten are the finest alternatives [58]. Wheat provides 8–17.5 % protein, primarily in the form of gluten, and is deemed safe to consume. Technologies have effectively isolated gluten from wheat while preserving its structure, allowing it to be easily combined with other substances to create meat substitutes. On the other hand, soy protein has acquired consumer acceptability as a superior meat substitute due to its high protein content, environmental benefits, and nutritional value, which includes lipids, carbohydrates, iron, zinc, calcium, and B vitamins [59,60].

A study conducted by Bohrer [56] on the protein content in conventional meat in comparison with plant-based meat alternatives revealed that PBMA contains approximately the same amount of protein as traditional meat. H analyzed beef patty, pork ham, meatballs, and chicken nuggets and estimated that beef patty contains 23.3 g of proteins, while plant-based patty contains around 16.8–25 g of proteins. PBMA of ham, meatballs, and nuggets was also found to have similar amounts of proteins and other nutritional components when compared to the conventional meat products. Figure 5 illustrates the comparative study on protein content in conventional meat and plant-based meat alternatives.

PBMA is also an alternative for such consumers who will not consume meat due to ethical beliefs. While considering the nutritional aspects, PBMA was found to balance all the nutrients in traditional meat. Currently, consumers also have an increasing consideration for animal welfare

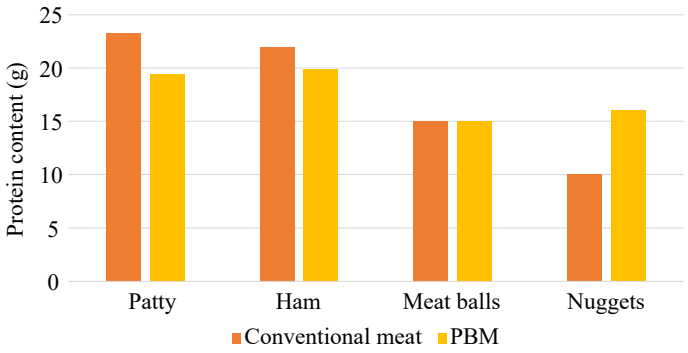


Figure 5. Comparison of protein content in conventional meat products and PBMA

and thus PBMA can show a great expansion in the market in the future [6]. Furthermore, plant food sources have a wide range of phytochemicals that play a significant role in human health. PBMA has less GHG emissions when compared with meat production and livestock handling. It has been stated that comparable amounts of protein, iron, and vitamin A can be acquired from delicately chosen plant-based foods at a lower carbon footprint when contrasted with meat consumption [35]. The lower carbon impression of plant-based meat analogs is promoted as a principal justification behind opting for PBMA [58].

Challenges of PBMA

Even though plant-based meat alternatives have good nutritive value, acceptability remains an obstacle among consumers. The major challenge for developing PBMA consists of meeting all the textural and sensory parameters of traditional meat. Various techniques including thermoplastic extrusion, spinning, and steam texturization are applied to plant proteins to develop texture and appearance [59]. Technologies are applied in extracting meat flavors from similar components and inducing them into plant meat. In addition, different flavor enhancers and fats are added during the manufacture of plant-based meat analogs [54].

Color is another important parameter apart from flavor for plant meat, which has a role in consumer perception [6]. Research has suggested that the PBMA should have a color resembling that of raw meat or cooked meat [59]. To bring the PBMA its particular color similar to conventional meat, beetroot juice, and tomato juice are added during its manufacture [54]. Some meat analogs are developed with added leghemoglobin, which has particular structural characteristics similar to hemoglobin that imparts a red color to meat. The addition of leghemoglobin imparts cooked meat color to meat substitute. Leghemoglobin was also identified to impart distinctive conventional meat flavor to PBMA [61].

During the development of PBMA, its safety should be ensured before marketing. Some anti-nutritional factors are present in meat analogs regardless of the many nutritional factors present in them. Some of the plant proteins can cause an allergic response in consumers. Therefore, ensuring the safety of plant meat is a great challenge. The challenges faced during the manufacturing of PBMA can be overcome in the future by applying advanced technologies [6].

Insect-based meat alternatives

In certain countries in Africa, Southeast Asia, and South America, the habit of eating insects, known as entomophagy, has a lengthy history extending back around 3000 years [62,63]. These societies have adopted the ingestion of insects as a means of meeting their daily protein and amino acid needs. With over 5.5 million recognized bug species on Earth, they have access to a mind-boggling 2000 insects for eating source [64,65]. Researchers discovered that insects such as grasshoppers, crickets, caterpillars, ants, bees, beetles, planthoppers, leafhoppers, and dragonflies are regularly consumed and provide a higher protein source than typical meat [66]. Insect food sources are equivalent or superior to conventional meat in terms of energy and protein [46]. Insects are also a rich source of iron, zinc, fat, and several vitamins [67].

It is worth mentioning that the protein level varies based on the insect species and stage of development, with the larval stage typically possessing a substantial amount of protein.

However, as a food component, the consumption of insects is very low among consumers because of the acceptability criteria. Recently due to the increasing global threats by meat consumption, meat alternatives are of much importance [68]. Currently, with this increasing demand for meat alternatives as a protein source, insect-based foods are an emerging trend. Research revealed that in the future the insect-based food market will steadily increase [69].

Benefits

Entomophagy is an environmentally friendly choice and it reduces GHG emissions and maintains the land degradation caused by the raising of animals for food [70]. Studies evidenced that insects are a great source of protein and thus it is a good alternative for meat. The protein percentage varies with species and stage of development. Some of the edible insect species and their nutritional composition are represented in Table 3.

Table 3. Edible insect species with their nutritional composition

Edible insects	Protein %	Fat %	Fiber %	Ash %	Energy content (kcal/100g)
Orthoptera **(grasshoppers, crickets)**	61.3	13.41	9.55	3.85	426.25
Blattodea **(cockroaches)**	57.30	29.90	5.31	2.94	—
Odonata **(dragonflies, damselflies)**	55.23	19.83	11.79	5.53	431.33
Diptera **(flies)**	49.48	22.75	13.56	10.31	409.78
Hemiptera **(true bugs)**	48.33	30.26	12.40	5.03	478.99
Hymenoptera **(ants, bees)**	46.47	25.09	5.71	3.51	484.45
Lepidoptera **(butterflies, moths)**	45.38	27.66	6.60	4.51	508.89
Coleoptera **(beetles, grubs)**	40.69	33.40	10.74	5.07	490.30

As compared with plant proteins, insect proteins have high bioavailability and more essential amino acids as well as proteins and thus insect proteins can serve as a major protein source in the diet. Some of the unique insect amino acids such as lysine, threonine, and tryptophan are only obtained from insect sources [71]. The utilization of insect proteins is beneficial not only due to their high nutritional content but also due to the fewer requirements of energy, water, land, and feed when compared with the rearing of animals for food [71,72].

Challenges

Not all insects are safe for human consumption and reports evidence that nutritional deficiencies and medical illness are also associated with the consumption of some species of insects. Ataxic syndromes were reported in Nigeria through the consumption of *Anaphe venata* [70]. The major challenge is the acceptability of insects as food for consumers. Mass production of insects is also a great challenge regarding the breeding and processing time of insects for consumption.

Food and Drug Administration (FDA) has implemented certain norms with regard to the processing of insects for a food source and proposed that insects for food sources should be cultivated specifically for the purpose of human consumption and manufacturers should ensure the 'wholesomeness' of a product, that is they should ensure that the product is free from parasites, microbes, and dirt [46].

Regardless of constant efforts to expand insect proteins in the market, their consumption may not turn into a standard eating choice [69]. Having skeptical attitude towards novel foods, consumers hesitate to consume insect proteins [68]. Consumption of insects remains the main challenge because people consider insects as unpleasing, dirty, harmful creatures and thus avoid their consumption. The consumers' unacceptance of insects as an alternative to meat is mainly due to food neophobia. Nonetheless, there are many antinutritional factors, toxic substances, and allergens present in insects that should be eliminated before their utilization as food, and thus insect protein utilization remains a challenging factor [6].

Fungi-based meat alternatives (Mycoprotein)

Mycoprotein is produced from a naturally occurring filamentous fungus *Fusarium venenatum*. In 1967, a strain of *F. venenatum* was found to be a potential source of protein. Later, *F. venenatum* was chosen as the most suitable organism for mycoprotein synthesis by the Rank Hovis McDougall (RHM) Company in England. The generic name mycoprotein was specified for the ribonucleic acid-reduced biomass comprising hyphae (cells) of the organism *F. venenatum* A3/5(ATCC PTA-2684) obtained using a continuous fermentation system. Following MFAFF certification, mycoprotein was marketed under the UK trade name "Quorn" [73–77]. It has nutritional benefits for human health being an excellent source of protein and fiber.

Mycoprotein production

The continuous flow system is the most frequent economic production method for biomass-related products, with a substantial dilution rate. In a constant flow environment (maintained at 28–30 °C and a pH of 6.0), fungi can multiply to the desired levels with minimal amounts of glucose and ammonium, i. e., carbon and nitrogen sources respectively. The biomass concentration and the flow rate can be assessed by evaluating and monitoring the growth in carbon dioxide (CO_2) levels. Following these conditions, a growth rate of 0.17 to 0.20 per hour and a production outcome of 300 to 350 kg per hour of *Fusarium venenatum* A3/5 biomass can be attained. The biomass is checked (every six hours) for hazardous contamination including mycotoxins. The harvested fungal biomass undergoes a step to reduce RNA content, including heating at 72 to 74 °C for 30 to 45 minutes to lower the RNA levels to below 2 %, making it safe for human consumption. Subsequently, in this RNA reduction process, the broth is subjected to an additional heat treatment at 90 °C and then centrifuged to obtain dry biomass with a solid content exceeding 20 % (W/V). Besides, the mycoprotein biomass undergoes a series of processes involving steaming, chilling, and freezing to achieve the meat-like texture characteristic of Quorn products (Figure. 6). This translates into a complex network of mycoprotein hyphae that reveals a high level of

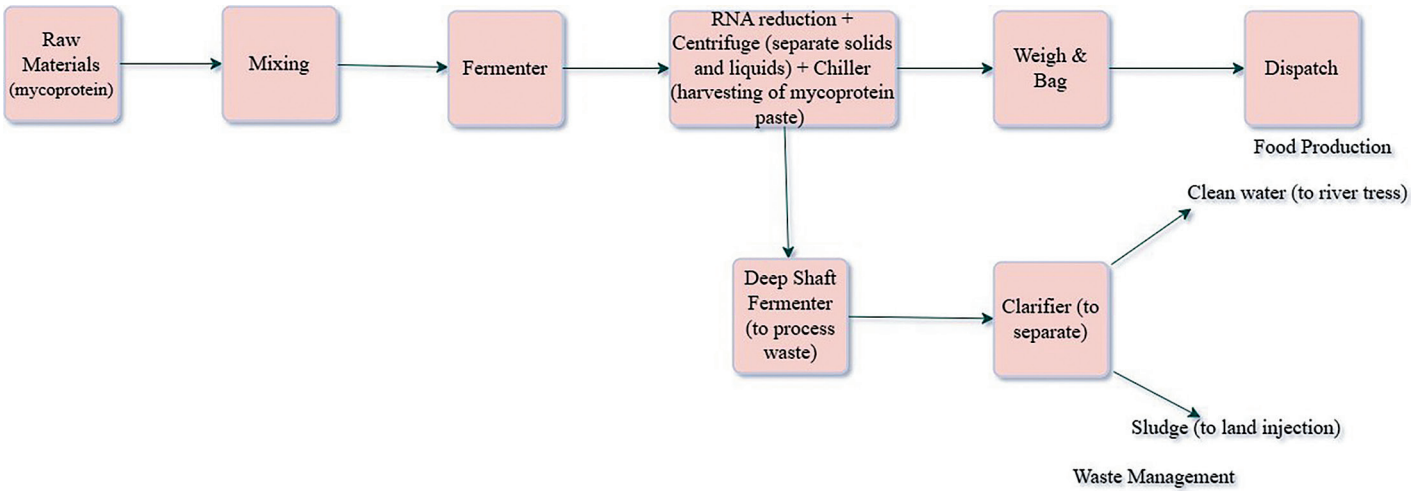


Figure 6. Process flowchart to produce Quorn mycoprotein [77]

fibrosity, analogous to the texture of chicken breast when viewed under a microscope. Presently, mycoprotein is used in a diverse array of products, ranging from frozen to refrigerated options, including Quorn mince, pieces, nuggets, steaks, sausages, fillets, fish fingers, and burgers [77].

Benefits

Mycoprotein is important for a healthy diet because of its high protein content, enhanced fiber, and low saturated fatty acid concentration. According to experimental investigations, mycoprotein may give various nutritional benefits, including increased satiety and better regulation of blood sugar and cholesterol levels [76]. If mycoprotein is to be used in main course dishes, it must be of high protein quality. Mycoprotein has all required amino acids. It contains 6 g of fiber per 100 g, indicating that it is "high in fiber" according to the European Commission. The natural dietary fiber of this fungal protein consists of 12 % soluble and 88 % insoluble fibers, with a small amount of chitin and a high concentration of glucan (forming a "fibrous chitin-glucan matrix" in the small intestine) [78,79].

The α-glucans (linear and branched), from grains and yeast help in fat metabolism and immune system function. Mycoprotein has an energy composition that is roughly one-third fat due to some easily available carbohydrates. Typically, mycoprotein contains less than 1.5 g of saturated long and short-chain fatty acids per 100 g solid. This fungal protein is high in monounsaturated and polyunsaturated fatty acids. It contains water-soluble B vitamins such as pyridoxine (0.1 mg), folate (114 μg), and cobalamin (0.72 μg). It also has a high concentration of zinc, phosphorus, calcium, iron, potassium, and other minerals [77,78].

Challenges

Mycoprotein, derived from the fungus *Fusarium venenatum*, faces multiple challenges in both its production and consumer uptake at present. A significant concern is the production cost. The existing techniques for farming mycoprotein tend to be quite costly, leading to prices that frequently align with those of conventional meat products. This mount price may demoralize consumers from considering mycoprotein as a feasible alternative, hence restricting its market reach and rate of economic growth. Another major challenge is the insufficient research on sensory attributes such as appearance, texture, and mouthfeel. Although mycoprotein is recognized for its meat-like texture, the lack of comprehensive studies on these sensory qualities can hinder consumer acceptance. A lot of people select meat substitutes based on their sensory experiences. Therefore, it may be difficult to develop a position in the market unless there is a more thorough understanding of how mycoprotein measures up against traditional meats in these aspects.

Moreover, the nutritional composition of the primary materials used in mycoprotein production presents a challenge. While lignocellulosic materials are suitable for fermentation, it is crucial to identify appropriate byproducts generated from agricultural and industrial processes that offer advantageous nutritional profiles for efficient products. This challenge is further complicated by the necessity to maintain environmental sustainability, as effectively utilizing agricultural waste could significantly lower the carbon footprint linked to mycoprotein production [80]. Consumer acceptance is also a concern, particularly regarding allergic reactions. Although mycoprotein is generally considered safe, there have been instances of adverse reactions, such as nausea and vomiting, in some individuals. This can lead to hesitation among potential consumers, especially those with allergies or sensitivities. Despite the rarity of such reactions, the perception of risk can influence consumer decisions [76].

Finally, mycoprotein encounters strong competition from an increasing variety of plant-based protein alternatives. As consumer preferences evolve towards a broader range of protein sources, mycoprotein needs to set itself apart through its taste, texture, and nutritional advantages to gain a larger market share. Tackling these challenges is essential for the future expansion and acceptance of mycoprotein as a sustainable and nutritious food option.

Microalgae-based meat alternatives

Microalgae are minute photosynthetic organisms that live mainly in aquatic environments, both freshwater and marine. They are mostly unicellular and belong to several taxonomic families, being a diverse and important part of ecosystems. Microalgae are essential not only due to their ecological functions but also due to their prospective applications in a variety of sectors. They are being investigated as sources of biofuels, nutraceuticals, and biopharmaceuticals because of their substantial amount of useful chemicals such as proteins, fatty acids, antioxidants, and pigments [81]. Furthermore, microalgae are regarded as a sustainable resource for food production, particularly in the creation of meat substitutes and other functional foods. Microalgae-based meat analogs are meat substitutes that use microalgae as a main ingredient. These products are intended to replicate the flavor, texture, and nutritional profile of traditional meat while providing a more sustainable and healthier alternative. Microalgae are noted for their high protein content, which can surpass 70 % in dry matter, and their rich composition of important amino acids, making them a viable resource for developing meat substitutes [82].

