ISSN 2414-438X (Print)
ISSN 2414-441X (Online)

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FOOD SYSTEMS
V.M. GORBATOV
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THEORY AND PRACTICE OF MEAT PROCESSING

2024, vol.9, no.2

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

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- improvement of technologies for meat product manufacture;
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Minister of Science and Higher Education of the Russian Federation

Federal State Budgetary Scientific Institution
"V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences"
(Gorbatov Research Center for Food Systems)

Theory and practice of meat processing
www.meatjournal.ru

Founder, Publisher and Printing Office:
Federal State Budgetary Scientific Institution
"V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences"
Talalikhina str. 26, Moscow, Russia, 109316

Editorial Office:
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The Journal is registered in the Federal Service on Supervision in the sphere of communication industry, information technologies and public communications.
The certificate of registration is
PI № FS 77 - 71611 of 13.11.2017
EL № FS 77 - 71609 of 13.11.2017
Founded in 2016

Free price
Frequency — 4 issues a year
Subscription index in the catalogue "Press of Russia" 38871
Signed print 30.06.2024
Released from press 02.07.2024
Circulation — 300 copies. Order № 544.

16+

ISSN 2414-438X (Print)
ISSN 2414-441X (Online)
DOI-prefix: 10.21323/2414-438X

EDITORIAL BOARD

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DOI: https://doi.org/10.21323/2414-438X-2024-9-2-100-107
Received 14.02.2024
Accepted in revised 03.04.2024
Accepted for publication 10.04.2024

Available online at https://www.meatjournal.ru/jour
Original scientific article
Open Access

MEAT COLD CHAIN MANAGEMENT IN MEXICAN SUPERMARKETS

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Keywords: *food safety, supermarkets, meat, cold chain, retail, temperature*

Abstract
The cold chain represents an important opportunity for the food industry to offer food safety and quality of perishable foodstuffs, mainly by maximizing shelf life through limiting bacterial growth. Especially meat products have a short shelf life due to their cold chain requirements. Refrigeration equipment must always be appropriately calibrated and monitored to provide the right temperature and ensure its correct performance. This study aimed to evaluate the cold chain at the retail link in supermarkets of the major commercial chains in Mexico during 2021 and 2022 by recording the surface temperature of pork, poultry, and beef cuts in open coolers. A general linear model was used for the statistical analysis in a completely randomized design. The study considered several factors, such as supermarket, season of the year, position inside the refrigerator, type of meat and environmental temperature. Cold chain breaks were detected in certain seasons of the year, in supermarkets, and even within the refrigerator; there were differences between positions where products were placed. The persistence of these variations in the cold chain represents a public health risk caused by the consumption of foods because a temperature increase can lead to a rise in the microbial load of meat products. Thus, meat cold chain requires integrated logistics management in order to maintain high quality of foods.

For citation: Arriaga-Lorenzo, P., Maldonado-Simán, E., Ramírez-Valverde, R., Martínez-Hernández, P.A., Tirado-González, D.N., Saavedra-Jiménez, L.A. (2024). Meat cold chain management in Mexican supermarkets. *Theory and Practice of Meat Processing*, 9(2), 100–107. https://doi.org/10.21323/2414-438X-2024-9-2-100-107

Funding:
The article was published as part of the Research Project Code: 23032-C-67 registered at Chapingo Autonomous University, General Directorate of Research and Postgraduate.

Acknowledgements:
The support of the National Council of Humanities, Science and Technologies of Mexico is gratefully acknowledged.

Introduction

Delivering safe foods with acceptable organoleptic characteristics is a food industry challenge due to the complexity of the food supply chain. Around one-third of the world's food production is wasted for different reasons, which adds up to about 1300 million tons per year [1,2]. Food supply chain involves stages that can be categorized as primary production, postharvest and storage, processing, distribution, and consumption [3]. Food wastage varies according to a link in the supply chain; for example, wastage of up to 931 million tons has been estimated for retail, households, and food services [4]. To minimize food wastage, shelf life of highly perishable foods can be extended by employing different packaging techniques. Nevertheless, usually they always need to be kept at a correct temperature [5]. Improper cold chain management results in a negative impact on food safety and a significant reduction in quality, which is reflected in economic losses, health risk, and lack of food availability [6].

Several observations in the retail sector indicate that cold chain management in facilities appears to be the weakest due to reported temperature abuses in food [7]. Having the time-temperature history is essential for food safety, as it can be used in identifying perishable foods that have been subjected to temperature control abuses and gives an opportunity to timely remove them from food displays [8] to minimize meat wastage caused by quality loss due to improper storage [9]. Since some years ago, different technologies have been developed which help in the cold chain monitoring, obtaining a large amount of data used to take pertinent actions. In this context, using the Internet of Things (IoT) allows the implementation of monitoring and traceability protocols by means of wireless sensors and RFID tags, which provide data in real time [10,11].

From animal slaughter to retail sales, meat products require adequate facilities and equipment to guarantee quality and safety [12]. They are achieved through refrigeration or freezing processes throughout the distribution chain after slaughter until reaching the final consumer [13]. The

cold chain is one of the main tools of the food industry, limiting bacterial growth, maximizing shelf life, and reducing food waste. This is relevant in the sanitary, environmental, and economic areas [14,15,16]. Strict monitoring must be implemented to preserve the cold chain integrity of sensitive food products, considering the temperature heterogeneity within the refrigeration display [8]. However, the cold chain must be implemented in conjunction with strict hygiene measures during handling of meat products to reduce contamination with microorganisms that may proliferate even under proper refrigeration temperatures, such as *Campylobacter jejuni* commonly found in poultry [17].

Temperature disturbance at any step of the food production chain represents a hazard that leads to product spoilage. The causes are various, such as interruptions of the refrigeration system itself, irregular temperature distribution due to uneven air distribution, exposure to ambient air, and everything related to incorrect settings in refrigeration systems. Maintaining control of the cold chain ensures the prolongation of the shelf life of fresh meat, so it is essential to avoid temperature fluctuations that lead to spoilage [18]. As a consequence, food spoilage leads to an unacceptable level of quality caused, in many cases, by a break in the cold chain [8]. These temperature variations promote the proliferation of a wide variety of microorganisms associated with a meat product type, which may include *Pseudomonas*, *Campylobacter*, *Moraxella*, *Enterococcus*, *Psychrobacter*, *Salmonella*, *Escherichia coli*, among other agents responsible for food-borne diseases [19].

Therefore, it is essential to consider a type of food within cold chain management so that refrigeration equipment provides the appropriate storage temperature [10]. To ensure the quality and safety of meat products, the Mexican authority established that meat products should be kept under refrigeration at all times at a temperature no higher than 4 °C according to NOM-213-SSA1–2018, which coincides with the Canadian, USDA, and FDA temperature limit [20,21,22,23]. Derens-Bertheau et al. [24] indicated that, along the cold chain, retail is one of the last three stages with the highest incidences of temperature violations. These temperature violations are caused by the lack of information and personnel training on the requirements of the products they handle [25].

In the face of various breaks in the cold chain, it has been indicated that the low efficiency of refrigeration equipment can influence maintaining the target temperature, as has been observed in open-type refrigerators [26]. Supermarkets widely use this type of equipment to display meat products in such a way as to make them accessible to consumers, because these refrigerators have no physical barriers and operate only with an air curtain that should maintain the proper temperature of products [27]. However, it is necessary to have properly calibrated equipment and monitor its performance to avoid temperature violations as a result of irregular distribution of airflow [28,29,30]. Despite the importance of monitoring throughout the cold chain, there needs to be more scientific evidence in Mexico that specifies the state of temperature management in supermarket refrigerators. Therefore, this research describes the temperature analysis in refrigerated open displays operated by some major chain supermarkets in Mexico to offer meat products to customers.

Objects and methods

Meat products, which temperatures were monitored in this research, were pork, poultry, and beef exhibited in refrigerated open displays for consumers' pick-up in four supermarkets located in an urban area to the northeast of the State of Mexico, Mexico. Pork and beef were in slices, while poultry was in whole pieces: breasts, legs, and thighs. Temperature was measured at three positions within the open display refrigerator: front, middle, and rear. Temperature recording was weekly at the same time for each supermarket for two consecutive years: 2021 and 2022. For each type of meat, 606 temperature records were obtained for an overall total of 3636 records during the whole monitoring. An infrared thermometer (GM320 MASIONE®, China) was used to record the temperature, pointing to the surface of the specific meat product. When temperatures in supermarket displays were taken, outside environmental temperature was collected from records of a nearby federal weather station (00015125 TEXCOCO [DGE]).

Statistical Analysis

The effects of year's season (winter, spring, summer, and autumn), meat type (pork, poultry, and beef), supermarket (1, 2, 3, and 4), position inside the refrigerator (front, middle, and rear), and their respective interactions were analyzed. The statistical analyses were performed using online SAS software. The analysis of variance (ANOVA) was performed considering season, position, supermarket, and meat as fixed effects under a general linear model, using a completely randomized design. A comparison of mean differences ($p < 0.05$) was performed with the LSMEANS function adjusted to Tukey. Environmental temperature was considered as a covariate using PROC Mixed and SGPLOT to obtain the effect on predicted and observed temperature in meat products. An orthogonal polynomial test was carried out to evaluate the linear and quadratic tendencies of the covariate (Proc Mixed) [31].

Results and discussion

All four variation sources included in the study influenced ($p < 0.05$) surface meat temperature (Table 1). Statistically significant differences were found for the effect of year's season ($p < 0.001$); winter, followed by summer, were the seasons with the highest temperatures in the products. Regarding the supermarket effect, differences in the cold chain management were highly marked between supermarket 1 and the rest with more than 5 °C differences. In other words, the temperature in supermarket 1 was around 1.3 times higher than the mean temperature in the other

three supermarkets. These results of variability are relevant, considering that all supermarkets evaluated in this study use the same type of refrigerator (open cabinet).

On the other hand, despite statistically significant temperature differences found between different meat types, they were tiny, showing cold chain breaks in beef. However, this is the statistical interpretation according to the temperature means. It can be observed in the minimum and maximum temperature data that the three meat types showed temperatures above 4 °C; the time during which the products were subjected to these conditions is unknown. Deficient cold chain management increases the growth risk of possible bacteria present in the different types of meat. Furthermore, it is necessary to consider the ability of different microorganisms including pathogens to grow at refrigeration temperature (4 °C), as is the case of *C. jejuni*, which causes gastroenteritis and is the main cause of Guillain-Barre Syndrome [32,33]. Upon the interpretation of statistical analysis, an effect of meat type was found to be very close to non-significant ($p = 0.05$). On the contrary, an effect of the meat product position in the refrigerator was significant. Temperature was rising from the rear to the front with the highest mean temperature of 5.84 °C.

Table 1. Single-effect temperature means

Variation source	Levels	Mean	Min — Max	S.E.	Significant differences
Season	Winter	4.18	–15.90 — 15.40	0.11	a
	Spring	3.95	–5.60 — 22.20	0.09	ab
	Summer	4.09	–6.10 — 26.00	0.09	a
	Autumn	3.73	–5.30 — 13.40	0.09	b
Supermarket					
	1	6.92	–1.50 — 14.40	0.09	a
	2	2.68	–5.60 — 9.20	0.09	c
	3	3.56	–6.10 — 26.00	0.09	b
	4	2.80	–5.90 — 12.70	0.09	c
Meat type					
	Pork	3.84	–6.10 — 22.20	0.08	b
	Poultry	4.00	–5.20 — 26.00	0.08	ab
	Beef	4.12	–5.90 — 20.30	0.08	a
Position					
	Front	5.84	–3.90 — 22.20	0.08	a
	Middle	4.58	–3.90 — 26.00	0.08	b
	Rear	1.54	–6.10 — 18.70	0.08	c

S.E. = standard error; means within the variation source with at least one literal in common are not statistically different

As for the seasonal effect on product temperature reported in this study, the highest temperature was in winter followed by summer. It is partially consistent with the results reported in the previous study by Baldera et al. [28], where fresh meat temperatures were higher during summer instead of winter.

Regarding open-type refrigerators, this study conducted in Mexico reports differences between refrigerators used by the supermarkets evaluated. However, the stores evaluated by Talbot et al. [34] use different refrigerator types. They mention that the global temperature means of various positions in the refrigerator did not show significant differences, but there were differences among the refrigerator types.

An effect of a supermarket on a temperature of meat products was analyzed. Cold chain management applied in supermarket 1 showed concerns in the three meat types with statistical differences; beef showed the highest temperature (Figure 1). The records indicate that this supermarket's maximum limit of 4 °C was not achieved in any meat type ($p < 0.01$). In contrast, none of the other supermarkets recorded violations of the temperature limit specified in the recommendations by NOM-213-SSA1–2018 in the tested meat types [20].

The results showed violations of current regulations concerning the permitted temperature for perishable foods such as meat in the Mexican supermarkets evaluated. In the same way Lundén, Vanhanen, Myllymäki, et al. [35] reported a higher rate of temperature violations in fish compared to minced meat, which shows that it is crucial to consider a product type to implement the proper refrigeration equipment and configuration consistently to achieve the right temperature. Therefore, according to Mexican legislation regarding temperature requirements for meat products, some supermarkets evaluated in this study sell meat that does not comply with the regulations, and as [8,36] point out, there is a high probability that a significant bacterial load can represent a health risk. Besides, shelf life of products is reduced with each degree of temperature above recommended.

Similarly, an effect of the position in the refrigerator was analyzed at different retail stores. Supermarkets 1, 3, and 4 presented temperatures above 4 °C in the front position. Whereas supermarkets 3 and 4 ensured the cold chain in the middle and rear positions, supermarket 1 did not achieve proper temperature in any position, contrary to supermarket 2, which successfully managed temperature in all three positions in the refrigerator (Figure 2). At the same time, the front and middle positions in the refrigerator showed temperature fluctuations that were out of the norm in all-year seasons (Figure 3). Based on this observation, it has been confirmed that monitoring of refrigeration equipment is essential to verify that it is correctly calibrated, since uniform temperature distribution is crucial to avoid disruption of the cold chain [37].

Temperature deviations in meat products can drastically affect food quality and safety, influencing meat structure, fracturing muscle fibers, and water loss [38]. It is of greater relevance, considering that various pathogens can be present in meat products, including *E. coli* [39]. In a study conducted in Slovakia, where the microbiological quality of ready-to-eat foods was evaluated, Lopašovský et al. [40] indicate that one of the main concerns in reducing food losses and public health risks focuses on maintaining control of refrigeration at all stages of the cold chain, thereby preserving control of food safety and quality. Due

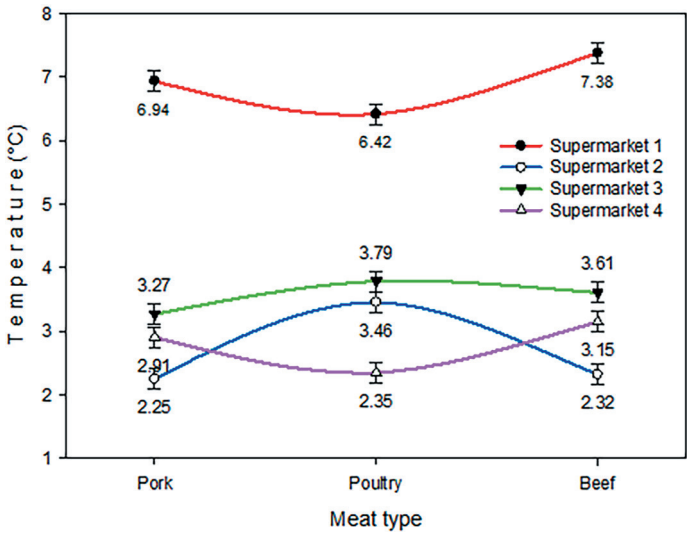

Figure 1. Temperature means of the three meat types in the supermarkets

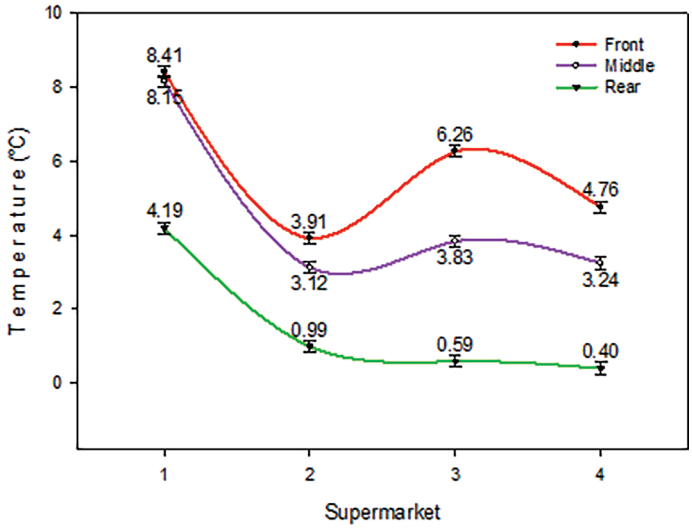

Figure 2. Temperature means of the three meat types in different positions in the refrigerator in the supermarkets

to cold chain breakage, bacteria colonies increase, and the product's spoilage is accelerated. It is reasonable to assume that there is the probability of cross-contamination during different stages of carcass processing after slaughter [41].

Cold chain breaks at retail distribution, particularly by supermarkets, can be attributed to a refrigerator type. Supermarkets often use open refrigerators, such as those examined in this study. This equipment facilitates consumer access to the products. An air curtain flow is used to prevent heat transfer from the environment. However, in many cases, it proves inefficient, having problems with maintaining the correct temperature at the front position as it is in the rear. Therefore, using refrigerators with doors, in many cases, helps to reduce this problem [27]. Likar and Jevšnik [42] reported that despite temperature fluctuations during food storage, retail stores did not have the corresponding documentation and system to control the cold chain. At the same time, this study shows that retailers are not well-informed about the importance of the food cold chain.

The cold chain monitoring in the three meat types showed breaks due to diverse factors related to a supermarket. With the reference 4 °C limit for meat products [20], a significant violation of up to 3.48 °C was reported for supermarket 1 during autumn; nevertheless, temperature fluctuations were observed during all seasons of the year (Figure 4). In this scenario, supermarket 3 had problems with maintaining its cold chain during the winter and spring. Considering the influence of the environmental temperature used as a covariate in the present study, it is possible to affirm its impact since it was found to have a linear effect ($p < 0.0001$), where it is predicted that for each 1 °C increase in environmental temperature, the meat products increase their temperature by 0.93 °C. However, a quadratic effect was also found, where the maximum point is predicted at 20.4 °C, and subsequently, meat product temperature is reduced by –0.02 °C for each 1 °C increase in ambient temperature. This phenomenon was observed in the four supermarkets (Figure 5). Figure 5 shows the general panorama that varied in each supermarket only in the temperature values of meat products kept, exceeding the values predicted and permitted by Mexican regulations.

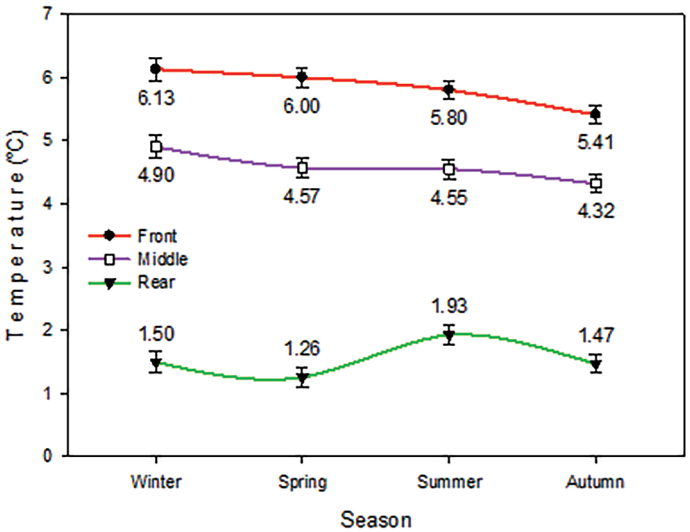

Figure 3. Temperature means of the three meat types in different positions in the refrigerator throughout the year

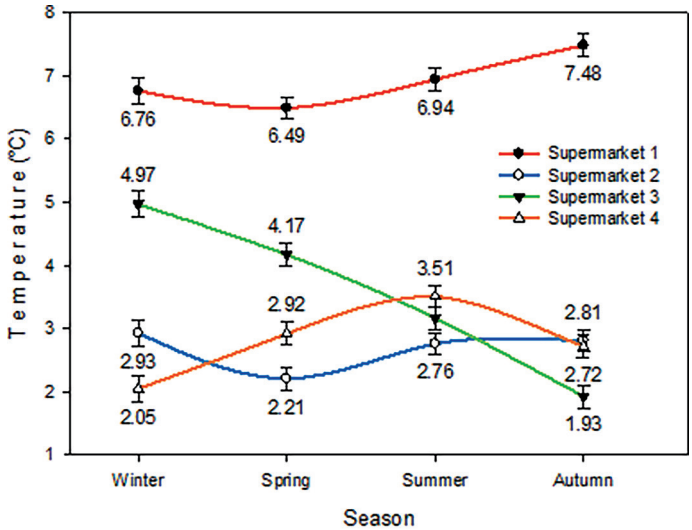

Figure 4. Temperature means of the three meat types in year seasons in the supermarkets

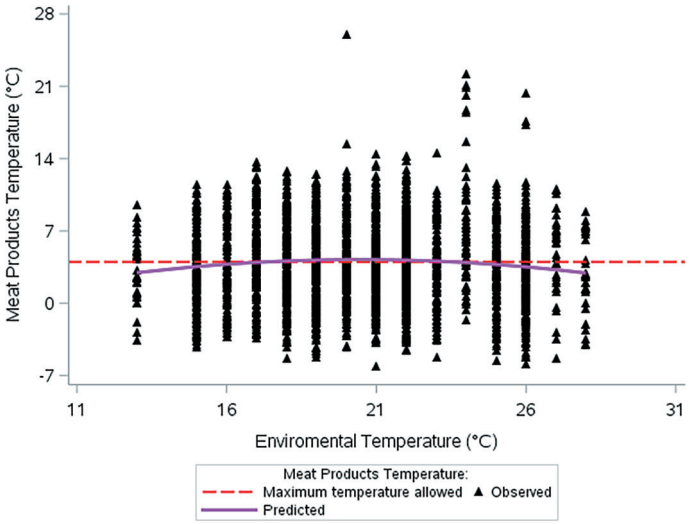

Figure 5. Effect of environmental temperature on meat products

This study did not evaluate an effect of a type of refrigerator used in the supermarkets (open or closed) on the cold chain. However, the difference in temperature control can be observed in the present study, given that supermarket 3 changed its open refrigerator display for refrigeration equipment with doors in the poultry (Figure 6A) and beef (Figure 6B) section at the beginning of August 2022. It can be observed that, after this date, the maximum temperature limit of 4 °C established by Mexican regulations was not exceeded during the rest of the study contrary to pork (Figure 6C), which remained on open shelves and showed temperature violations throughout the monitoring period. Bruckner et al. [43] noted that short temperature increases in fresh pork and poultry meat led to spoilage patterns that reduced shelf life during storage and influenced the growth of *Pseudomonas* spp. in these types of meat.

Consequently, it is necessary to have continuous temperature monitoring and temperature data exchange throughout the supply chain of products that are highly perishable to predict the remaining shelf life [44]. Nastasijević et al. [45] highlighted that meat is a fresh product with a short shelf life, so cold chain management is crucial to maintain quality and safety. To achieve successful management, retail establishments require special attention to weak points, such as the size and capacity of cold rooms, the initial temperature of incoming meat, the dimensions of cabinets, the procedures applied in meat handling, environmental temperatures, location of refrigeration equipment, light, and ventilation, among others [45]. Poor cold chain management can result in food waste and economic losses [46]. In the case of developing countries, food wastage may be mainly due to poor refrigeration infrastructure. Therefore, investment in better refrigeration can improve food availability and quality [46].

As for developed countries, large percentages of food waste are reported at the consumption stage [46]. However, it is estimated that 12% of the production of countries such as the USA, Canada, Australia, and New Zealand needs to be recovered during distribution and retailing [47]. For

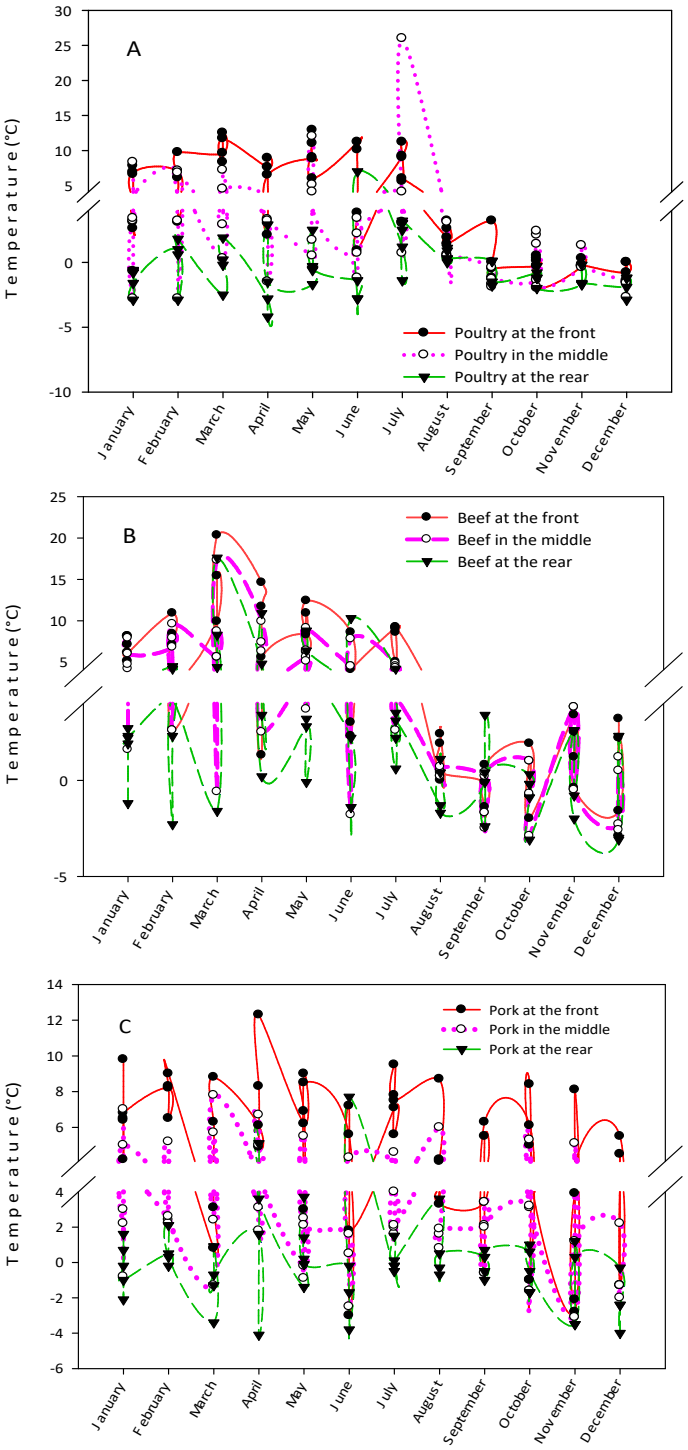

Figure 6. Effect of the change of refrigeration equipment in supermarket 3

food business operators to maintain effective temperature control of refrigeration equipment, it is necessary to know precisely about the type of equipment operating in the centers and, equally, the temperature parameters of the product, equipment alerts, and the situation, in which they are activated to solve the problem promptly [35]. Then, temperature monitoring must be performed on both the refrigeration equipment and the product it contains because there may be unfavorable variability between them [25].

Thus, temperature control in retail store refrigeration equipment is essential to avoid violations, particularly in the most sensitive products that require low temperatures

in storage processes. Undoubtedly, the use of an open refrigeration equipment represents a challenge to maintain proper temperatures [48], in addition to their inefficient use of electrical energy [49]. Therefore, a poor design of the equipment causes difficulties in maintaining the cold chain of every product and induces instability in temperature management in this type of equipment. Thereby, regular maintenance is required to ensure the recommended long-term performance.

It should be remembered that the cold chain not only is a tool to limit the growth of pathogenic bacteria, but also reduces the growth of microorganisms that deteriorate the product, thus maximizing its shelf life and reducing food wastage [50]. The magnitude of food waste produced worldwide has already been discussed, and its control has been a topic of great interest during the last decades because it can contribute to achieving another sustainable development objective [1]. However, food wastage needs to be addressed according to a geographic region, given that wastage exists worldwide but at different stages and magnitudes. In the case of Latin America, large amounts of food are lost in production, handling and storage, distribution, and consumption [51], attributed mainly to inadequate handling practices and food knowledge [52], such as the case of deficient cold chain management observed in this study.

Furthermore, climate change greatly influences food wastage at different stages. For the cold chain, the main impact is related to higher environmental temperatures, given that, at higher temperatures, it is a greater challenge to maintain the temperature within the established limits [53]. This problem is prevalent in developing countries, where infrastructure deficiencies such as refrigeration equipment have been detected [52]. Finally, it is important to highlight that several supermarkets use open refrigerators in the meat product areas, which do not have doors and, therefore, operate with an air curtain that prevents air and humidity from coming into contact with the products. Nevertheless, they could be inefficient in maintaining the cold chain and power consumption compared to equipment with doors, allowing for energy savings of 20 to 70% [27]. A suggestion put forward by Ashraf and Alanezi [54] indicate that a smart refrigerator would have the possibility to provide concrete information on food safety and leftovers.

Conclusion

This study detected significant temperature increases in major supermarket chains operating in Mexico. Only supermarket 2 achieved temperature means that complied with the Mexican regulations in force regarding different effects considered in the statistical analysis. All the other supermarkets showed temperature abuses for at least one season of the year, position in the refrigerator, or meat type. Particularly, the front position in the refrigerator showed the highest temperature abuses. The season of the year, related to the environmental temperature, may affect the performance of refrigeration equipment, and can lead to an accelerated growth of microorganisms. Finally, retailers face challenges in adequately managing refrigeration systems to guarantee the safety and quality of meat products. Knowledge of the product´s temperature range is necessary to choose the appropriate refrigeration equipment to ensure the cold chain integrity. In general, refrigerators with doors are recommended for better temperature control, as observed in supermarket 3, or the constant monitoring of open refrigerators to calibrate whenever necessary. High-quality meat products require integrated cold chain management.