Microalgae production

Figure 7 depicts various technological pathways for creating microalgae-based meat analogs. Both microalgal biomass and protein extracts can be evaluated for texturing purposes. While the direct use of microalgal biomass is a cost-effective option, certain species may have rigid cell walls and high oil content that can hinder texturing and lower nutrient bioavailability. Consequently, protein extracts can serve as a complementary material for extrusion

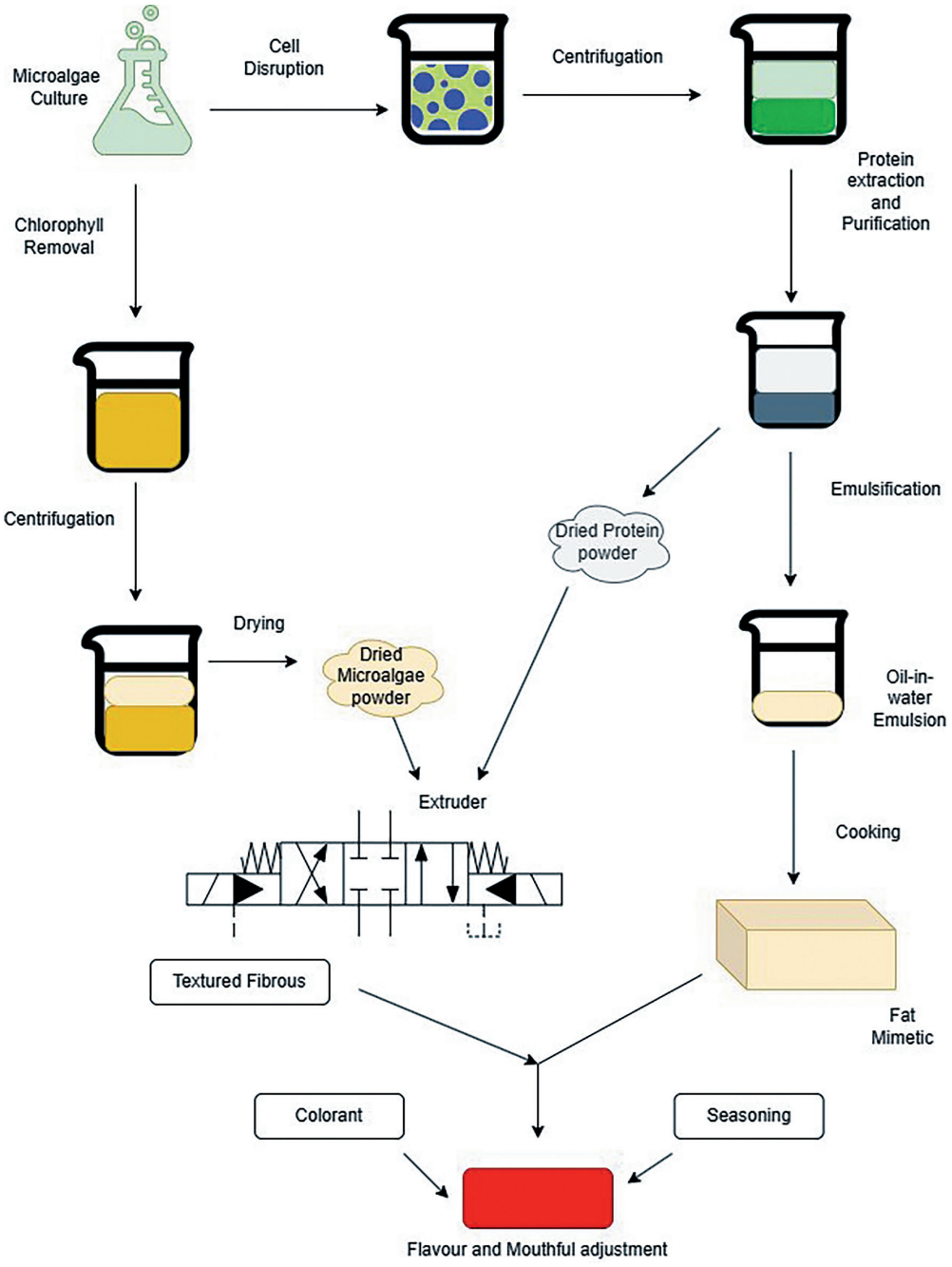


Figure 7. Schematic diagram of technical routes to produce microalgae-based meat

processes. After texturing, the resulting fibrous products and fat substitutes can be combined through emulsification [81]. Another critical aspect of producing meat analogs involves flavor adjustment. Different seasonings and colorants can be tested at various stages of food processing to identify beneficial chemical reactions. Additionally, the diverse compounds found in microalgae may serve as a source for novel chemical reactions, potentially leading to new flavors but also introducing new risks. Therefore, while microalgae as food ingredients offer numerous opportunities, they also pose risks that necessitate thorough investigation.

Microalgae biomass harvesting and processing

Harvesting microalgae biomass is a major challenge in production. Common ways include centrifugation, flocculation, and filtration [83].

Flocculation

A popular technique for harvesting microalgae is favored for its low cost, time efficiency, and high effectiveness in recovering algal biomass. This technique involves adding chemicals to aggregate microalgae cells, making them easier to separate. There are two main categories of flocculants: chemical flocculants and bio-flocculants. Studies indicate that bio-flocculants are safer, more economical, and environmentally friendly. Examples of bio-flocculants include acrylic acid and chitosan, which can occur naturally or be produced artificially [84,85].

Centrifugation

This method separates microalgae from the culture medium based on the density differences but can be energy-intensive. Harvesting microalgae through centrifugation is recognized for its high separation efficiency, exceeding

90 %. The most commonly used type of centrifuge for producing high-quality algae is the disk stack centrifuge. However, this method comes with significant operational energy demands [86]. According to pricing information, a typical dewatering centrifuge costs approximately RM 18,000, while a disk stack centrifuge is priced around RM 45,000 [84]. The high energy requirements associated with these centrifuges can lead to substantial operational costs. For instance, a disk stack centrifuge typically consumes about 1 kWh/m³ of feed. Given an average energy rate of RM 0.30 per kWh for industrial and agricultural applications, the cost to separate 1 m³ of feed would be RM 0.30, which is five times higher than the cost associated with flocculation. Similar to flotation methods, this high cost could be a disadvantage in the long term unless there is access to affordable and reliable renewable energy sources.

Flotation

Flotation methods, such as dissolved air flotation (DAF) and dispersed air flotation (DiAF), offer several benefits, including low initial capital costs, compact operational space, and rapid, efficient large-scale harvesting. These processes often utilize surfactants or flocculants to enhance harvesting effectiveness. However, a significant drawback is their high energy consumption, which leads to increased operational costs. Without access to affordable and reliable renewable energy, the energy expenses associated with these systems could become a major obstacle to their widespread adoption. Microalgae can also be harvested through membrane filtration techniques, which can be effective but may require pre-treatment to avoid clogging [83].

Benefits

Microalgae are gaining attention as a promising ingredient for meat alternatives because of their excellent nutritional profile, sustainable cultivation, and health advantages. It is a valuable source of carbon compounds that can be used in various applications, including biofuels, health supplements, pharmaceuticals, and cosmetics. They play a role in wastewater treatment and help mitigate atmospheric CO_2 levels. Besides, microalgae can be a source of several bioproducts, such as polysaccharides, lipids, pigments, proteins, vitamins, bioactive compounds, and antioxidants, which provide the consumer with a wide range of health benefits. The incorporation of microalgae in meat alternatives can improve their nutritional profile, making them a healthier option [87].

Lately, microalgae are regarded as a novel protein source for meat analogs. Microalgae proteins exhibit emulsifying, foaming, gelation, and solubility characteristics, comparable to those of other plant-based proteins such as soy, commonly used in meat formulations. Likewise, microalgae can enhance both the texture and flavor of meat alternatives. They are exceptional natural nutrient sources and can improve the overall quality of food products by diminishing the reliance on chemical preservatives. Microalgae production is regarded as more environmentally friendly than traditional cattle husbandry. Microalgae culture needs less land and water and can be farmed in a variety of settings, including non-arable terrain. Furthermore, microalgae can absorb carbon dioxide as they grow, helping to reduce greenhouse gas emissions. Studies have indicated that high moisture extruded microalgae products may be more sustainable than traditional meats such as pork and beef [87].

Challenges

Increasing the production of microalgae presents several challenges, particularly in optimizing both upstream and downstream processes. These challenges can be categorized into social inclusion, technological limitations, sensory properties, and commercial factors [88].

Consumer acceptance

One of the most significant challenges for microalgae-based meat substitutes is consumer acceptance. Consumers frequently form preconceived beliefs about algae, connecting them with unpleasant flavors or textures. This attitude can lead to reluctance to try microalgae products, regardless of their nutritional benefits. To overcome this obstacle, it is vital to educate customers about the positive impacts of microalgae and design marketing techniques that emphasize their environmental and medical advantages [83].

Technological limitations

The technological obstacles to converting microalgae into meat alternatives are enormous. Present techniques for texturizing plant biomass are still in the initial stages of research and development. This includes developing practical strategies to improve the texture and mouthfeel of microalgae, making them more palatable as meat alternatives. Furthermore, unpleasant scents and colors commonly seen in algae must be treated to improve consumer acceptance [88].

Sensory properties

Microalgae frequently have undesirable sensory characteristics, such as a grassy flavor or a fishy odor, which can repel customers. These sensory features can be unpleasant; thus, producers must devise methods to disguise or erase these flavors and colors. Creating formulations that blend the health advantages of microalgae with suitable sensory characteristics is critical for market success [83].

Commercial factors

Manufacturing microalgae-based meat replacements at a commercially viable scale is challenging. Scaling up production to meet customer demand while maintaining rates comparable with conventional meat products is difficult. Existing cultivation technologies may lack the requisite efficiency to enable the widespread use of microalgae in food products on a commercially feasible scale. To address this, continuous research and innovation are imperative to fully unlock the potential of microalgae in the realm of meat substitutes [82].

The overall benefits and challenges of each meat alternative are given in the Table 4 below.

Table 4. Benefits and challenges of meat alternatives

Meat Alternatives	Benefits	Challenges	References
In vitro meat	➢ Disease-free Meat: Production of healthy and sustainable meat with a favorable wholesome profile under controlled conditions. ➢ Animal Welfare: Elimination of the need for traditional animal slaughter, humane meat production ➢ Responsible Production: Potential to reduce nutritional deficiencies, foodborne illnesses, and antibiotic-resistant pathogen strains. ➢ Environmental Impact: Lowers greenhouse gas emissions, and depletion of water and land resources. ➢ Source of Protein: Cultured animal cells offer diverse protein sources. ➢ Availability: Meets the increasing global demand for meat while minimizing the strain on limited resources.	➢ Consumer Acceptance: Challenges to mimic natural meat flavor, texture, and nutritional composition without impacting safety or consumer acceptance as a meat substitute. ➢ Technological Challenges: The development of appropriate isolation methods for specific stem cells and suitable culture media is necessary. ➢ Health concerns: Plant-based media for cultivation may induce allergy reactions in some consumers. ➢ Industrial Scalability and Cost: Scaling up production in commercial bioreactors is crucial and not economically viable for large-scale production due to high manufacturing costs.	[36]
Plant-based meat	➢ Nutritional Benefits: Good source of vitamins, minerals, and protein (soy protein and wheat gluten). ➢ Eco-friendly: Moving away from traditional meat production can help conserve land, reduce greenhouse gas emissions, and minimize water usage. ➢ Beneficial for Health: Helps in weight management and supports heart health. ➢ Moral Implications: People can enjoy meat-like products by choosing plant-based substitutes while avoiding the ethical quandaries linked with animal farming and slaughter. ➢ Eclectic Taste: Mimic the taste and texture of traditional meats, offering a satisfying and familiar culinary experience.	➢ Material Selection and Handling: To achieve the desired sensory experience and nutritional value, it is necessary to selectively choose and process plant-based ingredients. ➢ Market Preference and Vision: To overcome ideas or judgments about their taste, texture, sugar, refined oil, dextrose, or modified cornstarch barriers. ➢ Reliability and Cost: Enhancing production capacity and optimizing distribution networks challenge plant-based meats to be more readily available and cost-effective for consumers. ➢ Breakthrough: New advancements are required to improve the flavor, texture, and nutritional value of plant-based meats.	[89]
Insect-based meat	➢ Sustainable Efficiency: Requires less feed and resources than traditional livestock as well as produces fewer greenhouse emissions. ➢ Nutrients: Rich source of protein, healthy fats, vitamins, minerals, and fiber, also provides essential amino acids and micronutrients, which make insect-based meat a potentially healthy food option. ➢ Food Sovereignty: Improve the availability of food, particularly in areas with limited access to traditional protein sources. ➢ Dependable Source: Insects are easy to breed and grow, making them a reliable supply of protein.	➢ Commercial Acceptance: Overcoming food neophobia, as well as altering consumer perceptions and attitudes toward insect-based proteins present significant challenges. ➢ Legal Basis: Establishing clear norms and standards for the production and sale of insect-based meat that includes addressing safety concerns, labeling requirements, and ensuring compliance with food regulations. ➢ Demand for Products: The rise in insect farming to meet the demand for insect protein creates logistical challenges. ➢ Technological lead: The development of efficient and automated rearing methods is essential to ensure a steady supply.	[90]
Mycoprotein-based meat	➢ Nourishment: High-protein, fiber-rich food that contains all of the essential amino acids required for good physical condition. ➢ Cardiac Wellness: Low in fat and cholesterol, which can contribute to better heart health. ➢ Ecological Footprint: Reduction in water and land resources requires a more sustainable option as the global population continues to grow. ➢ Adipose Reduction: Prevents overeating and supports weight management. ➢ Flexibility in Diets: Provides a meat-like texture that can appeal to those who are transitioning away from animal products or who have dietary restrictions.	➢ Hypersensitivity: Potential allergen, which can lead to dangerous reactions upon consumption. ➢ Manufacturing cost: The latest mycoprotein production methods are costly, leading to prices similar to traditional meats, which limits accessibility and adoption. ➢ Public Approval: Greater public awareness of mycoprotein's health and environmental advantages is needed, as many consumers may resist switching from traditional meat due to established preferences and cultural norms. ➢ Industrial Scale Production: Expanding production challenges in terms of texture, sustainability, and consumer acceptance have to be addressed effectively using ongoing technological innovations.	[76]
Microalgae-based meat	➢ Nutritional and Health Benefits: Enhanced nutritional profile and provides bio-active compounds, potentially diminishing the reliance on synthetic fortificants and chemical preservatives. ➢ Functionality: Improves texture and flavor and act as an emulsifier and foaming agent ➢ Sustainability: Captures CO_2 and reduce the carbon footprint of food production	➢ Low cell density: High cell densities are critical for economic feasibility because many microalgae species have minimal biomass concentrations in natural conditions. ➢ Production expenditure: The construction and operating costs of photobioreactors and harvesting methods can be prohibitively expensive. ➢ Contamination: Open systems are especially sensitive to pollution, which can reduce output.	[82]

SWOT analysis

Consuming meat is a vital part of diets worldwide and is deeply intertwined with food preferences, cultural customs, and economic activity. However, because of its popularity in contemporary diets, several intricate problems have emerged, demanding a methodical examination to comprehend its effects fully. The SWOT (Strengths, Weaknesses, Opportunities, and Threats) analysis provides a methodical framework for assessing the various facets of meat consumption (Table 5). This analysis attempts to shed light

Table 5. SWOT analysis of meat and meat alternatives

Parameters	Meat	Meat alternatives	References
Strength	➢ Nutrient Dense: High concentration of protein, lipids, vitamins (B_{12}, B_6, niacin), and minerals (iron, zinc), which are required for human health. ➢ Well-being: Meat consumption contributes to lower individuals' iron deficiency anemia (IDA) with increased bioavailability when compared with other food resources. ➢ Cultural Interest: Various regional and traditional diets around the world rely heavily on meat. ➢ Economic Effects: Millions of people worldwide depend on livestock for a living, especially in rural and agricultural areas. ➢ Consumer preference: A lot of people enjoy the flavor, texture, and satisfaction that comes with eating meat.	➢ Health Plan: Meat alternatives frequently have less saturated fat and cholesterol than traditional meats, which helps minimize the risk of cardiovascular disease. ➢ Environmental Conservation: Plant-based as well as lab-grown meat alternatives offer a reduced environmental impact of carbon dioxide emissions, utilization of land, and water use than traditional meat production. ➢ Ethical Dimension: Many customers pick meat alternatives because they are concerned about animal welfare and ethical issues associated with meat consumption. ➢ Revolution Equality: Continuous advancement in plant-based and lab-grown meat technology is broadening the diversity and availability of meat substitutes, appealing to a wider range of customers.	[92]
Weakness	➢ Medical Challenges: A disproportionate amount of red and processed meats has been related to an elevated risk of cardiovascular disease, specific malignancies (colorectal cancer), and other medical issues. ➢ Environmental Implications: The meat industry causes major releases of greenhouse gases, forest loss, contamination of water, and loss of biodiversity. ➢ Substantial Resources: Maintaining livestock takes a lot of water, feed crops (which may be utilized for human consumption), as well as land. ➢ Social Issues: Consumers are becoming increasingly concerned about animal welfare and the moral implications of industrial farming practices. ➢ Health Disorders: Health disorders were linked to excessive meat eating, particularly red meat. Diseases such as bovine tuberculosis have been transmitted to humans through the consumption of infected animal flesh, disrupting biodiversity.	➢ Nutrient Profile: Certain meat replacements may lack key critical elements present in meat, especially vitamin B12, iron, and zinc, while fortification attempts are being made to address this. ➢ Production Cost: Meat substitutes can be priced higher than traditional meats owing to costs associated with manufacturing and efficiencies of scale, and this might limit access, particularly among lower-income populations. ➢ Consumer Endorsement: Creating similar taste, texture, and overall sensory experience that comes from conventional meats can be difficult, limiting consumer acceptability and adoption. ➢ Market Adoption: Concerns regarding the implementation of processed foods, chemicals, including preservatives in some meat substitutes could discourage health-conscious shoppers.	[93]
Opportunities	➢ Human Demand on Nature: The increasing need for plant-based and lab-grown meat alternatives creates an opportunity to lessen the environmental impact of meat consumption. ➢ Health Trend: Flexible eating habits and vegetarian lifestyles are becoming more popular, as people become more aware of the health benefits of plant-based diets. ➢ Technical Developments: Innovations in agriculture and food technology may result in more environmentally friendly and effective meat production systems. ➢ Regulatory Compliance: Government initiatives such as supporting ethical farming and limiting meat consumption may open up chances for innovation and market expansion in other sources of protein.	➢ Commercial Demand: The growing public interest in plant-based diets and environmentally friendly choices is driving market expansion for meat alternatives. ➢ Technological Improvements: Innovative developments based on plants and cell-based farming is enhancing taste, texture, and nutritional profile of meat alternatives. ➢ Administrative Support: Federal initiatives encouraging sustainability and nutritious eating may offer incentives and support to meat replacement companies. ➢ Regional Expansion: There are opportunities to provide meat substitutes in countries where meat consumption has traditionally been strong, appealing to health-conscious and ecologically sensitive people.	[94]
Threats	➢ Industry Resilience: Established meat industries and cultural traditions may oppose transitions to diets based on plants and alternative proteins. ➢ Economic Consequences: Decreases in meat consumption could have a significant impact on economies that rely significantly on livestock production and exports. ➢ Consumer Trends: Challenging entrenched consumer habits and preferences for animal products may present problems for alternative protein markets. ➢ Socio-economic: Sociopolitical factors, such as lobbying efforts and public perception, may influence the implementation of programs that promote sustainable diets and reduce meat consumption.	➢ Industrial Development: Traditional meat industries may reject or strongly compete with meat alternatives, potentially altering market dynamics and customer preferences. ➢ Market Demand: Addressing mistrust and influencing consumer views of the flavor, texture, and nutritional value of meat alternatives continues to be a problem. ➢ Logistics Support: Reliance on specific crops or technology for meat substitutes may bring risks such as interruptions to the supply chain, climate change effects, and market volatility. ➢ Legal Barriers: Legislation and labeling requirements for meat alternatives may differ across locations, creating compliance issues and market entrance hurdles.	[91]

on the complications surrounding meat consumption by exploring the internal strengths and weaknesses of meat consumption, besides the external opportunities and threats from societal, environmental, and economic outlook, as well as the external possibilities and dangers that it faces. The following table examines meat consumption using a SWOT analysis, highlighting existing constraints and potential opportunities within the frame of developing food production.

Conclusion

Meat was identified as a stable food source but its impacts on health and the environment are yet to be considered. The current meat production system is found associated with environmental pollution, biodiversity loss, impacts on human health, GHG emission, etc., and thus meat alternatives are found to be a promising choice for reducing conventional meat production. The meat consumption trend was associated with high GHG emission that leads to global warming, which probably makes life harder on Earth. The ever-growing demand for meat and increasing population has led to a great increase in meat consumption trend. By considering its impacts, the trend should be decreased for the sustainability of life on Earth. Meat alternatives are a better option for reducing conventional meat consumption, but their acceptability as a meat substitute is a major problem. In-vitro meat and PBMA have already acquired a position in the global market but insect-based meat alternatives remain a major issue due to the low acceptability of insects as a food source by consumers, even though insects are a good source of proteins. In-vitro meat has acquired great acceptance among consumers and it was successfully developed as food for astronauts, but its huge production based on consumer demand remains a problem. Technologies have to be developed in the future for the further launch of meat analogs in the global market. Studies on meat alternatives are still going on for increasing their production and acceptance among consumers.

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AIR SANITATION IN CHICKEN PROCESSING USING SAEW: MIST VS FORCED-AIR

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Abstract
In the chilling step of chicken processing, air systems are used for cooling or draining. Sanitation also usually occurs during this step. However, an air sanitation system using SAEW has not yet been developed. This research describes evaluation of the air sanitation system using SAEW by comparing mist (SAEW-Mist) and forced-air (SAEW-Gas) against controls in terms of raw chicken quality during storage and the potential formation of trihalomethane. The air sanitation system using SAEW with both treatments reduced total microorganisms and Enterobacteriaceae, although they were not significantly different from the control ($P \geq 0.05$). However, SAEW treatments effectively slowed microbial growth over time, with SAEW-Mist showing better stability. NaOCl treatment caused higher microbial growth rates, greater porosity, and significant reductions in water holding capacity (WHC), likely due to protein denaturation. SAEW-Mist preserved WHC better and showed lower total volatile basic nitrogen (TVB-N) and lipid oxidation than SAEW-Gas and NaOCl. TVB-N and TBARS values in SAEW-Mist remained below the threshold for spoilage. Chloroform, a trihalomethane compound, was detected in NaOCl and SAEW-Gas samples but not in SAEW-Mist or control. SAEW-Mist also caused fewer physical and chemical changes during storage, produced no liquid waste, and did not generate trihalomethane. The mist-based system offers advantages such as reduced water usage, prevention of cross-contamination, and improved hygiene conditions. Therefore, SAEW-Mist can be proposed as part of an integrated and environmentally friendly sanitation approach in poultry processing facilities.

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Introduction

Chicken food safety is necessary due to high consumption. According to FAO [1] chicken has consistently been the most produced type of meat in the past four years. To ensure chicken food safety and extend shelf life, sanitation techniques, including chemical and system application, have become the focus of many studies [2–4]. The poultry industry mainly uses chemical disinfectants for microbial control. Sodium hypochlorite (NaOCl) is widely used as a sanitizing agent at a concentration of 200 ppm. It is preferred for its strong effectiveness and affordability [5,6]. Even so, the presence of organic matter with NaOCl higher ppm levels will enhance trihalomethane (THM) formation, which is a critical disadvantage. Chloroform, a marker of THM, has been found in broilers after NaOCl treatment, posing a carcinogenic risk [7]. Slightly Acidic Electrolyzed Water (SAEW) is considered in chicken processing due to its advantages over other sanitizing agents. It offers greater stability and high bactericidal efficacy with lower chlorine concentration. Moreover, it will affect the lower possibility of trihalomethane formation, and is less corrosive [4,5,7]. Rahman et al. [8] showed that chicken samples treated with SAEW had a lower total aerobic bacterial count (1.49 log CFU/g) than the control. Our previous research also showed a reduction of aerobic plate count by 1.36 log CFU/cm^2 after immersion in SAEW for five times [9].

Sanitation in chicken processing usually occurs in the chilling step. Generally, the step is performed using an immersion system or an air system. The immersion system is the most frequently chosen due to its efficiency [10]. However, our previous research showed that the samples treated using the immersion system had a higher microbial growth than the control [9]. Liu et al. [11] wrote that SAEW with immersion may damage egg cuticles. In contrast, SAEW with a sprayed mist did not affect the egg cuticle. Moreover, cooling carcasses with mist has some advantages

compared to immersion. It offers reduced water consumption and waste, as well as no cross-contamination [12]. Therefore, an air system should be further explored as a sanitation method in chicken processing.

In an air sanitation system, the liquid sanitation agent should be converted into gas phase. The transformation can occur when atomizing or forced-air vaporizing is used. The atomizing method utilizes spraying or ultrasonic misting. Ultrasonic misting of hypochlorite solution has effectively sanitized bacteria and viruses on solid surfaces. The mist contains fine HOCl droplets in aqueous form with higher densities than in gas form [13]. During mist production, the loss of free chlorine was around 11.7–13.2 % [14]. Dry mist with HClO at a concentration of 300 ppm reduced *Escherichia coli* and *Salmonella enterica* by more than 5 log after 60 s [15]. However, electrolyzed water with ACC more than 10 ppm could inactivate bacteria within 0.5 min [13]. Liu et al. [11] reported that using 50 ppm significantly reduced total culturable bacteria on eggshell compared to tap water. SAEW with 30–50 ppm was proven to eliminate *Staphylococcus aureus* after 3 hours of atomizing [16].

In forced-air vaporizing, gaseous HOCl is released along with the evaporation of water by flowing air through porous water-holding materials. A hypochlorite solution with a pH of 8.5 at 100 ppm resulted in an HOCl concentration of approximately 12 to 17 ppm after 2 hours with a flow rate of 2 m^3/min in a 75 m^3 room. An air sanitation system that uses chlorinated water in its gaseous state has demonstrated the capability to reduce *E. coli* cells on 0.9 % NaCl agar by more than 2.8 log CFU within 2 hours when positioned 3 meters from the vaporizer. Moreover, *V. parahaemolyticus* showed a reduction of 3.8 log CFU after being exposed to 20–50 ppb of gaseous HOCl at one meter from the vaporizer. Nevertheless, the research also indicated that the bactericidal effectiveness of HOCl diminishes in the presence of organic substances that consume chlorine [13]. Additionally, the research about an air sanitation system using SAEW has not been developed for chicken sanitizing, and its effect on the quality of raw chicken has not been investigated. Therefore, the purpose of this research is to evaluate an effect of different air sanitation systems using SAEW by comparing mist (SAEW-Mist) and forced-air (SAEW-Gas) against controls in terms of raw chicken quality during storage and the potential formation of trihalomethane.

Objects and methods

Preparation of SAEW solution

SAEW solution was produced by diluted electrolysis of saturated NaCl solution with tap water using a SAEW generator with three room-type electrolyte cells which was developed by Morinaga Milk Industry Co., Ltd. (Figure 1). Its characteristics include a pH range of 5.5–6.5, as measured by pH-meter D-51 (HORIBA, Ltd., Japan), and a total available chlorine content of 80 ppm, which was measured using a handy water meter AQUAB model AQ-202 (Sibata Scientific Technology Ltd, Japan).

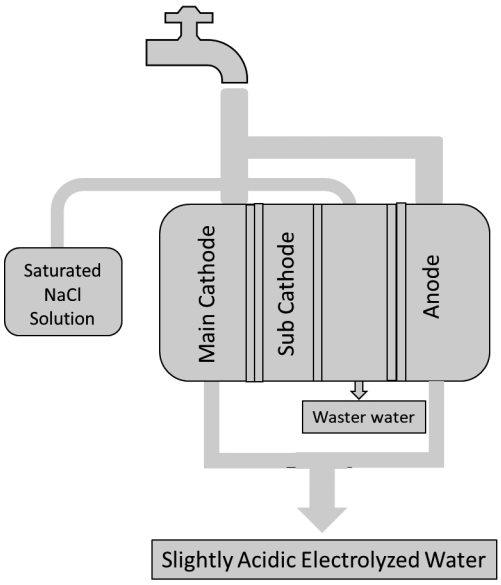

Figure 1. Three room-type electrolyte cells

Preparation of chicken samples

Chicken carcasses were transported from the Sanwa Oyadori factory to the laboratory in a cool box within 3 hours after slaughter. The chicken meat samples were cut from the thigh and breast parts closest to the gastrointestinal tract for microbiological analysis. James et al. [17] showed that the decay starts from the meat part nearest to the gastrointestinal tract. For other analyses, filleted thigh and breast parts were used. The chicken parts were stored at 4 °C before being used for the experiment.

Procedures for sanitizing

Cut chicken meat samples (120–150 g) were placed in the first rack of the chamber, positioned 30 cm above the fan in and exposed to gaseous SAEW generated either by forced-air vaporizing (SAEW-Gas) or ultrasonic misting (SAEW-Mist) for 120 min (Figure 2). The chamber was chilled by covering with a hose that was connected to a recirculating chiller, maintaining an internal temperature below 15 °C. Outside the chamber, the Rh was controlled with an automatic SALARI Pro dehumidifier Mitsubishi Electric MJ-P180RX-W (Mitsubishi Electric Corporation, Japan) to 50–60 %. For an air sanitation system using SAEW-Gas, the the speed of fan in and fan out was 5.3 m^3/min and 3 m^3/min, respectively, while SAEW-Mist was used to only fan out with a speed of 4.6 m^3/min. Negative control samples (Control) were chicken packed in sterilized plastic and placed together in the chamber during the operation of SAEW-Gas or SAEW-Mist for 120 minutes. For positive control samples (NaOCl), the sanitation system imitated the condition of the chicken factory with some modifications, in which chicken meat samples were immersed in 200 ppm of NaOCl solution two times with a ratio of 2:1 for 15 minutes each and drained for 15 min [18]. All samples were vacuumed, packed, and stored at 4 °C for 3 and 7 days.

Microbiological properties

Microbiological properties were evaluated before and after the treatment (0 days), 3 days, and 7 days of storage.

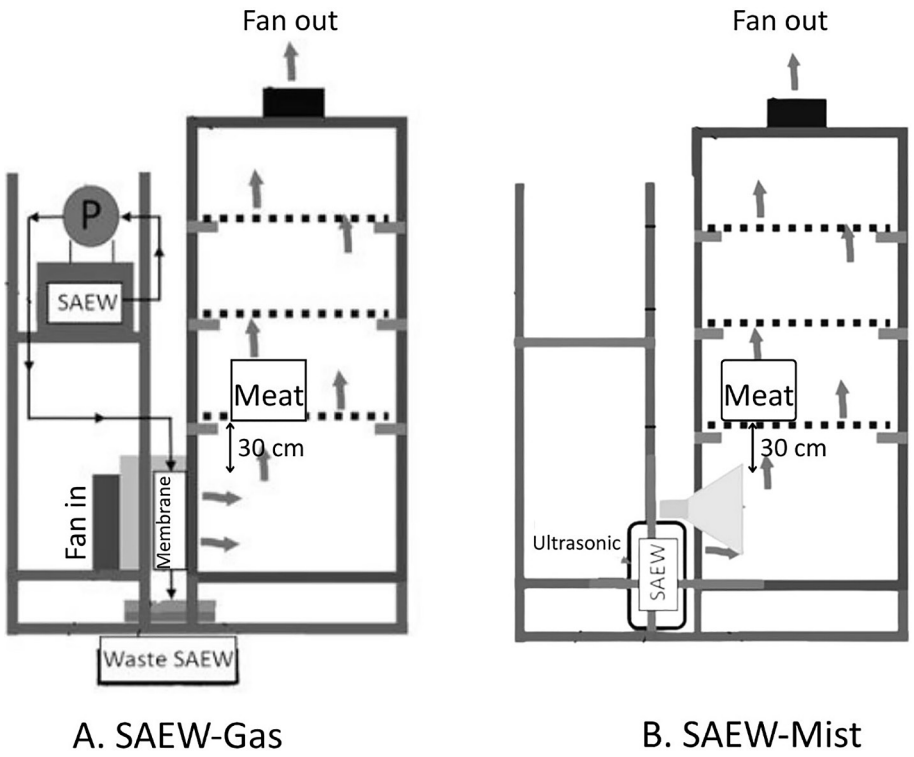

Figure 2. The air sanitation system

Chicken was swabbed in an area of 5 cm × 5 cm for 3 times with a sterilized wetted swab. Then, the swab was immersed in 10 mL of buffered peptone water (BPW), mixed with vortex TTM-1 (Sibata Scientific Technology, Ltd, Japan), and diluted serially with a ratio of 9:1 in sterile BPW. Total microorganisms and *Enterobacteriaceae* were evaluated by spreading 1 mL of aliquots of dilution to the top of 3M Petrifilm Aerobic Count Plate 6406 with the following incubation at 35 °C for 48 ± 2 hours and 3M Petrifilm Enterobacteriaceae Count Plate 6420 with the following incubation using CN-25C (Mitsubishi Electric Corporation, Japan) at 35 °C for 24 ± 2 hours [19,20].

Chemical properties

The chemical properties of the samples were evaluated using total volatile basic nitrogen (TVB-N) and lipid oxidation analyses. These chemical parameters were analyzed on 0, 3 and 7 days of storage.

TVB-N levels were determined using the Conway method. Chicken meat was cut into samples with a size of 1x1 cm and chopped using 100 mL portable mini food processor for 1 min. (WBLLGG, China). A 5-g portion of chopped meat was homogenized (Physcotron homogenizer NS-52K, Microtec, Japan) with 45 mL of trichloroacetic acid (TCA) at 17000 rpm for 30 seconds. The mixture was allowed to stand for 30 minutes. It was then filtered using Whatman No. 2 filter paper. One milliliter of the resulting filtrate was placed into the outer ring of a Conway dish. Meanwhile, 1 mL of 0.01 N boric acid solution containing methyl red and bromocresol green indicators was added to the inner chamber. Additionally, 1 mL of 50 % (w/v) potassium carbonate (K_2CO_3) solution was placed on the opposite side of the outer ring. The Conway dish was sealed and gently shaken to mix the sample and K_2CO_3. It was incubated (LTI-600SD, Tokyo Rikakikai, Japan) at 37 °C for 120 minutes. After incubation, 0.01 N sulfuric acid (H_2SO_4) was added to the inner chamber until a pink color appeared. The TVB-N was calculated as mg/100 g of sample [21].

Lipid oxidation was assessed using TBARS value. A 5-g portion of chopped meat obtained using the same method as for TVB-N was homogenized (Physcotron homogenizer NS-52K, Microtec, Japan) at 17000 rpm for 20 seconds in 10 mL of 10 % (w/v) trichloroacetic acid (TCA), then centrifuged (CN-1050, AS-ONE, Japan) at 4032 × g for 30 minutes over ice. The resulting mixture was filtered using Whatman No. 2 filter paper. From the obtained supernatant, 2 mL was collected and combined with 2 mL of 0.15 % (w/v) 2-thiobarbituric acid (TBA) solution. The mixture was vortexed and incubated at 70 °C for 2.5 hours in a water bath (Advantec Hotting Bath B-CS, Advantec Group, Japan). After cooling to room temperature for approximately one hour, the absorbance was measured using a spectrophotometer (V-630, JASCO Corporation, Japan) at 531 nm (maximum absorbance) and 600 nm (for nonspecific turbidity correction). The TBARS value was determined as equivalents of malondialdehyde (mg MDA eq/kg meat) [9,21].

Physical properties

The physical properties of the samples were evaluated in terms of water holding capacity (WHC) and color of chicken meat. Those parameters were analyzed for 0, 3, and 7 days. WHC was calculated as the percentage of the meat mass remained after centrifugation (CN-1050, AS-ONE, Japan) at 2800 × g for 10 minutes relative to the initial mass

of the meat [22]. The color was analyzed using CIE values for lightness (L*), redness (a*), and yellowness (b*). The meat was scanned using an EPSON GT-X980 (Epson, Japan) at a 24-bit color depth and 300 dpi resolution. The background was removed from the resulting image of meat using Canva BG Remover. The removed background image was calculated using Python 3.9.7 to get CIE values [23].

Muscle structure

Muscle structure was photographed for the meat samples only after treatments in SEM Hitachi TM4000 II (Hitachi High-Tech, Japan) using a 5kV voltage with the magnification of x100 and x1000. Before that, the samples were pretreated using one-time fixation. In one-time fixation, chicken meat was fixed using only 2.5 % glutaraldehyde in buffer phosphate solution pH 7.2–7.4 for one night. After fixation, meat samples were washed with buffered phosphate pH 7.4 two times for 10 min. Then samples was dehydrated by immersion in ethanol serially (25, 50, 70, 80, 90, 99, and 99 %) for 13 min each, and dried for 2 days using a freeze dryer (Eyela FDU-1200, Tokyo Rikakikai Co. Ltd, Japan) [24–26]. Porosity was calculated using images from one-time fixed samples by thresholding operation in open source software ImageJ [27].

Trihalomethane detection

Trihalomethane (THMs) was determined using GC–MS Shimadzu QP-5050 (Shimadzu Corporation, Japan) using the method 8260D [28]. The chopped meat (2 g) was weighed in a 20 mL glass vial; then, the 10 mL matrix modifying solution was added and the glass vial was sealed. The headspace solid phase extraction was done by injecting SPME fiber into the vial. Then the sample was mixed for 2 min at room temperature and heated at 85 °C for 40 min. During the heating, the sample was agitated for 10 min. After that, the fiber was removed and directly injected to GC injector manually [29]. Separations were performed on DB-5 capillary column (30 m × 0.25 mm I.D. × 0.25 µm film thickness). Injection occurred in spitless mode with helium as carrier gas (1.0 mL/min). The initial temperature of the GC–MS was set to 40 °C for 3 min and followed by an increase of 8 °C/min to 80 °C held for 3 min, and an increase of 6 °C/min to 140 °C. Then the temperature was increased 10 °C/min to 200 °C and held for 3 min.