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The authors declare no conflict of interest.

DOI: https://doi.org/10.21323/2414-438X-2024-9-2-108-116
Received 28.03.2024
Accepted in revised 18.04.2024
Accepted for publication 22.04.2024
Available online at https://www.meatjournal.ru/jour
Original scientific article
Open Access

CHEMICAL COMPOSITION AND BROILER MEAT QUALITY WHEN USING MELANIN

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Keywords: *broilers, chemical composition of meat, quality, fatty acid*

Abstract
The research was carried out to study an effect of the antioxidant melanin in the broiler nutrition on the chemical composition, antioxidant and technological properties of meat, and the fatty acid composition of abdominal fat. The experiment was conducted in the conditions of the physiological courtyard of the L. K. Ernst Federal Research Center for Animal Husbandry in 2023. Broilers (n = 27, N = 54) of the domestic broiler cross "Smena-9" were divided into two groups (control and experimental). Broilers of the control group were fed the basic diet, birds of the experimental group received the basic diet with the addition of water-soluble melanin at a dose of 1.42 mg /kg of bird weight from the 7th to 45th day of age. At the age of 45 days, the birds were slaughtered and the meat chemical composition, fatty acid content, and quality characteristics were determined. The addition of melanin to the diet led to an increase in the meat antioxidants in the breast by 18.75% ($p < 0.0001$) and in the thigh by 5.6%, and also resulted in an increase in reduced glutathione by 20.25%, glutathione peroxidase by 10.43%, catalase 17.35% in the breast compared with the control. The use of melanin in broiler diets contributed to an increase in the content of erucic and 8,11,14-eicosatrienoic acids in abdominal fat compared with the control (at $p = 0.02$ and $p = 0.07$, respectively), as well as to an increase in the moisture content in muscles and the enrichment of meat with the antioxidant.

For citation: Bogolyubova, N.V., Nekrasov, R.V., Zelenchenkova, A.A., Kolesnik, N.S., Lahonin, P.D., Rykov, R.A. et al. (2024). Chemical composition and broiler meat quality when using melanin. *Theory and Practice of Meat Processing*, 9(2), 108–116. https://doi.org/10.21323/2414-438X-2024-9-2-108-116

Funding:
This research was carried out with the financial support of the Russian Science Foundation, project No 22–16–00024.

Introduction

The poultry industry has received many benefits from achievements in the field of genetics, nutrition, and poultry keeping [1]. However, genetic selection based on productivity can lead to an increase in the number of metabolic disorders in the poultry body of modern genotypes [2], and a rapid growth rate is associated with the occurrence of meat quality defects [3]. Chicken meat is a source of protein, lipids, and minerals, which play an important role in basic human nutrition [4]. The quality and safety of food products is currently a very significant factor due to the enormous impact of products on human health and life expectancy [5,6].

The product quality and efficiency of poultry farming directly depend on the health of poultry [7]. The poultry meat quality is also influenced by the rearing systems [5,8].

Recently, the demand for organic food products has been growing due to their ability to reduce the risks of many diseases and improve the physical and mental well-being of consumers.

Since poultry of modern genotypes reacts acutely to stresses of various etiologies, metabolic disorders prevention and elimination is necessary to obtain high-quality poultry products [9,10].

One of the approaches to improving poultry health and meat quality is the use of various feed antioxidants [11–13].

Melanin is an irregularly shaped pigment, a product of the natural polymerization of dioxyphenol derivatives (tyrosine type) into a high-molecular compound under the action of tyrosinase in the presence of O_2. It is widely present in various organisms and has a wide range of biological effects, including the antioxidant activity [14,15]. Melanins are extensively used in medicine, pharmacology, cosmetology and other fields, but there is little information about the effect of this antioxidant on the composition and quality of meat and internal fat of broilers.

The purpose of the research was to study an effect of the adaptogen-antioxidant melanin in the broiler diet on the chemical composition, antioxidant and technological properties of muscle tissue, as well as the abdominal fat (FA) composition.

Objects and methods

Two groups of broilers (n = 27, N = 54) (control and experimental) of the domestic broiler cross "Smena-9" were formed in the physiological yard of the L. K. Ernst Federal Research Center for Animal Husbandry and an experiment was conducted. As the main ration for chickens of

all groups, full–fledged compound feeds were used, corresponding to the growing periods: up to 11 days — starting compound feed (up to 11 days), growth (12–26th day), finishing (27–45th day). From the 7th to the 45th day of life, water-soluble melanin was added to the main diet of poultry in the experimental group at a dose of 1.42 mg/kg of poultry weight. A highly concentrated water solution of melanin was prepared, which was sprayed onto the feed before feeding. On the 45th day, broilers (n = 27, N = 54) were slaughtered and parameters of the meat chemical composition were evaluated.

Moisture content

Moisture content of meat was determined according to GOST 33319–2015[1]. Purified sand and a glass rod were placed a weighing cup, dried at a temperature of 103 °C for at least 30 minutes, cooled at room temperature and weighed. A prepared crushed meat sample of about 5 g was placed in the weighing cup, weighed, and mixed with a glass stick. It was dried at a temperature of 103 °C for 2 hours, after which it was cooled to room temperature and weighed. The weighing cups were re-placed in a drying cabinet and kept at a temperature of 103 °C for 1 hour, after which they were cooled to room temperature and weighed. The weighing cups were dried to a constant weight (the discrepancy between two consecutive weighings did not exceed 0.1% of the sample weight). The moisture content in the samples was calculated using the equation:

$$X\,(\%) = \frac{(m_1 - m_2)}{m_1 - m} \times 100$$

Where:

X — mass fraction of moisture;
m_1 — the weight of the weighing cup with the analyzed sample, stick and sand before drying, g;
m_2 — the weight of the weighing cup with the analyzed sample, stick and sand after drying, g;
100 — conversion to a percentage;
m — the weight of the weighing cup with a stick and sand, g.

Fat content

The fat content of meat was determined according to GOST 23042–2015[2]. The method is based on the extraction of fat with a mixture of chloroform and ethyl alcohol using a filter separation funnel followed by separation of the extract, removal of the solvent and drying of the isolated fat. Chloroform and ethyl alcohol were mixed in a ratio of 2:1. About 2 g of the prepared sample was weighed in a glass. The analyzed sample was quantitatively transferred to a filter separation funnel, 20 cm^3 of the extraction mixture was poured, kept for 5 minutes, and extraction was carried out shaking the funnel for 2 minutes. The resulting extract from the dividing funnel was filtered using a water jet pump into the receiver. The extract was transferred from the receiver to a measuring flask. Extraction was performed two more times. After the end of the third extraction, the filter separation funnel and receiver were washed with an extraction mixture, which was collected into a measuring flask. The contents of the flask were brought to the mark with an extraction mixture and mixed. Then, 20 cm^3 of the extract was transferred to a weighing cup that had been dried at a temperature of 103 °C for at least 30 minutes and weighed beforehand. To remove the solvent, the weighing cup with the extract was placed in a water bath with a temperature of 40 °C and kept for 20 minutes until the smell of the solvent completely disappeared. The weighing cup with fat was placed in a drying cabinet, kept at a temperature of 103 °C for 15 minutes, cooled and weighed. The fat content in the samples was calculated using the equation:

$$X\,(\%) = \frac{(m_1 - m_2) \times 100}{m \times 20} \times 100 \qquad (1)$$

Where:

X — mass fraction of fat;
m_1 — the weight of the weighing cup with fat, g;
m_2 — the weight of the weighing cup, g;
100 — total volume of the extract, cm^3;
100 — percentage conversion factor;
m — mass of the analyzed sample, g;
20 — volume of the extract selected for evaporation, cm^3.

Ash content

The total ash content of processed broiler meat was determined according to ISO 936:1998[3]. Test portions (1 g) from samples of each group were weighted into preheated crucibles and incinerated overnight in a Muffle furnace at 550 °C until white ash free of carbon was obtained. The crucibles were removed from the Muffle furnace, cooled in a desiccator at a room temperature of 27 °C and reweighed. The ash content of the samples was calculated using the equation:

$$Ash\,(\%) = \frac{W_a}{W_s} \times 100 \qquad (2)$$

Where:

W_a — weight of ash;
W_s — weight of the sample.

Fatty acid composition

The fatty acid composition of abdominal fat was determined according to GOST R55483–2013[4]. A 15% solution of acetyl chloride in methanol, a saturated solution of potassium hydroxide in methanol, a saturated aqueous solution of sodium chloride, and a standard solution of a mixture of methyl esters of fatty acids were previously prepared. The

[1] GOST 33319–2015 Meat and meat products. Method for determination of moisture content. Retrieved from https://docs.cntd.ru/document/1200123927. Accessed February 06, 2024

[2] GOST 23042–2015 Meat and meat products. Methods of fat determination. Retrieved from https://docs.cntd.ru/document/1200133107. Accessed February 06, 2024

[3] ISO 936:1998 Meat and meat products. Determination of total ash Retrieved from https://docs.cntd.ru/document/1200098742. Accessed February 06, 2024

[4] GOST R55483–2013. Meat and meat products. Determination of fatty acids composition by gas chromatography Retrieved from https://docs.cntd.ru/document/1200103852/ Accessed February 06, 2024

analyzed sample (10 g) was placed in a flask with a ground stopper, filled with a mixture of 10 cm^3 methanol and 10 cm^3 chloroform and kept at room temperature for 24 hours for complete dissolution of lipids. Then, 3 cm^3 of a 15% acetyl chloride solution in methanol was added to the fat obtained after evaporation and the mixture was kept in a water bath at a temperature of 100 °C for 2 hours. After that, 1.25 cm^3 of a potassium hydroxide solution saturated in methanol was added to the mixture cooled to room temperature to a pH value of 5.0–6.0 and also 3 cm^3 of a saturated aqueous solution of sodium chloride and 3 cm^3 of hexane. The mixture was shaken and centrifuged until a transparent top layer of liquid was obtained. Finally, 1 cm^3 of a transparent upper hexane solution of methyl esters of fatty acids was placed in vials for use in a gas chromatograph.

Meat pH

The pH of meat was recorded using a Testo 205 pH meter (China).

Calcium content

The calcium content of meat was determined according to Methodological recommendations for chemical and biochemical studies of livestock products and feed[5]. The ash sample was boiled for 15 minutes with 5 ml of concentrated hydrochloric acid and 50 ml of water. The solution was transferred to a 100 ml flask. After cooling, it was brought to 100 ml with water and mixed. The next day, 0.1 ml of the tested ash solution was added to a test tube with 1 ml of caustic potassium, an indicator and a trilon. The solution was titrated with trilon B until the green color disappeared. The calcium content in the ash was determined by the formula:

$$Ca\ (\%) = \frac{a \times 0.04 \times 100 \times 100}{0.1 / c \times 1000} \quad (3)$$

Where:

a — the amount (ml) of 0.001 M of the trilon B solution used for titration of the test solution;
0.04 — the amount of calcium bound by 1 ml of 0.001 M trilon B solution, mg;
100 — dilution;
0.1 = volume of ash solution taken for titration, ml;
c — ash weight, g;
100 — conversion to %;
1000 — conversion of mg to g.

Phosphorus content

The *phosphorus* content of meat was determined according to Methodological recommendations for chemical and biochemical studies of livestock products and feed[5]. A sample of the analyzed substance was placed in a 100 ml Kjeldahl flask, 2.5 ml of concentrated sulfuric acid, 10 drops of a solution of hydrochloric acid were poured and burned until discoloration. After cooling, the contents of the flask were diluted with a small amount of water and transferred to a 100 ml volumetric flask, the solution was brought to the mark. The test solution (5 ml) was taken into a dry test tube, 1 ml of ammonium molybdenum solution was added and the test tube was shaken. After that, 0.25 ml of amidol solution was added and the test tube was put in a water bath at 37 degrees for 5 minutes. Then, the tube was placed in ice water and colorimetrated on a photometer with a wavelength of 635 nm. The phosphorus content was determined by the formula:

$$P\ (\%) = \frac{a \times 20 \times 100}{W_s \times 1000000} \quad (4)$$

Where:

a — the amount of phosphorus found on the calibration curve contained in 5 ml of the ash solution, mcg;
20 — recalculation to determine the phosphorus content in the mg of the sample;
100 — conversion to%;
W_s — weight of the sample;
1000000 — conversion of mcg to g.

Magnesium content

The magnesium content of meat was determined according to Methodological recommendations for chemical and biochemical studies of livestock products and feed[5].

The ash solution (5 ml) was placed in a 150 ml flask. Then, 20 ml of water, 2 ml of triethanolamine solution, 5 ml of trilon B solution, 2 drops of methylroth were added and neutralized with 255 M ammonia solution until yellow color appeared. After that, 10 ml of the ammonia buffer solution, 12 drops of 0.02% alcohol solution of methyl red and 5 drops of chromogen black were added. The color became green. The solution was titrated with magnesium sulfate until the color changed. The calculation of the magnesium content was carried out according to the formula:

$$Mg\ (\%) = \frac{(a - b) \times k \times 0.00012 \times 100 \times 100}{5 \times c} \quad (5)$$

Where:

a — amount of 0.01 M trilon B solution bound by the sum of calcium and magnesium ml;
b — amount of 0.01 N solution of trilon B (ml) bound with calcium, ml
0,00012 — the amount of magnesium corresponding to 1 ml of 0.01 N of trilon B solution, g;
k — correction to trilon B;
100 — the volume, in which the ash was dissolved, ml;
c — ash weight, g;
100 — conversion to %.

Water holding capacity

Water holding capacity was measured by pressing according to Grau and Hamm in the modification of Volovinskaya [16]; the amount of water-soluble antioxidants (AWSA) was determined on the device Tsvet-Yauza-01-AA (Khimavtomatika, Russia) by the amperometric method. The activity of glutathione peroxidase, catalase, the concentration of reduced glutathione were determined using

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

commercial Elabscience kits (Elabscience, China) on a Photometer Immunochem-2100 device (High Technology Inc., USA).

Ethics statement

Studies were carried out with approval by the bioethical commission of the L. K. Ernst Federal Research Center for Animal Husbandry (No. 3, May 27, 2022). The experiments were carried out in accordance with the requirements of the Federal Law of the Russian Federation[6], the Declaration of Helsinki[7], the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS No. 123, Strasbourg, 1986)[8].

Statistical analysis

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

Results and discussion

In thigh and breast meat of poultry treated with melanin, an increase in the moisture content was noted compared with the control (at $p < 0.001$ and $p < 0.0001$). In the experimental group, the protein content (at $p < 0.001$), ash (at $p < 0.001$) and the content of minerals calcium, phosphorus (at $p < 0.01$), fat (at $p > 0.05$) in the studied tissues decreased (Table 1).

The pH-45 in the breast of broilers slaughtered at 45 days of age was the same in all groups, and a day later it decreased by 0.13 units (the control), and by 0.14 units (the experimental group). The pH of the femoral muscle decreased from 6.04 to 5.54 units (by 0.5) in the control, and from 6.04 to 5.56 (by 0.48 units) in the experimental group. The water holding capacity (WHC) of thigh muscles in poultry of the control and experimental groups was at the same level and amounted to 58.79 and 58.36%, respectively. The addition of melanin to the broiler ration had a positive effect on the meat antioxidant content and, accordingly, the antioxidant capacity of muscle tissue. Thus, the AWSA in the breast of chickens treated with melanin was 18.75% higher ($p < 0.0001$) compared with the control, in the femoral muscle this difference was 5.6%. The content of reduced glutathione in the breast of the experimental group was 151.87 μM/g, which is 20.25% higher than in the control. There was a trend towards an increase in the concentration of glutathione peroxidase by 10.43% and catalase by 17.35% in the breast of chickens treated with melanin compared with the control (Table 2).

Table 1. Chemical composition of broiler meat at the age of 45 days,% (M ± SEM, n = 27)

Indicator	Group	
	Control	Experimental
Thigh meat		
Moisture	68.22 ± 0.43	70.23 ± 0.70**
Protein	22.18 ± 0.24	20.42 ± 0.41***
Moisture/protein	3.08	3.44
Fat	8.49 ± 0.44	8.29 ± 0.63
Ash	1.11 ± 0.02	1.06 ± 0.03
Calcium	0.05 ± 0.001	0.05 ± 0.0001
Phosphorus	0.17 ± 0.002	0.16 ± 0.0001
Magnesium	0.02 ± 0.0006	0.02 ± 0.0001
Breast meat		
Moisture	71.30 ± 0.29	73.15 ± 0.19***
Protein	26.05 ± 0.23	24.34 ± 0.04***
Moisture/protein	2.73	3.00
Fat	1.35 ± 0.09	1.30 ± 0.08
Ash	1.30 ± 0.02	1.21 ± 0.01***
Calcium	0.06 ± 0.001	0.05 ± 0.0001**
Phosphorus	0.19 ± 0.003	0.18 ± 0.0001**
Magnesium	0.03 ± 0.0007	0.03 ± 0.0001

Note: the differences are significant at: ** $p < 0.01$ *** $p < 0.001$ with the indicators of animals in the control group/

Table 2. Qualitative characteristics of broiler meat aged 45 days (M ± SEM, n = 27)

Indicator	Group	
	Control	Experimental
Breast pH-45	5.91 ± 0.05	5.86 ± 0.04
Breast pH-24	5.78 ± 0.03	5.72 ± 0.04
Thigh pH-45	6.04 ± 0.03	6.04 ± 0.05
Thigh pH- 24	5.54 ± 0.30	5.56 ± 0.43
Thigh WHC, %	58.79 ± 1.0	58.36 ± 1.46
AWSA in the breast, mg/g	0.16 ± 0.002	0.19 ± 0.01***
AWSA in the thigh, mg/g	0.18 ± 0.008	0.19 ± 0.01
Glutathione reduced in the breast, μM/g	126.30 ± 15.15	151.87 ± 7.31
Glutathione peroxidase in the breast, U/g	125.43 ± 8.60	138.52 ± 7.90
Catalase in the breast, U/g	6.11 ± 0.45	7.17 ± 0.72

Note: the differences are significant at: * $p < 0.05$, *** $p < 0.001$ — compared with the control group

The results of single factor analysis of variance show that the moisture ($p = 0.004$), protein ($p = 0.002$), fat ($p = 0.001$), calcium ($p = 0.006$), magnesium ($p = 0.05$) content in breast muscle and the protein ($p = 0.003$), ash ($p = 0.03$), phosphorus ($p = 0.02$), magnesium ($p = 0.01$) content in thigh muscle, pH-45 in the breast ($p = 0.00001$), and pH-24 in the breast ($p = 0.00001$) were associated with BW of poultry (Table 3).

[6] Federal Law of the Russian Federation dated December 27, 2018 No. 498-FZ "On the responsible treatment of animals and on amendments to certain legislative acts of the Russian Federation."Retrieved from https://docs.cntd.ru/document/552045936 Accessed February 06, 2024

[7] WMA Declaration of Helsinki — ethical principles for medical research involving human subjects Retrieved from https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/ Accessed February 06, 2024

[8] European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. Retrieved from https://rm.coe.int/168007a67b. Accessed February 06, 2024

Table 3. Relationship of the body weight (BW) with the chemical composition and quality of broiler muscle tissue (n = 54) (results of one-way analysis)

Indicator	BW	p-value
Thigh meat		
Moisture	***	0.004
Protein	***	0.002
Fat	***	0.001
Ash	n.r.	0.02
Calcium	***	0.006
Magnesium	*	0.05
Phosphorus	n.r.	0.20
Thigh pH-45	n.r.	0.10
Thigh pH- 24	n.r.	0.10
Thigh WHC	n.r.	0.95
Breast meat		
Moisture	n.r.	0.08
Protein	***	0.003
Fat	n.r.	0.35
Ash	*	0.03
Calcium	n.r.	0.70
Magnesium	*	0.01
Phosphorus	*	0.02
Breast pH-45	***	0.00001
Breast pH-24	***	0.00001
Glutathione reduced in the breast	n.r.	0.05
Glutathione peroxidase in the breast	n.r.	0.42

Note: the differences are significant at: * $p < 0.05$, ** $p \leq 0.01$; *** $p \leq 0.001$; n. r. — no relationship

The saturated fatty acid content (SFAs) in the studied abdominal fat samples was 21.21–21.50%, while monounsaturated fatty acids (MUFAs) were at a level of 33.03–33.65%, polyunsaturated fatty acids (PUFAs) at a level of 44.89–45.76%. The sum of n-6 fatty acids ranged from 40.93 to 41.73%, and n-3 from 3.87 to 3.93%.

The feeding factor had a positive effect on the content of erucic and 8,11,14-eicosatrienoic acids. The differences between the animal groups were significant, at $p = 0.02$ and $p = 0.07$, respectively (Table 4).

Poultry meat is a rich source of protein, which according to various sources comprises 23–25% in the breast and 18% in the thigh [17]. The main amino acids (AA) in poultry meat (asparagine, lysine, leucine, arginine, glutamine) determine the taste and technological properties of meat. The AA content is usually maintained, but may vary depending on a diet and its amino acid composition. Some experiments show that increasing the quantity of certain AAs in birds diets before slaughter leads to an increase in the AA content in tissues [17,18]. In our studies, the protein level in the breast was 24.34–26.05%, and in the thigh — 20.42–22.18%, and in the group of poultry that received melanin, the indicators were slightly lower than in the control. At the same time, the moisture content in both the pectoral and femoral muscles in the poultry of the experimental group was significantly higher (at $p < 0.001$ and $p < 0.0001$) than in the control, which may be more attractive in terms of consumer and culinary properties of chicken. An increase in the dietary properties of meat from poultry treated with melanin is indicated by a decrease in the fat content in the breast and thigh compared to poultry of the control group. Similar results were observed by other authors who established a direct relationship between the use of additional vitamins with the antioxidant properties in poultry diets and a decrease in fat deposition in tissues [19]. A possible mechanism of this action is the fact that an increase in the level of antioxidants in the diet is directly related to an increased level of the hormone T3 and the enzyme iodothyronine deiodinase in the blood. Thyroid hormones regulate the metabolic activity in animals and can stimulate the mobilization of fat reserves and reduce their deposition on the carcass [20].

The mineral content in the muscles of the broilers used in the experiment was 1.06–1.30%. In our studies, there was no difference between the groups in the content of individual mineral elements, both in the femoral and pectoral muscles of broiler chickens. As is known from the literature, feeding and other growing factors have little effect on these values if the feed intake meets the needs of animals [19].

pH is an important indicator that shows the mobilization of glycogen reserves, resulting in the formation of lactic acid in meat, which affects the isoelectric point of the main muscle proteins and determines their ability to retain or release water. The pH level of meat can affect its susceptibility to oxidation. The more acid in meat, the higher the risk of oxidation [21]. In our studies, we did not observe significant differences between the groups in this indicator. The pH 45 minutes after slaughter in both groups was at a level of 5.86–5.91 in the breast and 6.04 in the thigh. A day after slaughter, this indicator in the breast muscle decreased by 0.13 units in the control group, by 0.14 units in the experimental group, and by 0.5 and 0.48 units in the thigh muscle, respectively.

There was no difference between the groups in the WHC. Other authors have pointed out that the inclusion of antioxidants in the diet has a direct effect on the water holding capacity (WHC) of meat. By increasing the content of selenium yeast in feed cooking loss was reduced by 5% [22]. The same picture was obtained when zinc was added to the diet. The WHC can be affected by age, genotype, and other conditions. In broiler chickens slaughtered at the age of 35 to 63 days, juice losses after storage and cooking decreased by 1% and 0.6%, respectively [17].

An important characteristic in evaluation of broiler chicken growth and meat quality is a degree of correlation between the main parameters and live weight. High ($p < 0.05$) correlation of some important nutritional characteristics (protein, fat, calcium, phosphorus, magnesium content in thigh and breast muscles), as well as qualitative parameters (pH) with live weight has been experimentally established, which should be taken into account when raising poultry of modern crosses.

Table 4. Fatty acid composition of abdominal fat of broilers, % (M ± SEM, n = 27)

Indicator		Group		p-value
		Control	Experimental	
Caproic acid	$C_{6:0}$	0.0047 ± 0.0019	0.0042 ± 0.00199	0.70
Caprylic acid	$C_{8:0}$	0.0046 ± 0.0009	0.0037 ± 0.0004	0.59
Capric acid	$C_{10:0}$	0.0075 ± 0.0015	0.0061 ± 0.0009	0.81
Caproleic acid	$C_{10:1}$	0.0062 ± 0.0013	0.0052 ± 0.0004	0.54
Lauric acid	$C_{12:0}$	0.0118 ± 0.0012	0.0114 ± 0.0007	0.48
Tridecanoic acid	$C_{13:0}$	0.0025 ± 0.0005	0.0023 ± 0.0004	0.58
Myristic acid	$C_{14:0}$	0.2616 ± 0.0084	0.2498 ± 0.0065	0.41
Pentadecanoic acid	$C_{15:0}$	0.0630 ± 0.0032	0.0569 ± 0.0014	0.20
Cis-10-Pentadecenoic acid	$C_{15:1}$	0.0173 ± 0.0038	0.0120 ± 0.0008	0.28
Palmitic acid	$C_{16:0}$	15.7141 ± 0.3062	15.8129 ± 0.0297	0.74
Margaric acid	$C_{17:0}$	0.1338 ± 0.0039	0.1380 ± 0.0069	0.37
Stearic acid	$C_{18:0}$	4.7617 ± 0.0916	4.9493 ± 0.1268	0.41
Nonadecanoic acid	$C_{19:0}$	0.0209 ± 0.0015	0.0206 ± 0.0016	0.89
Heneicosanoic acid	$C_{21:0}$	0.0591 ± 0.0079	0.0549 ± 0.0026	0.96
Behenic acid	$C_{22:0}$	0.0298 ± 00025	0.0269 ± 0.0017	0.58
Lignoceric acid	$C_{24:0}$	0.0317 ± 0.0030	0.0309 ± 0.0024	0.99
Myristoleic acid	$C_{14:1}$	0.0442 ± 0.0050	0.0379 ± 0.0026	0.39
Palmitoleic acid	$C_{16:1}$	2.4910 ± 0.0098	2.4714 ± 0.1385	0.86
Cis-10-Heptadecenoic acid	$C_{17:1}$	0.0782 ± 0.0037	0.0743 ± 0.0027	0.48
Oleic acid	$C_{18:1}$	30.1205 ± 0.3989	30.7619 ± 0.4741	0.81
Gondoic acid	$C_{20:1}$	0.2038 ± 0.0110	0.2098 ± 0.0061	0.88
Cis-11,14-Eicosadienoic acid	$C_{20:2}$	0.1505 ± 0.0079	0.1564 ± 0.0072	0.51
Erucic acid	$C_{22:1}$	0.0063 ± 0.0010	0.0068 ± 0.0010*	0.02
Nervonic acid	$C_{24:1}$	0.0122 ± 0.0030	0.0105 ± 0.0026	0.45
Linoleic acid	$C_{18:2}$	41.5611 ± 0.7080	40.7790 ± 0.7647	0.84
Linolenic acid	$C_{18:3(n6)}$	0.1236 ± 0.0070	0.1135 ± 0.0067	0.46
Arachic acid		0.0988 ± 0.0058	0.0913 ± 0.0044	0.24
α-linolenic acid	$C_{18:3(n3)}$	3.8004 ± 0.0609	3.7362 ± 0.0779	0.77
Cis-8,11,14-Eicosatrienoic acid	$C_{20:3}$	0.0222 ± 0.0081	0.0124 ± 0.0020	0.34
Cis-11,14,17-Eicosatrienoic acid	$C_{20:3}$	0.1024 ± 0.0064	0.1065 ± 0.0070	0.07
Arachidonic acid	$C_{20:4}$	0.0239 ± 0.0019	0.0243 ± 0.0019	0.36
Cis-5,8,11,14,17-eicosapentaenoic acid	$C_{20:5}$	0.0152 ± 0.0029	0.0142 ± 0.0013	0.57
Cis-4,7,10,12,15,19-docosahexaenoic acid	$C_{22:6}$	0.0156 ± 0.0076	0.0088 ± 0.0031	0.63
The sum of acids				
The sum of SFA		21.2082 ± 0.3221	21.4592 ± 0.3276	
The sum of MUFA		33.0285 ± 0.4896	33.6546 ± 0.5954	
The sum of PUFA		45.7633 ± 0.7624	44.8863 ± 0.8274	
The sum of UFA		78.7918 ± 0.3221	78.5409 ± 0.3276	
n-6		41.7308 ± 0.7086	40.9292 ± 0.7643	
n-3		3.9336 ± 0.0677	3.8657 ± 0.0820	
MUFA/SFA		1.5592 ± 0.0170	1.5711 ± 0.0241	
n-6/n-3		10.6257 ± 0.1306	10.6318 ± 0.1763	

Note: the differences are significant at: * $p < 0.05$ — compared with the control group.

In our studies, chickens treated with melanin had a higher content of the amount of water-soluble antioxidants (AWSA) in the breast by 18.75% ($p < 0.0001$) and in the thigh by 5.6% compared to the control. The reduced glutathione content of the breast in the experimental group was 151.87 μM/g, which was 20.25% higher than that in the control. There was a tendency towards an increase in glutathione peroxidase by 10.43% and catalase by 17.35% in the breast of chickens treated with melanin compared with the control. Due to these facts, meat from poultry that received melanin was less exposed to oxidative effects during ageing. Other researchers have also observed improvements in meat quality and oxidative stability of broiler muscles when using various antioxidants in diets [23,24].

Chicken meat is rich in unsaturated fatty acids (FAs), which makes it more susceptible to lipid oxidation and the formation of volatile organic compounds. FAs of chicken meat consist of about one-third of SFAs, one-third of MUFAs and one-third of PUFAs [25].

The results of the study by Kanakri et.al [25] show that the content and ratio of fatty acids in diets largely determine their profile in meat and other tissue, which makes it possible to predict the poultry FA composition. The same opinion was reached by other researchers who showed that feeding the fat of insect larvae contributed to an increase in the proportion of SFA to the detriment of PUFA in breast and thigh with an increase in the ratio of n-6 FA/n-3 [26,27].

The use of melanin in broiler diets contributed to an increase in the content of erucic and 8,11,14-eicosatrienoic acids in abdominal fat compared with the control (at $p = 0.02$ and $p = 0.07$, respectively). Cis-11,14,17-eicosatrienoic acid belongs to omega-3 PUFAs, which are part of cell membranes and blood vessels, are not synthesize in the right amounts in the human body and are one of the necessary components of a full-fledged healthy diet.

The FA content in broilers can also be influenced by gender, genotype and the growing system. For example, it was found that a higher percentage of n-3 PUFAs was produced in slow-growing chickens compared to fast-growing ones [28]. Castellini et al. [29] showed that this content could increase in slow-growing birds raised in free range and in organic systems due to high grass consumption. At the same time, the consumption of herbs improved the antioxidant content of poultry meat and prevented the oxidation of PUFAs [30]. The energy source of the feed can also affect the FA composition of poultry meat. Diets with the high carbohydrate content will promote lipogenesis in the liver and, consequently, the synthesis of SFAs and MUFAs, whereas diets with a high fat content will rather contribute to the direct deposition of dietary fats in peripheral tissues [31].

Conclusion

The use of antioxidants in broiler diets not only has a positive effect on the health of fast-growing poultry, but also contributes to improving product quality. In our studies, it has been found that the inclusion of melanin in the diets of broilers of the Smena-9 cross contributes to an increase in the moisture content in the pectoral and femoral muscles, the enrichment of meat with antioxidants, and an increase in the content of erucic and eicosatrienoic acids in abdominal fat. The absence of the effect of melanin feeding on the WHC and pH of broiler muscle tissue requires further study.

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The authors declare no conflict of interest.

DOI: https://doi.org/10.21323/2414-438X-2024-9-2-117-124
Received 19.05.2024
Accepted in revised 31.05.2024
Accepted for publication 04.06.2024

Available online at https://www.meatjournal.ru/jour
Review article
Open Access

MICROBIOLOGICAL AIR CONTROL OF FOOD INDUSTRY ENTERPRISES: RELEVANCE, REGULATORY DOCUMENTS AND RESEARCH METHODS

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Keywords: *contamination, air, food products, maximum permissible level, pathogenic bacteria*

Abstract

A review of regulatory documents on air control was carried out; approaches to air sampling at food industry enterprises and methods of air disinfection were considered. It has been established that air is one of the important aspects in ensuring the quality and safety of food products. Air is a transport medium for pathogenic and spoilage microorganisms. The concentration of microorganisms and the area of their distribution in the air of industrial premises are influenced by the technological features of the products being manufactured and the design of the enterprise. The transfer of microorganisms at food industry enterprises occurs due to bioaerosols that are formed during high-pressure washing. The use of air filters allows reducing the level of microorganisms in the indoor air. Microbial air monitoring should be carried out during the technological process at critical control points (CCP). To assess air, it is advisable to select those microorganisms that cause spoilage of manufactured products or affected their safety. Passive and active air sampling methods have certain limitations. There are various methods of air disinfection, the main ones being fogging, ozonation and UV irradiation. The choice of the method should be made based on the characteristics of a particular enterprise. In Russian regulatory documents, microbiological indicators when assessing air at food industry enterprises are limited to total microbial count, yeast and mold count, while in foreign practice, the choice of indicators is based on those microorganisms that caused spoilage of finished products released from a particular enterprise. It is necessary to develop modern integrated approaches to ensure air control at food industry enterprises and establish regulatory documents on microbiological indicators and their permissible levels.

For citation: Yushina, Yu.K., Bataeva, D.S., Reshchikov, M.D., Grudistova, M.A., Makhova, A.A., Zaiko, E.V. (2024). Microbiological air control of food industry enterprises: Relevance, regulatory documents and research methods. *Theory and Practice of Meat Processing,* 9(2), 117–124. https://doi.org/10.21323/2414-438X-2024-9-2-117-124

Funding:

The article was published as part of the research topic No. FGUS-2024–0002 of the state assignment of the V. M. Gorbatov Federal Research Center for Food Systems of RAS.

Introduction

Air monitoring for microbial contamination, which is included in HACCP risk analysis system, is recognized as an important aspect of product quality control at food industry enterprises [1].

Air flows, particularly in industrial premises, transfer suspended droplets of liquid, solids and microorganisms (bacteria, spores, molds, yeasts, phages), which together represent aerosols [2] or bioaerosols [3] up to 50 μm in diameter [4,5]. Bioaerosols transport almost all microorganisms in indoor air. Although their reproduction in the air is difficult; they can survive in it by settling on dust particles [6]. The air itself does not promote the growth of microorganisms and acts only as a supporting medium or carrier until they settle on the surface of objects.

The purpose of this article is to establish the relevance of microbial air monitoring at food industry enterprises. To do this, it is necessary to review the regulatory documents on air control at food industry enterprises, analyze modern approaches to air sampling in industrial premises, and also focus on modern methods of air treatment.

Materials and methods

The object of the study was the publications of domestic and foreign scientists on the microflora of air at food industry enterprises and methods of air treatment, as well as regulatory documents establishing rules, general principles or characteristics of air at food industry enterprises. The data search was carried out in the ScienceDirect, Google Scholar, eLibrary and other open-source electronic databases. Combinations of keywords were used, such as microbiological composition of air, bioaerosols, microorganisms in the air, air disinfection, maximum permissible level. Keywords were used in English and Russian. In addition, the search for related articles was carried out using citation chains. Non-peer-reviewed, uninformative and duplicate sources, as well as those not related to the topic of research, were excluded from the search results.

Air microflora at agricultural enterprises

At food industry enterprises, bioaerosols may contain various microorganisms, including spores of *Bacillus* spp. and *Clostridium* spp., gram-positive *Micrococcus* spp. and *Staphylococcus* spp., molds *Penicillium* spp., *Cladosporium* spp., *Alternaria* spp., *Fusarium* spp., as well as yeasts *Saccharomyces* spp., *Torulaspora* spp., *Hanseniaspora* spp., *Pichia* spp. [7].

Microorganisms that are found in food raw materials, in moisture on equipment or in wastewater, become aerosols during food production or during washing process (spraying or splashing water) [8,9]. Then aerosols are transferred by air flows to objects located inside the enterprises (food products, raw materials, industrial environment objects) [10]. The smaller the size of an aerosol particle suspended in the air, the longer it stays in the air flow and the longer it may travel [3,11]. Brandl et al. [10] conducted a study to measure the concentration of aerosol particles, as well as the concentration of bacteria, yeast and molds in the air (at least 100 liters were sampled) of a milk powder processing plant. A correlation was established between the number of particles and the number of viable cells in the air. Bacterial counts were highly correlated with the total number of airborne particles of 1 to 5 μm, suggesting that a simple control system based on airborne particle counts could be implemented. The number of cultivated microorganisms on average was less than 100 CFU per 1 m^3 of air, but in the areas of finished product filling and packaging their number was higher. Based on the identification of the isolated bacteria, they were represented by 4 main types: *Firmicutes* (41%), *Acinobacteria* (28%), *Proteobacteria* (26%) and *Bacteroidetes* (5%). The dominant bacterial genera were *Staphylococcus* and *Bacillus*. Molds were also found, represented by *Penicllium cammberti, Penicillium glabrum* and *Scropulariopsis brevicaulis*. In conclusion, the authors indicated that the microbial composition of the bioaerosols was typical for this production.

Pearce et al. [12] assessed the concentration of *Escherichia coli* and *Salmonella* spp. in the air of a pig slaughter plant. An impactor type sampler was used to take air samples (volume of at least 100 liters). According to the data obtained, the concentration of microorganisms in the air increased during the slaughter of animals. Before the slaughter, the contamination was 1.58 to 2.49 lg CFU/m^3, and after 11 hours of slaughter, values of 2.74 to 3.61 lg CFU/m^3 were obtained. The lowest concentration of microorganisms was observed in the air of refrigeration chambers, and the highest was in the area of carcass bleeding and scalding. However, *Escherichia coli* counts in the air decreased as work progressed. At the same time, the lowest counts of *Escherichia coli* also remained in the refrigerators. *Salmonella* spp. were detected only in three air samples taken from the scalding area and the evisceration area.

Prendergast et al. [13] conducted a comparative assessment of microbial air contamination of two cattle slaughter plants with different designs. At both enterprises, the smallest number of microorganisms, i. e. about 1.0 lg CFU/m^3 of air, was observed before the start of the work process. On the slaughter line with a straight single-section structure, a decrease in air contamination was observed from the "dirty" to the "clean" zones. However, airborne contamination at the slaughter line with a winding two-section structure showed the opposite trend. For example, at the skinning site, the total microbial contamination of the air at the first enterprise was 3.49 ± 0.29 lg CFU/m^3, and at the second enterprise it was 3.03 ± 0.29 lg CFU/m^3, while the air contamination at the site, which immediately follows the carcass wet processing, decreased to 1.79 ± 0.29 lg CFU/m^3 and to 2.78 ± 0.29 lg CFU/m^3, respectively.

The results of studying the air of industrial premises show great variability in microbial contamination depending on a number of factors, such as the type of raw materials processed, design of industrial premises, manufacturing technologies and hygienic requirements. As a rule, less than 1% of aerosols settle in rooms with a high level of hygiene, because most of them are removed by the ventilation system and retained by filters [14,15]. Particles of 1 to 20 μm, which are easily dispersed directly around the aerosol generation zone, are of particular concern.

Regulatory documents on air control

In Russia and other countries, there is not enough information about the maximum permissible levels (MPL) of microorganisms in the air of food industry enterprises during the technological process.

The document developed in 1995 [16], "The procedure for sanitary and microbiological control in the production of meat and meat products" provides only permissible levels of mold content in refrigeration chambers at meat industry enterprises.

To assess air quality of industrial premises at fish and marine invertebrates processing enterprises, "Instructions for sanitary and microbiological control of food production from fish and marine invertebrates" was developed in 1991 [17]. According to this document, to assess the sanitary state of air in industrial premises, two indicators are standardized: total microbial count and mold count. To determine them, it is proposed to use two methods of air sampling: sedimentation and aspiration. The same document provides the MPL of standardized indicators for each method.

As a part of production control, in workshops for the production of pasteurized canned foods, air condition is determined based on total microbial count and mold count, as well as the presence of coliforms in 1 m^3 of air. This is reflected in the "Instructions on the procedure and frequency of monitoring the content of microbiological and chemical pollutants in meat, poultry, eggs and their processed products" developed in 2000 [18].

"Instructions for sanitary and microbiological control of carcasses, poultry meat, poultry products, eggs and egg products at poultry and poultry processing enterprises" [19] developed in 1990 also reflects the requirements for

the microbiological state of air in industrial premises during poultry processing and establishes three indicators: total microbial count (TMC), mold and yeast count.

The sanitary condition of the air in refrigeration chambers was determined by the total number of molds and the number of *Cladosporium* and *Thamnidium*, which contribute to the spoilage of meat products, especially meat. However, SP 4695–88 "Sanitary rules for refrigerators" [20] expired in 2021. Instead, in terms of sanitary and epidemiological requirements for refrigeration equipment (refrigeration chambers) in relation to product safety and requirements for the processes of its production, storage, transportation, sale, operation, application (use), the following documents have been established: SanERR2.3/2.4.3590–20 "Sanitary and epidemiological requirements for the organization of public catering" [21], SP 2.3.6.3668–20 "Sanitary and epidemiological requirements for the conditions of operation of retail facilities and markets selling food products" [22], SP 2.4.3648–20 "Sanitary and epidemiological requirements for organizations of education and training, recreation and health improvement of children and youth" [23]. However, none of these documents contain requirements for the microbiological quality of air in refrigeration chambers. Also, instead of SP 4695–88, in relation to sanitary and microbiological methods for studying refrigeration equipment (refrigeration chambers), according to the Decree of the Government of the Russian Federation N1850 dated November 16, 2020 [24], methodological recommendations MR4.2.0220–20 "Methods of sanitary and bacteriological investigation of microbial contamination of environmental objects" [25] may be applied. However, these MR only apply to the assessment of washouts. Thus, the documents introduced to replace SP 4695–88 do not contain information on assessing the air of refrigeration chambers.

In 2022, MR2.3.0279–22 "Recommendations for the implementation of production control over the compliance of manufactured products with standards, technical regulations and specifications" was developed [26]. However, this document did not reflect the control of the sanitary condition of air in industrial premises.

Among foreign sources, we can highlight the "Guidelines on air handling systems in the food industry — Air quality control for building ventilation" [27], which was developed by the European Hygienic Engineering & Design Group (EHEDG). In this guide, the authors draw attention to the importance of monitoring the state of air at food industry enterprises, which includes physical factors such as temperature, humidity, as well as biological factors, i. e. the number of microorganisms. However, there are no recommendations on methods of air sampling and the study of microorganisms, as well as on their permissible levels in indoor air. Another document that addresses the topic of assessing air quality in food industry is the environmental management guide developed by 3M [28]. The authors consider air as one of the important routes for fungal spore transfer and recommend monitoring its quality using the sedimentation method. Guided by this document, the manufacturer should know that sampling points and frequency are indicated in the production control program and they are specific for each enterprise. Taking a closer look at the issue of the pathogenic microorganism spread at the enterprise by aerosol, the authors concluded that a more appropriate strategy for solving the problem would be to identify the sources and locations of aerosol formation rather than monitoring the air for the presence of pathogens in aerosols.

Air sampling methods

The stage of air sampling for microbiological assessment is also important. Air sampling methods may be divided into two categories: passive and active ones.

Passive method

The passive or sedimentation method is based on the ability of microorganisms under the influence of gravity and under the influence of air flow (together with dust particles and aerosol droplets) to settle on the surface of the nutrient medium in open plates. The number of microorganisms present is measured in CFU/m^2/t, where t is a unit of time. It is known that small and light particles remain in the air longer than large and dense ones. In addition, if the air flow rate exceeds the rate of deposition, the particles will remain suspended for indefinite period. Even indoors, air flow is subject to slight temperature fluctuations, so the volume of air in a passively collected sample will be unknown. The combination of these and other factors has a significant impact on the representativeness of the sample obtained by the passive method [29].

Active method

In the active sampling method, air sampler physically draws a predetermined volume of air and passes it through a particle collection substrate, which may be a liquid, solid medium, or a nitrocellulose membrane. The number of microorganisms present in the sample is measured in CFU/m^3 of air.

There are several types of active samplers, with the most popular being impactors, impinger and electrostatic samplers.

Impactors

Inertia of particles is used to facilitate collection. The air sample is passed through a series of nozzles that direct the air with particles toward a plate containing a dense nutrient medium positioned perpendicular to the nozzle outlet. The plate deflects air flows by 90°, while part of the air passes by through the space between the plate and the walls of the device. Particles with sufficiently low inertia are carried away by air flow and do not settle on the nutrient medium. However, particles with higher inertia cannot follow the 90° curve of the air flow and, under the influence of centrifugal force, hit the dense nutrient medium or

membrane filter. Thus, the efficiency of particle capture by an impactor primarily depends on the diameter and density of particles, the diameter of the nozzle, and also on the air flow rate [29]. One of the main advantages of using impactors for air sampling to identify microorganisms is their ease of use. For example, after taking air samples, plates with a dense nutrient medium are transferred to a thermostat without intermediate steps. However, the collision of microorganisms with a dense nutrient medium may harm them, including loss of cultivability [30,31] and even loss of membrane integrity [32], which reduces the proportion of culturable microorganisms. However, ease of use and extensive reference information make agar-based impactors the preferred tool in many studies [33].

Impinger

Impingers direct the flow of air containing particles through nozzles that exit into a chamber containing liquid. When particles in air come out of the nozzles, they enter the collection chamber. The distance from the nozzle outlet to the liquid surface, together with the air flow rate, influences the diameter of the particles collected. The collection of airborne microorganisms into the liquid prevents the collected particles from drying out, but the shear forces in the air, combined with the turbulence caused by forcing air into the chamber, may cause them to lose viability. This bioefficiency (the ability of the sampling device to maintain the viability of the bioaerosol during and after sampling) may also be reduced by evaporation, re-aerosolization (loss of previously collected particles), and particle adhesion to internal walls of the collection chamber [34,35].

Other sampler types

A special type of bioaerosol impactors are fungal spore traps. Most of these impactors are disposable devices with a single circular nozzle or slit that directs airborne particles toward a glass slide with an adhesive surface. After sampling, such impactors are disassembled and the slide is examined under a microscope.

A less popular sampling method is electrostatic deposition. Upon entering an electrostatic sampler, bioaerosol particles are electrically charged and then pass through an electric field, where they are separated from the air flow and deposited on charged plates. Despite active research on the natural charge of bioaerosol particles, the efficiency and design of electrostatic precipitators, there is concern that the electric field affects microbial viability. Therefore, more extensive research is needed for their widespread implementation in industrial practice [36,37].

Air sampling to control pathogenic microorganisms

Pearce et al. [12] conducted a study aimed at isolating *Salmonella* from air samples taken at different stages of pig slaughter and processing. Passive and active (manual impactor) sampling methods were used. Samples were collected on non-selective agar (PCA). The procedure for enriching microorganisms was carried out by adding agar from a plate to a buffered peptone solution with further incubation at a temperature of 37 °C for 24 hours. After incubation, an aliquot of the enriched culture of 0.1 cm^3 was transferred to 10 cm^3 of Rappaport Vassiliadis (RV) medium and incubated at a temperature of 42 °C for 24 hours. After the incubation, the RV medium was swabbed onto brilliant green agar (BGA) and then incubated at 37 °C for 24 hours. The grown black colonies were determined as *Salmonella* using biochemical and serological tests. Pathogenic microorganisms (*Salmonella* spp. and *Listeria* spp.) found in the air of food industry enterprises as a part of aerosols [38] are very often subject to significant stress, leading to their damage and/or death.

In the other work, the researchers collected air samples from several cattle, pig and sheep slaughter and processing plants. As in the previous case, the impaction and sedimentation methods were used, and the target indicators were *Salmonella* and *Listeria*. To detect *Salmonella*, air sampling was carried out in parallel on two nutrient media: non-selective PCA agar and selective BGA agar [38]. PCA enrichment procedure was carried out in the same way as by Pearce et al. [12], except that the volume of the buffered peptone solution was 200 cm^3. BGA agar plates were incubated with air sample at 37 °C for 24 hours. After incubation, typical colonies were selected and cultured on BGA and XLD agars and incubated under the same conditions. In both cases, after incubation, typical colonies were selected, transferred to MacConkey agar and incubated under the same conditions, followed by colony identification using API 20E biochemical tests. To detect *Listeria*, air sampling was carried out in the same way as for detecting *Salmonella*. LSA agar with selective additive SR140 was used as a selective medium. The contents of non-selective media plates were transferred to Listeria enrichment broth (LEB) and incubated at 30 °C for 48 hours, after which they were passaged onto LSA agar. Selective medium plates were incubated under the same conditions. After incubation, typical colonies were selected and determined as *Listeria* using API Listeria biochemical test and Listeria test kit.

In a study by Dobeic et al. [39], in order to detect *Listeria* in the air of slaughterhouses, polycarbonate filters were used as a substrate for collecting cells of microorganisms of this genus, placed on the bottom of plates and soaked in 2 cm^3 of the primary enrichment Fraser broth with half the concentration of antibiotics. The moistened filter material served as a trap for dust particles, aerosol and possible bacterial cells. The samples were delivered to the laboratory within several hours. Before incubation, an additional 8 cm^3 of Fraser broth with half the concentration of antibiotics was added to each plate and the contents were gently shaken. Cultures were incubated at 30 °C for 24 hours. Subsequently, 0.1 cm^3 of inoculum was transferred into 10 cm^3 of the secondary enrichment Fraser broth with a full concentration of antibiotics. Using a loop for subculture, inoculum was also taken from the primary enrich-

ment medium onto selective media: ALOA agar or Palcam agar, followed by incubation at 37 °C for 24 to 48 hours. The same procedure was repeated with the culture obtained in secondary medium after 48 h of incubation. Up to five representative colonies of *Listeria* spp. grown on ALOA and Palcam agars were transferred to blood agar to determine hemolytic activity. Final identification was made using the Listeria API kit.

Air purification and disinfection methods

Modern ventilation systems are designed to ensure pure air both entering and leaving industrial premises. Separate air filtration systems should be used to reduce the risk of cross-contamination between different production areas. Any ventilation systems must have filters and insulated panel casing consisting of a frame and various fixed and removable access panels. Air treatment is achieved using HEPA filters. Primary air filters protect the mechanical elements of the air flow system from heavy contamination throughout many years of operation. Secondary filters are used to remove fine particles down to levels necessary to maintain process hygiene. Rigid cellular filter ensures that the selected level of air purity is maintained throughout the entire life of the filter. To ensure the overall efficiency of the system, it is necessary to use a sealed filter mounting. Tertiary filters provide the best protection in units where maximum particle control is required. These are typically HEPA filters or ultra-low penetration air (ULPA) filters [16]. The required degree of filtration largely depends on the technology of the product being manufactured. For example, the presence of HEPA filters reduces the number of molds indoors by 30 times [11]. Maintaining the purity of input-outlet equipment is mandatory for its effective functioning at food industry enterprises [17]. The required efficiency of input-outlet equipment in the ventilated area should be set in accordance with the maximum permissible level (MPL) of microorganisms. In the air of industrial premises, the species composition of microorganisms and their numbers may vary greatly [40]. To provide consumers with safe and high-quality products, the manufacturer must be interested in the effectiveness of regular cleaning and disinfection procedures. However, due to the increasing resistance of microorganisms to various disinfectants, there is an urgent need to introduce additional approaches to air disinfection, in particular, fogging, ozonation and UV irradiation.

Fogging method is based on spraying a disinfectant to form an aerosol with a given particle size. Various commercial systems are available on the market, both static, which are integrated into the premises' communications network, and the most commonly used, mobile. The effectiveness of this method of air disinfection using various disinfectants based on quaternary ammonium compounds [41], peracetic acid [42], hydrogen peroxide [41] has been confirmed by a number of scientific studies.

Ozonation is based on the use of ozone gas. Ozone is a strong oxidizing agent; it has an antimicrobial effect and is effective against bacteria, fungi, viruses and protozoa. As for the spectrum of action, each microorganism is inherently sensitive to ozone. Bacteria are more sensitive than yeasts and molds. Gram-positive bacteria are more sensitive to ozone than gram-negative microorganisms, and spores are more resistant than vegetative cells. In the US, the permissible level of ozone exposure in the workplace is 0.1 ppm, as adopted by the Occupational Safety and Health Administration (OSHA). This is the concentration at which a person may be continuously exposed to ozone under normal operating conditions for 8 hours per day or 40 hours per week without any adverse effects. The short-term exposure limit is 0.3 ppm, which means exposure lasting less than 15 minutes no more than 4 times per day with intervals of at least 1 hour between each short-term exposure. Safety aspects must always be taken into account, especially when ozone gas is used in refrigerating chamber, rooms or enclosed spaces. In such situations, it is necessary to accurately control the concentration at various critical points and establish appropriate safety intervals before opening to avoid risk to human health. Ozone is a toxic gas that must be monitored in the workplace when used to disinfect equipment and installations. A wide range of ozone sensors are available to monitor its levels. These are typically UV analyzers equipped with a cell that measures concentrations from 0.1 to 100 ppmv, which trigger an alarm as soon as the ozone concentration rises above 0.1 ppm [43]. Ozone treatment is performed after washing procedures, since its bactericidal activity decreases in the presence of residual organic compounds. Portable units are used to form ozone from atmospheric oxygen. Ozone interacts with surfaces and equipment, so before use, it is necessary to ensure that the materials used at the enterprise are resistant to ozonation. The effectiveness of ozonation is confirmed by a number of scientific studies. Thus, Serra et al. [44] revealed a 10-fold reduction in airborne viable molds in a cheese ripening chamber when ozonation was used for 20 weeks. Masotti et al. [40] assessed the effectiveness of ozonation in a dairy packaging facility over a period of 5 weeks. The authors found that there was no growth of bacteria and fungi in 92% of air samples taken after air treatment with ozone 3 days a week for 3 hours.

UV is capable of destroying molecular bonds in DNA and thereby inactivating microorganisms. Short-wave UV radiation (254 nm) has been shown to reduce microbial load both in the air and on solid surfaces free of organic residues [45]. The effectiveness of UV irradiation depends on many different parameters, such as intensity, exposure time, lamp location and air flow patterns.

The disinfecting ability of UV is well known and widely used in medical and veterinary practice, as well as in the disinfection of air, surfaces and instruments [46]. The microbial status of the air in egg incubation cabinets has also been improved using UV light installations [45]. UV light has been shown to be able to reduce airborne microbial counts by 4 log units [47].

The susceptibility of airborne microorganisms to UV radiation depends on temperature and relative humidity. For example, as relative humidity increases, UV radiation becomes less effective [48]. Uniform distribution of the required UV dose in large volumes of air is a major challenge given the current state of technology [49]. Today, UV inactivation of bioaerosols is considered an additional method to standard cleaning and disinfection procedures.

Conclusion

Based on a review of regulatory documents on air control at food industry enterprises, consideration of modern approaches to air sampling in industrial premises, as well as modern methods of air treatment, the following conclusions were made.

It has been established that air is one of the important aspects in ensuring the quality and safety of food products. Air is a transport medium for most pathogenic and spoilage microorganisms. The concentration of microorganisms and the area of their distribution in the air of industrial premises are influenced by various factors, including the technological features of the products being manufactured and the design of the enterprise. The transfer of microorganisms at food industry enterprises occurs due to bioaerosols that settle on the surface of equipment, finished products or raw materials. The formation of bioaerosols is caused by procedures involving the use of water or air under high pressure. The use of air filters allows reducing the level of microorganisms in the indoor air. Microbial air monitoring should be carried out during the technological process at critical control points (CCP). To assess air, it is advisable to select those microorganisms that cause spoilage of manufactured products or affected their safety.

Existing air sampling methods, both passive and active, allow the collection of air samples, but with certain limitations. The passive (or sedimentation) method makes it possible to capture larger particles settling under the influence of gravity. To use the active method based on forced particle settling, a special device is necessary.

There are various methods of air disinfection, the main ones being fogging, ozonation and UV irradiation. These methods have both a number of advantages and a number of disadvantages, so the choice should be made based on the characteristics of a particular enterprise.

Currently, in Russian regulatory documents, microbiological indicators when assessing air at food industry enterprises are limited to total microbial count, yeast and mold count. At the same time, in foreign practice, the choice of indicators is based on those microorganisms that caused spoilage and influenced the safety of finished products released from a particular enterprise.

Thus, we believe that it is necessary to develop modern integrated approaches to ensure air control at food industry enterprises and establish regulatory documents on microbiological indicators and their permissible levels.

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

The authors declare no conflict of interest.

DOI: https://doi.org/10.21323/2414-438X-2024-9-2-125-134
Received 13.02.2024
Accepted in revised 02.06.2024
Accepted for publication 07.06.2024
Available online at https://www.meatjournal.ru/jour
Review article
Open Access

DIVERSITY OF *SATE* (SATAY) AS INDONESIAN ANCIENT FOOD

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Keywords: *ancient food, diversity, food system, sate, food security*

Abstract
Sate (satay) dishes have a wealth of ingredients and spices spread throughout Indonesia, which produces a diversity of types and flavors of satay. In sate dishes, the way pieces of meat are served on skewers was affected by Arabic culture, which influence is most visible in the development of Indonesian food culture. On average, sate is made using grilling, which is an ancient type of cooking technique survived into modern times. For centuries, wood and charcoal have been some of the oldest human-made fuels as important ingredients for cooking and heating in ancient times and even today. Apart from being an everyday food, sate is Indonesia's gastronomic culinary cultural heritage with a wide diversity that needs to be preserved because it functions as a national identity and has excellent potential for developing culinary tourism.

For citation: Rahmah, L., Sari, N.I.P., Ansori, A.N.M. (2024). Diversity of *sate* (satay) as Indonesian ancient food. *Theory and Practice of Meat Processing*, 9(2), 125–134. https://doi.org/10.21323/2414-438X-2024-9-2-125-134

Acknowledgements:
We thank LPPM Akademi OTTIMMO for providing support in carrying out this research and the OTTIMMO Academy Lab for providing the opportunity, permission, place and support for the implementation of this research. We also thank Jalan Tengah, Indonesia (http://jalantengah.site/), for editing the manuscript.

Introduction

Indonesia is an archipelago with a large number of races and cultures that influence the diversity of traditions and food [1]. It has a wealth of natural resources and a large population [1]. The diversity of Indonesian customs and ceremonies, as well as food diversity is very well known [2]. Indonesia is a place for various types of ethnicities with a high contribution to its culinary delights [3]. Maintaining and preserving Indonesia's cultural heritage is our responsibility as a young generation because it is a legacy from our ancestors [4]. Traditional culinary delights with history and high quality must be fully exploited and promoted as assets that are part of the country.

Regional specialty products are obtained from different local plants and livestock used in their preparation resulting in traditional dishes acquiring certain taste values that cannot be recreated in other regions [5].

Sate dish or satay is an Indonesia's culinary heritage dish with a huge variety of ingredients and spices spread throughout Indonesia, which produces many types and flavors of satay [6]. Mostly, an ancient technique, such as charcoal grilling is a common *sate* cooking method in Southeast Asian countries [7]. From ancient times until now, wood and charcoal fuels have been used and are one of the oldest human-made fuels for cooking and heating [8,9].

The *sate* cooking method of grilling is a complex physico-chemical phenomenon. Food processors using grilling techniques usually rely on their intuition and experience during the process. These intuitions and experiences come from habits and heritage passed down by ancestors from generation to generation [10].

Research on mapping Indonesian food culture is expected to serve as an initial contribution to increasing market awareness of Indonesian cuisine and its richness, especially through exploration of social and cultural aspects [11]. Writing an article about *sate* in Indonesia aims to support the preservation of gastronomic culinary heritage foods that are an important part of tourism development in Indonesia.