Statistical analysis

This research used randomized complete block design, in which all treatments were applied in each replication. Each experiment was conducted in triplicate, with replications performed at different times. The data from all replicates were subjected to descriptive analysis to calculate means and standard deviations, shown as error bars in the figures. Additionally, repeated measures and one-way analysis of variance were performed to determine significant differences between treatments. Means that showed significant differences were further analyzed using Tukey's Honestly Significant Difference (HSD) test. The means within repeated measurements were analyzed using Bonferroni correction. Data analysis was performed using Microsoft Excel 365 and JASP [30].

Results and discussion

Figure 3 illustrates total microorganisms and *Enterobacteriaceae* before and after treatment. An air sanitation system with SAEW-Gas and SAEW-Mist showed no significant differences compared to the control ($P \geq 0.05$). However, SAEW-Gas and SAEW-Mist reduced total microorganisms by 0.23 ± 0.07 and 0.20 ± 0.084 log CFU/cm^2 and *Enterobacteriaceae* by 0.49 ± 0.46 and 0.32 ± 0.33 log CFU/cm^2, respectively, in all replications. In contrast, the total microorganisms and *Enterobacteriaceae* of the control samples increased. Gaseous HOCl has been proven to have bactericidal action. Gas is more effective against pathogens due to its ability to penetrate surface irregularities [31]. However, chlorine-consuming organic matter reduced HOCl effectiveness [13]. Additionally, the loss of ACC during misting also caused lower antibacterial activity of SAEW-Mist [14].

Immersion with 200 ppm of NaOCl solution reduced total microbial counts by 1.20 ± 0.70 log CFU/cm^2, and they were significantly different before and after treatment ($P < 0.05$). Still, the reduction was not significantly different from SAEW-Gas and SAEW-Mist treatments. Byun et al. [2] showed that *Salmonella enteritidis* biofilm on chicken skin treated with 200 ppm NaOCl was not significantly different from that on the untreated sample. On the other hand, chlorine levels used in poultry processing are limited

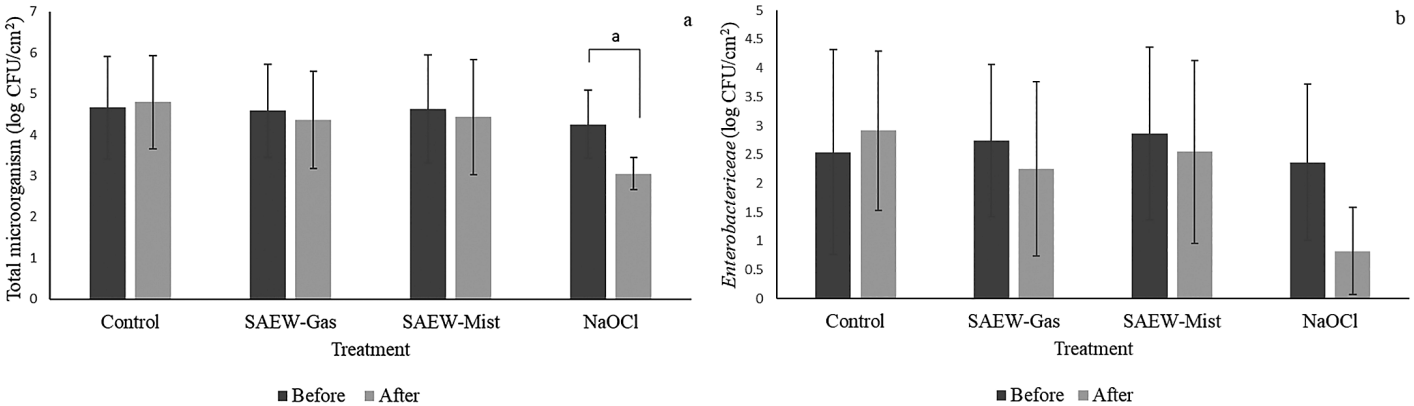

Figure 3. Total microorganisms (a) and *Enterobactericeae* (b) in raw chicken before and after treatment.
[a] shows significant differences within treatment ($P < 0.05$)

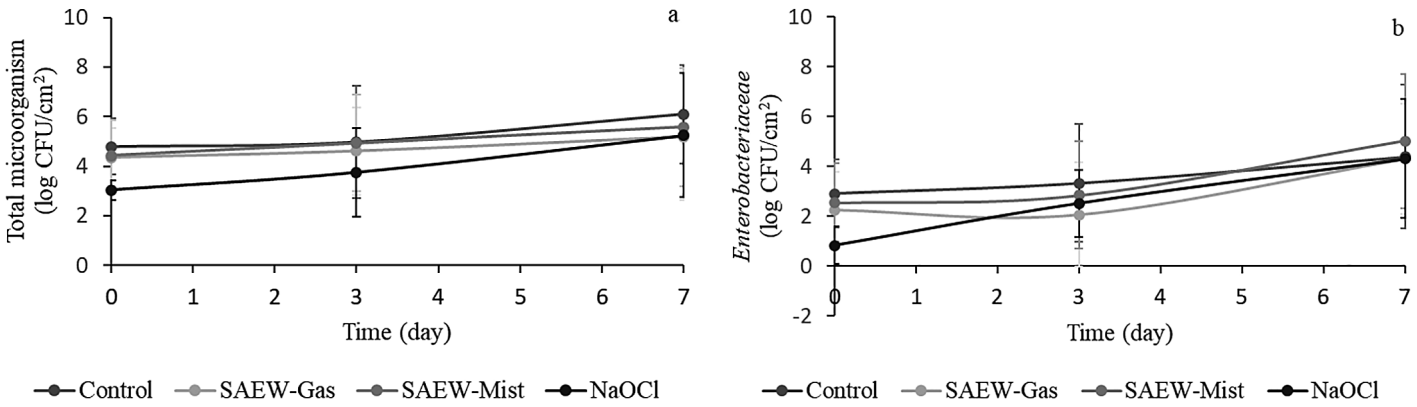

Figure 4. Total microorganisms (a) and *Enterobactericeae* (b) in raw chicken during storage. There was no significant difference between all treatments ($P \geq 0.05$)

to a maximum of 50 ppm in the USA because of the formation of trihalomethanes. Kartikawati et al. [9] showed that immersion in 30 ppm of SAEW repeated three times with a total time of 9 min could reduce total microorganisms to 1.25 ± 0.14 log CFU/cm^2, which was slightly higher than NaOCl treatment.

Table 1. Growth rate and porosity of raw chicken

Treatment	Total microorganisms, log CFU/cm^2.day	*Enterobacteriaceae*, log CFU/cm^2.day	Porosity, %
Control	0.19 ± 0.12	0.21 ± 0.22	12.36 ± 5.16
SAEW-Gas	0.12 ± 0.20	0.31 ± 0.10	12.85 ± 3.54
SAEW-Mist	0.16 ± 0.14	0.36 ± 0.16	14.47 ± 4
NaOCl	0.32 ± 0.33	0.50 ± 0.26	15.17 ± 3.02

Values are means ± standard deviations. There was no significant difference between all treatments ($P \geq 0.05$).

There were no significant differences in the growth of total microorganisms and *Enterobacteriaceae* between treatments (Figure 4). NaOCl treatment showed the highest growth rate of total microorganisms and *Enterobacteriaceae* of 0.32 ± 0.33 and 0.50 ± 0.26 log CFU/cm^2.d, respectively, with the highest porosity of 15.17 ± 3.02 %, even though they were not significantly different from those of other treatments (P ≥ 0.05) (Table 1). During the storage, NaOCl treatment resulted in the highest growth rate, since the immersion system caused damage to the muscle bundle (Figure 6), leading to higher porosity and growth rate. Immersing salmon in 100 ppm HOCl solution also resulted in higher total microorganisms than the control after 7 days of storage. The treatment likely caused increased cell lysis. Cell lysis released nutrients that became available for microbes [32]. Our previous research also showed that the muscle bundle was broken with an immersion system, which led to the increased growth rate [9]. SAEW-Mist had a higher growth rate of total microorganisms and *Enterobacteriaceae* than SAEW-Gas. However, the growth rate of total microorganisms for SAEW-Gas and SAEW-Mist was lower than that of the control. These results demonstrated that an air sanitation system could reduce microorganisms while slowing the growth rate.

Figure 5 shows that TVB-N and lipid oxidation in raw chicken increased in all groups during storage. TVB-N is widely used as an indicator of freshness and overall quality. This relates to protein and nonprotein breakdown by bacteria and enzymes [33]. However, the increases in these parameters were not significantly different between all treatments ($P \geq 0.05$). The highest TVB-N after 7 days of storage was observed in the negative control. In contrast, the lowest TVB-N on 0 to 7 days was in the NaOCl treatment, which was 6.63 ± 0.66 mg/100 g and increased to 11.75 ± 3.08 mg/100 g. Lower TVB-N in raw chicken may result from chlorine binding to protein, forming insoluble and less volatile precipitate forms [34]. The SAEW-Mist

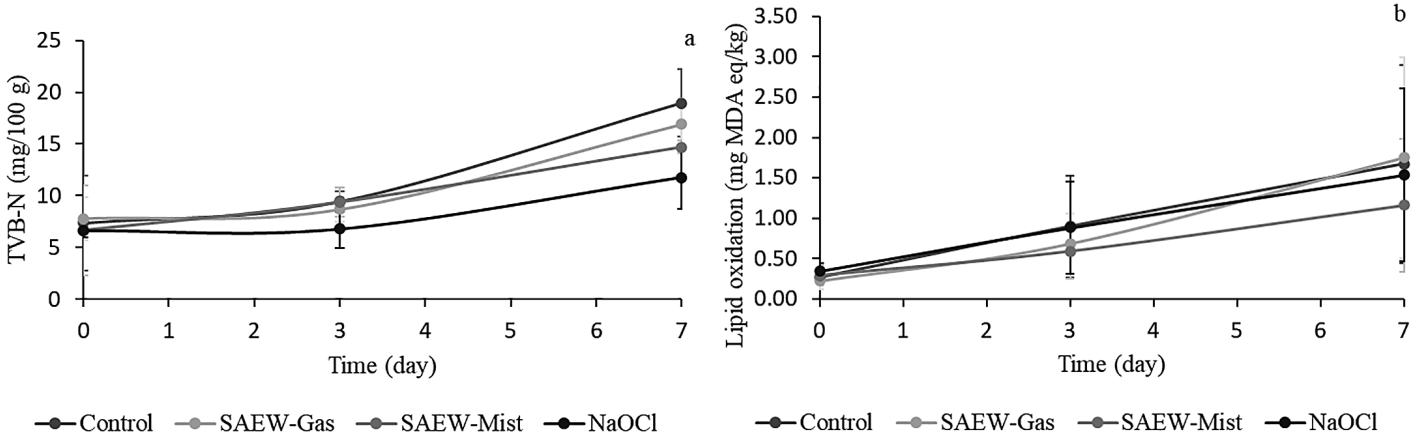

Figure 5. TVB-N (a) and lipid oxidation (b) in raw chicken during storage. There was no significant difference between all treatments ($P \geq 0.05$)

treatment showed lower TVB-N values and smaller change during storage compared to SAEW-Gas. This indicates that chlorine was incorporated into proteins in the aqueous form more than in the gaseous one. Since chlorine-based sanitizing agents were used, treated samples had lower TVB-N values during storage. Additionally, all the sanitizing treatments reduced microorganisms in meat that prevent protein decomposition [6]. The 15 mg/100 g TVB-N threshold appears to be the most commonly used standard in the literature [35]. Therefore, SAEW-Mist and NaOCl treatments maintained acceptable quality even after 7 days of storage.

Lipid oxidation products are often evaluated through TBARS analysis [32]. After treatment, the lipid oxidation values of samples were 0.22 ± 0.33 to 0.34 ± 0.10 mg MDA eq/kg. NaOCl treated samples showed the highest values, while SAEW-Mist had the lowest. However, the differences among the treatments were not significant ($P \geq 0.05$). SAEW-Mist maintained the lowest lipid oxidation on both day 3 and 7. Meanwhile, SAEW-Gas resulted in the highest lipid oxidation after 7 days of storage. The phenomenon of lipid oxidation can occur due to the presence of oxygen [36]. SAEW-Gas was generated using a forced-air system, which introduces additional oxygen. The destruction of lipids which is shown by non-round shape of the lipid in the SAEW-Gas treated sample is shown in Figure 6 at 1000x magnification. As an oxidant, chlorine interacts with lipids and increases lipid oxidation. Lipid oxidation threshold of 0.6–2.0 mg MDA/kg may cause off-flavors detectable by untrained consumers [36]. SAEW-Mist maintained lipid oxidation levels below this threshold after 3 days storage, unlike other treatments. Previous findings have shown that electrolyzed water can prevent lipid oxidation during storage [9,37].

WHC and color changes of raw chicken during storage are presented in Figure 7. WHC describes the ability of meat to retain water content. Water loss or retention affects the economic value of meat during processing and storage [21]. This property depends on interactions between muscle proteins and water molecules. Denaturation of proteins can disrupt these interactions and reduce WHC significantly [38]. The air sanitation systems with both SAEW

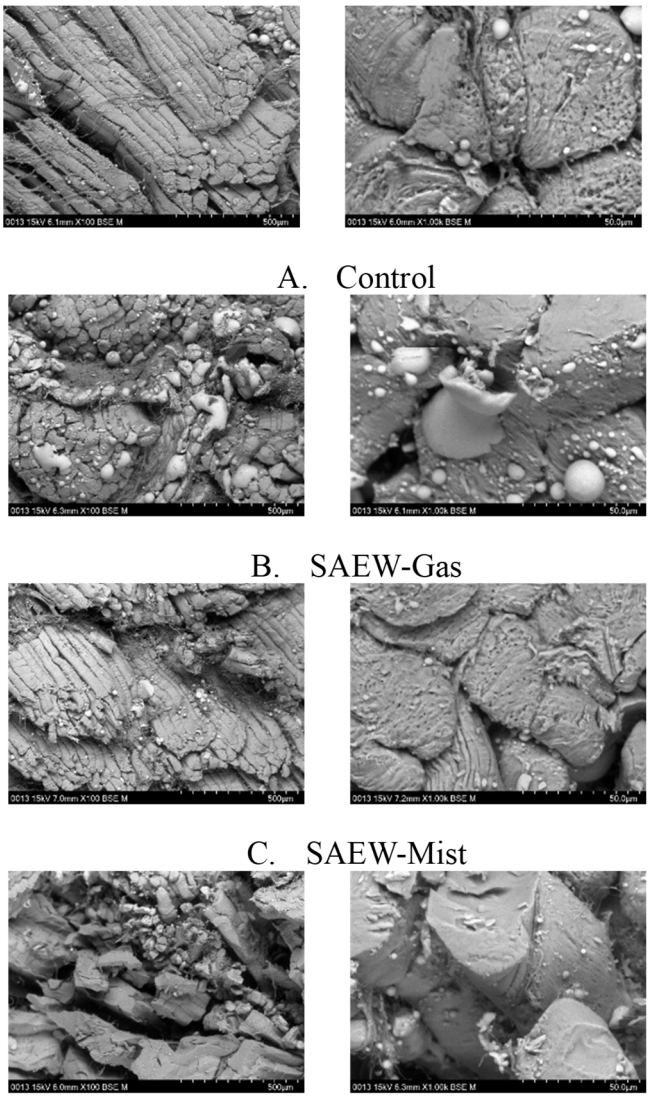

Figure 6. Muscle structure of raw chicken (Left: 100x magnification; Right: 1000x magnification)

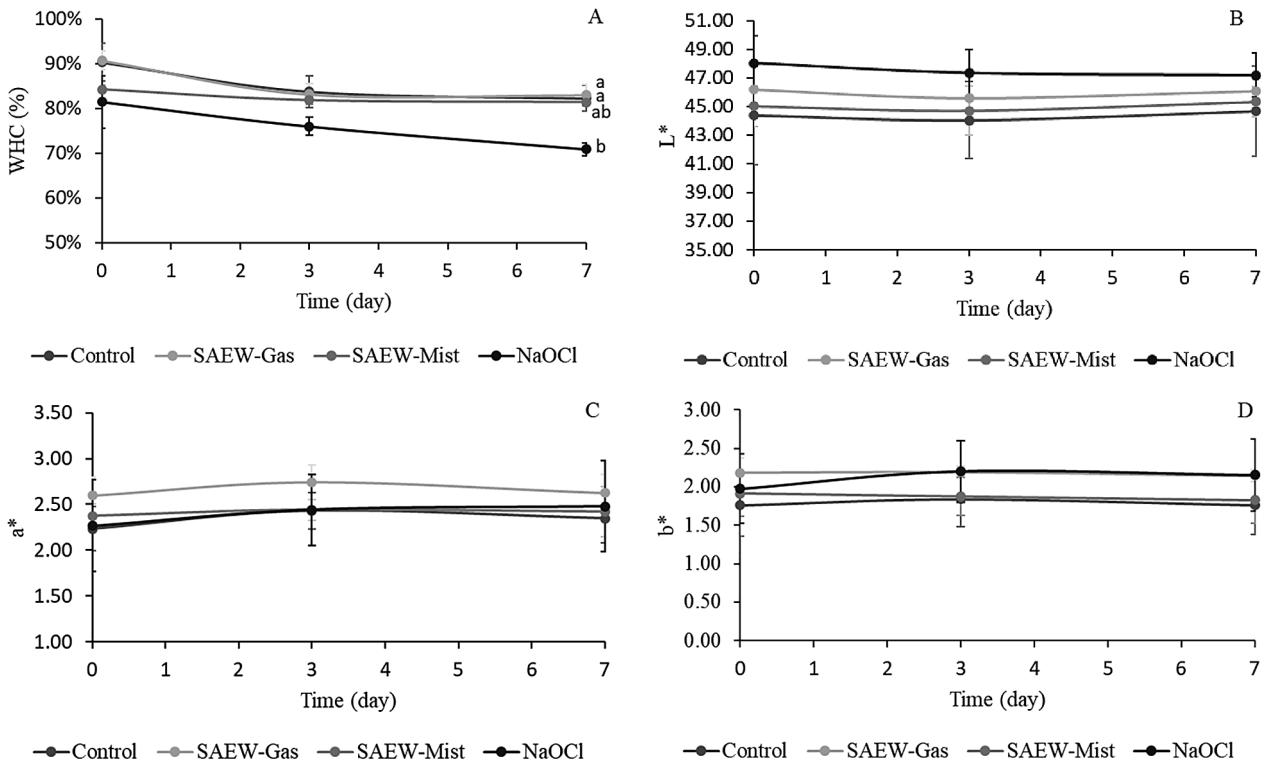

Figure 7. WHC (A) and color (B, C, and D) of raw chicken during storage.
Values with different letters (a and b) are significantly different ($P < 0.05$)

Mist and Gas were not significantly different compared to the negative control. Immersion in 200 ppm NaOCl reduced WHC after treatment and during storage resulting in values that were significantly different from the control and SAEW-Gas treatment ($P < 0.05$). The lower WHC after NaOCl and SAEW-Mist treatment was due to increased moisture content absorption [39]. Carciofi and Laurindo [40] also showed that water uptake by poultry carcasses occurred within 10–15 minutes of immersion, which in turn may reduce the water holding capacity due to the higher amount of free water within the tissue. The porosity of raw chicken, as shown in Table 1, followed a similar trend to the WHC result. Kong et al. [41] also reported that larger gaps between muscle bundles are associated with lower WHC, further supporting this observation.