Objects and methods

This research aims to explore the historical traces of *sate* and various types of *sate* that exist in Indonesia. The methodology used in this research includes analysis of food ethnographic literature, history, culinary traditions and types of *sate* from relevant articles and book chapters containing reviews, summaries and the authors' thoughts.

This article uses offline and online literature sources. Offline literature consists of cookbooks, recipe books and food history books (revealed from Perpustakaan Nasional Republik Indonesia, Jakarta, Indonesia). Meanwhile, online literature was collected from national and international journals. Data was processed using Microsoft Excel (Microsoft Corporation, USA) and presented in the

form of tables, pie charts and maps. To verify the data and strengthen the literature study, several small field observations were carried out by examining satay recipes and taking photos of satay.

Results and discussion

Indonesian food satay, which is usually called *'sate'*, has a history of syllables originating from one of the Chinese tribes, namely Minann, in whose dialect the word *'sa tae bak'* appeared, which means 'three pieces of meat'. There are other sources that say satay originates from the Middle East because it comes from the Tamil word *'catai'*, which means meat. Satay first appeared in the 19th century. Initially, Indonesian people cooked satay by boiling it. However, after the arrival of Tamil and Gujarati Muslim traders in Indonesia and the introduction of grilled kebabs, satay began to be cooked by grilling. Gradually, satay has developed with the application of special ingredients and processing into a traditional Indonesian food. With its distinctive taste from the aroma of Indonesian spices, *sate* is able to penetrate geographical boundaries. Therefore, *sate* is considered one of Indonesia's gastronomic culinary heritage [12].

With the culture of eating *rijsttafel*, *sate* is served not only simply but also luxuriously on plates. The modern *sate* recipe first appeared in the book "Kokki Bitja" (1857) written by Nonna Cornelia. Recipes for some types of *sate* are still written using the old Indonesian spelling. The word *sate* is written as *'sesate'*. Some of the recipes include: *sesate Bandang* (milkfish satay), *sesate Babi* (pork satay), *sesate Ajam* (chicken satay), *Sampi* (beef satay) [13]. Furthermore, a large book on national cuisine entitled "Mustika Rasa: Indonesian Recipes from Sabang to Merauke" was published in 1967. This book was published by the Department of Agriculture, Republic of Indonesia. There are 1500 various "Indonesian" recipes with influences from Chinese, Arabic, Indian and European flavors. The book is written using Indonesian, a mixture of old and new spellings. For example, the word *'sesate'* (old spelling) was changed to *sate* (new spelling). Several satay recipes have been written including *sate Ajyam* (chicken satay), *sate Babi* (pork satay), *sate Bandeng* (milkfish satay), *sate Kerang* (shellfish satay), *sate Lilit, sate Madura, sate Padang and sate Pusut.* In the 1967 book Mustika Rasa, it not only contains food recipes but also food chemicals. By consuming a diversity of foods, there is the potential to increase consumption of various food chemicals such as carbohydrates, fats, proteins, water, ash, and various vitamins, thereby preventing humans from malnutrition. In the chapter there is a photo of a child suffering from kwashiorkor who recovered due to consuming adequate nutrition. The chapter on food chemicals is included as one of the government's efforts to eradicate malnutrition [13].

In the world of culinary tourism, Indonesia is famous for its food diversity. *Sate* has a taste that comes from a mixture of different spices in each tourist destination in Indonesia and each seller has its own uniqueness [6]. Sate skewers as a means of serving food, such as beef *sate*, were initially made manually by hand with less-than-optimal results, then the manufacturing process for making these skewers was developed using a semi-automatic machine. *Sate* skewer products made from bamboo trees are still abundantly available in Indonesia [14].

Sate is one of the most popular foods in Indonesia, served everywhere, from street food to luxury restaurants in hotels [15]. One element of Indic culture that was popular during the colonial period was the food culture called *rijsttafel*. If interpreted literally, *rijst* means rice and *tafel* means table, combined into a "rice dish". The impression can be seen from the way indigenous food culture is packaged through the concept of table settings and dishes with a touch of the Western style [16]. As the country of origin of *sate*, Indonesia has various types of *sate* recipes. Typical Indonesian *sate* is served with a sauce with a thick texture in the form of spices, peanut sauce, or others, usually accompanied by a condiment of sliced cayenne pepper, red onion and cucumber. It is eaten with processed rice such as *nasi, lontong,* or *ketupat*. Variations are usually named based on the region of origin of the *sate* recipe, type of meat, ingredients, or manufacturing process. Several types of *sate* typical of regions in Indonesia are *sate Madura*, *sate Padan*, *sate Ponorogo* and *sate Kelinci* [17].

In Indonesia, *sate* sellers roam the streets and alleys of every city and announce their presence with wooden sticks that they hit on their portable grills. When the orders start coming in and *sate* has been grilled, smoke and a sweet aroma come out and attract other buyers. Ginger, coriander and palm sugar are the main flavors. The tamarind in the marinade acts as a powerful tenderizer, making even very chewy meat tender [18]. For centuries, grill charcoal fuel has been one of the oldest human-made fuels. It was an important ingredient for cooking and heating in ancient times and even today charcoal is used in grilling cooking techniques to make *sate* [8,10].

One of Indonesia's dominant tourism assets is culinary tourism because Indonesia has a great variety of cultures, agriculture, and plantations that affect culinary diversity in each region so that domestic and foreign tourists are interested in visiting regions in Indonesia to taste the typical food and drinks of the region [62].

The way of eating and serving food, the function of food and the composition of spices are characteristics of a region. Using regional specific features can produce Indonesian culinary delights with regional excellence that cannot be separated from the identity of the region of origin. An example of *sate* that uses the name of the original region is *sate Madura*, which is famous for its peanut sauce [6]. Some other examples of using regional names for *sate* are: *sate Padang* (the name of the city in West Sumatra province), *sate Blora* (the name of the city in West Central Java province), *sate Ponorogo* (the name of the city in East Java province), *sate Makassar* (the name of the city in South Sulawesi province) [25,42,58,75].

Table 1. Types of *sate* according to provinces in Indonesia

No	Island	Province	Sate	References
1	Sumatra	Nanggroe Aceh Darrussalam	*Gurita*	[19]
2		Nanggroe Aceh Darrussalam	*Dongdong*	[20]
3		Nanggroe Aceh Darrussalam	*Matang*	[21]
4		North Sumatra	*Kerang*	[22]
5		West Sumatra	*Danguang-Danguang*	[23]
6		West Sumatra	*Lokan*	[24]
7		West Sumatra	*Padang*	[25]
8		South Sumatra	*Cucuk Manis*	[26]
9		South Sumatra	*Pentul*	[27]
10		South Sumatra	*Ayam Kampung*	[28]
11		Riau Islands	*Ikan Senapelan*	[29]
12		Jambi	*Rang Kayo Hitam*	[30]
13		Bengkulu	*Pancah Daging*	[31]
14		Bengkulu	*Gembolo*	[32]
15		Lampung	*Tuhuk*	[33]
16	Java	West Java	*Maranggi*	[34]
17		West Java	*Terpedo*	[3]
18		West Java	*Udang*	[35]
19		West Java	*Kalong*	[36]
20		West Java	*Jando*	[3]
21		Banten	*Bandeng*	[15]
22		Banten	*Bebek Cilegon*	[37]
23		DKI Jakarta	*Asem*	[38]
24		DKI Jakarta	*Lembut*	[39]
25		DKI Jakarta	*Taichan*	[40]
26		Central Java	*Balibul*	[41]
27		Central Java	*Loso*	[6]
28		Central Java	*Blora*	[42]
29		Central Java	*Suruh*	[43]
30		Central Java	*Ambal*	[44]
31		Central Java	*Bumbon*	[45]
32		Central Java	*Buntel*	[46]
33		Central Java	*Kere*	[46]
34		DI Yogyakarta	*Telur Puyuh*	[47]
35		DI Yogyakarta	*Ratu*	[48]
36		DI Yogyakarta	*Petir*	[49]
37		DI Yogyakarta	*Sor Talok*	[50]
38		DI Yogyakarta	*Klatak*	[51]
39		DI Yogyakarta	*Kikil*	[52]
40		East Java	*Klopo*	[53]
41		East Java	*Kelinci*	[54]
42		East Java	*Karak*	[55]
43		East Java	*Gebug*	[56]
44		East Java	*Komoh*	[57]
45		East Java	*Madura*	[17]
46		East Java	*Ponorogo*	[58]
47	Nusa Tenggara	Bali	*Kakul*	[59]
48		Bali	*Languan*	[60]
49		Bali	*Plecing*	[3]
50		Bali	*Lilit*	[61]
51		Bali	*Lilit Lindung*	[59]
52		West Nusa Tenggara	*Rembiga*	[62]
53		West Nusa Tenggara	*Bulayak*	[62]
54		West Nusa Tenggara	*Tanjung*	[62]
55		West Nusa Tenggara	*Ampet*	[3]
56		West Nusa Tenggara	*Pusut*	[63]
57		East Nusa Tenggara	*Kerang Belawar*	[64]
58	Kalimantan	West Kalimantan	*Manis*	[65]
59		West Kalimantan	*Kuah*	[66]
60		West Kalimantan	*Tulang*	[67]
61		East Kalimantan	*Payau*	[68]
62		East Kalimantan	*Babi*	[69]
63		North Kalimantan	*Ikan Pari*	[70]
64	Sulawesi	North Sulawesi	*Ragey*	[71]
65		North Sulawesi	*Kolombi*	[72]
66		Gorontalo	*Tuna*	[73]
67		West Sulawesi	*Tambulinas*	[74]
68		South Sulawesi	*Makassar*	[75]
69		Southeast Sulawesi	*Pokea*	[76]
70	Papua	Papua	*Ulat sagu*	[77]

Figure 1. Map of *sate* diversity in Indonesia

The Lombok area, West Nusa Tenggara, has a wealth of traditional culinary delights with great potential to be developed to support the development of tourist attractions, especially culinary tourism. Some of the *sate* included in culinary tourism are (a) *Sate Bulayak* is a meat and offal based dish seasoned with sasak spices, grilled using coconut shells or coir and served with peanut sauce (pounded with garlic, coriander and chili), (b) *Sate Tanjung* comes from skipjack or langoan fish. The savory taste of fish meat and coconut milk and the spicy taste obtained from spices are very pronounced when enjoying *Sate Tanjung*. and (c) *Sate Rembiga* is made from diced meat or offal that is marinated in a mixture of ground spices based on shallots, cayenne pepper, sugar, candlenut, shrimp paste, and several other spices [62].

It can be concluded from Table 1 and Figure 2 that the largest number of variations of *sate* in Indonesia is on the island of Java (44%) and the island of Sumatra (21%). Java Island has the largest and most diverse population. It has a large library of traditional foods. With the combination of various tribes that have entered the island of Java, there is more and more exploration of new menus. This makes the food on the island of Java increasingly diverse. Javanese people usually serve satay on special occasions such as sacred weddings, social gatherings and baby births. This satay dish is made as a main dish with meat as a main ingredient. A sweeter taste compared to that in other regions is a characteristic of the culinary arts of the people of the island of Java, Indonesia [78].

The arrival of the Arabs gave several distinctive features to Indonesian culinary delights, namely satay, which is made using a skewer from lamb or goat meat and served whole on the skewer. The spices available range from seed, fruit, root, bark, or vegetative substance, and the most common include coriander seeds, pepper, nutmeg, cumin, and cloves. Either grated, chopped, or dried, these spices, together with other fresh ingredients, play a part as a seasoning for the purpose of flavouring the food (in Indonesian language, it is called bumbu) [79]. However, what makes the presentation of typical Indonesian satay different is the use of peanut sauce served with satay originating from the Java region. India has also influenced Indonesian culinary delights, as seen from the Sumatran cuisine, which serves meat with vegetables using spices such as cloves and nutmeg to provide a distinctive taste [11].

It can be seen from the pie chart in Figure 3 that on average a type of raw materials most widely used are mammals (51%), such as cattle and goat. Since the beginning of human civilization, meat consumption and livestock farming have been inseparable because they have a major impact on the environment. Meat provides high quality protein, vitamins and minerals while offering pleasure when consumed [80]. The variety of *sate* is a form of protein-type functional food. Functional food is food which wide variety will enable consumers to optimize their diet because it is nutritionally balanced and provides a real opportunity to reduce the risk of malnutrition and improve physical health [81].

The ingredients used for *sate* are more specific with the use of halal ingredients. Indonesia is a country where the majority of the population is Muslim. The life of a Muslims is always connected with the concept of halal in their daily life, especially in food [82]. Indonesia plays an important role in halal food production because it is one of the largest countries in Asia, thus guaranteeing halal culinary standards in Indonesia. This halal culinary has been developed as an important part of Indonesia's food security strategy [83].

Several types of *sate* made from mammals include *sate Balibul, sate Kelinci, sate Terpedo, sate Jando, sate Ampet. Sate Balibul* is a local Indonesian food in Central Java made from lamb under five months of age, which is popular because it has a soft texture [41]. *Sate Kelinci* made from rabbit is one of the typical foods of the city of Batu, one of the cities located in the province of East Java, Indonesia, which has superior tourism. *Sate Kelinci* is very popular as a culinary specialty that is sought after by tourists. The rabbit meat is obtained from local breeders in Batu City. Rabbit meat is popular because of its high protein content and low fat and cholesterol content. Thus, this meat is healthier to consume [84].

Moreover, Indonesia's culinary traditions are shaped by its wealth of natural resources. The ingredients used to make food are not only meat but also offal. The use of offal has created a myriad of recipes for dishes made from

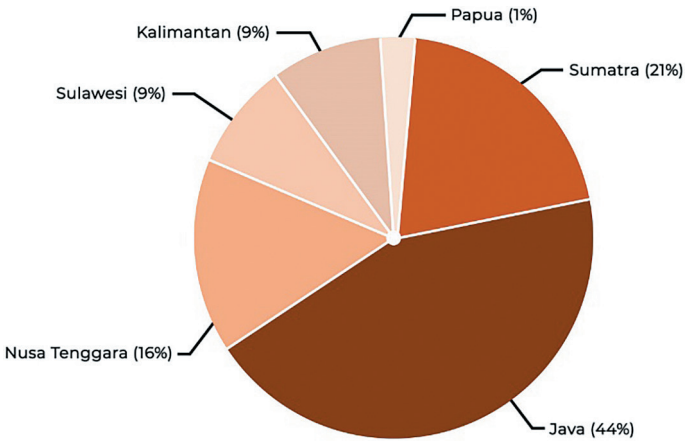

Figure 2. Pie chart of *sate* types according to provinces in Indonesia

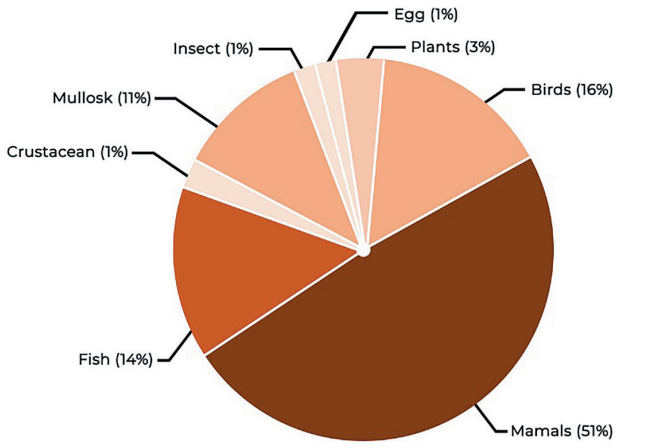

Figure 3. Pie chart of *sate* types according to the main ingredients

offal, resulting in culinary diversity throughout the country. There are several *sate* that use beef offal, including *sate Terpedo* (cattle penis), *sate Jando* (cattle nipple), *sate Ampet* (cattle liver, intestine, tripe) [3]. In the Banten province, especially the city of Cilegon, there is a typical satay dish made from duck, namely *sate Bebek Cilegon* [37].

Figure 4 shows *sate Madura*, which is a type of *sate* made from chicken. *Sate Madura* is one of the popular Indonesian satays that can be found easily in almost all regions of Indonesia, especially in big cities on the island of Java, such as Jakarta, Bandung and Surabaya. *Sate Madura* is usually made from grilled chicken and then served with peanut sauce plus a few slices of shallot [17].

Apart from satay that is made from meat, there is also a unique satay made from quail eggs, namely *sate Puyuh*, which comes from Yogyakarta. Quail satay is usually sold in angkringan (comes from the Javanese word *"angkring"*, which means a tool and place for selling food, around which the handle is curved upwards) [47].

It can be seen from the pie chart in Figure 5 that the most frequently used ingredient preparation process is cutting. *Sate* is a food, which main ingredient is meat cut into small pieces. They are skewered using bamboo which is cut into thin pieces, grilled using charcoal and seasoned with spices. In Indonesia, *sate* is usually served with rice or lontong [15]. *Sate* consists of boneless pieces of chicken, beef, pork or fish cut into cubes and skewered using coconut leaves or bamboo [7].

There is a unique ingredient preparation technique, namely chopping used, for example, for *sate Bandeng* and *sate Lilit* (Figure 6). One of the processed fish *sate* that is characteristic of Banten region is *sate Bandeng*, which is made from milkfish skewered on bamboo sticks. The process of making milkfish *sate* consists of several stages. Milkfish is cleaned and then loosened slowly, flesh and bones are separated from the skin. Flesh is mixed with spices, then put back into the milkfish skin and grilled [15]. *Sate Lilit* is *sate* from the island of Bali, which is famous in the international culinary world. *Sate lilit* is made from chopped chicken, seasoned with spices, then shaped into sere (a type of Indonesian spice with an elongated shape that functions to replace the sweeker) and grilled. *Sate lilit* is served almost in every cultural event for consumption and as an offering to ancestors [61].

It can be seen from the pie chart in Figure 7 that a seasoning base with the use of ground spices is most widely used. Ingredients such as chili peppers are also found in some dishes, showing traces of culinary exchange with the

Figure 4. *Sate Madura, which is famous for its peanut sauce*

Figure 6. *Sate Lilit*

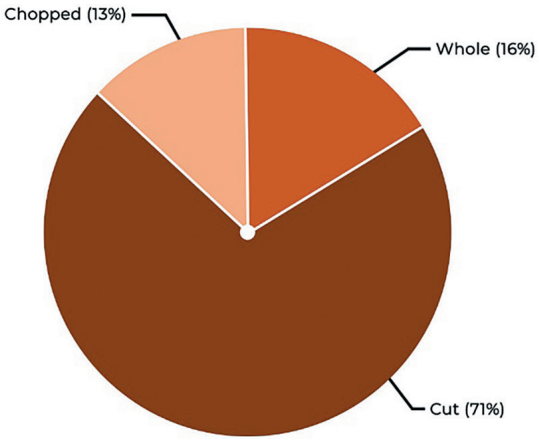

Figure 5. Pie chart of *sate* types according to ingredient preparation

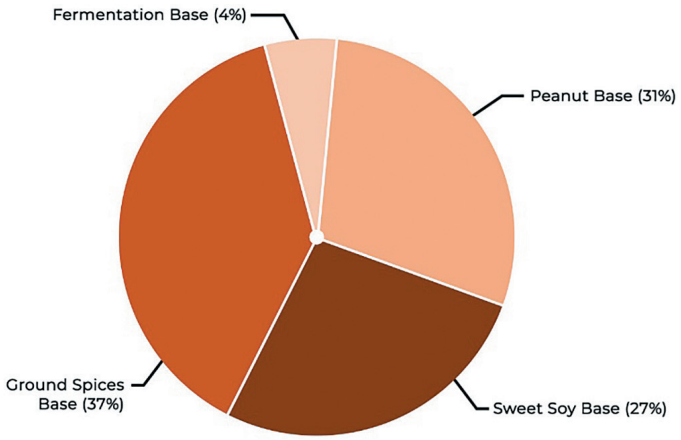

Figure 7. Pie chart of *sate* types according to a seasoning base

Colombians [3]. Mortar and pestle or stone grinder are the most important equipment so far. They are used to grind, crush and make a paste of spices, which are very important in Indonesian taste. It is necessary to choose a mortar that is slightly curved, heavy, sturdy and has a rough texture. If available, it is advisable to purchase mortar and pestle carved from volcanic rock as they are harder and, therefore, more durable. This is because many spices are hard and require a lot of effort to grind them by hand [85]. The example of *sate* that uses the ground spices base is *sate Padang*, which has become an icon of culinary tourism in Sumatra Island. *Sate Padang* is also made from pieces of chicken skewered with bamboo and grilled. It is served with a delicious spicy curry sauce typical of Padang city [86].

Sate Padang (Figure 8) is *sate* that uses seasoning based on ground spices. The use of different herbs, spices and ingredients in Indonesian cooking has become a characteristic that differentiates food from one place and another in Indonesia. There is a strong connection between the way people prepare food and ingredients that are present and available in their region [87]. It is an interesting fact that the use of local spices in the marination of grilled beef for *sate* can inhibit/lower the levels of toxic and harmful HCAs [7].

Taste, aroma and texture of grilled meat are influenced by herbs or spices used. Other factors, characteristics of meat, parameters of the cooking process, such as time and temperature, and the water content remaining after the grilling process, also have an influence [88]. In the process of cooking food, the most important aspects are time and temperature [10]. The purpose of cooking meat products is to reduce the moisture content and kill microorganisms to increase the storage time, as well as to improve taste and texture of food [89]. Cooking techniques in Indonesia are slightly different from other cultures and cuisines. The heat source in many homes is still a simple wood fire, although some modern homes use kerosene and gas stoves. Cooking containers are also simple, made of sheet iron or aluminum. Most Indonesians use very low heat in cooking, which means the food takes longer to cook. The key in preparing Indonesian dishes lies in mixing and blending spices; therefore, it is important to use only fresh ingredients when preparing Indonesian dishes [87].

It can be seen from the pie chart in Figure 9 above, that the final cooking technique with grilling is the most widely used. Cooking techniques in Indonesia are very simple, including those used to cook *sate,* such as boiling, frying and grilling. Different cooking methods had different effects on the nutrition value of chicken meat [90].

When it comes to braising, it's important to know whether cooking of meal should be started with cold or hot liquid. Meat such as chicken or beef should be added to the boiling liquid. This will close the pores and prevent meat juices from escaping, which causes meat to dry out. Frying involves using a lot of oil. Oil should be heated at 160–180 °C (325–350 °F), then food should be added and fried until dry. Grilling is a very popular cooking method,

Figure 8. *Sate Padang*

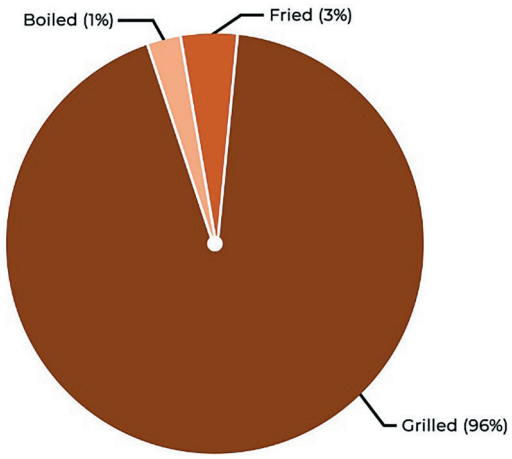

Figure 9. Pie chart of *sate* types according to the final cooking technique

which is widely used to make *sate*. *Sate* is always cooked with burning charcoal, making sure the fire is very low and the heat is very high by using a sturdy handheld bamboo fan. It is important to avoid dripping of meat juices into the fire as this will cause the flames to jump and add a very unpleasant burnt taste to the food [85].

Figure 10 is a picture of the process of grilling *sate* in Indonesia using charcoal as fuel. Charcoal production is carried out naturally through an artificial manufacturing process that has existed since ancient times. For centuries, humans have used the trial and error approach in production of charcoal producing various types of charcoal from natural organic materials ranging from wood and sawdust to waste materials, such as coffee husks cotton stalks, shells and bones [91].

The world now has a variety of cooking methods from ancient to modern, as well as a combination of ancient and modern methods. One of the oldest cooking methods is grilling. The grilling method uses dry heat and can be defined as a cooking process, during which raw materials are exposed to a heat source that uses thermal radiation for primary heat transfer. Grilling is not something easy because there are various variables that are difficult to control thoroughly [10].

Figure 10. *Sate* grilling process in Indonesia

Indonesia has several foster villages. One of them is *Kampung Oase Ondomohen,* which is located in the city of Surabaya, East Java Province, Indonesia and has the potential for the sustainable regional development. The village was giving this name because of the local initiative to process waste into products such as briquettes from tree branches and used charcoal from making *sate Klopo Ondomohen* [53].

Sate Klopo is a typical satay made from meat wrapped in coconut, then skewered and grilled using briquettes [53]. To cook beef on the grill, a charcoal fire over medium heat is prepared. Beef is grilled for two to five minutes (time will depend on how hot the fire is) until cooked through and started to show crispy dark brown spots. It is necessary to carefully turn the skewers over and continue grilling two to five minutes more until the other side is lightly browned. Meat should not be cooked for too long, because it will dry out [18]. Other cooking techniques used are frying and boiling. In Indonesia, these two techniques are applied using a tool called a *wajan* or *kuali* in Javanese, which has a shape similar to a wokpan. [93]. Although in Indonesia *sate* is usually cooked by grilling, there are also some types cooked by boiling, namely *sate Kerang* and frying, namely *sate Pentul* [22,27].

Conclusion

Sate (satay) dishes have a diversity of types and flavors obtained from various ingredients, including spices. In *Sate* dishes, the way the pieces of meat are served on skewers was influenced by Arabic culture, which is most visible in the development of Indonesian food culture. On average, *sate* is made using the grilling cooking technique, which is an ancient type of cooking technique survived into modern times. For centuries, wood and charcoal have been some of the oldest human-made fuels as important ingredients for cooking and heating in ancient times and even today.

Apart from being an everyday food, *sate* is Indonesia's gastronomic culinary cultural heritage with a wide diversity that needs to be preserved because it functions as a national identity and has excellent potential for developing culinary tourism.

The largest number of variations of *sate* in Indonesia is on the island of Java. Java Island has the largest and most diverse population. This makes the food on the island of Java increasingly diversified. *Sate* in Indonesia has a variety of food sources of protein, from mammals, which are most commonly used, to insects, which are very rarely found. In terms of preparation, the protein ingredients used are usually cut, but some are chopped and some are even served whole. The diversity of spices in Indonesia results in the great variety of seasoning bases for *sate*, some of which are often prepared using ground spices base, sweet soy base, peanut base and most rarely using the fermentation base.

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The authors declare no conflict of interest.

DOI: https://doi.org/10.21323/2414-438X-2024-9-2-135-144
Received 21.03.2024
Accepted in revised 06.06.2024
Accepted for publication 10.06.2024

Available online at https://www.meatjournal.ru/jour
Original scientific article
Open Access

MEAT PRODUCTS WITH BEETROOT EXTRACT REDUCE DNA DAMAGE IN MOUSE INTESTINES

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Keywords: *Beta vulgaris L, meat, genotoxicity, cancer, methylmethanesulfonate, single-cell gel electrophoresis, functional food, chemoprevention, mice*

Abstract
Red beetroot (Beta vulgaris L.) is known as the popular vegetable in Russian cuisine, as well as a plant food that protects human health. Beetroot is rich in chemical compounds with antioxidant, anticarcinogenic, anti-inflammatory and other health-beneficial properties. Using the DNA-comet method (alkaline version), the effect of dry red beetroot extract was assessed as part of a meat product on spontaneous and induced DNA damage and presence of "abnormal comet" indicators in cells of various parts of the gastrointestinal tract (GIT) and liver of male F_1 mice C.B.A. x C57 Bl /6. The obtained results showed that intraperitoneal administering of alkylating agent methyl methanesulfonate (MMS) to the mice at a dose of 40 mg/kg of the animal body weight statistically significantly increased the numerical value of DNA damage (% DNA in the tail) and the number of atypical DNA-comet in the liver, stomach, small and large intestines. Introduction of meat product with added red beetroot extract (20 g/kg of finished product) into the diet of the animals did not provide any significant effect on the scope of DNA damage caused by MMS in liver cells, but it led to a statistically significant decrease of this parameter by 58%, 59% and 48% in cells of the stomach, duodenum and rectum, respectively. The significant decrease of 29–54% in the release of atypical DNA-comet in the cells of all organs studied has been also confirmed, which proves decreasing in the cytotoxic effect of MMC in the gastrointestinal tract and liver. Thus, the antigenotoxic and cytoprotective effects of a meat product with addition of dry red beetroot extract have been recorded. This finding is able to have significant practical application, since the complications and issues in the gastrointestinal tract often occur and secondary tumors are induced in its parts during the chemotherapy of cancer located beyond the gastrointestinal tract. This result shows the potential ability of the developed meat product in protecting gastrointestinal tract cells from the genotoxic and cytotoxic effects of alkylating anticancer drugs.

For citation: Lisitsyn, A.A., Zhanataev, A.K., Chernukha, I.M. (2024). Meat products with beetroot extract reduce DNA damage in mouse intestines. *Theory and Practice of Meat Processing,* 9(2), 135–144. https://doi.org/10.21323/2414-438X-2024-9-2-135-144

Introduction

According to WHO data presented by the UN, in 2022 about 20 million people in the world were diagnosed with cancer and about 10 million patients succumbed to cancer. Among the top five of most common cancers there are colorectal cancer (9.6%) and gastric cancer (4.9%), they are noted as the types of cancer most often leading to death in patients [1].

Based on various experimental approaches, it has been demonstratively proven that the trigger of carcinogenesis is genotoxic cell damage, i. e. DNA damage.

Stochastic depurination and deamination of bases, the hydrolysis of the n-glycosyl bond, disruptions in meiotic and mitotic recombination or topoisomerases functioning, exposure to random thermal fluctuations and many other spontaneous processes lead to the typical DNA damage; chemical modification or loss of purine and/or pyrimidine bases, their oxidative damage, various cross-links, single-strand and double-strand breaks of DNA chain. Single-strand DNA breaks are considered to be the predominant damage [2]. The overwhelming majority of spontaneous DNA damages are fixed by cellular repair systems [3].