In terms of color, the NaOCl treated sample was the brightest with the L* value of 48.02 ± 1.93, although the difference was not significant compared with other treatments. Muscle proteins are generally classified into three main groups: sarcoplasmic proteins, myofibrillar proteins, and stromal or connective tissue proteins. Myofibrillar proteins, which form long, fibrous structures, constitute the majority of skeletal muscle proteins, contributing approximately 60 % to 70 % of the total protein content [42]. Che et al. [43] wrote that the denaturation of myofibrillar and sarcoplasmic proteins caused lower WHC and led to higher L* values. The positive correlation between L* values of poultry meat and WHC was also reported in previous research [44]. However, although SAEW-mist had lower WHC than SAEW-Gas, it showed higher L* values. SAEW-Gas had higher TVB-N values, suggesting greater protein denaturation, which may contribute to increased L* values. SAEW-Mist exhibited color values similar to the control. At day 0, L*, a*, and b* values were 45.03 ± 1.44, 2.38 ± 0.20, 1.91 ± 0.30 for SAEW-Mist, and 44.39 ± 3.42, 2.23 ± 0.24, 1.76 ± 0.40 for the control, respectively. SAEW-Gas showed the highest a* value among all treatments, although it was not significantly different ($P > 0.05$). This may be attributed to the use of a forced-air system, which enhances surface myoglobin oxygenation and increases a* values [45]. In addition, SAEW-Gas also had higher b* values than SAEW-Mist. According to Kong et al. [41], increased b* values may be associated with lipid oxidation.

Table 2. Trihalomethane formation (chloroform) on raw chicken

Treatment	Chloroform, µg/kg
Control	ND
SAEW-Gas	3.43 ± 3.81
SAEW-Mist	ND
NaOCl	5.46 ± 5.62

Values are means ± standard deviations and not significantly different ($P \geq 0.05$)

Chloroform was detected in NaOCl and SAEW-Gas at levels of 5.46 ± 5.62 and 3.42 ± 3.81, respectively (Table 2). One of the trihalomethanes that is usually detected in chicken is chloroform. The ACC types of NaOCl solution is mostly ClO^- that drive to trihalomethane formation [6]. The high standard deviation indicates variations in results across different replications. These variations likely occurred due to differences in meat quality resulting from varying harvest times. As shown in Figure 4, the control group had a total *Enterobacteriaceae* count of 2.54 ± 1.78 log CFU/cm², reflecting variations in sample quality among replicates. *Enterobacteriaceae* are also

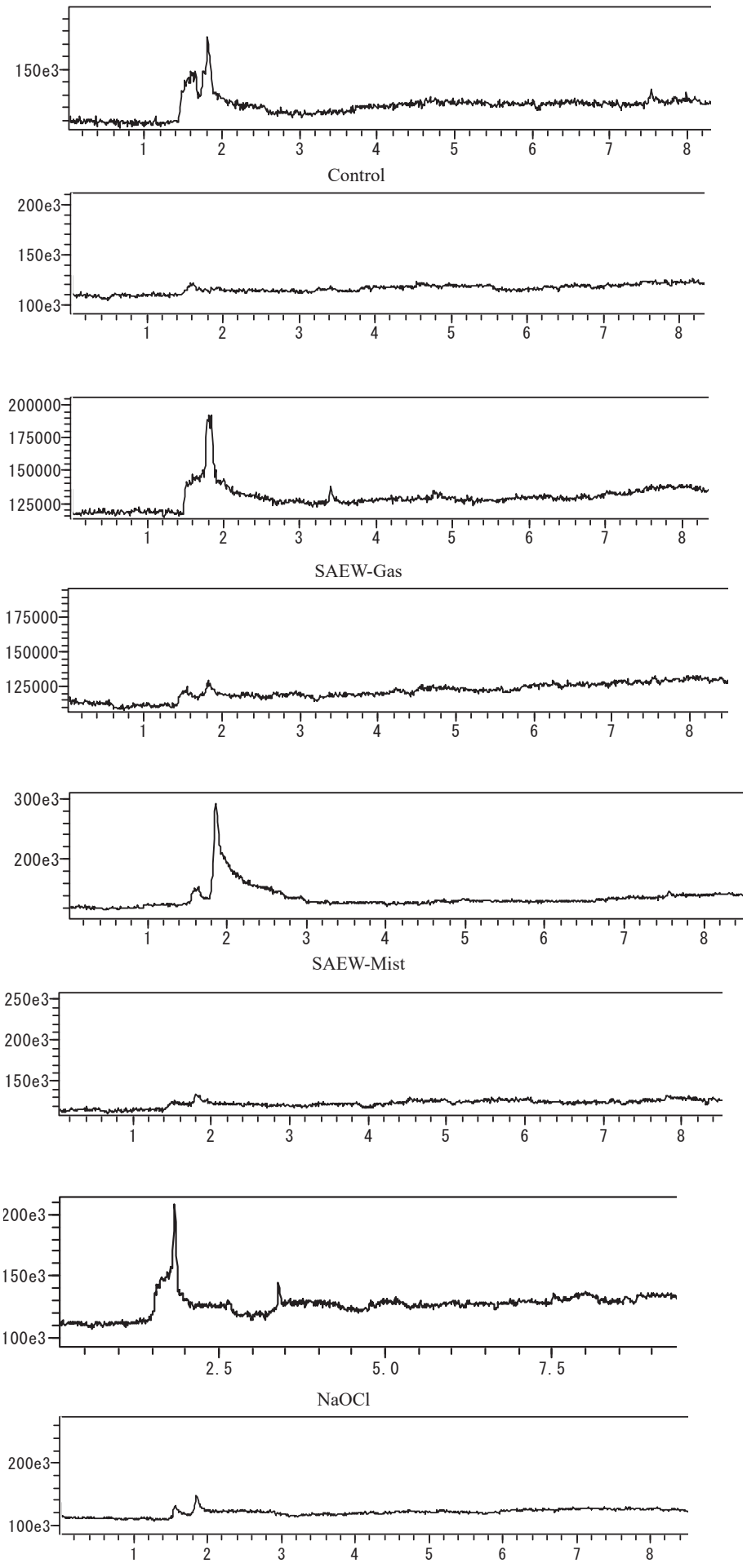

Figure 8. Trihalomethane detection in raw chicken from different replications

known as predominant histamine-producing bacteria in fish [46]. Meat quality can change depending on storage conditions and handling practices. Seasonal factors may also influence meat quality at the time of sampling [47]. Therefore, inconsistent meat conditions could affect the consistency of experimental results. The retention time of chloroform was recorded between 3.33 to 3.58. Some replications showed another peak with retention time 1.40 to 1.66. This peak was identified as propenamide (Figure 8). Protein through hydrolysis, rearrangement, and decarboxylation, can eventually give rise to formation of propenamide [48]. The presence of propenamide indicates the deterioration of meat. NaOCl and SAEW-Gas samples with propenamide peak also showed chloroform peaks. In contrast, control and SAEW-Mist samples showed no chloroform peak. This occurred even when propenamide peaks were present in those samples. The quality of the initial meat treated with a chlorine-based sanitation system may influence potential of trihalomethane formation. However, SAEW-Mist did not generate trihalomethanes. Therefore, SAEW-Mist can be considered a safer option for air sanitation systems.

Compared to an immersion sanitation system using NaOCl, the air sanitation system using SAEW-Gas and SAEW-Mist showed a lower microbial reduction. However, those treatments resulted in a slower microbial growth rate during storage. SAEW-Mist caused fewer chemical and physical changes throughout the storage period. This treatment also did not generate trihalomethanes. Moreover, the air system with mist reduces water usage and minimize waste. It also prevents cross-contamination and improves hygienic production conditions during the chilling process [12]. Therefore, atomized SAEW (SAEW-Mist) can be proposed for air sanitation in chicken processing. It is especially suitable for the chilling step as part of an integrated sanitation system.

Conclusions

This study demonstrated that air sanitation systems using SAEW-Gas and SAEW-Mist were able to reduce total microorganisms and *Enterobacteriaceae*, although the reductions were not significantly different from the control groups. However, both treatments effectively slowed microbial growth during storage. SAEW-Mist showed better consistency in maintaining microbial, chemical, and physical quality. Compared to air sanitation system, the NaOCl immersion system caused higher porosity and faster microbial growth due to cell lysis and protein denaturation. SAEW-Mist also showed lower levels of TVB-N and lipid oxidation throughout storage, remaining below the threshold for off-flavor detection. Furthermore, SAEW-Mist produced no detectable trihalomethanes, unlike NaOCl and SAEW-Gas, which had potential of trihalomethane formation. The SAEW-Mist treatment offers additional benefits, including reduced water usage, elimination of liquid waste, and lower risk of cross-contamination. These advantages support the potential of SAEW-Mist as a safe, effective, and environmentally friendly alternative for air sanitation during the chilling step in chicken processing. Therefore, incorporating SAEW-Mist into integrated sanitation systems may improve product safety and quality while minimizing environmental impact.

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EFFECT OF BITTER ORANGE OR SWEET ORANGE JUICES ON THE CHARACTERISTICS OF BEEF SAUSAGES

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Abstract
This study evaluated the effects of replacing water with bitter orange and pear orange juices on the properties of beef sausages. Three formulations were prepared: control with water (F1) and two experimental formulations with bitter orange juice (F2) and pear orange juice (F3). Moisture, protein, lipid, ash, carbohydrate content, pH, water activity, shear force, and instrumental color were analyzed. Formulations complied with legislation for moisture (< 70 %), protein (> 12 %), and lipid (< 30 %) content. Moisture content ranged from 55.96 % (F3) to 60.32 % (F1), with the reduction in F2 and F3 attributed to the soluble solids in the juices. Protein content remained stable across formulations (22.24–23.39 %), reflecting the consistent meat proportion. Lipid content varied significantly, with F1 (15.25 %) showing the lowest value and F3 (19.29 %) the highest due to altered lipid retention influenced by acid-matrix interactions. F2 exhibited the highest acidity (0.40 g 100 mL^{-1}) and the lowest pH (4.68), leading to the lowest shear force (56.68 N). Due to particularities in the preparation, meat and fat portions were analyzed for colorimetry. Lighter sausages with lower redness (a^*) values in F2 (5.20) and F3 (4.48) compared to F1 (8.50), and higher yellowness (b^*) in F2 (7.26) were observed for the meat portion. The total color difference (ΔE^*) values indicated noticeable differences, particularly for F2 and F3 in the meat portion and F3 in the fat portion. These findings demonstrated that orange juices can modify the properties of beef sausages, enhancing their functional attributes, offering opportunities for the development of new food products.

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Introduction

Meat is a highly perishable product, with its shelf life strongly influenced by storage conditions. Throughout history, humans have sought to preserve its quality, leading to the development of preservation techniques that have evolved from rudimentary methods to advanced technologies [1].

Sausages are spiced meat products encased in natural or artificial casings made from a variety of meats, including beef, pork, poultry, lamb, or fish. Available globally in butcher shops, they are prepared through cooking, curing, maturing, or as fresh products [2,3]. Sausages rank among the oldest processed foods, with hundreds of varieties shaped by local ingredients, climate, and cultural traditions. While their production initially relied on traditional practices, understanding the scientific principles of their physical, chemical, and biological processes is a more recent advancement. This knowledge has improved control over sensory qualities, nutritional value, and safety [4].

Maracaju sausage, originating from the Maracaju region in Mato Grosso do Sul, Brazil, began as a method of meat preservation and evolved into a cultural tradition. It is made from premium beef cuts such as striploin, rump, and brisket. It includes up to 30 % fat and is seasoned with salt, pepper, garlic, and bitter orange juice (*Citrus aurantium*). Recognized with a Geographical Indication (GI) by the Brazilian Institute of Intellectual Property (INPI), it is protected against origin and production falsification [5].

Bitter orange is a member of the Rutaceae family commonly found in domestic orchards. Cultivated from seeds, the medium-sized plants produce thick-skinned, acidic fruits [6]. Native to Maracaju, bitter orange is more acidic than sweet orange, which dominates global citrus production, accounting for nearly 50 % of total citrus output [7]. In Brazil, the pear orange (*Citrus sinensis*) is the most cultivated variety, used for both fresh consumption and juice production [8].

The use of citrus fruit juices, fibers, and extracts in sausages has varied effects on the products' physicochemical, sensory, and technological properties, depending on the type and concentration of the citrus used. Extracts from grapefruit, lemon, tangerine, and orange peel can increase the yellow intensity of sausages due to the presence of

natural pigments such as carotenoids and flavonoids, without negatively affecting the typical red color of cured sausages [9].

The use of citrus fibers or orange juice residue can add citrus sensory notes, in addition to increasing characteristic acidic flavors and aromas, especially at higher concentrations [10]. The addition of citrus fibers may also make the product firmer and less elastic, particularly at high levels (e. g., 2%) [11]. Citrus fibers and juice residue help reduce lipid oxidation, which extends shelf life and maintains the red color for a longer period [10,11].

Moreover, citrus extracts can serve as natural reducing agents, potentially replacing synthetic additives such as sodium ascorbate without compromising curing efficiency or oxidative stability [9]. The addition of citrus fibers does not significantly alter the yield, emulsion stability, or sensory characteristics at moderate levels [11,12]. Specifically, orange juice/residue reduces oxidation, adds flavonoids, and improves shelf life, while imparting a slightly citrus flavor to sausages [10,11].

While recent research often focuses on the valorization of citrus by-products like peel and bagasse extracts for their rich bioactive compounds and to address waste management [12–15], the direct use of orange juice in food formulations, such as in Maracaju sausages, is also a viable method to leverage its natural acidity and antioxidant properties.

Given this context, this study aimed to investigate the specific effects of bitter orange and sweet orange juices, which are integral to the traditional preparation of Maracaju sausage, and to evaluate how they influence the physical, chemical, and microbiological characteristics of this beef product.

Objects and methods

Beef sausages

The beef sausages were prepared following the traditional Maracaju sausage manufacturing process, using three different formulations: F1 (control with water), F2 (traditional recipe with bitter orange juice), and F3 (with pear orange juice), as detailed in Table 1.

For that, the beef brisket was cleaned and stored together with the beef fat at 4 °C for 30 min. Then, the meat was chopped into 1×2 cm rectangles and the fat into 1×1 cm cubes. Meat, fat and the other ingredients were weighted according to the sausage formulation and then mixed in a CAF M60 mixer (CAF Máquinas, Brazil) for 10 min at 4 °C. Each mixture was embedded using an EP-8 manual filler (Picelli, Brazil), in natural bovine casings hydrated for 1 h in water before embedding and packaged in a spiral. Sausages were immediately utilized for the analysis.

Beef (brisket) and beef fat were purchased at the market (Maracaju, Mato Grosso do Sul, Brazil). Bitter oranges (*Citrus aurantium*) were harvested (Maracaju, Mato Grosso do Sul, Brazil), selected and washed, and the bitter orange juice obtained by manually squeezing. Commercial pasteurized orange juice from pear orange (*Citrus sinensis*) was purchased from the local commerce (Dourados, Mato Grosso do Sul, Brazil). The condiments were supplied by Cavenaghi Eireli (Dourados, Mato Grosso do Sul, Brazil).

Table 1. Formulations of beef sausages containing water (F1), bitter orange juice (F2) or pear orange juice (F3)

Ingredient, %	Formulation		
	F1	F2	F3
Beef (brisket)	67.70	67.70	67.70
Beef fat	20.32	20.32	20.32
Water	8.81	—	—
Bitter orange juice	—	8.8l	—
Pear orange juice	—	—	8.81
Salt	1.76	1.76	1.76
Pepper	0.85	0.85	0.85
Garlic	0.35	0.35	0.35
Scallion	0.18	0.18	0.18

Microbiological analysis

To assess microbiological analysis, duplicate 25 g samples were aseptically transferred into a stomacher bag containing 100 mL of sterile distilled water containing 0.1% peptone (1% for *Salmonella spp.* determination). Samples were homogenized for 1 min. Ten-fold serial dilutions were prepared using sterile 0.1 peptone solution (9 mL) and spread plated (0.1 mL) in duplicate onto broths and/or agars for detection of typical colonies, biochemical confirmation and identification, and plate counting for thermo-tolerant total aerobic mesophilic microorganisms, *Escherichia coli*, and *Salmonella spp.*, in accordance with the methodology described elsewhere [16].

Water activity and pH

Water activity of the samples was determined in triplicate using a hygrometer Aqualab, model CX-2, series 3 (Decagon Devices, Inc., United States) at 25 °C with 1 ml of sample. pH of the samples was measured in triplicate using a digital pH meter model HI99163 (Hanna Instruments, Brazil) [17].

Titratable acidity

For the determination of the titratable acidity, the titration apparatus was a standard laboratory burette and stand setup. The equipment consisted of a 50 mL glass burette (Schott Duran, Germany), a magnetic stirrer model: C–MAG HS7 (IKA, Germany), and a stand. It was used for the chemical titration of 10 g of sample diluted in 90 ml of distilled water in a 250 mL beaker, using phenolphthalein as a final indicator of the reaction until the appearance of the pink color. The titrant used was 0.1 N sodium hydroxide and titratable acidity was expressed in g 100 mL^{-1} [18]. The procedure was carried out in triplicate.

Shear force

Texture analysis was carried out using a texture analyzer Model TAXTplus (Stable Micro Systems, Surrey, England) calibrated with a standard weight of 5 kg. Sausages were equilibrated at room temperature (28–30 °C) before

analysis. Cylindrical samples of 20 mm diameter and 30 mm length were cut, placed in the texture analyzer, and submitted to a cutting/shearing test (speed of 1.0 mm s^{-1}, distance of 30 mm) using a Warner-Bratzler shear blade (1 mm thick) to determine the shear force (N). A minimum of 10 replicates of each treatment were analyzed [19].

Proximate composition

Moisture, crude protein, and crude ash contents were determined in triplicate according to the methods described by AOAC (2012) [20]. Moisture was determined by the oven drying method at 105 °C until constant weight (method 950.46), protein by the Kjeldahl method (method 928.08) utilizing a conversion factor of 6.25, ash by using the muffle oven technique (method 920.153), and crude fiber by chemical digestion (method 978.10). The lipid content was obtained in triplicate by the extraction method with cold organic solvent [21]. The carbohydrate content was estimated by difference.

Instrumental color

The color [CIE L^*(lightness), a^* (redness), b^* (yellowness)] was evaluated using a colorimeter Chroma Meter CR410 (Konica Minolta Inc., Japan), with measurements standardized with respect to the white calibration plate [22]. After the transversal cut of the sausages, five readings were made for the portion containing meat and another five readings for the portion containing fat, for each formulation.

The hue saturation index or chromaticity (C^*) and the hue angle (h°) were obtained from Equations 1 and 2.

$$C^* = \sqrt{\left(a^*2 + b^*2\right)}. \quad (1)$$

$$h° = \frac{\tan - 1b^*}{a^*}. \quad (2)$$

The total color difference (ΔE^*) was obtained using Equation 3. For $\Delta E^* < 1.5$, the sample color was considered almost identical to the original. For $1.5 \leq \Delta E^* \leq 5$, the color difference was considered distinguishable. For $\Delta E^* > 5$, the color difference was considered evident.

$$\Delta E^* = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2}. \quad (3)$$

Statistical analysis

Statistical results were evaluated through analysis of variance (ANOVA) and Tukey's test for comparison of means, at a level of 5 % of significance, using the statistical software Statistica 7.0. The results were presented as means ± standard deviations.

Results and discussion

The mixture of ingredients and the corresponding beef sausages can be observed for F1 (control with water) (Figure 1A and Figure 1D, respectively), F2 with bitter orange (*Citrus aurantium*) juice (Figure 1B and Figure 1E, respectively) and F3 with pear orange (*Citrus sinensis*) juice (Figure 1C and Figure 1F, respectively).

Microbiological analysis

To evaluate the microbiological quality of the fresh beef sausages, microbiological evaluations were carried out in triplicate for aerobic mesophilic bacteria, *Escherichia coli* and *Salmonella spp*. According to Brazilian Legislation, all samples met the legal standards for sausage from meat from butcher's animals (Table 2), which limits *E. coli* to 1×10^2 CFU/g and aerobic mesophilic bacteria to 1×10^6 CFU/g, beyond the absence of *Salmonella* spp. in 25 g of the product [23]. The absence of *Salmonella* spp. is important due to the serious food poisoning caused by these microorganisms [24].

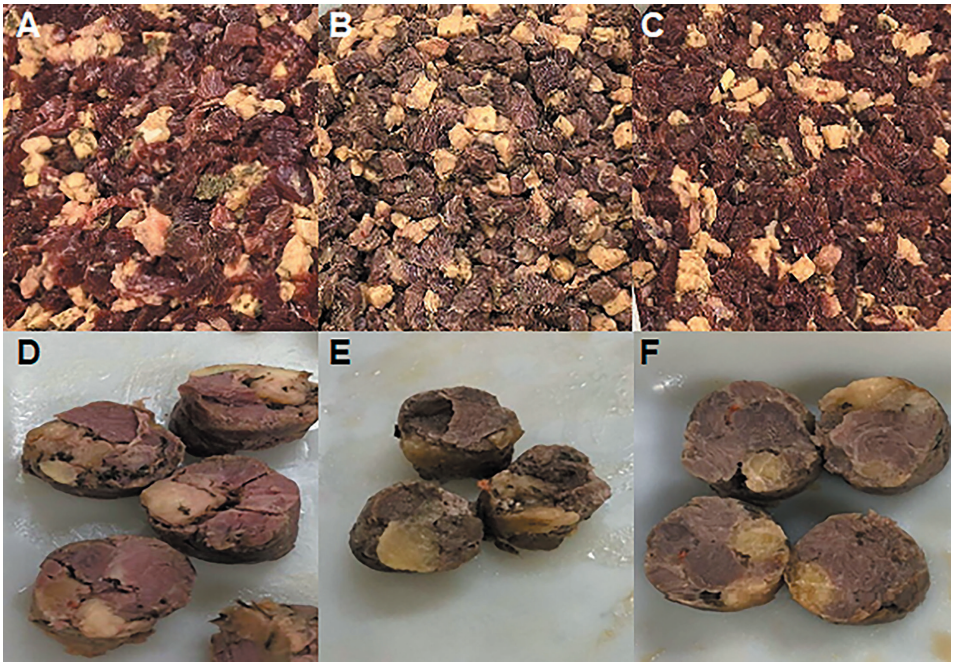

Figure 1. Photos of the raw materials (A, B, C) used in the formulations of beef sausages and the cross-sections (D, E, F) of the respective sausages after stuffing, for F1, F2 and F3, respectively

Table 2. Microbiological analyses of the beef sausages containing water (F1), bitter orange juice (F2) or pear orange juice (F3)

Determination	Formulation		
	F1	F1	F1
Aerobic mesophilic bacteria (CFU g^{-1})	6.4×10^{3a}	6.2×10^{3a}	6.4×10^{3a}
Escherichia coli (CFU g^{-1})	3.1×10^{1a}	2.5×10^{1a}	2.9×10^{1a}
Salmonella spp.	Absence	Absence	Absence

CFU: colony-forming units. Means with the same letter in the same line do not differ statistically at 5 % ($p > 0.05$). *p*: probability.

Water activity, pH, titratable acidity and shear force

Table 3 presents the results of determinations of water activity, pH, titratable acidity and shear force of the beef sausages. The water activity varied from 0.965 (F3) to 0.983 (F1), showing significant differences between them ($p < 0.05$). This range of water activity is in line with research by Akbar et al. [25], which verified water activity in cooked beef sausages prepared with different fat sources ranging from 0.96 to 0.97 after one day of storage. Water activity is the aspect utilized to determine the viability of microbial growth in food. For fresh sausages, the recommendation is a water activity below 0.92 [26]. In this sense, the values obtained here are propitious to bacterial growth, which inspires attention during production and storage.

Table 3. Water activity, pH, titratable acidity and shear force of the beef sausages containing water (F1), bitter orange juice (F2) or pear orange juice (F3)

Determination	Formulation		
	F1	F2	F3
Water activity	0.983 ± 0.001^{a}	0.971 ± 0.002^{b}	0.965 ± 0.001^{c}
pH	5.09 ± 0.07^{a}	4.68 ± 0.20^{b}	4.73 ± 0.10^{b}
Titratable acidity (g 100 mL^{-1})	0.25 ± 0.02^{b}	0.40 ± 0.03^{a}	0.26 ± 0.02^{b}
Shear force (N)	64.33 ± 2.40^{a}	56.68 ± 1.51^{b}	61.04 ± 2.52^{a}

Means with the same letter in the same line do not differ statistically at 5 % ($p > 0.05$). p: probability.

The pH value for F1 was 5.09, which differed significantly ($p < 0.05$) from F2 and F3. These two formulations did not differ from each other ($p > 0.05$), with pH values of 4.68 and 4.73, respectively (Table 3). Normal pH values for meat products typically range from 5.4 to 6.2 [27] and the pH indicated for fresh sausages is 5.6 [26]. All samples (F1, F2, and F3) had pH levels below this range. de Carvalho et al. [28], analyzing Cuiabana sausage, reported pH values between 5.61 and 5.83. Unlike Maracaju sausage, Cuiabana sausage contains milk, which contributes to a slight pH decrease. In contrast, the presence of orange juice in Maracaju-type sausages more significantly reduces pH, explaining the difference between the control (F1) and the formulations with orange juices (F2 and F3).

For total titratable acidity, F2 differed significantly ($p < 0.05$) from F1 and F3, which did not differ from each other ($p > 0.05$) (Table 3). Although the samples exhibited low acidity levels (0.25–0.40 g 100 mL^{-1}), these values were within acceptable limits [2]. The formulation containing bitter orange (F2) showed the highest acidity (0.40 g 100 mL^{-1}), while F1 and F3 (with sweet orange) presented the lowest value (0.26 g 100 mL^{-1}). The higher acidity in F2 aligns with findings that bitter orange can have acidity levels up to 45 times higher than sweet orange [29].

When comparing pH and total titratable acidity, a clear relationship is observed: higher acid concentrations in the juice increased H^{+} ion availability, lowering the pH and making the sausages more acidic (Table 1). However, since pH measures the strength of acid dissociation rather than the total acid content, total titratable acidity provided a more accurate assessment of bitter orange's effect on enhancing food acidity [30].

The shear force results showed that F1 and F3 required the application of a greater force to be cut. There was a significant difference ($p > 0.05$) between samples F1 and F3 in relation to F2, probably due to the lower pH and higher acidity which may led to the denaturation of proteins. So, it can be assumed that the amount of bitter orange juice added produced a higher reduction in shear force and a reduction in cohesion due to the tenderization of the meat, which involves both uptake of liquid and solubilization of connective tissue collagen [31]. One of the main factors that affect the texture of the sausage is the protein structure [32]. In finely comminuted products, the salt-soluble meat proteins myosin and actin form a three-dimensional cross-linked matrix that can entrap water and fat. When sausages are made from minced meat rather than finely chopped meat, the shear forces are lower because the meat is mixed, not chopped. This mixing process results in a weaker, less cohesive three-dimensional, cross-linked matrix. Moreover, raw briskets present higher shear forces than the thick flank, which is directly related to the collagen content [33] and in some cases to the presence of actomyosin bridges [34] in the cuts. As example, some other authors reported a shear force of 12.3 N for beef sausages prepared with minced meat [35].

Proximate composition

The results of the proximate composition analysis for the sausages are presented in Table 4.

Table 4. Proximate composition of the beef sausages containing water (F1), bitter orange juice (F2) or pear orange juice (F3)

Determination (%)	Formulation		
	F1	F2	F3
Moisture	60.32 ± 0.06^{a}	57.37 ± 0.19^{b}	55.96 ± 0.16^{c}
Protein	23.39 ± 0.39^{a}	22.99 ± 0.58^{a}	22.24 ± 0.73^{a}
Lipids	15.25 ± 0.35^{b}	$17.74 \pm 0.01^{a, b}$	19.29 ± 1.25^{a}
Ash	0.78 ± 0.24^{b}	$1.50 \pm 0.23^{a, b}$	2.16 ± 0.32^{a}
Carbohydrates*	0.26	0.40	0.35

Means with the same letter in the same line do not differ statistically at 5 % ($p > 0.05$). p: probability. *Calculated by difference.

Moisture

Samples F1, F2 and F3 differed ($p < 0.05$) from each other for moisture, as observed (Table 4). In F1, the added water (8.81 %) directly increases the moisture content. In F2 and F3, water is replaced by orange juices, which contain soluble solids (e. g., sugars, organic acids, and minerals) that reduce the amount of free water in the final product, which explains the lower moisture content in F2 (57.37 %) and F3 (55.96 %) compared to F1 (60.32 %). It possibly occurred due to water-solute interactions, where the compounds in the juices, particularly acids and salts, interact with proteins and other matrix components, reducing water retention and increasing water release during processing. Thus, it is possible that F2 and F3 presented lower moisture values due to the pH values below the isoelectric point of the myofibrillar proteins, causing the protein to denature, and consequently reducing moisture.

The obtained values were in accordance with the limit of 70 % established by the Brazilian legislation [2]. For comparison, a 59.30 % moisture content for fresh beef sausage made at very similar conditions was reported in [36], coinciding with the result obtained for sample F1 (control) (Table 4). Other authors report an average value of 64.6 % for the moisture content of a low-calorie beef sausage, which was attributed to the fat replacement and reduction and to the addition of ice [37].

Protein

The protein content of F1, F2 and F3 did not differ significantly ($p > 0.05$) from each other (Table 4), ranging between 22.24 % and 23.39 %. It occurred because all formulations contained the same amount of beef brisket (67.74 %) as the primary source of protein and the juices contributed with negligible amounts of protein. Thus, the protein content remained stable across F1, F2, and F3. While the acids in the juices may cause slight protein denaturation, this did not significantly impact the total protein content measured.

The protein contents were well above the minimum limit established for sausages, which is 12 % [2]. The brisket beef has 17.6 to 22.20 % protein [38], which agrees with the protein content of the sausage (Table 4). Literature reports 20.65 % of protein for fresh beef sausage prepared with 65 % beef [36]. For Cuiabana sausages prepared with the rear cut of cattle, the average value was 16.53 % of protein content [28].

Lipids

The lipid content of F1, F2 and F3 differed significantly ($p < 0.05$) from each other (Table 4). Despite the identical fat proportion (20.32 %) in all formulations, the presence of juices, especially pear orange juice in F3, may affect lipid extraction, distribution, and retention during emulsification and/or cooking. Acidic compounds and their contents in the juices could have altered the protein structure, decreasing the binding of lipids to the matrix and resulting in higher apparent lipid content in F3 (19.29 %) compared to F2 (17.74 %) and F1 (15.25 %). Moreover, the lower pH in the formulations with juices influences emulsion stability, potentially impacting lipid distribution in the final product.

All formulations were within the limit of 30 % lipids established by the Brazilian legislation [2]. For comparisons, an average of 15.5 % of lipids was reported for fresh beef sausage with 15.0 % sheep fat tail [36]. Only 5.75 % of lipids was recorded in Cuiabana sausage because milk and mozzarella cheese were the main sources of fat [28].

Ash and carbohydrates

The ash content differed significantly from each other ($p < 0.05$), as can be seen in Table 4. The significant increase in ash content in F2 (1.50 %) and F3 (2.16 %) can be attributed to the mineral contribution of citrus juices, which have a higher concentration of salts compared to water. The carbohydrate content obtained by difference was 0.26, 0.40 and 0.35 % for F1, F2 and F3, respectively (Table 4).

The legislation does not establish limits for the analysis of mineral matter and the carbohydrates content cannot surpass 7 %, including 2 % starch [2]. Shahin [36] reported ash content value of 1.92 % and carbohydrates content of 2.63 % in fresh beef sausages. These values are higher than those found here (except for ash in F3) and are due to the differences in the added ingredients, which have mineral content, including sodium nitrite and nitrate, sodium polyphosphate and various spices, and the addition of 5 % starch as reported by these authors. Another example is the carbohydrate content of 3.70 % found for Cuiabana beef sausages formulations made with rump and 2.28 % for formulations made from the fillet cover. These higher values were explained due to the addition of milk and mozzarella cheese, which are rich in the carbohydrate lactose [28].

Instrumental color

The colorimetric characterization of beef sausages (Table 5) revealed significant differences between formulations containing water (F1), bitter orange juice (F2), and pear orange juice (F3), particularly in the meat and fat portions.

In the meat portion, lightness (L^*) values were significantly higher for F2 (52.49), indicating that bitter orange juice enhanced the lightness of the meat compared to F1 (43.21) and F3 (45.64). The redness (a^*) values were highest for F1 (8.50), with F2 (5.20) and F3 (4.48) showing significantly lower redness, suggesting that the inclusion of orange juices reduced the red tones in the meat. Yellowness (b^*) was greater in F2 (7.26) compared to F3 (4.41), with F1 (5.18) showing an intermediate value. These differences indicate that bitter orange juice contributed to a more yellowish hue. Chroma (C^*) values followed a similar trend, with F3 (6.38) showing the lowest saturation, significantly differing from F1 (10.04). Hue angle (h°) was highest in F2 (53.79), indicating a shift towards a more yellowish tone. The color difference (ΔE^*) for F2 (10.06) and F3 (10.69) was above 5, indicating noticeable differences from the control (F1) (Table 5).

Table 5. Instrumental color analysis of the beef sausages containing water (F1), bitter orange juice (F2) or pear orange juice (F3)

Parameter	Formulation (portion)					
	F1 (meat)	F2 (meat)	F3 (meat)	F1 (fat)	F2 (fat)	F3 (fat)
L^*	43.21 ± 3.52^{b}	52.49 ± 2.89^{a}	45.64 ± 2.72^{b}	65.49 ± 3.3^{a}	68.26 ± 3.63^{a}	66.65 ± 3.72^{a}
a^*	$8.50^{a} \pm 1.62^{a}$	5.20 ± 1.94^{b}	4.48 ± 0.50^{b}	7.15 ± 1.07^{a}	4.78 ± 1.18^{b}	2.89 ± 0.98^{b}
b^*	$5.18 \pm 1.47^{a, b}$	7.26 ± 3.33^{a}	4.41 ± 2.03^{b}	$20.29 \pm 1.07_{a}$	18.56 ± 2.36^{a}	18.98 ± 3.36^{a}
C^*	10.04 ± 1.38^{a}	8.98 ± 3.69^{a}	6.38 ± 1.75^{b}	21.70 ± 0.96^{a}	19.21 ± 2.18^{a}	19.21 ± 3.40^{a}
$h°$	31.04 ± 9.68^{c}	53.79 ± 7.66^{a}	42.45 ± 9.55^{b}	70.72 ± 7.78^{b}	75.26 ± 4.39^{b}	81.50 ± 2.60^{a}
ΔE^*	—	10.06	10.69	—	4.03	39.52

Means with the same letter in the same line do not differ statistically at 5 % ($p > 0.05$). p: probability; L^*: lightness; a^*: chroma a^* (intensity of red); b^*: chroma b^* (intensity of yellow); chroma C^*: hue saturation index; $h°$: hue angle; ΔE^*: color variation.