The induction of DNA breaks and chemical modification of the macromolecule increases dramatically under the influence of chemical genotoxicants. They can have both exogenous and endogenous origin.

The reactive oxygen species (ROS), which cause oxidation of bases and DNA breakage, are generally acknowledged as endogenous genotoxicants. ROS are the products of normal cell metabolism that normally are not dangerous. However, under the conditions of oxidative stress, when the production of ROS increases extremely and it quantity exceeds the compensatory capabilities of the body's antioxidant protection, oxidative damage to DNA bases, single-strand and double-strand breaks of the macromolecule occur.

Oxidative stress goes along with many human diseases. These are viral and bacterial infections, bronchial asthma, diabetes, malignant neoplasms and many other diseases. Oxidative stress is also induced by smoking, physi-

cal stress, emotional stress, chronic alcoholism and other harmful addictions. In all diseases accompanied with the oxidative stress, increasing of the level of DNA damage has been recorded, which is proven by DNA-comet method, and these diseases are associated with carcinogenic risk increase [3,4,5,6].

Exogenous genotoxicants are usually divided into physical, biological and chemical agents or, depending on the source of origin, classified as household, industrial, medicinal and other genotoxicants.

Natural, industrial or diagnostic ionizing radiation, as well as natural ultraviolet radiation, is the reason of breaks of single-strand and double-strand DNA chains, DNA-DNA cross-links, DNA-protein cross-links and their other modifications [3,5].

Breaks of single-strand and double-strand DNA breaks, as well as various modifications of this molecule, are caused by numerous chemical factors. This are fumes and smokes of various origin, industrial emissions, exhaust gases from internal combustion engines, certain pesticides and medications. For example, the chemotherapeutic agents bleomycin and cisplatin cause DNA damage by their interfering with the activity of the enzyme DNA topoisomerase and alkylating DNA bases [3,5,6].

Chemical genotoxicants are able to interact directly with DNA — these are DNA-reactive genotoxicants; or they are able to provide damaging effect indirectly, without penetrating the cell nucleus and without direct interaction with DNA — these are DNA-non-reactive genotoxicants. The individual representatives of each of these groups require metabolic activation in order to obtain a genotoxic function, and are denoted as indirect genotoxicants.

Several mechanisms make up the action of majority of genotoxicants. Most often, this is the induction of oxidative stress and alkylating effect, which is typical for more than 90% of known chemical genotoxicants [3].

The assurance in the presence of connection between the genotoxic and carcinogenic activity of chemical compounds originates from two groups of facts. First, most known carcinogens have genotoxic properties. For example, the genotoxicant cyclophosphamide induces tumors of the bladder and hematopoietic system; and azothiopril induces tumors of the skin and lymphatic system. Secondly, patients suffering from hereditary DNA repair defect syndromes have a tenfold higher risk of developing tumors in comparison with healthy people [3].

Thus, the prevention of genotoxicity can serve as an efficient method to combat the socially significant disease as cancer. Attention is focused on the idea that up to one third of the cancer cases can be prevented by mere change of nutrition. The attempts to create food products for cancer prevention have been so far based on the idea of the unconditional benefits of food antioxidants for the human health [7].

Most antioxidants feature antigenotoxic properties, it means that they are capable to reduce the damaging effects of genotoxicants in experiments or in the clinic. This corresponds well with the concept of oxidative stress as the driving mechanism of genotoxic damage. Several dozens of natural compounds and plant products have been identified that possess antigenotoxic properties and are able to reduce the risk of malignant tumors. These are, in particular, vitamins of groups A, C, E, natural flavonoids, phenols, epigallates, catechins, etc. Based on natural antigenotoxic compounds or food raw materials that contain them, it is possible to create functional food products for the prevention of genotoxicity and associated cancer conditions [3,8,9].

In this regard, red beetroot (*Beta vulgaris L.*) deserve the most serious attention. This plant belongs to the botanical family *Amaranthaceae* and is commercially cultivated. It perfectly combines nutritional and medical value. Beetroot is rich in proteins (1.68 g), carbohydrates (9.96 g), fat (0.18 g), amino acids (1.22 g), fatty acids (0.12 g), phytosterols (0.03 g), minerals (0.48 g) and fiber (2.0 g) per 100 g fresh weight. It contains vitamins in amount of 4.8 mg per 100 g of fresh weight, and in dry beet extract there are such biologically active substances as betalains (3.9 g / 100 g), betacyanins (2.1 g / 100 g), betaxanthins (1.9 g/100 g) and phenols (0.19 g/100 g) [10].

The preventive and therapeutic effects of beetroot in many diseases are traditionally related with its profound antioxidant activity [10,11]. It is worth to note that antioxidant activity is inherent in beetroot not only in their fresh form, but also as part of processed food products. For example, Ali et al. [12] proved that fruit jelly sweets consisting of 75% strawberries and 25% beetroot have high antioxidant activity (more than 52%).

The possibility of using beetroots as part of functional food products with preventive properties, and the grounded feasibility of their production, were previously determined in the independent works [7,10,11]. In these works, as well as in many other researches, it was underlined that the mechanisms of the protective effects of beetroot and the technology for its use as the ingredient of the functional food are just at the very beginning of their development.

Earlier beetroot was already considered by us [13] as a possible source of antigenotoxicants. Methodological achievements that made it possible to use the method "DNA-comet" to assess DNA damage *in vivo,* in particular, to record DNA damage in cells of the various parts of the gastrointestinal tract (GIT) [3,4] opened up the future prospects for researching the use of beetroots for reducing the genotoxic effect in GIT cells, which function is exceptionally important in view of the above-cited information on genotoxicity as a trigger of carcinogenesis and the statistical prevalence of gastrointestinal types of cancers.

The purpose of this study is to assess the effect of red beetroot extract as part of a meat product on DNA damage in the cells of stomach, duodenum, rectum and liver of the experimental mice.

Objects and methods

Object of study and chemical reagents

The object of research was a ready-to-eat meat product — a boiled sausage. The product is based on the recipe of the sausages "Piknichok", developed in accordance with state standard GOST R 58110–2018[1]. The recipe for the experimental product contained poultry meat (chicken breast), pork back fat, water, salt, spices and dry beet extract (Specification No. KW/1001–11/2005, manufacturer: Parzew 14 63–220 Kotlin, Poland), in amount of 20 g per 1 kg of raw meat.

The recipe for the control food product was fully analogous to the recipe for the sausages "Piknichok".

The experimental sample and the control sample were produced on the same day on the same equipment from the same batch of the raw materials. The product was developed according to the modes described in GOST R58110–2018[1].

The alkylating agent — methyl methanesulfonate (MMS), produced by the company Sigma (USA), was used as a genotoxicant.

Fluorescent dye — SYBR Green I (Thermofisher, USA).

While running the lysis and electrophoresis of isolated cells, the following reagents produced by the company AppliChem GmbH (Germany) were used: ris-HCl (pH 10), NaCl, EDTA-Na_2, Triton X-100, dimethyl sulfoxide, NaOH.

Animals

For the experiment the male mice of hybrids F1 CBAxC57Bl/6 raised in "Stolbovaya" nursery (the branch of the Scientific Center of Biomedical Technologies of Federal Medical and Biological agency of Russia) were used.

Two weeks before the beginning of the experiment, the animals were kept in the vivarium of the Federal State Budgetary Institution "Federal Research Center for Original and Prospective Biomedical and Pharmaceutical Technologies" on a twelve-hour light regime, 5 animals per each group in polycarbonate cages (235 × 155 × 140). They had free access to water and feed for the rodents "Profgryzun" (Russia). At the beginning of the experiment, the weight of the mice varied in the range of 20–22 g, and their age was 8–9 weeks.

The experimental researching, parameters of environment, housing and keeping the animals were approved by the Bioethical Commission of the Scientific and Research Institute of Pharmacology n. a. V. V. Zakusov, which is a structural subdivision of the Federal State Budgetary Institution "Federal Research Center for Original and Prospective Biomedical and Pharmaceutical Technologies" and conformed with GOST 33215–2014[2].

[1] GOST R58110–2018 "Sausage cooked goods from poultry meat (offal) for children nutrition. Specifications" Retrieved from https://docs.cntd.ru/document/1200159009 Accessed February 14, 2024

[2] GOST 33215–2014 "Guidelines for accommodation and care of animals. Environment, housing and management" Retrieved from https://docs.cntd.ru/document/1200127789 Accessed February 14, 2024

DNA-comet method

The method is used in its neutral and alkaline versions. In the first case, only double DNA breaks are recorded. In the alkaline version, the method allows to reliably detect single-strand and double-strand DNA damage, which are the predominant damage that happens under the influence of physical and chemical genotoxicants [3,4]. Moreover, during alkaline denaturation, the numerous chemical modifications of DNA, collectively known as alkaline-labile sites, are implemented as the single-strand DNA breaks, which allows integrative assessing of DNA macromolecule damage in the cell in comparison with the corresponding control sample [4,14,15].

The DNA damage was tested with the DNA-comet method in its alkaline version. The method is based on measuring the electrophoretic mobility of DNA of the individual cells in agarose gel. During the electrophoresis, the DNA of a cell has formed more or less pronounced figure, consisting of a nucleus and a tail, which in general looks like a comet (Figure 1). The generally accepted indicator of DNA damage is the DNA content in the comet tail, where DNA gets if it has been loosened and has acquired mobility as a result of single-strand and double-strand breaks [4].

The method is applicable to cells of any tissue. The main problem when studying the another tissue sample is to obtain high-quality microscopic preparations of isolated cells suitable for digital analysis. This problem has been successfully solved in this work in regards to gastrointestinal tract cells, as illustrated by the images shown below in the Figure 1. The procedure of isolating the gastrointestinal tract cells is described below.

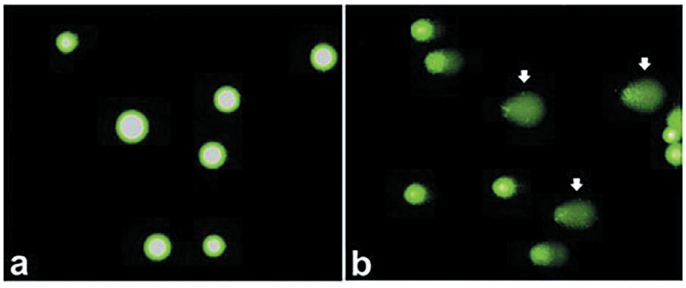

Figure 1. Digital images of the preparations of DNA-comets of rectal cells: a) undamaged cells b) typical DNA-comets (red arrows) and atypical comets (vaguely expressed nucleus and diffuse tail) are marked with white arrows. SYBR dye Green I, magnification × 200

Preparation of meat product and genotoxicant for their administering to the animals

Samples of the meat product weighing 5 g were finely cut with scissors and ground in a porcelain mortar with 10 ml of distilled water until a homogeneous mixture was obtained.

Methylmethane sulfonate (MMS) was administered as distilled water solution.

Experimental groups

Four experimental groups were formed, 5 animals per group. The animals in the control group got intragastric

administering of distilled water at a rate of 10 ml/kg, three times, with an interval of 24 hours.

The animals of MMS group got the intragastric administering of the distilled water in the same mode. The last administering was combined with an intraperitoneal injection of an alkylating genotoxicant — methylmethanesulfonate (MMS) at a dose of 40 mg/kg of animal body weight. The choice of genotoxicant dose was based on own experience and literature data [15,16].

The resulting suspension of the meat product was administered to the animals of the experimental groups "MMS + Mstd" (sample of the meat product without beetroot extract (*std*) and "MMS + M*be*" (sample of a meat product with beetroot extract (*be*) intragastrically at rate of 10 ml/kg, which corresponded to 100 mg of red beetroot extract per kilogram of animal body weight. The suspensions were administered three times, with an interval of 24 hours. The last administering was combined with an intraperitoneal injection of MMS at a dose of 40 mg/kg.

Preparation of cell suspensions, cells lysis and electrophoresis

Three hours after the last administering of the solutions, the animals were euthanized by decapitation. The liver, stomach, duodenum and rectum were taken out as quickly as possible and crushed in 3 ml of phosphate-buffered saline pre-cooled down to a temperature of 4 °C containing 20 mM EDTA-Na_2, and of 10% dimethyl sulfoxide (pH 7.5). The tissue samples were pestled in the glass vials with a glass pestle to obtain cell suspensions and kept for 1–2 minutes for sedimentation of large fragments of tissue.

30 µl of a suspension of cells from one or another part of the gastrointestinal tract was added into the prepared glass tubes containing 120 µl of 1% solution of low-melting agarose in phosphate-buffered saline heated up to temperature 42 °C (in microthermostat "Termit", "DNA-technology", Russia). After resuspension, 30 µl of cell suspension in low-melting agarose was applied onto the slides coated with 1% universal agarose, placed under coverslips, and put onto ice. The next actions were carried out under yellow light, the solutions used were cooled down to the temperature of 4 °C.

After 5–10 minutes of exposure to low temperature, after the agarose got solidified, the coverslips were taken off and the microslides were placed into a glass cuvette (Schiffendecker type) for lysis, which lasted for 1 hour in a buffer containing 10 mM Tris-HCl (pH 10), 2.5M NaCl, 100 mM EDTA-Na2 1% Triton X-100 and 10% dimethyl sulfoxide of 4 °C.

After completion of lysis the microslides were kept for 20 min in electrophoresis buffer containing 300 mM NaOH, 1 mM EDTA-Na2 (pH>13) and were transferred to a horizontal electrophoresis chamber (Bio — Rad (USA), Sub-Cell type, model 192, 25 x 10 cm), filled with fresh buffer of the same composition. Electrophoresis was run for 20 minutes (field strength was 1 V/cm, current ~300 mA). Then the preparations were washed with phosphate-buffered saline and fixed with 70% ethyl alcohol for 15 minutes.

Staining and analysis of the microslides

The obtained preparations were stained with fluorescent dye SYBR Green I (dilution 1:10000 in TE buffer (pH 8.5) in 50% glycerol) for 30 minutes long in the dark room right before microscopy. For microscopy (×200) an epifluorescence microscope Mikmed-2 12T (Lomo, Russia) with a digital camera of high-resolution (VEC-335, EVS, Russia) was used.

The digital images of DNA-comets were computed via CASP 1.2.2 software [17]. In accordance with the existing recommendations [4], DNA damage was assessed by its percentage in the "tail" of DNA-comets (% of DNA in the tail). At least 200 DNA comets were analyzed per each experimental point. As an additional indicator the atypical DNA-comets were taken into consideration also. They featured vague, undefined nucleus and a peculiar diffuse tail. They are regarded as an indirect indicator of cytotoxicity [18].

Assessment of antigenotoxic effect

The antigenotoxic effect (AE) was expressed as a percentage and was calculated using the following formula:

$$AE = 100 - \frac{\%\ \text{of DNA in the tail}_{s+g}}{\%\ \text{of DNA in the tail}_{g}} \times 100, \qquad (1)$$

where: % of DNA in the $tail_{s+g}$ — parameter % of DNA in the tail, recorded during the assessment of the mice that received sausage and genotoxicant; $\%DNA_g$ — parameter % of DNA in the tail, registered after the action of the genotoxicant.

Statistical processing

While confirming the distribution normality and dispersion homogeneity with the help of the Shapiro-Wilk test and Bartlett's test, respectively, the data obtained from the experimental groups were compared pairwise out in accordance with the Mann-Whitney test.

Otherwise, in accordance with existing recommendations [19], the original values were logarithmically converted, followed by the Mann-Whitney test.

When comparing the proportions of atypical DNA-comets, Fisher's exact test was used.

Results and discussion

In a control series of experiments the spontaneous levels of DNA damage in cells of certain parts of the gastrointestinal tract and liver were determined, as well as the number of atypical DNA comets (ADC) was found, which phenomena are the evidence of cytotoxicity according to the overwhelming majority of opinions [4,18]. The relatively larger amount of ADC observed in the cells of the analyzed parts of the gastrointestinal tract compared to liver tissue is explained by the physiologically determined intensive renewal of cells of the gastrointestinal mucosa, which undergo intensive exfoliation into the lumen of intestines (Table 1).

Table 1. The influence of control and experimental samples of meat product on the genotoxic and cytotoxic effects of methyl methanesulfonate in the mice organs *in vivo*

Group/ organ	Control		MMS40 mg/kg		MMS + M_{std}		MMS + M_{be}	
	% of DNA in the tail	ADC (%)	% of DNA in the tail	ADC (%)	% of DNA in the tail	ADC (%)/ RE	% of DNA in the tail/AE	ADC (%)/ RE
Liver	0.6 ± 0.2	0.4 ± 0.6	7.4 ± 2.2[a]	6.5 ± 1.9[b]	7.3 ± 1.9 —	3.6 ± 1.0[c] ↓44.6%	6.2 ± 0.5 —	4.0 ± 1.7 ↓38.5%
Stomach	1.7 ± 0.4	13.2 ± 4.5	12.8 ± 2.9[a]	32.7 ± 1.0[b]	11.5 ± 1.9 —	20.0 ± 6.7[c] ↓38.8%	5.4 ± 0.8[d] ↓57.8%	19.3 ± 5.2[c] ↓41.0%
Duodenum	0.7 ± 0.2	19.6 ± 1.5	14.0 ± 2.5[a]	28.0 ± 3.8[b]	13.4 ± 0.7 —	25.7 ± 8.0 —	5.7 ± 1.2[d] ↓59.3%	20.3 ± 3.1[c] ↓28.5%
Rectum	0.7 ± 0.3	7.9 ± 4.9	14.6 ± 4.3[a]	29.7 ± 4.6[b]	13.9 ± 5.0 —	18.5 ± 7.5[c] ↓37.7%	7.6 ± 2.6[d] ↓48.0%	13.6 ± 4.2[c, e] ↓54.2%

Notes:
M_{std} — the sample of a standard meat product; M_{be} — sample of a meat product with beetroot extract; AE — a antigenotoxic effect; RE — reduction in the number of atypical comets, ADC — atypical DNA-comets; ↓ — decrease of ADC level in comparison with the effect of MMS;
[a] — $p < 0.01$ compared with the "Control" group (as per Mann-Whitney test); [b] — $p < 0.01$ compared with the "Control" group (Fisher's test); [c] — $p < 0.05$ compared with the "MMS40 mg/kg" group (Fisher test); [d] — $p < 0.01$ compared with the "MMS40 mg/kg" group (Mann-Whitney test); [e] — $p < 0.05$ compared with the "MMS+ Mst " group (Fisher test).

Alkylating genotoxicant MMS, recommended by the OECD as the positive control sample when using the DNA-comet method [16], demonstrated the profound DNA damaging and cytotoxic activity in all parts of intestine and liver researched. The results characterizing DNA damage during the use of the genotoxicant significantly exceeded the values recorded in the control sample by at least 11 times. In its turn, the number of ADC also significantly exceeded the control values in all versions of the experiment; the maximum increase in the yield of ADC under the influence of MMS was found in the liver, where 6.5 ± 1.9% of abnormal comets were recorded in comparison with 0.6 ± 0.2% in the control sample.

The results that mark the effects of MMS in the liver coincide with the data of historical control run by the Research Laboratory of Genetic and Reproductive Toxicology of Federal State Budgetary Institution Research Institute of Pharmacology n.a V. V. Zakusov "Federal Research Center for Original and Prospective Biomedical and Pharmaceutical Technologies" [3], data on the effects of genotoxicant action in the cells of the gastrointestinal tract were obtained for the first time.

Supplementing the diet of mice with the control meat product without beetroot extract provided no effect on the levels of MMS-induced DNA damage in the stomach, small intestine, rectum, and liver. Among the same animals of the same group, the significant decrease of MMS cytotoxicity was observed in the cells of liver, gastric and rectal mucosa, by 44.6%, 38.8% and 37.7% respectively. This effect can be hypothetically explained by enriching the animals' diet with substances in the base meat of the cooked sausage, which substances improve the constructive and energy metabolism in animals. Meanwhile, it is important to underline that the addition of the meat component into the diet provided no effect on DNA damage, which indicates the absence of antigenotoxic activity in the studied control sample.

A different situation was observed after feeding the animals with the sausage with beetroot extract added. In the mucosal cells of all examined parts of their intestine a significant decrease in DNA damage was recorded. In stomach cells the genotoxic effect of MMS was significantly diminished by 57.8%, in duodenal cells by 59.3%, and in rectum by 48.0% respectively. Eating of a meat product with added beetroot extract did not anyway affect DNA damage of liver cells.

ADC accounting showed that consumption of a meat product with added beetroot extract significantly diminished the cytotoxicity of MMS in all organs under research. In the liver, a decrease in ADC was noted by 38.5%, in the stomach by 41.0%, in the duodenum by 28.5%, and in the rectum by 54.2%. It is worth to point out that the last result in the list above, recorded in the rectum, was significantly higher than the result recorded when consuming a meat product without beetroot extract, which directly proves the role of beetroot extract in the cytoprotective protection of liver and gastrointestinal cells.

So, in result of the experiments conducted it was found that poultry meat products enriched with beetroot extract possess profound antigenotoxic activity in the cells of various parts of the gastrointestinal tract, except for the liver cells. Significantly more expressed cytoprotective activity of a meat product enriched with beetroot extract was also revealed in comparison with the food product, not enriched with beetroot extract, in terms of ADC count in the liver and rectum cells.

It is logically reasonable to consider the data obtained in the experimental part of this study in the context of known information about the consumer and biological properties of red beetroot.

Historical review shows that beetroots have been long used not only as a food product and a valuable source of dye pigments of plant origin, but also as a folk curative remedy in Persian and Arabic medicine, in Serbia and Germany to prevent metastases and/or treat patients who suffer from gastrointestinal tumors. Evidence-based medicine data on the beneficial effects of red beetroot for the prevention and treatment of tumors is insufficient, but this

plant features antioxidant, anti-inflammatory and other functions potentially beneficial for the patients with cancer of certain stages, including using it for prevention of the chemotherapy side effects. [20]. Moreover, individual clinical studies have confirmed the benefits of beetroot for managing blood pressure and endothelial dysfunction, improving cognitive function and aiding for recovery after physical activity among the athletes [20,21,22].

In the predominant majority of cases whole variety of beetroot's positive health effects of are attributed to their antioxidant properties. The antioxidant activity of beetroot juice is higher than that of many other juices (tomato, carrot, orange, pineapple) and it only slightly ranks below to pomegranate juice [7].

However, two facts are left beyond the focus of due attention. Firstly, antioxidants, especially phenolic ones, tend, depending on their concentration, do typically change their antioxidant effect to the opposite pro-oxidant effect, i. e. they demonstrate the inversion of their effect. Perhaps this is the feature that explains the heightened mortality among the cancer patients treated with antioxidants, as well as the ability of these antioxidants to boost metastasis [3,7,23,24]. Secondly, antioxidant activity as a chemical property and the antioxidant status of an organism, organ or tissue are fundamentally different concepts, one is purely chemical concept inherent to a substance, the other concept is biological one; it is the antioxidant status of a body that determines the efficiency of its protection from the harmful effects of oxidative stress, which today is considered as one of the most widespread pathogenic mechanisms [3,25,26]. If to add to this effect the obvious genetic heterogeneity of the mankind and the diversity of dietary preferences among the people, it is easy to imagine that additional consumption of exogenous antioxidants can provide positive effect for some individuals and be negative for the others, or within one organism it can provide positive effect on functioning of one organ and be negative for another one. Many authors paid attention to the probable negative effect of antioxidants and the inadmissibility of their consumption [25,26]. It is therefore obvious that enriching food with antioxidants not in all cases would lead to the desired beneficial health-improving effect, and the declared health-improving and cancer-preventive properties of the developed functional products, as noted by the academicians Lisitsyn and Oganesyants of the Russian Academy of Sciences [27], must be proven in special researches and studies, similar in their program to preclinical and clinical studies of the medical drugs. This condition is especially relevant for the compounds which antioxidant functions were demonstrated *in vitro*, in model chemical systems.

The significant benefit of red beetroot is that its positive effect on the efficiency of functioning of mammals antioxidant system has been demonstrated *in vivo*. In particular, there is data [28] that in the liver of rats that were consuming beetroot extract for 7 days in a row and while this diet got injected with carcinogenic carbohydrate tetrachloride (CCl_4), a significant decrease in peroxidation products was observed in comparison with the corresponding positive control. The same authors point out the protective antioxidant properties of beetroot juice (8 ml/kg of rat's body weight for 28 days) in rats against the oxidizing effects of scopolamine and the famous carcinogen 7.12-dimethylbenzaantracene.

As possible mechanisms of the antioxidant effect of beetroot, the capability to maintain the antioxidant function of tissues is considered which function is due to restoration of glutathione, the important endogenous antioxidant, and affecting the antioxidant defense enzymes glutathione peroxidase and catalase, which decompose peroxides down to water and thereby prevent the formation of highly reactive forms of oxygen [29].

Based on the concept of genotoxicity of the products formed by oxidative stress, antioxidant and antigenotoxic properties are considered as mutually conjugated. Despite this, the research of the antigenotoxicity of beets *in vivo* is represented by only few studies with controversial results

In some studies beetroots did not affect the induction of DNA damage [28], in some other studies beetroot juice, administered orally to rats at rate of 8 ml/kg per day for 28 days, reduced by 20% max. the damage of DNA caused by a single injection of the carcinogen N-nitrosodiethylamine%, but increased the damaging effect of carbon tetrachloride in rat blood leukocytes by almost 40% [30,31].

So, only the data obtained in this study convincingly demonstrate the ability of dry beetroot extract added as an ingredient of a meat product to diminish induced genotoxicity and for the first time demonstrate the functional manifestation of this ability in the cells of gastrointestinal tract.

The Table 2 presents the information we described in detail earlier [13], characterizing the content of natural compounds in beetroot which showed antigenotoxic activity in experiments or in direct observations in humans.

Most of the potential antigenotoxicants are found in beetroot in the quantities insufficient to form doses which have an antigenotoxic effect. Ascorbic acid the most well-known and widespread antigenotoxicant in plant materials is present in beetroot in amount of 13 mg/100 g, which is definitely not sufficient to demonstrate the antigenotoxic effect which is observed in doses of tens of milligrams per kg of animal weight [13]. Though there are two exceptions — betaine and betanin. However, betaine is synthesized in the body from choline, which suggests a dynamic balance between its intake and removal from the body for maintaining a physiological optimum. There are more grounds and reasons to consider betanin as the compound responsible for the antigenotoxicity.

Betanin (betanidin-5-O-β- glucoside) — is the representative of N- heterocyclic betalain pigments. This is a red dye known as E162; it is used in majority of countries and regarded as harmless. Earlier we described betanin in detail [13].

Table 2. Potential antigenotoxicants and their content in red beetroot.

ANTIMUTAGENS IN BEETROOT					
PHENOLS AND POLYPHENOLS		VITAMINS VITAMIN-LIKE SUBSTANCES		BETALAINS* (betacyanins and betaxanthins)	
Compound	Contents per 100 g of beets	Compound	Contents per 100 g of beets	Compound	Contents per 100 g of beets
4-hydroxybenzoic acid	12 mg	Ascorbic acid (vitamin C)	3.6÷13 mg	Betanin (betanidin 5-O-β-glucoside)	50–60 mg
Vanillic acid	5.1 mg	Betaine	Up to 500 mg		
Katekhin	38 mg	Carotene	0.01÷20 μg		
Quercetin	no data	Pantothenic acid (vitamin B_5)	130÷155 μg		
Kaemferol	no data	Pyridoxine (vitamin B_6)	67 μg		
Coffee shop acid	7.1 mg	Retinol (vitamin A)	2 μg		
Rutin	0.25 mg	Tocopherol (vitamin E)	40 μg		
Saponins	no data	Thiamine (vitamin B_1)	0.31÷31 μg		
Chlorogenic acid	1.8 mg	Folic acid	73÷109 μg		
Epicatechin	0.39 mg				

Note:
*** — some betalains are phenolic compounds, in particular — isobetanin, prebetanin and neobetanin, vulgaxanthin I, vulgaxanthin II and indicaxanthin.**
**** — Dry beet extract contains 3.976 g of betalains / 100 g of extract.**

It is noteworthy that the vast majority of studies engaged in betanin actually used beetroot extract. For example, in the study [29], betanin produced by ABCR GmbH is specified as the compound under study and even its structural formula is given, whereas according to the manufacturer's catalogue it is beetroot extract with the same name. Commercially available products marketed as "betanin" " (E162) are red beetroot extracts with dextrin, and consist of betalains and some non-coloring substances like minerals, organic acids, vitamins, etc. [32]. Being isolated in the form of a separate compound betanin is unstable and gets easily destroyed under the influence of heat, light and oxygen. The instability creates certain challenges for its storage and experimental works [33]. In this regard, we emphasize that in commercial products and in experimental studies, the name "betanin" is used to denote red beetroot extracts.

For the first time this work demonstrates the possibility of targeted protection of gastrointestinal tract cells from the damaging effects of alkylating agent *in vivo* as per the framework of the accepted methodology of searching and studying antigenotoxicants. The aggregate of the achieved results is shown in the Figure 2.

The action of the predominant majority of anticancer medications is based on their ability to damage DNA of tumor cells, but they affect healthy cells too. Cancer chemotherapy entails large number of side effects, including the development of new tumors, which formation is provoked by genotoxic effects of the applied chemotherapy [34,35]. Actively proliferating tissues, including gastrointestinal cells, are the most sensitive to the iatrogenic effects of chemotherapy; DNA damage plays significant role in these tissues damage [36,37,38,39].

The application of antigenotoxicants or cytoprotectors, usually antioxidants, for prevention of the side effects of chemotherapy has long been discussed [40]. However, while protecting healthy cells, they inevitably compromise the efficiency of chemotherapy [41], moreover, antioxidants taken *per se* out of the natural complexes are characterized by inversion of their effects [3,23,24,42].

The problem of selective protection of the healthy cells from the genotoxic effects of chemotherapy while keeping up the antitumor effect within the tumor growth area still has no practical solution [37], although theoretically the solution lies in the path of creation of selective antigenotoxicants. They should protect healthy tissues that are most vulnerable to the genotoxic effects of chemotherapy, and should not provide protection for tumor cells. The obtained data indicate that the developed meat product with dry beetroot extract protects the cells of gastrointestinal tract from genotoxic effects. The similar effect has not been recorded for liver, so the consuming the meat product with a dry beetroot extract solves the task of antigenotoxic protection of gastrointestinal tract cells.