For the fat portion, lightness (L^*) values were similar across formulations, ranging from 65.49 (F1) to 68.26 (F2), indicating that all formulations were bright. Redness (a^*) was highest in F1 (7.15), with F2 (4.78) and F3 (2.89) showing significantly lower values, particularly F3, which exhibited the least redness. Yellowness (b^*) values were consistent across formulations, with F1 (20.29) and F2 (18.56) showing no significant differences, while the values were lower in F3 (18.98), indicating a similar yellow hue across all formulations. Chroma (C^*) values were also similar between formulations, ranging from 19.21 (F2 and F3) to 21.70 (F1). The hue angle (h°) was significantly higher in F3 (81.50), indicating a greater shift towards yellow. The color difference (ΔE^*) was notably higher in F3 (39.52), indicating a significant color variation compared to the other formulations (Table 5).

The color of the meat portion showed a shift from red to yellow due to the orange juices, with a slight grayish tone due to the salt. In contrast, the fat portion exhibited greater differences, especially between F3 and the other formulations, with a shift from red to yellow. The inclusion of orange juices, particularly pear orange juice, caused notable changes in both the meat and fat portions, with pear orange juice leading to the most significant color alterations. The color differences may also be attributed to a limited degree of fat dispersion, commonly observed in other sausages. This is particularly due to the spread of oily lipids, which contributes to an increase in lightness [25]. These changes may influence consumer perception, as color plays a key role in sensory evaluation [39].

Conclusion

The replacement of water with orange juices in beef sausage formulations significantly influenced the product's physicochemical properties. All formulations complied with Brazilian legislation regarding moisture, protein, and lipid content. The addition of orange juices resulted in lower water activity and reduced moisture content due to the presence of soluble solids. This effect, combined with lower pH values caused by the juices, contributed to protein denaturation and altered matrix interactions. While protein content remained consistent across all formulations due to the fixed meat content, lipid content showed significant variation, with higher lipid retention observed in formulations containing pear orange juice. These differences were attributed to acid-matrix interactions during emulsification and cooking. Bitter orange juice, with its higher acidity, resulted in the lowest shear force, which is likely due to enhanced protein denaturation, leading to softer product consistency. In terms of color, orange juices caused noticeable changes, with lighter tones and shifts from red to yellow observed in both the meat and fat portions. Bitter orange juice led to a more pronounced yellowish hue in the meat, while pear orange juice caused the most significant color differences overall. These color alterations, alongside the structural modifications, could influence consumer acceptance and sensory perception.

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INFLUENCE OF MODULATED STRESS ON THE COMPOSITION AND QUALITY OF THE BROILERS MEAT

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Abstract
The purpose of this work is to conduct comprehensive studies aimed at studying the effect of modulated stress of cage density in the poultry houses on the body of domestic cross-breed of the broilers "Smena-9", in particular -on the chemical composition, some qualitative characteristics and antioxidant status of their muscle tissue. An experiment was conducted in the conditions of the physiological yard of the Federal Research Center for Animal Husbandry named after Academy Member L. K. Ernst in 2023 on 2 groups of the broilers (n = 40, N = 80) (control group and experimental group) of the domestic cross-breed of the broilers "Smena-9". The control group was kept under the conditions of the cage density recommended for this cross-breed (Stress-). The poultry cage density in the experimental group (Stress +) was increased by 10 % from the 21st day of the poultry's life in order to create stress conditions. To study the effect of modulated stress on the composition and quality of meat, we conducted poultry slaughterings at the age of 24 (n = 10, N = 20), 34 (n = 10, N = 20) and 52 (n = 10, N = 20) days. Stress led to significant changes in the pH of the breast flesh: 45 minutes after slaughter, the index was 5.55 versus 5.59 units (p = 0.004), and 24 hours later — 5.44 versus 5.60 units. (p = 0.08). The values of the WHC of the experimental group were also lower than those in the control group on the 34th and 52nd days. The stress factor under study was not critical for the development of acute oxidative stress; the greatest changes were observed in age dynamics. There is a decrease in the pH of the breast flesh depending on the age aspect (p < 0.05) in both groups. On day 52, there was a significant (p < 0.01) decrease in TAC content in the breast of poultry of both groups, in the heart muscle in the control group (p = 0.06) and the experimental group (p < 0.001), there was an increase in the activity of SOD and catalase. The data obtained will allow the development of ways to regulate the quality of poultry products.

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Introduction

Poultry meat products are a popular product among the population due to their relatively low cost and rich chemical composition [1]. Poultry meat is a rich source of omega-3 fatty acids, and its use in human nutrition provides a positive effect on vascular health [2].

Since 2016, poultry meat has occupied a leading position in the structure of meat products consumption in the Russian Federation, and the volumes of this production are growing annually [3].

In terms of broilers meat production, Russia is one of the leading countries, occupying the fifth place in the world ranking. In January-September 2024, poultry meat production in Russia (in live weight) exceeded the figure for the same period last year by 2.4 % and amounted to 5.2 million tons [4].

To meet the growing demand for poultry meat, selection and breeding work is continuously carried out to improve the efficiency of broiler poultry farming, aimed at obtaining fast-growing breeds. But poultry health and the quality of poultry products may negatively correlate with the level of metabolic processes [5,6]. In addition, the impact of thermal, physiological, process stresses, including transport stress affecting the body leads to a decrease in the quality of poultry products [7,8]. There are occurrences of various myopathies, changes in the texture and taste of meat, which leads to a decrease in consumer demand [9]. It is known, for example, that heat stress causes a decrease in protein content and an increase in fat in chicken meat [10]. An explanation for this mechanism is given in the work of Zaboli et.al. [11]. It has been shown that an increase in corticosterone secretion causes an increase in protein breakdown and an increase in fat deposition [11]. In another study, cage density stress in poultry negatively affected breast muscle pH [12]. Under the influence of stress, the color of muscle tissue can change towards the development of a darker shade of color [13]. Thus, a higher cage density contributes to the change in color of the

chicken breast and the production of a paler coloring [14]. These researchers also assessed liver color because of its important role in fatty acid synthesis. Broilers kept at cage density of 29 kg/m^2 had the lowest relative liver weight and featured red liver color, while broilers who were bred at cage density of 37 kg/m^2 had a more yellow liver color [15]. Both chronic and short-term heat stress can contribute not only to negative color changes, but also to a decrease in muscle pH, water-holding capacity (WHC), and juiciness of meat [16]. In the study of Liu et.al [16] it was noted that chronic heat stress reduces the density of muscle fibers, increases the content of connective tissue and leads to intracellular vacuolization. Transcriptome analysis in this study showed that the effect of stress on meat quality was associated not only with metabolism and oxidative stress, but also with impaired transduction, violated immune system, cell growth and cell death.

In the Liao et al. [17] experiment, the pre-slaughter transportation of broilers led to the generation of reactive oxygen forms, increased antioxidant capacity in the mitochondria of the pectoral muscle, thus contributing to the development of meat PSE.

The purpose of this work is to conduct comprehensive studies aimed at studying the effect of modulated stress of cage density in the body of domestic cross-breed broilers "Smena-9" on the chemical composition, some qualitative characteristics and the antioxidant status of muscle tissue (thighs and breasts). The data will be useful for developing effective strategies aimed at improving poultry products derived from fast-growing poultry crosses.

Objects and methods

2 groups of broilers ($n = 40$, $N = 80$) (control group and experimental group) of the domestic cross-breed of broiler "Smena-9" were formed in the physiological yard of the Federal Research Center for Animal Husbandry named after Academy Member L. K. Ernst and an experiment was conducted on those two groups. As the main ration for chickens of all groups, total mixed ration made of compound feeds were used, in due relevance to the poultry growing periods: from 0 till 11th day — starting compound feed (till 11th day), during the period of growth (12–26th day) — growth mix was given, and the finishing period (27–52th day) — the poultry received the finishing ration. (Table 1).

The broilers were kept in the specialized cages for BB-1 broilers (Stimuli Group LLC, Russia). When determining the normal and increased cage density, we were guided by the relevant standards for working with meat cross-breed of poultry "Smena 9"[1]. The control group was kept under the conditions of the recommended cage density for this cross (Stress-). The poultry cage density in the experimen-

[1] Efimov, D.N., Egorova, A.V., Emanuilova, Zh.V., Ivanov, A.V., Konopleva, A.P., Zotov, A.A. et al. (2020). Manual for working with poultry of meat cross-breed "Smena 9" with autosexing maternal parent form. Sergiev Posad: All-Russian Research and Technological Poultry Institute, 2020.

Table 1. Composition and nutritional value of concentrates for broilers

Item	Unit	Starting compound	Growth compound	Finishing compound
Wheat	%	35.00	40	55
Corn	%	24.00	20	10
Wheat bran	%	—	—	5
Full-fat soybeans	%	—	9	5
Soybean meal	%	26.00	9	10
Sunflower cake	%	5.00	14.4	10
Sunflower oil	%	0.60	—	—
Fish meal	%	1.70	—	—
Feed yeast	%	3.00	3	3
Lysine monochlorhydrate 98 %	%	0.20	0.2	0.2
Feed methionine 98 %	%	0.30	0.3	0.3
Monocalcium phosphate	%	1.20	1.2	1.2
Feed chalk	%	1.80	1.8	1.8
Salt	%	0.20	0.2	0.2
Premix P5–1	%	1.00	1	1
Nutritional values				
Metabolizable energy	Kcal/100g	308.00	313.00	315.30
Metabolizable energy	MJ/kg	12.90	13.10	13.20
Dry matter	%	89.42	89.28	91.47
Crude protein	%	22.91	21.03	19.98
Crude fat	%	3.21	4.92	3.73
Linoleic acid	%	1.64	2.61	2.01
Crude fiber	%	4.32	5.41	5.12
Crude ash	%	3.41	3.10	3.07
NES	%	52.15	50.82	55.69
Starch	%	34.32	34.74	38.72
Sugar	%	3.30	2.83	2.79
Lysine	%	1.28	1.03	0.97
Methionine	%	0.63	0.61	0.58
Methionine + cystine	%	0.96	0.93	0.87
Threonine	%	0.79	0.70	0.64
Tryptophan	%	0.28	0.25	0.24
Arginine	%	1.35	1.25	1.13
Valin	%	1.03	0.96	0.91
Histidine	%	0.55	0.50	0.46
Glycine	%	0.97	0.92	0.84
Isoleucine	%	0.96	0.87	0.81
Leucine	%	1.48	1.36	1.25
Phenylalanine	%	1.02	0.91	0.86
Tyrosine	%	0.71	0.62	0.58
Ca	%	1.04	0.95	0.95
P	%	0.74	0.71	0.74
Na	%	0.13	0.11	0.11
Cl	%	0.22	0.21	0.21
Vitamin A	mln. IU/t	12.00	12.00	12.00
Vitamin D3	mln. IU/t	4.00	4.00	4.00
Vitamin E	%	30.00	30.00	30.00
Vitamin K	%	4.00	4.00	4.00
Vitamin B1	%	4.00	4.00	4.00
Fe	%	40.00	40.00	40.00
Cu	%	20.00	20.00	20.00
Zn	%	100.00	100.00	100.00
Mn	%	120.00	120.00	120.00
Se	%	0.30	0.30	0.30

tal group (Stress +) was increased by 10 % from the 21st day of the poultry's life to create stress conditions. The length of the poultry cage was 0.99 m, the width was 0.61 m, and the cage area was 0.60 m^2. On a weekly basis, the area of the cage for poultry of the experimental group was adjusted using sliding plywood partitions as the live weight of the poultry increased (the standard for poultry weight was the value of the cage area "minus" 10 %).

Table 2. Poultry cage density in groups 2, 3 and 4 (Stress +)

Live weight, kg	The norm of head/ m^2, according to the manual	Number of heads in a group (roosters and hens)	Stress + (density increased by 10 %)	Cage area, m^2	Frontal feeding area width, cm
0.3–0.4	72	17	79.2	0.21	2.09
0.4–0.5	66	17	72.6	0.23	2.28
0.5–0.7	61	17	67.1	0.25	2.47
0.6–0.8	55	12	60.5	0.20	2.74
0.7–0.9	50	12	55	0.22	3.01
0.8–1.0	46	12	50.6	0.24	3.27
0.9–1.1	42	12	46.2	0.26	3.58
1.1–1.2	38	12	41.8	0.29	3.96
1.3–1.4	32	12	35.2	0.34	4.70
1.5–1.6	28	7	30.8	0.23	5.38
1.7–1.8	26	7	28.6	0.24	5.79
1.9–2.0	23	7	25.3	0.28	6.55
2.1–2.2	21	7	23.1	0.30	7.17
2.3–2.6	17	7	18.7	0.37	8.86

To study the effect of modulated stress on the composition and quality of meat, we performed poultry slaughterings at the ages of 24 ($n=10$, $N=20$), 34 ($n=10$, $N=20$) and 52 ($n=10$, $N=20$) days and the following parameters were evaluated: chemical composition of meat (dry matter (GOST 33319-2015[2]), fat (GOST 23042-2015[3]) and ash (GOST 31727-2012 (ISO 936:1998)[4]). Crude protein content was calculated. Fatty acid composition of abdominal fat (GOST R55483-2013[5]) (gas chromatograph GC2010, Shimasu, Japan), meat pH — using a Testo 205 pH meter (China); water holding capacity — these values were assessed by pressing method according to Grau and Hamm in the modification of Volovinskaya; the amount of water-soluble antioxidants (AWSA)- was assessed on the device Tsvet-Yauza-01-AA (Khimavtomatika, Russia) by the amperometric method. The activity of glutathione peroxidase, catalase, the concentration of glutathione reduced, total antioxidant status (TAS), superoxide dismutase (SOD) were assessed, using commercial Elabscience kits ("Elabscience Biotechnology, Inc.", China) on the Immunochem-2100 device (High Technology, Inc., USA).

The content of mineral components was determined using the following methods. The calcium content of meat was determined according to Methodological recommendations for chemical and biochemical studies of livestock products and feeds[6] The phosphorus content of meat was determined according to Methodological recommendations for chemical and biochemical studies of livestock products and feeds[6] The magnesium content of meat was determined according to Methodological recommendations for chemical and biochemical studies of livestock products and feeds[6].

The methods are described in more detail in our previous publication [18].

Studies were carried out with approval by the bioethical commission (No. 3, May 27, 2022). The experiments were carried out in accordance with the requirements of the Federal Law of the Russian Federation[7], the Declaration of Helsinki[8], the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes[9]

Mathematical and statistical processing of the results was implemented with the help of Microsoft Office Excel 2003, STATISTICA 10 (Statistica 13RU, StatSoft, USA) using methods of variance and factor analysis, involving the Dunnett's test and Tukey's test, t-test. The differences were considered as statistically significant at $p<0.05$, highly significant at $p<0.01$; $p<0.001$.

Results and discussion

Poultry meat is an important source of high-quality proteins, fats and minerals [19]. In recent years, due to the active development of industrial poultry farming and the emergence of fast-growing poultry crosses, attention has been paid to the poultry meat product quality. Food quality is a complex concept that includes a set of properties closely related to physical and chemical characteristics, texture, and taste that satisfy the consumer [20]. Consumers are increasingly paying attention to the quality, safety

[2] GOST 33319-2015 "Meat and meat products. Method for determination of moisture content". Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200123927 Accessed May 17, 2025 (In Russian).

[3] GOST 23042-2015 «Meat and meat products. Methods of fat determination». Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200133107. Accessed May 17, 2025 (In Russian).

[4] GOST 31727-2012 (ISO 936:1998) «Meat and meat products. Determination of total ash». Moscow: Standartinform, 2013. Retrieved from https://docs.cntd.ru/document/1200098742. Accessed May 17, 2025 (In Russian).

[5] GOST R55483-2013 «Meat and meat products. Determination of fatty acids composition by gas chromatography». Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200103852/ Accessed May 17, 2025 (In Russian).

[6] Drozenko, N.P., Kalinin, V.V., Raetskaya, Yu. I. (1981). Methodological recommendations for chemical and biochemical studies of livestock products and feed. Dubrovitsy, 1981.

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

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

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

and nutritional value of meat, directly linking them with an animal's welfare.

The stress of poultry cage density leads to the development of heat stress, which provides a negative impact on feed consumption and digestion efficiency, meat quality and livestock safety [21].

The analysis of the chemical composition of the meat of the "Smena 9" cross-breed broilers in age dynamics (Table 3) showed that there were no significant changes in the chemical composition of the thigh flesh both in the control group and the group under the influence of the factor being studied. Changes in the moisture content, basic organic substances and mineral substances were not significant on the 24th, 34th and 52nd days taken into account. With age, there was a certain trend ($p = 0.07$) to accumulate Ca in the poultry meat of the control group, in contrast to the experimental group, where these changes were not noted. At the end of the experiment, there were no differences in the ratio of moisture and protein in poultry thigh meat (3.63 versus 3.62, respectively in the control group and experimental group).

According to [22] various cage densities practiced during the first 10 days of poultry growing do not significantly affect slaughter yield parameters, abdominal fat content, carcass yield, color and pH values in breast and thigh meat. It is concluded that growing broiler chickens with dividing the area of the poultry house in half during the first 10 days of their lives (36 chickens/m^2) can help increase overall farming productivity due to ease of maintenance, efficient heating and better control of livestock.

On the other hand, in [23] it was noted that live weight, breast weight, leg weight, and fat weight increase linearly along with age. Conversely, the ratio of water to protein, breast fat, and breast collagen values decrease linearly along with age.

It is noted that the age of poultry slaughter is reflected in the amino acid composition. For example, amino acids, peptides, and phospholipids predominated in young chickens, especially in the pectoral muscles of Lueyang blackbone chickens. The study of the amino acid composition of muscles is of great practical importance in assessing the quality of meat, as it determines the taste properties. We plan to study this issue in our further research [24].

In our studies, some age-related changes also occurred in the muscle tissue of the poultry breast of the control group. Thus, breast fat and magnesium levels decreased in the broilers of the control group at the age of 34 days ($p < 0.05$). In the experimental group of the broilers, these changes were leveled, apparently, under the influence of the stress factor. Stress negatively affects the quality of broiler breast meat, including the fat content. Some studies [25] show that chronic heat stress (CTS) increases fat deposition in broilers. CTS affected meat quality parameters such as acidity (it decreased), tenderness, and water holding capacity (it increased). Stress contributed to a decrease in subcutaneous and intramuscular fat in Arbor Acres broilers, while increasing the abdominal fat content in Beijing You chickens [8].

The poultry is very sensitive to oxidative reactions. Transport, feed, veterinary and temperature stresses increase the level of oxidative reactions in cells of various tissues, which leads to the occurrence of oxidative stress. At the end of the experiment, a lower ratio of moisture and protein was found in poultry breast meat exposed to the influence of stress (3.01 in the experimental group versus 3.10 in the control group), which is probably related to the stressful effects of housing conditions.