Moreover, it is known that DNA damage plays a leading role in the occurrence of colorectal cancer, which affects younger and younger people on incrementally larger scale

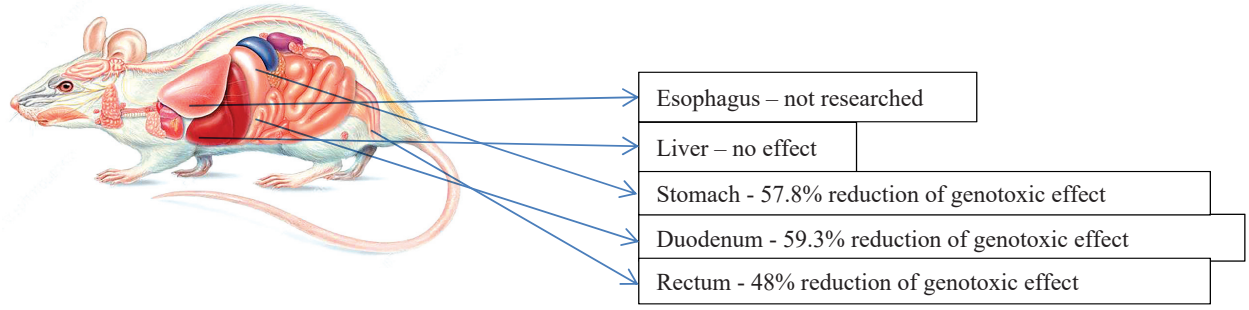

Figure 2. Antigenotoxic effect of the experimental meat product with addition of red beetroot extract.

[35]. Prevention of this disease with the help of antigenotoxic functional products with addition of beetroot extract can be considered as significant scientific direction at the intersection of food technologies and technologies of preventive medicine.

It is crucially important that beetroot extract, one of the few natural red dyes, has no dosage limitations; there is no upper limit for its consumption. Its application is limited only to current tasks and requirements to the organoleptic properties of the product, where it is used as a dye. This feature attaches particular value to the beetroot in terms of its use as part of functional antigenotoxic food products.

It is important to point out a large number of studies that demonstrate antioxidant, anti-inflammatory, antitumor, hypotensive properties of red beetroot, as well as its positive effect on cognitive functions [10], and now antigenotoxicity in the gastrointestinal tract as well. However, beetroot have not still taken their respected place among the raw materials for functional products, losing out to more exotic and expensive vegetables and fruit. This situation is caused by insufficient attention of the researchers to this valuable vegetable and the lack of evidence-based medicine data in the clinical setting demonstrating the beneficial effects of beetroot on human health. Those researches are impossible without the development of functional food products with addition of beetroot and beetroot products; this study is a fragment of such research.

Conclusion

The results of the conducted studies demonstrated the manifestation of antigenotoxic and cytoprotective properties of red beetroot extract (*Beta vulgaris*) used as part of a meat product. Taking into consideration the fact that dry extract of red beetroot is a widely used food production additive E162 ("Beetroot Red"), the revealing of this peculiar property of this food additive ensures it using in the composition of the functional and specialized food products. The above-specified food additive is allowed for use without restrictions, so the restrictions can only be determined by organoleptic aspects and consumer's perception. It is necessary to note that up to this date the antigenotoxic and cytoprotective properties of red beetroot in gastrointestinal tract cells *in vivo* have not been proven.

So, the above-presented results demonstrate the protective effect of a meat product — a boiled sausage — enriched with beetroot extract added in amount of 20 g per 1 kg of raw meat, on DNA damage induced by alkylating agent in stomach, duodenum and rectum cells. The obtained data confirm the anticarcinogenic properties of beetroot and reveal the mechanism of their actuation through the manifestation of antigenotoxic activity, provide the grounds for further research of the functional antigenotoxic food products based on beetroot extract and, in the future, their clinical tests. It was established that standard technological methods and modes of the boiled sausages production do not provide any negative impact on the studied properties of the red beetroot betanin.

The discovered capability of targeting the antigenotoxic effect of dry beetroot extract as part of a meat product opens up the opportunity of it using in economically efficient and natural diets aimed to prevention of primary and secondary gastrointestinal cancer.

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The authors declare no conflict of interest.

DOI: https://doi.org/10.21323/2414-438X-2024-9-2-145-152
Received 08.04.2024
Accepted in revised 19.06.2024
Accepted for publication 20.06.2024

Available online at https://www.meatjournal.ru/jour
Original scientific article
Open Access

ECONOMIC IMPACT OF EDIBLE OFFAL REJECTION AT EL-QUREIN SLAUGHTERHOUSE, EGYPT

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Keywords: *slaughterhouse, carcass, economic loss, offal rejection, parasitic diseases*

Abstract

The thorough examination of animal carcasses and organs intended for human consumption is crucial for food safety. However, it is not always conducted adequately due to time constraints during the slaughter process. A cross-sectional study was undertaken to identify the key reasons for the rejection of edible offal in slaughtered animals at El-Qurein slaughterhouse in Sharkia province, Egypt, and to evaluate the associated economic losses during the period from August, 2020 till July, 2021. The study examined 1,218 organs (406 livers, 406 lungs, and 406 hearts) from various animals (cattle, buffaloes, camels, sheep and goats), among which 138 organs (78 livers, 38 lungs, and 22 hearts) were rejected, primarily due to lesions caused by Cysticercus ovis, fascioliasis, Cysticercus tenuicollis, pneumonia, and hydatid cysts. The financial loss at the slaughterhouse due to organs rejection over the twelve-month period totaled 1,113,905.24 Egyptian pounds (equivalent to 71,865 USD). The high frequency of meat rejection and resulting financial impact exceeded the region's local revenue. This study highlights the persistent prevalence of parasitic diseases, resulting in significant detrimental economic repercussions in Sharkia province, Egypt. Furthermore, the present work underscores the necessity for the development of an effective control program addressing the causes of meat rejection in the region.

For citation: Ras, R., Mahmoud, A.F.A., Hafez, A. El-S.E., Ghazaly, E.E.I., Shata, R.H.M., El-tahlawy, A.S. (2024). Economic impact of edible offal rejection at El-Qurein slaughterhouse, Egypt. *Theory and Practice of Meat Processing*, 9(2), 145–152. https://doi.org/10.21323/2414-438X-2024-9-2-145-152

Acknowledgements:

The authors express gratitude for the support received from the Department of Food Hygiene, Safety, and Technology at the Faculty of Veterinary Medicine, Zagazig University.

Introduction

The sustainable rise in the global number of human population necessitates a corresponding proportional increase in the production of animal-derived food products, fostering a balanced growth in both developed and developing countries [1]. In Egypt, cattle, camels, sheep, and goats are the vital sources of red meat, offering crucial protein for human diets [2,3]. Acknowledged for its nutrient richness, red meat contains high-quality proteins, trace elements, and vitamins, contributing significantly to dietary balance [4]. Additionally, the edible offal like livers, kidneys, and tongues despite their lower consumption rates compared to meat, hold the considerable economic value and gastronomic appeal [5]. However, persistent challenges arise from infectious diseases and climate change, thus hindering the meat industry's efforts to maintain red meat's dietary importance [6].

Meat and edible offal often harbor various contaminants, including pathogens, posing health risks and potential negative impacts [7,8]. Widespread bacteria in food of animal origin pose transmission risks to humans during meat handling, preparation, cooking and consumption, as well as during rejection procedures at the slaughterhouse [9]. Diseases like bovine tuberculosis, and parasitic infections such as cysticercosis, hydatidosis, and fascioliasis further threaten human health and meat safety [10,11,12, 13,14]. Consequently, ensuring meat safety and hygiene is a critical concern for all parties concerned, including producers, distributors, and consumers.

In developing countries, the slaughterhouses play a crucial role in providing data on disease prevalence, public health importance, and financial losses due to organ rejection [15,16]. Stringent meat inspection regulations, that include pre- and post-slaughter analyses, are vital for protecting consumers from foodborne hazards [17,18]. Veterinarians conduct thorough examinations to assess carcass quality and safety, addressing potential health risks [19]. However, certain diseases, like fasciolosis, hydatidosis, and cysticercosis, inflict significant economic losses through partial organ or carcass rejection, impacting both public health and the economy [20].

In Egypt, numerous studies have been conducted at various slaughterhouses across various regions of the country, with a primary focus on the epidemiological aspects of specific disease conditions such as parasitic and bacterial diseas-

es [21,22]. Nevertheless, there is currently a lack of available data on the primary causes of rejection and the corresponding economic losses in Sharkia province slaughterhouses in Egypt. Therefore, the objectives of this study were to identify the major causes of total edible organs (lungs, liver, and heart) rejection and to assess the associated direct financial losses as a result of total edible organs rejection at El-Qurein slaughterhouse in Sharkia province, Egypt.

Objects and methods

Samples collection and preparation

The present research was carried out at El-Qurein slaughterhouse in Sharkia province, Egypt at the period from August, 2020 till July, 2021. Sharkia province is situated in the eastern region of the Nile delta in Egypt, with geographical coordinates ranging from approximately 30.7 °N to 31.63 °E. Furthermore, it has the second-largest agricultural area within the country, following Beheira province, boasting a cultivated plant area covering 824,098 acres.

Research project

The cross-sectional study was carried out to determine the major causes of lungs, livers, and heart rejection and to evaluate the direct financial losses. In total 220 randomly selected cattle, in particular: 89 buffaloes, 40 camels, 47 sheep, and 10 goats were subjected to ante-mortem and postmortem examinations following the guidelines outlined in Ministerial Decision No. 517/1986 issued by the Egyptian Ministry of Agriculture and Land Reclamation (MALR) [23].

Antemortem inspection

All animals were observed while resting and moving within the lairage pens. Furthermore, their general behavior, visual cleanliness, symptoms of diseases, nutritional status, and any other irregularities were monitored according to standard ante-mortem inspection procedures. Animals deemed suitable for slaughter, being free from any diseases or abnormalities, were then sent for slaughter [24].

Postmortem meat inspection

The thorough postmortem examination was carried out, involving visual inspection, palpation, and systematic incision of each internal organ, with particular attention given to the liver, lungs, and heart, in order to detect cysts, various types of adult parasites, and any other abnormalities.

Assessment of financial loss

As local visceral organs such as lungs, livers, and hearts were assessed for rejection, only the direct losses associated with rejection were calculated. Meanwhile, the average prevailing market prices of each organ were determined by surveying the prices in the shops of the various butchers who purchase the meat from the slaughterhouse. These variables were then input into the following formula to determine the annual financial loss resulting from rejection of the organs deemed unsuitable for human consumption due to gross pathological lesions [25].

Thus, the estimated annual economic loss (EL) due to organ/carcass rejection from local market was defined by the following formula:

$$EL = S_{rx} \times C_{oy} \times R_{oz} \quad (1)$$

Where,

S_{rx} — annual animal slaughter rate of the slaughterhouse;
C_{oy} — average cost of each liver/lung/heart/carcass;
R_{oz} — rejection rates of liver/lung/heart/carcass.

Ethics statement

The search protocol has been revised and approved by the Institutional Animal Care and Use Committee, Zagazig University, Egypt, with an approval number (ZU-IACUC/2F/286/2022).

Statistical analysis

The data of infected organs were analyzed with *Chi-square (χ2)* tests using IBM SPSS Statistics for Windows software version 21. *P-values* ≤ 0.05 were considered statistically significant.

Results and discussion

Rejection rate of edible organs of the slaughtered animals

According to the findings outlined in the Table 1, a total of 1,218 edible organs were examined, consisting of 406 livers, 406 lungs, and 406 hearts sourced from various slaughtered animals including cattle, buffaloes, camels, sheep, and goats. Among these organs, 138 were deemed unsuitable for consumption, with 78 livers, 38 lungs, and 22 hearts being rejected. This resulted in rejection rates of 0.19 (19%), 0.09 (9%), and 0.05 (5%) for rejected livers, lungs, and hearts, respectively, attributed to various lesions.

Table 1. Rejection rate of edible organs of animals slaughtered at El-Qurein slaughterhouse during 2020 and 2021

Animal species	No. of examined edible organs			No. of rejected edible organs			Rejection rate, (%)		
	Liver	Lung	Heart	Liver	Lung	Heart	Liver	Lung	Heart
Cattle	220	220	220	52	3	5	0.24 (24%)	0.01 (1%)	0.02 (2%)
Buffaloes	89	89	89	11	26	2	0.12 (12%)	0.29 (29%)	0.02 (2%)
Camels	40	40	40	2	9	2	0.05 (5%)	0.23 (23%)	0.05 (5%)
Sheep	47	47	47	12	0	13	0.26 (26%)	0.00	0.28 (28%)
Goats	10	10	10	1	0	0	0.10 (10%)	0.00	0.00
Total	406	406	406	78	38	22	0.19 (19%)	0.09 (9%)	0.05 (5%)

The recent study indicates that there were no reasons for rejection noted during the antemortem inspection of the animals prior to slaughter. However, all rejections were attributed to diseases identified during postmortem

inspection. Table 2 provides a summary of the postmortem findings from the animals inspected and slaughtered at El-Qurein slaughterhouse in the years 2020 and 2021.

In the investigated slaughterhouse, out of a total of 220 cattle slaughtered, the rejection rates for liver, lung, and heart were 0.24 (24%), 0.01 (1%), and 0.02 (2%), respectively. According to the study, the highest proportion of liver rejection was caused by fasciolosis, accounting for 20.45%, while abscesses were the least common cause, accounting at 3.18%. *Cysticercus bovis* was identified as the primary cause for heart rejection, accounting for 2.27%, and hydatidosis was the leading cause for lung rejection, with a portion of 0.91%. Additionally, this research indicated that the incidence of liver rejection in buffaloes was 0.12 (12%), while for lungs and hearts, it was 0.29 (29%) and 0.02 (2%), respectively. The study identified fasciolosis as the primary cause of liver rejection, accounting for 7.87% of cases. Pneumonia was identified as the predominant reason for lung rejection, constituting 15.73% of cases, while pericarditis was the leading cause of heart rejection, representing 2.25% of cases.

Moreover, the primary reasons for rejecting the camel edible organs such as the liver, lung, and heart were hydatid cyst and abscess (2.5% each), hydatid cyst (12.5%), and *Cysticercus dromedary* (5%), respectively.

Regarding sheep carcasses, the primary reasons for liver, lung, and heart rejection were *Cysticercus tenuicollis*, accounting for 17.02% of liver rejections, and *Cysticercus ovis*, responsible for 27.66% of heart rejections. Lungs showed no signs of lesions.

Concerning goat carcasses, *Cysticercus tenuicollis* (10%) was the primary reason for liver rejection, whereas the lungs and heart showed no abnormalities.

The rejection rate of cattle liver was significantly higher than lung and heart (P-values ≤ 0.05). Moreover, the rejection rate of liver in goats was significantly higher than lung and heart (P-values ≤ 0.05).

Causes of liver rejection

In the recent research, it was observed that the liver manifested the most significant number of abnormal conditions, totaling 78 cases. Among the various animals studied, cows had 52 liver lesions, buffaloes had 11, camels had 2, sheep had 12, and goats had 1. The main reasons for liver rejection were identified as fasciolosis (accounting for 28.32%), *Cysticercus tenuicollis* (27.02%), necrosis (6.55%), abscess (3.62%), and hydatid cyst (2.5%). Lower obtained rate of fascioliasis in cattle was recorded by Tembo and Nonga [26] in Tanzania (4.5%) and Assefa and Tesfay [27] in Ethiopia (9.26%). Moreover, in the study of Jaja et al. [28], the prevalence of fascioliasis among cattle carcasses in South Africa was equal to 5.95%, 4.48%, and 2.7% for the years 2010, 2011, and 2012, respectively. While, according to Denbarga et al. [29], Mohammed et al. [16] in Ethiopia, Cadmus and Adesokan [30] in Western Nigeria, and Dukundane et al. [31] in Gicumbi districts of Rwanda, the higher prevalence of fasciolosis in cattle carcasses with percentages of 86.4%, 36.06%, 29.44%, and 90%, respectively was noted.

Fasciolosis significantly contributes to the onset of various liver conditions in buffaloes, such as bile duct hyperplasia, hepatitis, and fatty degeneration. The prevalence of fascioliasis in Egyptian slaughterhouses is notable, likely due to the abundance of larvae and favoring climatic conditions. This parasitic infection is widespread across several African nations and typically doesn't result in mortality in livestock. However, it does lead to diminished productivity in animals and the discard of infected livers in slaughterhouses [32].

Concerning *Cysticercus tenuicollis*, lower rates of 7.81%, 5.73%, and 0.71% were observed in sheep liver through the investigation of Bayu et al. [33], Mandefro et al. [34], and Dejene et al. [35] in Ethiopia, respectively. The presence of *Cysticercus tenuicollis* is linked to the extent of pasture contamination caused by unrestricted dogs' movement and the husbandry practices as well as grazing habits of these animals. These factors play a role in facilitating the transmission cycle among ruminants, dogs, and other wild canines [33].

Causes of lung rejection

The combined total of 38 lesions were identified in the lungs. Specifically, cows had 3 lesions, buffaloes had 26 lesions, and camels had 9 lesions. The primary reasons for lung rejection included pneumonia (20.73%), hydatid cysts (17.9%), tuberculosis (9.44%), and abscesses (5%). These results were nearly similar to the previous studies conducted by Mummed and Webb [36], and Cadmus and Adesokan [30] who documented that the prevalence of pneumonia cases among cattle carcasses in Ethiopia and Western Nigeria was equal to 22.68% and 21.38%, respectively. Lower prevalence of pneumonia cases was detected in Nigeria by Raji et al. [15] with a rate of 8.79%. In another study performed by Jaja et al. [37], lower rates of pneumonia (1.09%, 2.21%, and 0.77%) were recorded at three Namibian slaughterhouses. In contrast to that, Abdel-Rassol et al. [38] showcased a greater proportion of 47.4% in Egypt.

Hydatidosis prevalence rates vary across various regions, as evidenced by studies conducted in Tanzania by Tembo and Nonga [26], in Kenya by Kere et al. [39], in Tanzania by Komba et al. [40], and in Egypt by Abd El-Aziz et al. [41] who reported lower incidence rates of 3.1%, 5.3%, 0.04%, and 0.038%, respectively. In contrast, Kerala et al. [19] found a significantly higher prevalence in Southern Ethiopia (23.04%), along with Denbarga et al. [29] (82.5%) and Mulatu et al. [42] (20.05%) in other parts of Ethiopia. This variance in occurrence could be attributed to factors such as geographical location, the population of dogs, and the standards of the livestock sanitation [43,44].

Concerning lung tuberculosis prevalence, Okeke et al. [45] found a comparable bovine tuberculosis rate of 9.1% in Nigeria. Furthermore, in Tanzania, Komba et al. [40]

detected a lower prevalence of 1.87%. Moreover, Shitaye et al. [46] reported that the prevalence of bovine tuberculosis in slaughterhouses across various regions of Ethiopia varied from 3.5% to 5.2%. In another study conducted by Woldemariyam et al. [47], the prevalence of bovine tuberculosis in cattle carcasses slaughtered at the Debre Birhan municipality slaughterhouse in Ethiopia was recorded at 4.7%. Alternatively, Dechassa [48] reported a greater incidence of bovine tuberculosis at rates of 24.7% in Ethiopia, while Cleaveland et al. [49] observed a rate of 20% in Tanzania.

Causes of heart rejection

The combined heart lesions observed in cows, buffaloes, camels, and sheep totaled 22 cases, with respective occurrences of 5, 2, 2, and 13. The primary reasons for heart rejection were cysticercosis, accounting for 34.93% of cases, and pericarditis, which constituted 2.25% of cases. In comparison with the obtained results, a decreased occurrence of cysticercosis (0.27%) was identified at the Adigrat municipal slaughterhouse in the northern part of Ethiopia by Assefa and Tesfay [27], while EDO et al. [50] reported a lower rate (0.9%) in Adama municipal slaughterhouse, Ethiopia. Furthermore, Dyab et al. [51] documented a prevalence of 12.6% in El-Minia governorate slaughterhouses, Egypt, between June 2017 and May 2018. On the other side, Aziz et al. [52] conducted a study in various slaughterhouses located in Sohag governorate, Egypt, which revealed a higher prevalence rate of 64.43%. Similarly, Rabi and Jegede [53] observed a higher prevalence rate of cysticercosis in the heart, reaching 66%, within the Kano slaughterhouse, Nigeria. As for pericarditis, a lower incidence rate (1.6%) was reported by EDO et al. [50] on the basis of bovine carcasses slaughtered at Adama municipal slaughterhouse, Ethiopia. However, other studies by Mummed and Webb [36], Ahmed et al. [54], Raji et al. [15], and Madzingira et al. [55] observed higher prevalence rates of pericarditis (4.59%, 8%, 17.06%, and 19.7%) in various regions including Ethiopia, Egypt, Nigeria, and Namibia, respectively.

Table 2. Major causes of rejection of each edible organs of the animals slaughtered at El-Qurein slaughterhouse during 2020 and 2021.

Livestock species	Rejected organs	Disease	Infection rate (%)
Cattle	Liver (52/220)	*Fascioliasis* (45/220)	20.45%
		Necrosis (7/220)	3.18%
	Lung (3/220)	*Hydatid cyst* (2/220)	0.91%
		Tuberculosis (1/220)	0.45%
	Heart (5/220)	*Cysticercus bovis* (5/220)	2.27%
Buffaloes	Liver (11/89)	*Fascioliasis* (7/89)	7.87%
		Necrosis (3/89)	3.37%
		Abscesses (1/89)	1.12%
	Lung (26/89)	*Pneumonia* (14 /89)	15.73%
		Localized Tuberculosis (8/89)	8.99%
		Hydatid cysts (4/89)	4.49%
	Heart (2/89)	*Pericarditis* (2/89)	2.25%
Camels	Liver (2/40)	*Hydatid cysts* (1/40)	2.5%
		Abscesses (1/40)	2.5%
	Lung (9/40)	*Abscesses* (2/40)	5.0%
		Hydatid cysts (5/40)	12.5%
		Pneumonia (2/40)	5.0%
	Heart (2/40)	*Cysticercus dromedary* (2/40)	5.0%
Sheep	Liver (12/47)	*Cysticercus tenuicollis* (8/47)	17.02%
		Necrosis (4/47)	8.51%
	Lung (0/47)	0	0
	Heart (13/47)	*Cysticercus ovis* (13/47)	27.66%
Goats	Liver (1/10)	*Cysticercus tenuicollis* (1/10)	10%
	Lung (0/10)	0	0
	Heart (0/10)	0	0

Financial losses due to rejections

The total economic impact resulting from the rejection of meat over the span of a year was assessed to be 1,113,905.24 Egyptian pounds, equivalent to USD 71,865 as detailed in the Table 3. In a study performed by Ciui et al. [56], the combined monetary losses stemming from the rejection of edible parts over a two-year period were calculated to be EUR4,021,717.3 at a cattle slaughterhouse located in southeastern Germany. In addition, Mohammed and Maky [11] undertook a study that lasted for two years (2017–2018) in both northern and southern regions of Egypt. Their investigation revealed a financial deficit of 4,529,010 Egyptian pounds (USD 383,063) attributable to meat rejection across three slaughterhouses. The primary factors leading to meat rejection were tuberculosis, icterus, pneumonia, hydronephrosis, parasitic cysts, and fasciolia-sis. The economic impact observed in this study surpasses the results reported in a previous study conducted in Egypt by Ahmed et al. [54], where they estimated an annual financial loss of 36,480 Egyptian pounds resulting from the rejection of organs from slaughtered male cattle at the Ismailia slaughterhouse. Moreover, Yibar et al. [1] undertook a survey within the slaughterhouses located in Bursa province, Turkey, revealing a financial deficit of USD 245,483 due to the rejection of organs and carcasses over a span of six months in two slaughterhouses. Hydatidosis and fasciolosis served as the primary factors for organ rejection, whereas tuberculosis and jaundice were identified as the primary causes leading to carcass rejection. Furthermore, Amuamuta et al. [57] conducted the research at the Bahir Dar municipal slaughterhouse in Ethiopia, determining that the rejection of liver and lung tissues resulted into a substantial economic loss amounting to USD 9,257.914. Primary reasons for rejection included fasciolosis and hydatidosis, which inflicted significant economic losses due to their prevalence. Furthermore, a yearly financial deficit amounting to USD 11,155.52 linked to zoonotic metacestodes found in cattle slaughtered at Yabello municipal slaughterhouse in Ethiopia during the timeframe spanning December 2017 to March 2018 was documented by Beyene and Hiko [58], where *Cysticercus bovis* and hydatidosis were the predominant causes of organ rejection. Moreover,

Table 3. Rejection rates, price, and annual financial loss of rejected organs of the livestock slaughtered at El-Qurein slaughterhouse during 2020 and 2021.

Item	Average weight (kg)	Price[a]	Cost[b] (C_{oy})	Rejection rate[c] (R_{oz})	Annual slaughter rate[d] (S_{rx})	Annual loss estimation (EL)
Cattle liver	8	200	1600	0.24	1,849	710,016
Buffalo liver	8	200	1600	0.12	143	27,456
Camel liver	8	200	1600	0.05	1,708	136,640
Ovine liver	1.2	210	252	0.26	47	3,079.44
Caprine liver	0.9	210	189	0.10	10	189
Cattle lung	8	60	480	0.01	1,849	8,875.2
Buffalo lung	8	60	480	0.29	143	19,905.6
Camel lung	8	60	480	0.23	1,708	188,563.2
Ovine lung	1.2	60	72	0.00	47	0
Caprine lung	1.2	60	72	0.00	10	0
Cattle heart	2.5	60	150	0.02	1,849	5,547
Buffalo heart	2.5	60	150	0.02	143	429
Camel heart	2.5	60	150	0.05	1,708	12,810
Ovine heart	0.5	60	30	0.28	47	394.8
Caprine heart	0.5	60	30	0.00	10	0
Total estimated loss						1,113,905.24 EGP (71,865 USD)

(a) Price: Average price of organs at the local market (Egyptian pound/kg); (b) Cost (Coy): Average cost of organs at the local market (Egyptian pound), calculated as Average weight × Price; (c) Rejection rate (Roz): The proportion of organs rejected during inspection; (d) Annual slaughter rate (Srx): The number of animal species slaughtered annually at El-Qurein slaughterhouse; (EL) Annual loss estimation: The financial loss due to rejected organs, calculated as Annual slaughter rate × Cost × Rejection rate.

1 US dollar was equal to 15.50 Egyptian pounds

Abd Elaziz et al. [41] reported financial losses amounting to approximately EGP 11,712.5 for fascioliasis, EGP 32,940.0 for cysticercosis, and EGP 2,410 for hydatidosis, resulting from the rejection of liver and lung tissues at a local Egyptian slaughterhouse situated in Cairo governorate. Differences in economic losses across the studies may stem from the variations in factors such as livestock numbers, disease occurrence rates, estimation methods, the scope of loss items analyzed, disparities in livestock productivity and market prices.

Conclusion

Substantial financial losses have been incurred to the Egyptian slaughterhouses due to various pathological conditions. The research highlights fascioliasis, parasitic infections, and pneumonia as the predominant ailments significantly impacting the Egyptian economy. It's evident that implementing preventative measures such as regular administration of anti-parasitic drugs and stringent monitoring of sanitation protocols, especially in Sharkia province, is imperative. Ensuring the thorough meat inspection and proper disposal of rejected meat are essential for safeguarding of the public health. Focus should be directed towards dealing with frequently identified illnesses in the slaughterhouses. Implementing the anti-parasite monitoring programs at farms is crucial for minimizing the risk of infectious diseases spreading and reducing financial losses. Furthermore, the construction of well-equipped slaughterhouses and comprehensive training for the slaughterhouse staff are the essential steps. Finally, more research is needed for better understanding the causes of pathological findings, particularly at the slaughterhouse level, to enhance our understanding of the zoonotic risks posed to the consumers.

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The authors declare no conflict of interest.

DOI: https://doi.org/10.21323/2414-438X-2024-9-2-153-159
Received 25.05.2024
Accepted in revised 25.06.2024
Accepted for publication 28.06.2024

Available online at https://www.meatjournal.ru/jour
Original scientific article
Open Access

COMBINED EFFECTS OF DIFFERENT TEMPERATURE-TIME MODES ON THE MECHANICAL CHARACTERISTICS OF SOUS-VIDE AND CONVENTIONAL OVEN-COOKED CAMEL MEAT

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Keywords: *camel meat; sous-vide; conventional oven; mechanical properties*

Abstract

Camel meat was subjected to sous-vide and conventional oven cooking at different combinations of temperature (70, 80, 90, and 100 °C) and time (30, 60, 90, 120, 150, and 180 minutes). The influence on the mechanical properties (shear force, penetration force, and texture profile) were studied. In general, our results revealed significant differences ($p \leq 0.05$) between the sous-vide and conventional oven cooking methods for most of the studied parameters. Increasing the sous-vide cooking temperature-time combination resulted in lower shear and penetration forces. Moreover, a clear decline in meat hardness, chewiness, and gumminess was observed. Sous-vide cooking depends on water for cooking, where the thermal conductivity coefficient is high and uniform. The textural changes during sous-vide cooking made the meat more homogenous and tender. Conversely, the conventional oven method depends on dry air heat where the thermal conductivity coefficient is low in comparison with sous-vide cooking. The elevation of the penetration force, hardness, chewiness, and gumminess along with the increasing temperature-time values combination was obvious for the conventional oven-cooked meat.