The spectral characteristics of indoor lighting can also affect the quality of poultry meat. For example, it was found that higher rates of juice loss were found in breast meat samples obtained from the broilers grown under neutral and cold LED lighting. At the same time, the levels of fatty acids changed [26].

The results of the study indicate that prolonged exposure to stress on the broiler's body significantly affects the metabolism of the pectoral muscle of fast-growing broiler chickens. Apparently, the pectoral muscle, compared with the femoral, is more deeply involved in the energy and protein metabolism of the stressed broilers, which may have important consequences for energy homeostasis in the body and growth indicators [27]. This is also shown in our work. The greatest changes in the chemical composition of muscle tissue are found in the breast meat. It can be concluded that breast is an indicator of changes in metabolic status of the body.

Magnesium is one of the essential macronutrients for the body of animals and poultry. The lack of magnesium in the diet of chickens causes a slowdown and then cessation of growth, excessive excitability and death of the poultry. Despite its involvement in numerous biological functions, magnesium (Mg) supplementation is usually overlooked in the broiler diet. Magnesium supplementation implements a clear interaction with the absorption/accumulation of Calcium in the blood and its accumulation in the pectoral muscles. The addition of magnesium can provide a positive effect on certain meat quality indicators, such as WHC and color. The magnesium supplement protects against protein oxidation in the liver and plasma of the broilers. This effect may be associated with increased catalase activity in such tissues. Magnesium supplementation [28] reduces the incidence of myopathies by almost 2 times. In our studies, the broilers of the experimental group at the age of 34 days had a higher magnesium content in the breast flesh ($p < 0.05$) in comparison with the control group.

Numerous studies have shown that intensive genetic breeding and improved feeding programs have increased the growth rate, feed digestion efficiency, and meat productivity of broiler chickens. Unfavorable conditions of housing can negatively affect the quality and composition of poultry meat [13].

An important feature of meat is an improper change in the pH value during autolysis, which leads to a significant change in quality parameters. For example, one study

compared the initial pH of the pectoral muscle of broiler chickens exposed to stress and that of the poultry that were not exposed to it. The stressed poultry showed a higher initial pH (6.24 vs. 6.10, $p < 0.0001$) [29].

In general changes in meat acknowledged as PSE are related to temperature, pH and postmortem glycolysis, and in addition, muscle tissue type, poultry genotype and stress factor play a major role. pH measured at 24 hours after slaughter is considered a predictor of technological qualities [30]. Lu et al. [31] emphasized that increased mitochondrial energy production during early heat stress inevitably increased the level of reactive oxygen species and caused permanent oxidative stress, therefore lowering the pH at 24 hours.

It should also be noted that changes in the chemical composition of the breast of broilers exposed to stress of cage density at the age of 34 days (13 days after the onset of stress) are observed in an increase in fat and magnesium levels (Table 3). By day 52nd, significant differences between the groups were observed only with respect to magnesium content. At the same time, the fat level also tends to increase in the group of poultry with stress ($p = 0.13$). The qualitative characteristics of broiler meat of the experimental groups in age dynamics are presented in the Table 4. We found that the stress to which the poultry was subjected led to significant changes in the pH of the breast flesh. So, 45 minutes after slaughter, the concentration of hydrogen ions in breast meat was 5.55 versus 5.59 units ($p = 0.004$), and after 24 hours — 5.44 versus 5.60 units. ($p = 0.08$). In general, it is considered that the optimal pH level of broiler breast meat (measured 24 hours after poultry slaughter) is 5.5–6.2, a value of 5.44 indicates signs of poor-quality meat of PSE category. At the same time, there is a decrease in the pH of the breast flesh in the age aspect ($p < 0.05$) in both the control and experimental poultry groups. The age-related decrease in pH is probably associated with the intensive growth of poultry in the final fattening period.

Since red muscle fibers contain more myoglobin and hemoglobin and less glycogen compared to white, they are less susceptible to developing PSE. We found a more pronounced decrease in the pH of thigh flesh in the age-related aspect in the group of poultry exposed to stress. A significant decrease in pH ($p < 0.05$) was noted in this group both 45 minutes after slaughter and within 24 hours, which confirms the negative effect of the stress factor being under study. In contrast to the experimental group, in the control group the pH of the thigh flesh decreased with age only 24 hours after slaughter.

Oxidative stress occurs when there is an excess of oxidants and a deficiency of enzymatic and non-enzymatic antioxidants. Oxidative stress can cause damage to the cell membranes, causing disruption of the integrity of the structure, and then the nucleus, which leads to cell death [32].

We have studied the content of water-soluble antioxidants combination in breast, thigh, liver and heart flesh, as well as the content of glutathione in breast flesh. We found that there were no significant differences between the control group samples and the experimental group samples on the 24th, 34th and 52nd days. This indicates that the studied factor (a decrease in content value as a chronic factor of influence) was not critical for the development of acute oxidative stress. There was no more significant consumption of antioxidants in the experimental group. At the same time, a detailed analysis of the age-related dynamics of the content of the studied antioxidants in organs and tissues indicates that there were no changes in the content of TAS in both breast and thigh flesh along with age, as well as in the concentration of glutathione in breast flesh ($p > 0.05$). At the same time, on the 52nd day there was a significant ($p < 0.01$) decrease in TAS in the liver of both control groups samples and experimental group samples, as well as an accumulation of TAS in the heart muscle: there was a trend of its accumulation in the control group ($p = 0.06$), while in the experimental group it was significantly higher ($p < 0.001$). We assume that the genetic factor played a significant role in this case, and the content factor we are studying is secondary. The same can be noted in the content of enzymes in the flesh of the breast (SOD, catalase). No intergroup differences were found on the 24th, 34th, and 52nd days being considered.

The study reports that stress can increase the activity of antioxidant enzymes in the liver and serum [33], as well as the expression of superoxide dismutase and catalase genes in the spleen [34]. Other authors have observed a significant increase in the level of reduced glutathione in the liver of broilers exposed to heat stress [35].

It has been shown that regardless of the duration of stress, it can cause a deterioration in the quality of meat, a decrease in water holding capacity, changes in color, texture, smell, and shelf life shortening.

WHC is one of the most important indicators of meat quality, as lean meat contains 75 % water, which can be lost during slicing, cooking, or storage. These losses directly affect the sensory properties (for example, reduce the juiciness) during processing and delivery of meat to the consumer [36]. WHC which directly affects the color and tenderness of meat, is one of the most important functional properties of raw meat. Increasing the water content in muscles, which increases their tenderness, juiciness, elasticity and appearance, improves the quality and economic value of meat [37]. The disruption of protein structures and their denaturation (under the influence of high temperatures and low pH) leads to a decrease in their ability to hold water, which determines the color, soft structure and increased wateriness in PSE meat. This leads to an increase in drip loss or a decrease in moisture retention. In our study, there were no significant changes in the WHC value in both the experimental poultry group and the control group, although there was a slight downward trend in the values of this parameter with age, both in the flesh of the breast and in the flesh of the thigh. At the same time, the values of the WHC of the experimental group were lower than in the control group on the 34th and 52nd days, which is probably due to the effect of the

Table 3. Chemical composition of broiler meat of experimental groups in age dynamics,% (M ± SEM, n = 10)

Indicator	Age 24 days			Age 34 days			Age 52 days			p-value (the values of the control group in age dynamics)	p-value (the values of the experimental group in age dynamics)
	Group		p-value	Group		p-value	Group		p-value		
	control	experimental		control	experimental		control	experimental			
Breast meat											
Moisture	74.87 ± 0.24	75.10 ± 0.32	0.58	75.10 ± 0.17	74.72 ± 0.19	0.22	73.96 ± 0.26	73.26 ± 0.22	0.12	0.44	0.35
Protein	23.07 ± 0.13	22.65 ± 0.27	0.18	23.14 ± 0.17	23.34 ± 0.25	0.52	23.87 ± 0.27	24.34 ± 0.16	0.22	0.84	0.72
Fat	0.82 ± 0.22	1.056 ± 0.24	0.49	0.64 ± 0.09	0.74 ± 0.12*	0.22	0.99 ± 0.06	1.20 ± 0.15	0.13	0.04	0.61
Ash	1.23 ± 0.02	1.20 ± 0.02	0.14	1.12 ± 0.01	1.20 ± 0.04	0.11	1.18 ± 0.01	1.19 ± 0.01	1.0	0.56	0.69
Calcium	0.057 ± 0.002	0.054 ± 0.003	0.39	0.05 ± 0.002	0.06 ± 0.001	0.55	0.059 ± 0.001	0.056 ± 0.002	0.79	0.76	0.74
Phosphorus	0.197 ± 0.002	0.191 ± 0.002*	0.04	0.17 ± 0.004	0.18 ± 0.003	1.0	0.172 ± 0.03^{C}	0.168 ± 0.03	0.50	0.15	0.10
Magnesium	0.031 ± 0.002	0.031 ± 0.001	1.00	0.025 ± 0.001	0.026 ± 0.0011***	<0.001	0.029 ± 0.001	0.031 ± 0.001*	0.05	0.008	0.72
Thigh meat											
Moisture	75.01 ± 0.51	74.22 ± 0.74	0.39	74.56 ± 0.32	74.34 ± 0.29	0.40	74.38 ± 0.50	73.11 ± 0.80	0.71	0.85	0.86
Protein	17.26 ± 1.57	18.61 ± 0.24	0.41	19.37 ± 0.17	19.16 ± 0.23	0.29	20.48 ± 0.20	20.21 ± 0.21	0.06	0.14	0.90
Fat	4.81 ± 0.63	6.17 ± 0.73	0.18	5.08 ± 0.43	5.40 ± 0.33	0.65	4.08 ± 0.42	5.65 ± 0.64	0.37	0.52	0.83
Ash	2.79 ± 1.78	1.00 ± 0.017	0.33	1.0 ± 0.01	1.1 ± 0.04	0.14	1.05 ± 0.01	1.03 ± 0.02	0.60	1.0	0.62
Calcium	0.04 ± 0.001	0.05 ± 0.006	0.21	0.04 ± 0.001	0.05 ± 0.001	0.23	0.05 ± 0.002	0.052 ± 0.004	0.23	0.07	0.41
Phosphorus	0.160 ± 0.004	0.165 ± 0.003	0.31	0.15 ± 0.003	0.15 ± 0.003	0.70	0.152 ± 0.002	0.141 ± 0.004	0.42	0.41	0.48
Magnesium	0.024 ± 0.001	0.026 ± 0.001	0.43	0.02 ± 0.001	0.04 ± 0.02	0.42	0.024 ± 0.001	0.024 ± 0.0003	0.44	0.61	0.51

* the differences between the control and experimental groups are statistically significant at $p < 0.05$, *** at $p < 0.001$.

Table 4. Qualitative characteristics of broiler meat of the experimental groups in age dynamics, % (M ± SEM, $n = 10$)

Indicator	Age 24 days			Age 34 days			Age 52 days			p-value (the values of the control group in age dynamics)	p-value (the values of the experimental group in age dynamics)
	Group		p-value	Group		p-value	Group		p-value		
	control	experimental		control	experimental		control	experimental			
Breast pH-45	6.19 ± 0.08	6.20 ± 0.11	0.83	6.20 ± 0.03	6.10 ± 0.02	0.55	5.59 ± 0.04	5.55 ± 0.06*	0.004	0.007	0.0005
Breast pH-24	5.81 ± 0.15	5.76 ± 0.12	0.80	5.47 ± 0.19	5.49 ± 0.04*	0.02	5.60 ± 0.03	5.44 ± 0.11	0.08	0.13	0.28
Thigh pH-45	6.20 ± 0.09	6.08 ± 0.11	0.27	5.96 ± 0.07	5.91 ± 0.08	0.14	6.00 ± 0.04	5.95 ± 0.03	0.25	0.62	0.04
Thigh pH- 24	5.98 ± 0.14	5.93 ± 0.15	0.56	5.83 ± 0.05	5.65 ± 0.04	0.20	5.56 ± 0.04	5.52 ± 0.06	0.14	0.05	0.05
TAS in the breast, mg/g	0.256 ± 0.03	0.238 ± 0.05	0.18	0.084 ± 0.004	0.090 ± 0.04	0.73	0.140 ± 0.008	0.149 ± 0.01	0.44	0.08	0.07
TAS in the thigh, mg/g	0.238 ± 0.03	0.209 ± 0.03	0.35	0.209 ± 0.012	0.213 ± 0.01	0.50	0.204 ± 0.02	0.196 ± 0.02	0.13	0.52	0.11
TAS in the liver, mg/g	0.839 ± 0.07	0.829 ± 0.05	0.67	0.776 ± 0.08	0.941 ± 0.06	0.38	0.116 ± 0.01	0.121 ± 0.02	0.50	0.01	2×10^{12}
TAS in the heart, mg/g	0.187 ± 0.01	0.193 ± 0.01	0.18	0.258 ± 0.03	0.272 ± 0.03	0.64	0.505 ± 0.03	0.455 ± 0.02	0.43	0.06	4.6×10^{9}
Reduced glutathione in breast, μM/g	32.07 ± 12.87	39.96 ± 1.64	0.83	83.63 ± 7.36	67.24 ± 2.49	0.32	82.57 ± 19.12	58.49 ± 14.53	0.48	0.79	0.51
SOD in the breast, Units/g	8.13 ± 2.30	7.14 ± 0.25	0.89	12.96 ± 0.68	10.65 ± 2.11	0.64	11.50 ± 0.15	11.80 ± 0.94	0.98	0.02	0.65
Catalase in the breast, Units/g	17.22 ± 1.51	24.02 ± 3.69	0.07	28.02 ± 1.75	20.07 ± 2.75	0.09	26.49 ± 0.95	22.16 ± 3.22	0.57	0.63	0.74
Breast WHC, %	61.06 ± 1.30	62.12 ± 0.90	0.79	60.80 ± 0.75	58.33 ± 0.45	0.12	57.88 ± 0.92	56.29 ± 0.75	0.72	0.08	0.76
Thigh WHC, %	59.55 ± 0.72	62.04 ± 1.04	0.10	58.93 ± 1.65	56.96 ± 1.31	0.91	60.75 ± 0.88	55.32 ± 1.48	0.28	0.56	0.35

* differences between the control and experimental groups are statistically significant at $p < 0.05$.

stress factor, which also correlated with the values (decrease) of pH during this period.

In another study, on the contrary, the WHC of broiler chicken meat was higher under the influence of heat stress, as evidenced by their lower losses during draining compared with the control group (not exposed to stress) [25]. An increase in the WHC of meat has also been observed by other researchers when the poultry was exposed to high cage density in poultry rearing [38].

Cluster analysis (Figure 1) indicates the mutual dependence of the studied parameters and can have a predictive aspect to identify factors leading to deterioration in product quality. The dendrogram shows the presence of a close correlation between the pH values and the AWSA in organs and tissues, which correlation must be taken into account in further programs for rearing and feeding intensively growing young poultry. In addition, WHC of the tissues is closely related to the activity of antioxidant enzymes (SOD, catalase).

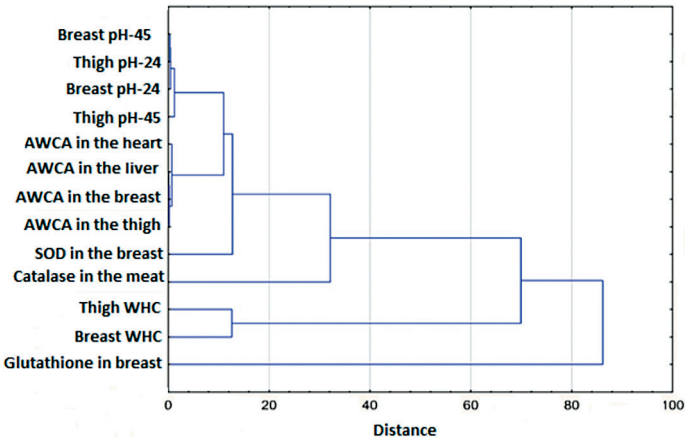

Figure 1. Results of cluster analysis on the correlation between antioxidant content in meat in various tissues and the distance

The correlation between qualitative parameters of broiler chicken muscle tissue and antioxidant capacity has also been shown in the works of other authors. For example, a decrease in WHC in muscles was accompanied by a decrease in lipid peroxidation products [39]. According to the authors, a decrease in the WHC index is an undesirable phenomenon, since breast muscles with higher lightness and lower ability to hold water are prone to the formation of meat of PSE category [40]. Other researchers have found negative correlations between the pH of broiler breast meat and WHC [41].

Given the close correlation between the antioxidant status of the body and the quality of meat, research aimed at developing and using various sources of antioxidants in nutrition is promising, especially during periods of the poultry exposure to climatic, technological and feed stresses. The use of dietary sources of antioxidants may be a good strategy for the prevention and control of oxidative stress. Positive effects of antioxidant applying include increased live weight gain, slaughter yield, and improved quality of meat. The use of antioxidants in feed may reduce muscle lipid breakdown and improve meat stability.

Conclusion

As a result of comprehensive studies, it has been established that the modulated stress conditions of poultry cage density provide an impact on the composition and quality parameters of the muscle tissue (thighs and breasts) of broiler chickens. Changes were noted only in the pectoral muscle of the poultry and depended on the age of the poultry, that is, on the duration of exposure to stress. The effect of modulated stress affected the fat content (causing its increase) and minerals (it changed the content of magnesium and phosphorus). At the age of 34 days, stress affected the final pH (pH 24), causing it to increase in the pectoral muscle. The level of WHC tended to decrease in the muscles of broiler chickens at both 34 and 52 days of age. In conclusion, improving the quality of broiler chicken meat depends on various factors. Any violation of these factors can negatively affect the quality of the meat. The strong influence of genotype on meat quality, as well as the importance of feeding and poultry housing conditions, cannot be overlooked. Cage density is a crucial factor in the poultry production, and maintenance stress can lead to decreased product quality, which consumers perceive as tough meat. Understanding these factors is essential for studying these mechanisms and developing approaches for producing broiler chicken meat that not only meets economic demands but also promotes human nutrition. To improve meat quality exposed to stress in the future, it will be necessary to explore various feed additives based on natural antioxidants that can prevent oxidative stress and enhance product quality. Using the above factors, it is possible to achieve the desired meat composition and product type properties, as well as to ensure high quality of meat.

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