For citation: Fickak, A.A., Othman, M.B., Hobani, A.I., Mohamed, G.M., Al- Ghamdi, S., Alfaifi, B. et al. (2024). Combined effects of different temperature-time modes on the mechanical characteristics of sous-vide and conventional oven-cooked camel meat. *Theory and Practice of Meat Processing,* 9(2), 153–159. https://doi.org/10.21323/2414-438X-2024-9-2-153-159

Introduction

Sous-vide or "under vacuum" cooking is a method of cooking food inside the heat-stable vacuumed pouches under the controlled temperature and time conditions [1]. Unlike sous-vide cooking, convection cooking involves using a conventional oven with a heating element to raise the temperature to a preset level, and hot air circulates within the oven's cavity. Convection cooking uses higher temperature settings than sous vide cooking. This is caused by the fact that air does not conduct heat as well as water does. Because of the longer cooking time at a higher temperature, the food may be over-cooked on the outside and undercooked in the center. Sous-vide cooking has been endorsed worldwide for meat cooking, possibly for its simplicity and potential in enhancing meat tenderness [2,3,4] and for its ability to improve the sensory characteristics in a range of meat varieties, particularly firm meat cuts, due to the uniform thermal dispersion during the vacuumed cooking process [5,6]. Moreover, the growing consumer awareness towards healthy and quality food products has changed the consumers' preferences for high quality, freshly preserved food, with minimum volume of additives, and with the lowest degree of food processing [7]. Various studies have been carried throughout the recent few years for better understanding of the effect of sous-vide cooking on meat quality characteristics such as physical, and biochemical properties [8,6,9,10,11]. However, meat texture remains the most important parameter of consumptive quality of meat [12]. The recommended sous-vide cooking temperature-time combinations vary for different types of meat, for example, a temperature-time combination around 58–63 °C for 10–48 hours is recommended for beef, pork and lamb [13]. Camel meat is another type of red meat, and it is known to have similar characteristics as beef [14], in fact, it has nutritive advantage over beef or lamb due to its low proportion of intramuscular fat, low cholesterol content, and high iron content [15]. Very insufficient research is available about the use of novel processing techniques such as sous-vide which can improve the consumer acceptability of camel meat. Camel meat is tougher than almost all other types of red meat [14] and hence, may require different sous-vide cooking temperature-time combinations. It has been widely noted that consumers are ready to pay more for meat with high-quality characteristics that satisfy their eating aspirations on a continuous basis [16,17]. Meat palatability and quality characteristics, particularly the mechanical characteristics (shear force, penetration

force, and texture) are affected by a complex series of processing parameters such as processing temperature and time [18]. Structural changes of meat tissue occur during the various thermal processes. Accordingly, the meat proteins denaturize [19] and cause structural changes (fiber shrinkage, aggregation, solubilisation of collagen and connective tissues, formation of sarcoplasmic and myofibril gel) [20]. Hence, the mechanical characteristics of meat are altered [21]. The tenderness of meat is inversely proportional to shear force [20]. Previous studies on sous-vide cooking were mainly focused on beef [22,23,24], goat [25], and chicken meat [26]. However, fewer studies investigate the effects of sous-vide cooking on camel meat quality, particularly the mechanical characteristics. The selection of accurate sous-vide cooking temperatures and duration of exposure is fundamental and may greatly improve the tenderness and overall qualities of the tough camel meat. This study is aimed at evaluating the combined effects of different temperature-time modes on the mechanical characteristics of sous-vide and conventional oven-cooked camel meat.

Objects and methods

Preparation of meat samples

Camel meat samples (shoulder cutlets muscle: Latissimus dorsi) were prepared from healthy male camels between 6/7 months old, obtained from a company specialized in selling fresh camel meat (Umm Al Hammam Butchery — Riyadh, KSA), the meat was delivered within 24 hours after slaughter. *Longissimus dorsi* shoulder cutlets samples were prepared and shaped into similar sizes (8 × 6 × 1.3 cm chops) as described by Palka and Daun [27], and Dawood [21]. The prepared samples were then placed in plastic bags and stored in deep freeze at –20 °C for 72 hours prior to processing.

Cooking methods

Two different cooking methods were used in this study: sous-vide and conventional oven cooking. Before cooking, the prepared samples were thawed for 24 hours in the refrigerator at 3 °C [19]. For the sous-vide cooking the thawed samples were placed in sous-vide cooking pouches, (20.3×15.2 cm and 85 μm thickness) obtained from the company Sous-vide Supreme, Broomfield — USA, and vacuum sealed by a vacuum device as described by Baldwin et al [5]. The samples were then placed in a thermostatic circulating water bath (GFL Water bath, Model1083, Germany) at different temperatures (70, 80, 90, 100 °C) and time duration (30, 60, 90, 120, 150 and 180 minutes) combinations as described by Garcia-Segovia et al [23]. For the conventional oven cooking, a forced convection oven (Drying oven Binder, Model E240. Germany) was heated up to 100 °C, the thawed samples were then placed inside and cooked at the temperatures-time combinations defined above. For analysis accuracy, three replicates were cooked for each treatment mode [23].

Mechanical characteristics analysis

The influence of cooking on the meat texture profile, shear force, and penetration force was determined using a TA HDi Texture Analyzer, HD3128. Cooked meat samples were cut into 1 cm^3 chunks [27]. The direction of cutting was parallel to that of the fiber.

Texture profile analysis

The texture profile components (hardness, gumminess and chewiness) were analyzed by using a circular piston with diameter of 75 mm. The apparatus was set to have a depth penetration of 10 mm pressing and a piston speed was set at 1.5 mm/second, and the time between two test operations was set to 10 seconds.

Shear and Penetration tests

The test procedure followed the method described in ASAE Standards (2000), specifically in ASAE S368.4 DEC99 [28]. The samples were compressed with 'V' shaped craft knife (Craft knife HD/Bs) down to 75% of their original height at distance of 22 mm down, and a column speed of 1.5 mm/s. The curves of dependence of shear force, and the penetration force vs time were generated.

Statistical analysis

The study was arranged in a complete random design (CRD) with a 3-way factorial design of 2 × 4 × 6. The statistical analysis of the data was performed using ANOVA in GLM (SPSS Software for Windows, version 27.0. software program provided by IBM Corp 2020).

The data were analyzed using ANOVA in GLM (SPSS Software for Windows, Version 27.0. Armonk, NY, USA: IBM Corp 2020). The data were assessed under normal distribution, and the mean values were used to represent the results. If any significant differences were observed at $p \leq 0.05$, a post hoc analysis was conducted using the least significant difference (LSD) method.

Results and discussion

Effects of cooking methods on the mechanical properties of camel meat

The mechanical qualities of camel meat are the key factors that influence consumer satisfaction. Thus, the effect of cooking methods and different temperature-time combinations on the mechanical properties of camel meat are investigated. These properties include texture profile (hardness, chewing and gumminess), shear force, and penetration force. The results (Table 1) show that the mechanical characteristics were significantly ($p \leq 0.05$) influenced by the cooking methods at all tested temperature-time combinations.

Textural profile analysis (TPA)

The TPA statistics analysis (Table 1) shows that the hardness, chewiness, and gumminess were significant ($p \leq 0.05$) affected by the cooking time and by the effect of its interaction with temperature of this cooking method. Only

the hardness and chewiness were significantly ($p \leq 0.05$) affected by the cooking temperature.

The TPA results for the sous-vide cooked camel meat (Figures 1A, 1C and 1E) show an apparent decreasing of meat hardness, chewiness, and gumminess. This is possibly due to using water in this method, which has a high thermal conductivity coefficient, and results into rapid heat distribution that causes changes in the composition of protein fibers [29]. Extending the temperature-time combination causes collagen gelatinization, which creates the paths through which the dissolved muscle fat can get released and displaced, potentially acting as a moisture sealer during cooking [20,29,30]. Accordingly, the highly fat-lined meat structure shrinks less during cooking and remains juicier [31]. Furthermore, meat with a substantial amount of connective tissue will become tender if cooked for extended periods in moist-heat cooking [20,27].

In contrast, the conventional oven cooking method TPA results (Figures 1B, 1D and 1F) shows an increase in hardness, chewiness, and gumminess. This is possibly explained by the changes in the myofibrillar proteins and connective tissue during the conventional cooking [32]. Initially, when being conventionally heated, meat proteins denature, resulting in texture hardening, possibly due to changes in the tertiary structure and increased collagen fiber concentration in cross sections [33]. According to Hostetler and Landmann [34] the myosin denatures and coagulates during cooking, causing shrinkage of the myofilaments, tightening of the myofilaments microstructure and shortening of sarcomeres [35].

Shear force and penetration force

The results (Table 1) show the values for the shear force and penetration force for the sous-vide and conventional oven-cooked camel meat. The shear force and penetration force were significantly ($p \leq 0.05$) affected by the cooking

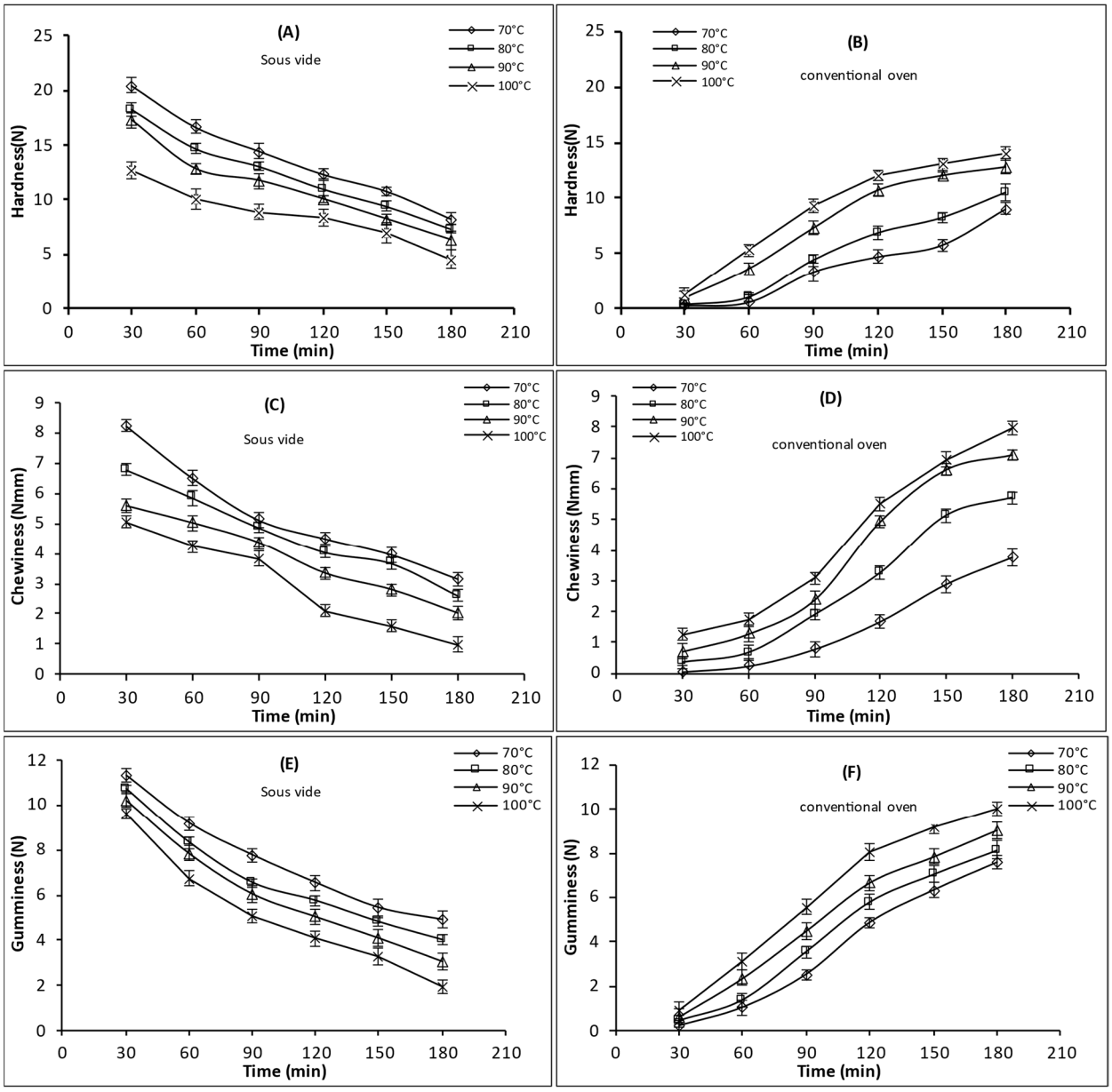

Figure 1. Effect of the cooking method (sous-vide and conventional oven) on the camel meat hardness (A and B), chewiness (C and D) and gumminess (E and F)

Table 1. Influence of the cooking method on the mechanical properties of camel meat

Treatments	Mechanical properties				
	Shear force	Penetration force	Hardness	Chewiness	Gumminess
Cooking method:					
Sous vide	36.36^{b}	1.38^{a}	11.88^{a}	4.18^{a}	6.07^{a}
Electric oven	45.35^{a}	1.14^{b}	6.57^{b}	3.12^{b}	4.51^{b}
Cooking temperature (°C):					
70	49.14^{a}	1.30^{a}	8.92^{b}	3.41	6.17^{a}
80	41.97^{b}	1.28^{a}	9.26^{a}	3.71	5.54^{b}
90	40.77^{b}	1.24^{b}	9.49^{a}	3.79	3.79^{c}
100	31.53^{c}	1,21^{b}	8.89^{b}	3.68	5.63^{b}
Cooking time (min):					
30	49.14^{a}	1.17	8.97^{b}	3.50^{c}	4.93^{b}
60	45.20^{b}	1.19	8.12^{c}	3.19b^{c}	4.78^{b}
90	43.15^{c}	1.20	9.07^{b}	3.29^{c}	4.90^{b}
120	40.01^{d}	1.26	9.53^{b}	3.60^{b}	5.58^{a}
150	37.09^{e}	1.31	9.31^{b}	4.19^{a}	5.83^{a}
180	30.52^{f}	1.42	10.38^{a}	4.05^{a}	5.71^{a}
SEM	1.82	0.08	0.87	0.42	0.58
Main effects	**P-values**				
Cooking method	< .001	< .001	0.04	< .001	0.01
Cooking temperature	< .001	< .001	0.03	NS	0.03
Cooking time	< .001	NS	0.01	< .001	0.04

$^{a, b c, d, e, f}$ **Means within the same column with different superscripts are significantly different. SEM, standard error mean; *p-value*, probability level (considered significant when $p \leq 0.05$); NS, not significantly different.**

methods, cooking time and by the cooking temperature. However, the sous-vide cooked meat was less resistant to the shear force than conventional oven cooked meat (Figures 2A and 2B). This can be related to the constant temperature during the sous-vide cooking by means of water bath, which allows the breakdown of the perimysium the tissue that supports the surrounding of the fiber bundle within the muscle. As the meat tissues break down, the meat becomes more tender and the shear force required to cut the meat is reduced [17].

Unlike the shear force, constant increase in penetration force was observed in regards to the conventional oven cooked meat (Figure 2D). In contrast, the penetration force decreased constantly along with the increasing temperature-time values combinations, the similar results were obtained in the preceding studies where sous-vide cooked meat (Figure 2C) showed lower penetration force values [36,37]. The penetration force appears to be very sensitive to changes in connective tissue caused by the dry heat during the conventional oven cooking [37], and hence, increasing the meat toughness, and subsequently increasing the penetration force [38]. Our results agree with Bouton et al. [39] work on the mechanical properties of cooked meat samples. In addition, Lorenzo et al. [40] reported on the dependence between the penetration force values and thermal losses caused during conventional oven cooking, where samples with high thermal loss demonstrated the highest penetration force values.

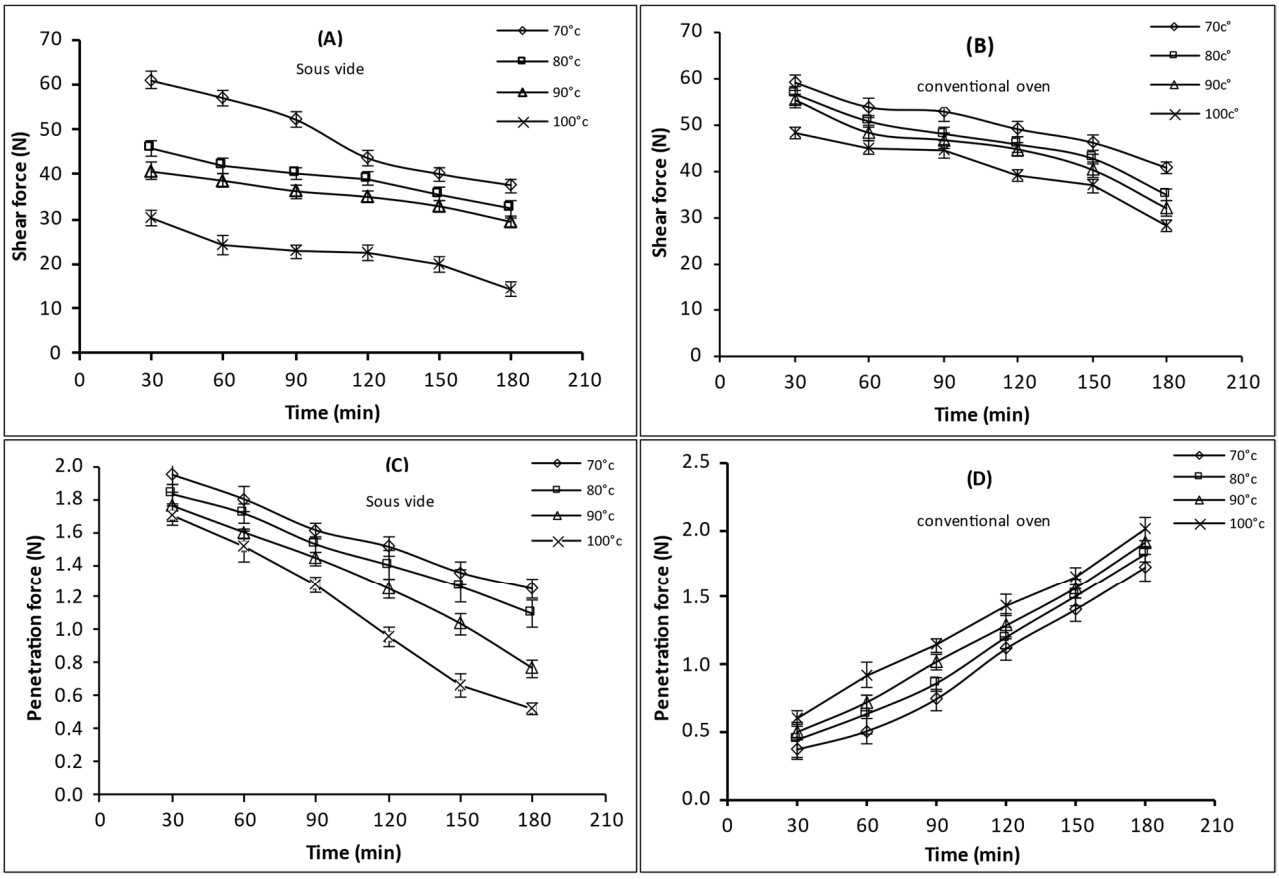

Figure 2. Effect of sous-vide and conventional oven cooking on camel meat shear force (A & B) and penetration force (C and D)

Conclusion

In conclusion, both cooking temperature and cooking time appears to significantly impact the mechanical properties of sous vide and conventionally cooked camel meat. The obtained results showed that increasing the sous vide cooking temperature-time values combination decreased the TPA values (hardness, gumminess chewiness), penetration force and shear force. It-is opposite to conventional oven cooking, where all tested parameters increased, except for the shear force which decreased. In addition, the meat cooked using the sous vide method exhibited lower shear force compared to that of the conventional oven. This can be attributed to the uniform cooking temperature, which rapidly breaks down the perimysium tissue supporting the fiber bundles within the muscle, resulting in more tender and juicy meat with a natural flavor profile that may lack the depth of flavor that comes from exposure to high dry heat provided by the conventional oven cooking.

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The authors declare no conflict of interest.

DOI: https://doi.org/10.21323/2414-438X-2024-9-2-160-168
Received 15.05.2024
Accepted in revised 14.06.2024
Accepted for publication 22.06.2024
Available online at https://www.meatjournal.ru/jour
Original scientific article
Open Access

THERMAL STABILITY AND DIGESTIBILITY OF A BIOPOLYMER SYSTEM FOR THE DELIVERY OF MINOR NUTRIENTS IN ENRICHED MEAT PRODUCTS

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Keywords: *supramolecular complex, enriched meat products, eicosapentaenoic acid, docosahexaenoic acid, vitamin D3, in vitro digestion, oxidation*

Abstract
The study examined thermal stability and digestibility of a biopolymer delivery system for the liposomal form of minor nutrients (omega-3 polyunsaturated fatty acids, vitamin D3, essential oil of clove buds) in enriched meat products. A fraction of encapsulated liposomes in the biopolymer delivery system, i. e. in the supramolecular complex with sodium caseinate (SC), was more than 74%. The difference between the number of bound liposomes before and after freeze-drying is statistically insignificant. The study of the fatty acid composition in samples of enriched meat product containing a supramolecular complex (EPSC) and enriched meat product containing components of the supramolecular complex (EPC) showed that the total omega-3 fatty acids content in EPC was 0.079 ± 0.002 g/100 g, while in EPSC it was 0.207 ± 0.002 g/100 g. The data obtained made it possible to state that EPSC sample was a source of omega-3 fatty acids. Product fortification with the supramolecular complex made it possible to meet the daily requirement of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) by 70%, and vitamin D3 by 470%. A study of the in vitro digestion of EPC and EPSC enriched meat products, it was revealed that in both samples, release of fat in the gastric phase was almost identical, in contrast to the intestinal phase, where the released fat in EPSC was found to be 2 times higher than in EPC. This indicates that the use of physiologically functional ingredients in encapsulated form to fortify meat products is more effective and does not violate the general principles of lipid digestion. At the same time, the mass fraction of released fatty acids in the intestinal phase was higher by 74.4% and 48.5%, respectively, when using physiologically functional ingredients in the form of a supramolecular complex in comparison with a product containing these ingredients in their native form. Use of high temperature treatment did not affect the bioavailability of EPA and DHA, as well as the organoleptic parameters or oxidative stability of EPSC.

For citation: Semenova, M.G., Aslanova, M.A., Galimova, A.R., Fedulova, L.V., Antipova, A.N., Martirosova, E.I. et al. (2024). Thermal stability and digestibility of a biopolymer system for the delivery of minor nutrients in enriched meat products. *Theory and Practice of Meat Processing,* 9(2), 160–168. https://doi.org/10.21323/2414-438X-2024-9-2-160-168

Funding:
The article was published as part of the research topic No. FGUS-2024–0003 of the state assignment of the V. M. Gorbatov Federal Research Center for Food Systems of RAS (development of a meat product, analysis of fatty acids and modelling of digestion: Aslanova M. A., Bero A. L., Fedulova L. V.) and with the support from the Russian Science Foundation: grant No. 21–16–00085, https://rscf.ru/проект /21–16–00085/ (development of a supramolecular complex and characterisation of its physicochemical properties: Semenova M. G., Galimova A. R., Antipova A. S., Martirosova E. I., Zelikina D. V.).

Acknowledgements:
The authors acknowledge Lipoid GmbH (Germany) for providing a free sample of soybean phosphatidylcholine.

Introduction

Currently, due to the lack of various bioactive substances (BAS) in the human diet, product fortification with physiological and functional ingredients is becoming increasingly popular.

Long-chain omega-3 polyunsaturated fatty acids (PUFAs), which are mainly concentrated in fish oil, represent one of the most physiologically important nutrients. The deficiency of these nutrients among children and adults in the Russian Federation is quite high with a prevalence of 80% [1]. Therefore, it is of the utmost importance to develop the production of fortified, functional and specialised food products with the aim of increasing daily consumption of PUFAs [2].

The biochemical functions of omega-3 PUFAs are associated with the transmembrane transmission of synaptic signals, the synthesis of prostaglandins, as well as vasodilatory, antithrombotic and antiatherogenic effects. These functions underlie the successful functioning of the cardiovascular, central nervous, visual systems and

the regulation of lipid metabolism [3]. However, the use of polyunsaturated fatty acids in food production is complicated by their low solubility in aqueous media (due to their hydrophobic nature) and high susceptibility to oxidation and degradation (due to the presence of unsaturated carbon-carbon bonds) [4]. The incorporation of PUFAs into meat products is of paramount importance, given that the lipid profile of meat is characterized by a high content of saturated fatty acids and a low content of PUFAs [5]. At the same time, the negative effect on organoleptic parameters is a limitation for application of PUFAs in quantities to achieve their claimed physiological effect [6,7]. This is associated with increased oxidative processes during heat treatment and storage. Unlike PUFAs [8], vitamin D3 does not affect the taste of the product, but has low solubility in aqueous media, including body fluids of the gastrointestinal tract, due to its hydrophobic nature [9]. One way to overcome these problems is to use the encapsulated forms of these bioactive substances in food products [10]. This approach can help to slow down the oxidation of PUFAs, increase the bioavailability of vitamin D3, neutralize undesirable odors and extend the shelf life of food products, thereby improving their quality and safety [11].

Phospholipid liposomes are a form of unique membrane-forming constructs for encapsulating hydrophobic bioactive substances (BAS), which are considered as a promising form for BAS delivery to cells and tissues [12]. This is due to the similarity of their bilayers to the cell membrane, as well as their ability to form mixed micelles with bile salts in the small intestine [13,14]. In addition, it is important that after entering the body, liposomes are metabolized and do not accumulate in the body. However, along with these properties, phospholipid liposomes have a number of disadvantages, i. e. instability of liposome structure and the associated uncontrolled release of loaded bioactive substances, as well as the tendency of liposomes to autoxidize with atmospheric oxygen due to the presence of PUFAs in them, especially at high temperatures in food production and during storage. It is now well known that additional encapsulation of phospholipid liposomes with loaded bioactive substances using food biopolymers may help solving these problems in practice [15].

A number of works are devoted to the use of encapsulated bioactive substances in the composition of meat products, in which the following food biopolymers were used as an encapsulating agent: sodium alginate, chitosan, maltodextrin, carrageenan, inulin, sodium caseinate, and whey protein concentrate [16]. Mohamed K. Morsy studied the stability of functional beef hamburgers enriched with microencapsulated fish oil [17]. Jimenez-Martin studied the effects of microencapsulated omega-3 PUFAs on the oxidative stability and sensory properties of frozen chicken nuggets [18]. Solomando investigated the effect of fish oil microcapsules in meat systems on the bioavailability of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) using *in vitro* models [19]. All these studies focused on the effect of microencapsulation on the bioavailability of the introduced components, as well as on the physicochemical and sensory properties of short-term heat-treated meat products. In this regard, it is of interest to study the effect of prolonged high-temperature exposure, on the preservation and bioavailability of the liposomal form of minor nutrients, in the form of a biopolymeric delivery system in the production of enriched meat products.

The purpose of this study was to study thermal stability and digestibility of a biopolymer system for the delivery of omega-3 PUFAs and vitamin D3 in liposomal form as a new functional ingredient for meat product fortification.

Objects and methods

The objects of the research were as follows:

— the *biopolymer supramolecular complex* consisting of phosphatidylcholine (PC) liposomes, with an amount of 0.156% w/v, 99.5% purity (Lipoid GmbH, Germany) coated with food-grade sodium caseinate (SC), with an amount of 2.44% w/v (88% protein, 3% carbohydrates, 1.5% fat, Targis Moloko LLC, Russia);

The following biologically active substances were encapsulated into bilayers of phosphatidylcholine (PC) liposomes:

- fish oil (FO), in the amount of 0.146% w/v ("Omega-deti Omega-3 Concentrate", Ruskaps, Russia), containing 20% DHA, 27.6% EPA according to gas-liquid chromatography data [20]);
- vitamin D3, in the amount of 5×10^{-6}% w/v ("Vitamin D3, drops" food supplement, Mirolla LLC, Russia), D3 content 250 μg/ml;
- essential oil of clove buds (EOC), in an amount of 0.006% w/v (IP Repicheva T. D., Russia).

Supramolecular complex (SC-PC-FO-EOC-D3) was prepared in an aqueous medium and then freeze-dried using AK 4–50 freeze-dryer (Proflab, Russia).

The supramolecular complex was formed by electrostatic and hydrophobic interactions, as well as by the formation of hydrogen bonds between the functional groups of sodium caseinate (SC) and PC-FO-EOC-D3 liposomes.

Previously obtained data indicated that the degree of encapsulation for fish oil and the essential oil of clove buds in a liposome bilayer reached 100 ± 1%, while for vitamin D3 it was 82 ± 2% [21].

The use of sodium caseinate for liposome encapsulation is due to its amphiphilic nature, which promotes the formation of various non-covalent bonds with phosphatidylcholine liposomes [22].

— *samples of heat-treated meat products*:

1) enriched meat product containing components of the supramolecular complex (EPC): sodium caseinate (SC) 2.75 g, phosphatidylcholine (PC) 0.156 g, fish oil (FO) 0.146 g, essential oil of clove buds (EOC) 0.006 g, vitamin D3 0.017 g;

2) enriched meat product containing a supramolecular complex (EPSC).

The technology of meat products was as follows: after grinding in a grinder, raw meat (54 g/100 g) was blanched for 5 minutes at 80 °C and homogenized to obtain a homogeneous finely ground paste, to which the recipe components were added, i. e. water (40 g/100 g), butter (3 g/100 g), nutmeg (0.05 g/100 g), supramolecular complex (3 g/100 g) or individual components included in the complex: sodium caseinate (SC) 2.75 g, phosphatidylcholine (PC) 0.156 g, fish oil (FO) 0.146 g, essential oil of clove buds (EOC) 0.006 g, vitamin D3 0.017 g. After mixing, the paste was packed in glass jars and heat treated at a temperature of 120 °C for 30 minutes.

The amount of functional ingredients added, i. e. EPA+DHA and vitamin D3, is determined by meeting at least 15% of physiological needs for adults established in Methodological recommendations of the MR 2.3.1.0253–21[1] and the TR CU 022/2011[2].

Determination of fat mass fraction in enriched meat products was carried out using the Soxhlet method in accordance with the GOST 23042–2015[3].

To assess the degree of lipid peroxidation, the peroxide value (PV) was determined by a method based on the reaction of the primary products of fat oxidation with potassium iodide, followed by titration and quantitative determination of the released iodine according to GOST 34118–2017[4], and the thiobarbituric acid value was determined by a method based on the reaction of thiobarbituric acid with malonic dialdehyde and the subsequent measurement of the absorbance of the product of this reaction on a spectrophotometer according to the GOST R 55810–2013[5].

The composition of fatty acids was determined by gas chromatography according to the GOST R 31663–2013[6] on an Agilent 7890A automatic gas chromatograph (Agilent Tech., USA) with a flame ionization detector. To determine fatty acids, a Supelco SP 2560 100 m × 0.25 mm × 0.2 mkm chromatographic column (Supelco, USA) was used;

Determination of vitamin D3 mass fraction was carried out according to the GOST 32307–2013[7];

An organoleptic analysis was carried out according to the GOST 9959–2015[8].

Determination of supramolecular complex particle size using laser light scattering in a dynamic mode

The effective hydrodynamic radius for sodium caseinate, PC-FO-EOC-D3 liposomes and SC-PC-FO-EOC-D3 supramolecular complex was determined in aqueous buffer solutions by dynamic laser light scattering using an LS-01 apparatus (Scientific Instruments, Russia), having a He-Ne laser with a vertically polarized beam (λ = 633 nm). All measurements were carried out in a thermostatic cell at 25 °C. The method is based on the analysis of autocorrelation function G(t) of fluctuations in the intensity (number of photons) of scattered light, which correspond to fluctuations in the local concentration of matter particles caused by their Brownian motion.

$$G(t)=\Sigma_j A_j A_{(j+n)} \quad (1),$$

where $t = n\tau$ is the delay time equal to the time interval of $\tau = 10^{-7}$ seconds, during which the intensity of light scattering is measured, multiplied by n which is the correlator channel number; A_j is the number of photons on the j-channel of the autocorrelator (high-speed computer). The light scattering intensity itself has a very strong fluctuation, but the product of the light scattering intensities $A_j A_{j+n}$ from two intervals separated by a fixed time period (the delay time t) is well averaged using an autocorrelator if the measurement procedure is repeated 10^5 to 10^6 times. Thus, j_0 corresponds to the start of measurements.

The time-correlation function may be associated with the time-correlation function of the electric field $g(t)$ of the scattered wave of light (determined by fluctuations in the number of photons in time) according to Seigert relation, chosen to interpret the empirical autocorrelation function in most cases:

$$G(t) = A + [Bg(t)]^2 \quad (2),$$

where

$$g(t) = exp(-D_{trans} q^2 t) \quad (3),$$

or

$$ln\, ln\, g(t) = -D_{trans} q^2 t \quad (4),$$

where

D_{trans} is the translational diffusion coefficient, m²/s;

t is time, s;

$q = \frac{4\pi}{\lambda} sin\frac{\theta}{2}$ is wave scattering vector, m⁻¹.

The effective hydrodynamic radius R_h was calculated from the time-correlation function of the light scattering intensity,

[1] Methodological recommendations MR 2.3.1.0253–21 "Norms of physiological needs for energy and nutrients for various groups of the population of the Russian Federation". Moscow: Garant, 2021. Retrieved from https://www.garant.ru/products/ipo/prime/doc/402716140. Accessed April 15, 2024. (In Russian)

[2] TR CU 022/2011 Technical Regulations of the Customs Union "Food products regarding their labeling". Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/902320347. Accessed April 15, 2024. (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 April 15, 2024. (In Russian)

[4] GOST 34118-2017 "Meat and meat products. Method for determination of peroxide value". Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/1200146654 Accessed April 15, 2024. (In Russian)

[5] GOST R 55810-2013 "Meat and meat products. Method for determination of tiobarbituric acid reactive assay" Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200107008. Accessed April 15, 2024. (In Russian)

[6] GOST R 31663-2013 "Vegetable oils and animal fats. Determination of methyl esters of fatty acids by gaz chromatography method". Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200104486. Accessed April 15, 2024. (In Russian)

[7] GOST 32307-2013 "Meat and meat products. Determination of fat-soluble vitamins by high performance liquid chromatography" Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200107182. Accessed April 15, 2024. (In Russian)

[8] GOST 9959-2015 "Meat and meat products. General conditions of organoleptical assessment" Moscow: Standartinform, 2016. Retrieved from https://docs.cntd.ru/document/1200133106. Accessed April 15, 2024. (In Russian)

which was measured at the scattering angle θ = 90°. Then, from it, the dependence of the natural logarithm of the time-correlation function of the electric field $g(t)$ on the delay time (t) was calculated using DYNALS Release 1.5 software (all rights belong to A. Golding and N. Sidorenko). Then, from the tangent of the angle of initial section, translational diffusion coefficient D_{trans} was found. Next, the value of R_h was calculated from D_{trans} using Stokes-Einstein equation:

$$D_{trans} = \frac{kT}{6\pi\eta R_h} \quad (5),$$

where

k is Boltzmann's constant, $1{,}38 \cdot 10^{-23}$ J/K;

T is absolute temperature, K;

η is the dynamic viscosity of the liquid (in a dilute solution, usually it is solvent), Pa · s;

R_h is hydrodynamic radius, m.

The calculation results were presented as the size distribution of light-scattering particles in the form of a histogram, the ordinate axis of which was the light scattering intensity values, and the abscissa axis was the size (hydrodynamic radius) of the particles in microns.

Determination of the degree of liposome encapsulation with sodium caseinate

PC-FO-EOC-D3 liposomes not bound in the supramolecular complex with sodium caseinate before and after freeze-drying, as well as their control variant (without protein), were extracted from their aqueous solutions with diethyl ether (Kuzbassorgkhim LLC, analytical grade, Russia). 3 ml of ether was added to 1 ml of sample solution, stirred, kept for 30 minutes at a room temperature and then left for 24 hours at a temperature of 5–7 °C. The upper organic phase was separated and the absorbance value of extracted lipids was measured using SF 2000 spectrophotometer (Spectrum, Russia) at λ = 210 to 215 nm. Diethyl ether was used as a reference solution (the method was developed by the Laboratory of Functional Properties of Biopolymers at Emanuel Institute of Biochemical Physics of Russian Academy of Sciences) [20].

The efficiency of encapsulation of PC-FO-EOC-D3 liposomes with sodium caseinate in their supramolecular complex was calculated using the equation:

$$E = \frac{A_{control} - A_{SC}}{A_{control}} \times 100 \quad (6),$$

where

E is the efficiency of liposome encapsulation with sodium caseinate (SC), %;

$A_{control}$ is absorbance value measured for the diethyl ether extract of PC-FO-EOC-D3 liposomes from the control buffer solution, taken as 100%;

A_{SC} is absorbance value measured for the diethyl ether extract of non-SC-encapsulated liposomes in SC-PC-FO-EOC-D3 supramolecular complex solution.

In total, 3 independent experiments were carried out, the experimental error in which did not exceed 5%.

Simulation of experimental in vitro digestion

The release of fat and omega-3 fatty acids, EPA and DHA, from SC-PC-FO-EOC-D3 supramolecular complex and enriched meat products was assessed under conditions simulating *in vitro* digestion.

To simulate digestion process of the sample, the Infogest 2.0 digestion model was used in accordance with the method [23]. First, to simulate chewing, the sample was crushed in a porcelain mortar. Then 5 g of the test sample was mixed with 3.5 ml of simulated oral fluid (pH 7) consisting of 0.5 ml of α-amylase 1500 U/ml (Sigma, China), 25 μl of 0.3 M calcium chloride (PanReac, Spain), 10.6 ml of phosphate-buffered saline (PanReac, Spain) and 0.09 ml of 6 M hydrochloric acid (Component-Reaktiv, Russia). The mixture was thoroughly mixed at 250 rpm at 37 ± 1 °C for 2 minutes using ImmunoChem-2200 thermal shaker (Helena Biosciences Europe, USA). After that, a sample was taken for further research.

Simulation of gastric digestion was done by mixing 10 ml of liquid oral contents with 7.5 ml of simulated gastric juice (pH 3) consisting of 1.6 ml of porcine pepsin 25,000 U/ml (PanReac, Spain), 1 ml of lipase 1200 U/ml (ABBOTT LABORATORIES, Russia), 5 μl of 0.3 M calcium chloride (PanReac, Spain), 0.8 ml of 1 M hydrochloric acid (Component-Reaktiv, Russia) and 10.0 ml of phosphate-buffered saline (PanReac, Spain). The mixture was thoroughly mixed at 250 rpm at 37 ± 1 °C for 120 minutes using ImmunoChem-2200 thermal shaker (Helena Biosciences Europe, USA). At the end of the incubation time, samples were taken for further studies.

Next, intestinal digestion was simulated, for which 20 ml of gastric chyme was mixed with 10 ml of simulated intestinal fluid (pH 7), which contained 5.0 ml of porcine pancreatin 800 U/mg (PanReac, Spain), 1.5 ml of preserved bovine bile (Samson-med, Russia), 40 μl of 0.3 M calcium chloride (PanReac, Spain), 10.6 ml of phosphate-buffered saline (PanReac, Spain). The mixture was thoroughly mixed at 250 rpm at 37 ± 1 °C for 120 minutes using ImmunoChem-2200 thermal shaker (Helena Biosciences Europe, USA). Then samples were also taken for further research.

In parallel with the simulation of experimental digestion of the studied samples, a similar experiment was carried out, where instead of 0.5 g of the sample, distilled water was added to take into account the determined indicators of the introduced reagents (background).

After simulation, the resulting substance was centrifuged on LISTON C2201 (LISTON, Russia) at 3500 rpm for 15 minutes to sediment undigested particles. The supernatant was collected and frozen at minus 40 °C to inactivate the enzymes for at least 12 hours.

The fatty acid composition of the obtained samples was determined. Results were resented as the percentage of released fat, EPA and DHA relative to the initial content (before digestion) at the end of each digestion stage.

Statistical analysis

Statistical analysis was performed using Mann-Whitney U-test ($p<0.05$) and STATISTICA 10.0 software.

Results and discussion

The results of measuring the particle size of SC-PC-FO-EOC-D3 supramolecular complex and its components separately, i. e. sodium caseinate (SC) and PC-FO-EOC-D3 liposomes, measured by the dynamic laser light scattering showed that the average value of the hydrodynamic radius R_h for liposomes was 60 nm, for sodium caseinate it was 180 nm, and for supramolecular complex it was 210 nm (Figure 1).

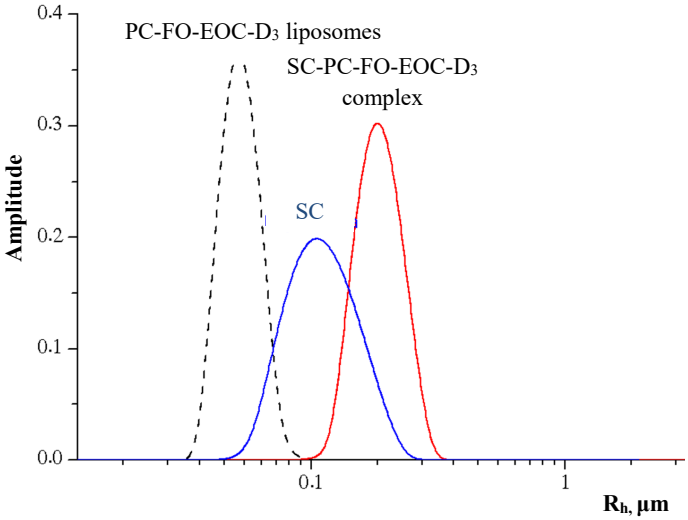

Figure 1. Size distribution for the studied samples: native sodium caseinate (blue), PC-FO-EOC-D3 liposomes (black dotted line) and their supramolecular complex, SC-PC-FO-EOC-D3 (red) in an aqueous medium (pH 7.0, 25 °C)

The shift of the complex peak from the peaks of the protein solution and liposomes to the region of a larger hydrodynamic radius indicates the successful formation of a submicron-sized complex in an aqueous medium.

The results of evaluating the effectiveness of liposome encapsulation with sodium caseinate in their supramolecular complex before and after freeze drying are presented in Table 1.

Table 1. The degree of liposome encapsulation with sodium caseinate in their supramolecular complex before and after freeze drying

Parameters	Free liposomes, %	Bound liposomes, %
The degree of liposome encapsulation in supramolecular complex before freeze drying	23.9 ± 0.3^b	76.1 ± 0.7^a
The degree of liposome encapsulation in supramolecular complex after freeze drying	25.3 ± 0.1^a	74.7 ± 0.9^a

Note: Statistical analysis was performed using Mann-Whitney U-test ($p < 0.05$). Different lowercase letters indicate statistically significant differences in parameter values determined for the two samples (comparing the values in the same column).

The degree of liposome encapsulation in supramolecular complex was more than 74%, while the difference between the number of bound liposomes before and after freeze drying was statistically insignificant.

The results of total fat and omega-3 PUFA (EPA and DHA) content in SC-PC-FO-EOC-D3 supramolecular complex and in thermally processed meat products, i. e. enriched meat product containing components of the supramolecular complex (EPC) and enriched meat product containing a supramolecular complex (EPSC), are presented in Table 2.

The results (Table 2) indicate that samples of enriched meat products differ slightly in fat content. When omega-3 fatty acids, EPA and DHA, were added in equal amounts to the formulations of EPC and EPSC samples, the content of ∑EPA+DHA in finished products was 21.6% and 70% of the adequate daily intake for adults and children over two years of age, respectively, which is 250 mg DHA+EPA per day[9]. Probably, the identified difference is associated with the oxidation and further degradation of fatty acids introduced into EPC sample in a free (non-encapsulated) form. The data obtained indicate the advantage of using SC-PC-FO-EOC-D3 supramolecular complex for fortification of meat products subjected to heat treatment and are consistent with the work [24].

A study of the fatty acid composition of the samples showed that the sum of all omega-3 fatty acids in EPC was 0.079 ± 0.002 g/100 g, and in EPSC it was 0.207 ± 0.002 g/100 g. The data obtained allow to state that EPSC sample as a source of omega-3 fatty acids, because in accordance with the technical regulations TR CU 022/2011 "Food products regarding their labeling", a product in which the amount of omega-3 fatty acids is at least 0.2 g per 100 g may be labeled as a "source" of omega-3 fatty acids.

The vitamin D3 content in EPC and EPSC samples was 231% and 470% of the recommended daily intake, respectively[10]. Despite the fact that the research results showed an excessively high content of vitamin D3 in both samples, its amount does not exceed the maximum permissible daily intake [25]. The results are consistent with those obtained

[9] Methodological recommendations MR 2.3.1.0253–21 "Norms of physiological needs for energy and nutrients for various groups of the population of the Russian Federation". Moscow: Garant, 2021. Retrieved from https://www.garant.ru/products/ipo/prime/doc/402716140. Accessed April 15, 2024. (In Russian)

[10] TR CU 022/2011 Technical Regulations of the Customs Union "Food products regarding their labeling". Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/902320347. Accessed April 15, 2024. (In Russian)

Table 2. Total fat and omega-3 fatty acids (EPA and DHA) content in the studied samples

Parameters	SC-PC-FO-EOC-D_3	EPC	EPSC
Fat, g/100 g of sample	10.76 ± 0.79	4.2 ± 0.4^a	4.6 ± 0.8^a
EPA, g/100 g of sample	1.136 ± 0.01	0.029 ± 0.002^b	0.101 ± 0.003^a
DHA, g/100 g of sample	2.025 ± 0.01	0.025 ± 0.001^b	0.074 ± 0.002^a
∑EPA+ DHA, g/100 g of sample	3.161 ± 0.01	0.054 ± 0.001^b	0.175 ± 0.002^a
Vitamin D_3, μg/100 g of sample	210.49 ± 31.57	11.6 ± 2.9^b	23.5 ± 5.9^a

Note: Statistical analysis was performed using Mann-Whitney U-test ($p < 0.05$). Different lowercase letters indicate statistically significant differences in parameter values determined for the two samples (comparing the values in the same row).

by Rabelo et al. [26], who believe that encapsulating vitamin D3 in colloidal nanocarriers may maintain its effectiveness, biological activity and bioavailability, thereby increasing its physiological benefits.

The effectiveness and bioavailability of supramolecular complex and enriched meat products in an in vitro model simulating the processes in the gastric and intestinal phases of digestion was determined by studying the content of released fat and omega-3 fatty acids, EPA and DHA, directly from SC-PC-FO-EOC-D3 supramolecular complex (Figure 2), and from EPC and EPSC samples (Figure 3).

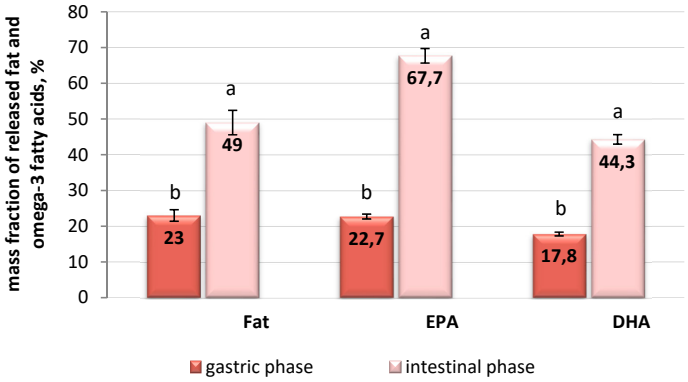

Figure 2. Mass fraction of released fat and omega-3 fatty acids during in vitro digestion of SC-PC-FO-EOC-D3 supramolecular complex, %

Note: Data scatter bars show the standard deviation from the average value of the measured parameter. Statistical analysis was performed using Mann-Whitney U-test ($p < 0.05$). Different lowercase letters indicate statistically significant differences in parameter values measured in the gastric and intestinal phases.

The results showed that most of the fat released from SC-PC-FO-EOC-D3 supramolecular complex during the intestinal phase (49%), while during the gastric phase, it was 23% (Figure 2).

Since lipolytic enzymes (pancreatic lipase, phospholipase and sterol esterase) are secreted at the intestinal level producing most of the hydrolyzed lipid compounds, fat found in the gastric phase is due to partial hydrolysis and dissolution of the superficial fat of the capsule, as a result of the gastric lipase action, which probably also indicates the effectiveness of liposome encapsulation in a supramolecular complex with sodium caseinate.

These results are consistent with the studies by Solomando et al. [27], who showed that during digestion, depending on the nature of the lipid compound, about 10% to 30% of fats are hydrolyzed in the gastric phase and 50% to 90% of fats are hydrolyzed in the intestinal phase.

The results of bioavailability studies of EPA and DHA fatty acids in SC-PC-FO-EOC-D3 supramolecular complex showed an increase in their content by 2.9 and 2.4 times, respectively, in the intestinal phase relative to the data obtained in the gastric phase.

When studying in vitro digestion of EPC and EPSC meat products, it was revealed that the release of fat for both samples in the gastric phase was almost the same, in contrast to the intestinal phase, where in EPSC, released fat was found to be 2 times higher than that of EPC (Figure 3).

This indicates that the use of physiologically functional ingredients in encapsulated form for meat product fortification is more effective and does not violate the general principles of lipid digestion.

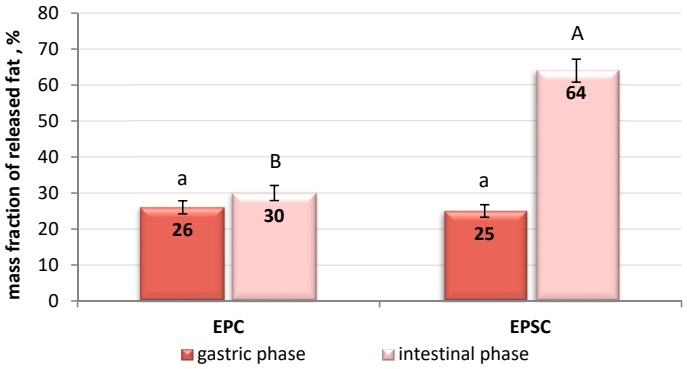

Figure 3. Mass fraction of released fat during in vitro digestion of enriched foods, %.

Note: Data scatter bars show the standard deviation from the average value of the measured parameter. Statistical analysis was performed using Mann-Whitney U-test ($p < 0.05$). Different letters indicate statistically significant differences in parameter values, enriched meat product containing components of the supramolecular complex, EPC, and enriched meat product containing a supramolecular complex, EPSC, namely lowercase letters in the model gastric environment and capital letters in the model intestinal environment.

Regarding the release of EPA and DHA from enriched meat products when simulating in vitro digestion, the mass fraction of released EPA and DHA in the intestinal phase was 3.9 and 1.9 times higher, respectively, when using physiologically functional ingredients in the form of SC-PC- FO-EOC-D3 supramolecular complex compared to a product containing these ingredients in their native form (Figure 4).

The relatively low bioavailability of EPA and DHA from non-encapsulated fish oil is due to the higher release of these fatty acids in the gastric phase by gastric lipase.

Lipid oxidation is a common type of spoilage in foods containing fats and oils, resulting in undesirable changes in taste, discoloration, and reduced nutritional value. Wang et al. [28] and Solomando et al. [29] believe that the use of encapsulation not only protects the polyunsaturated fatty acids (EPA and DHA) in fish oil from oxidation, but also increases their solubility and hides any undesirable odors.

The results of using encapsulation in enriched meat products are presented in Table 3.

Table 3. Parameters of fat oxidation in enriched meat products

Parameters	EPC	EPSC
Peroxide value, meq/kg	1.6 ± 0.2^{a}	1.1 ± 0.1^{b}
Thiobarbituric acid value, mg of MA/kg	< 0.039	< 0.039

Note: **Statistical analysis was performed using Mann-Whitney U-test ($p < 0.05$). Different lowercase letters indicate statistically significant differences in parameter values determined for the two samples (comparing the values in the same row).**

Data analysis showed that the use of encapsulation led to a decrease in peroxide value by 1.5 times in EPSC relative to EPC. No changes in thiobarbituric acid value were detected. The results correlate with the data on the sensory

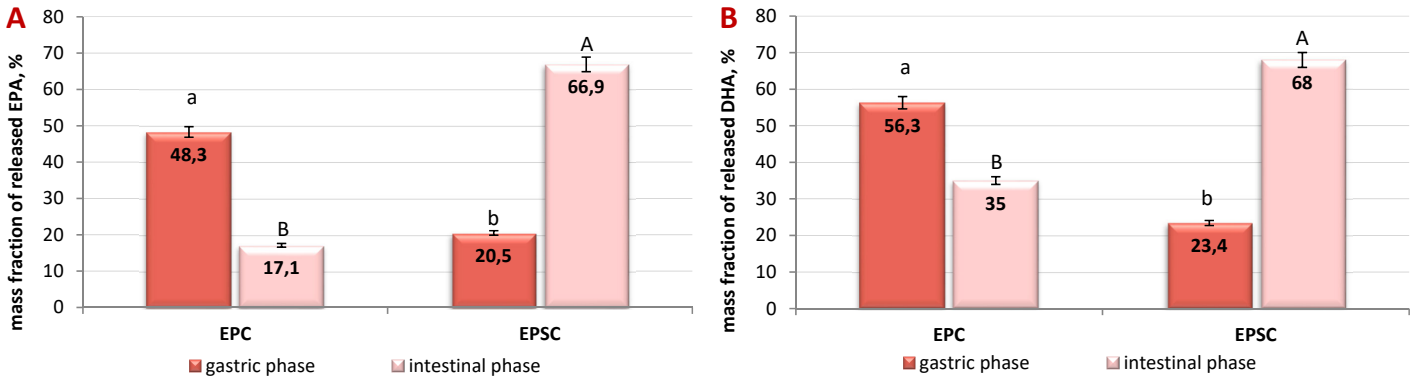

Figure 4. Mass fraction of released omega-3 fatty acids (A — EPA; B — DHA) during in vitro digestion of the studied samples, %.

Note: Data scatter bars show the standard deviation from the average value of the measured parameter. Statistical analysis was performed using Mann-Whitney U-test ($p < 0.05$). Different letters indicate statistically significant differences in parameter values, enriched meat product containing components of the supramolecular complex, EPC, and enriched meat product containing a supramolecular complex, EPSC, namely lowercase letters in the model gastric environment and capital letters in the model intestinal environment.

evaluation of the samples. It is also important to note that additional inhibition of PUFA autoxidation in the composition of the fats was due to adding one of the most effective plant antioxidants to the liposomes, i. e. essential oil of clove buds [20].

Sensory evaluation of the enriched meat products showed that EPSC had an intense meaty taste and no taste or smell of fish oil, while EPC had a weak taste and smell of fish oil, which intensified when heated. It was also noted that the presence of SC-PC-FO-EOC-D3 supramolecular complex in the composition of the products had a stabilizing effect on the texture of the product.

A number of studies have shown that, in general, the addition of encapsulated fish oil has a little effect on the sensory characteristics of meat products.

Solomando et al. [29] noted that the addition of fish oil microcapsules to boiled and dry-cured sausages had a slight effect on taste and color parameters.

In studies by Aquilani et al. [4] and Solomando et al. [5], a slight reduction in odor and taste intensity was observed in hamburgers fortified with microencapsulated fish oil. But during storage, these products showed no changes in sensory characteristics and no increase in rancidity, in contrast to the control product without fortification [5].

In addition, Jiménez-Martín et al. [18] and Solomando et al. [29] noted that the use of fish oil microcapsules resulted in increased salt taste in fortified chicken nuggets and dry-cured sausages, which may be related to the hypothesis that increasing the oil phase improves mixing with saliva, thereby accelerating the transport of salt to taste buds [5]. According to Solomando et al. [29], this hypothesis may be used to develop salt-reduced meat products fortified with fish oil microcapsules without reducing taste perception.

Conclusion

EPSC fortification with physiologically functional ingredients (fish oil, vitamin D3, essential oil of clove buds) in encapsulated form allows meeting the daily requirement of EPA+DHA by 70%, and vitamin D3 by 470%, respectively, as well as claiming the product as a "source" of omega-3 fatty acids and "a product with a high content of vitamin D3" in accordance with the legislation of the Russian Federation. When simulating the digestion of the studied meat product, it was revealed that the use of physiologically functional ingredients in encapsulated form had a positive effect on the bioavailability of EPA and DHA. At the same time, the use of heat treatment did not affect their bioavailability, as well as sensory parameters and oxidative stability of the meat product.

Thus, the use of functional ingredients in encapsulated form for meat product fortification helps improving their lipid profile.

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The authors declare no conflict of interest

DOI: https://doi.org/10.21323/2414-438X-2024-9-2-169-179
Received 06.05.2024
Accepted in revised 22.06.2024
Accepted for publication 25.06.2024
Available online at https://www.meatjournal.ru/jour
Review article
Open Access

BIODEGRADABLE MEAT PACKAGING: MICROBIAL SAFETY AND CONTROL OF ENVIRONMENTAL POLLUTION

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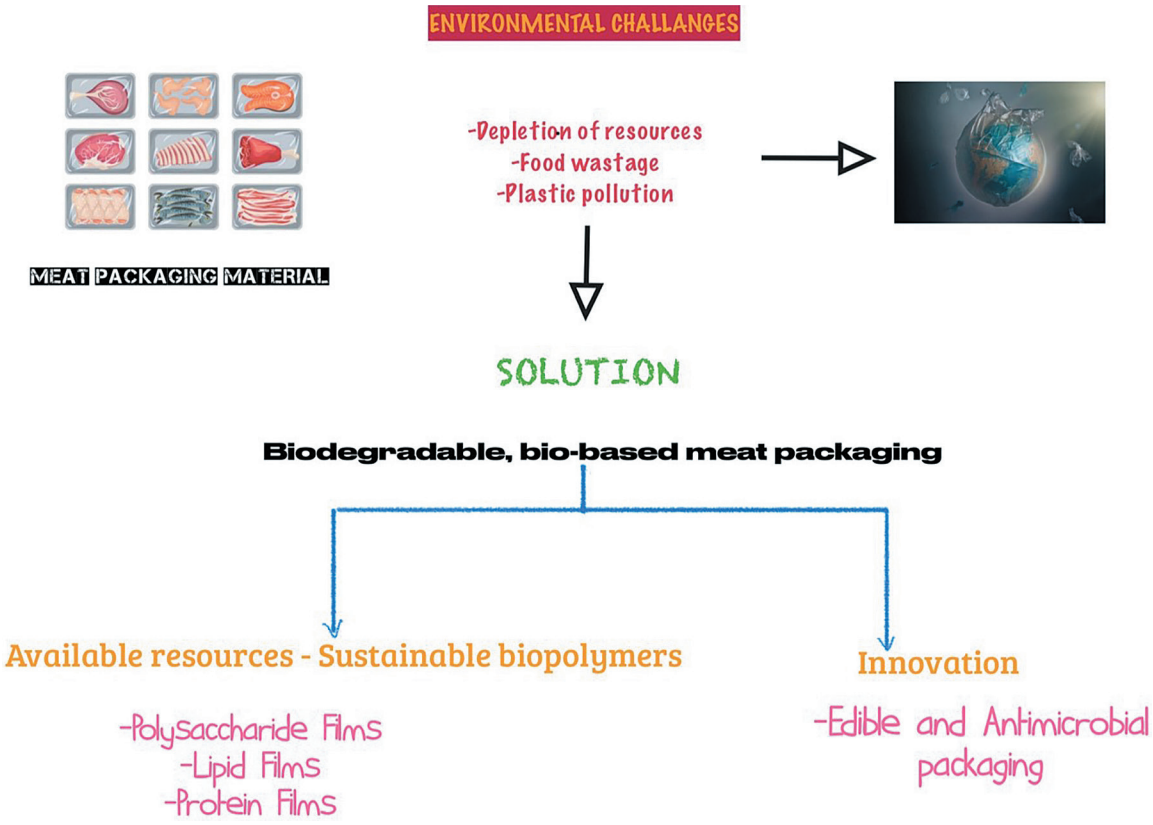
Keywords: *meat and meat products, antimicrobial, biodegradable, edible film*

Abstract
Plastic fragments from packaging material not only pollute the environment but also contaminate food material, causing detrimental health effects. The ultimate solution to this "white" pollution is biodegradable food packaging material. These films can be produced using proteins, polysaccharide and lipid-based materials and can enhance the shelf life of perishable commodities like meat and meat products by incorporating the natural antioxidant and microbial compound in packaging matrix, like essential oils. Essential oils of the aromatic plants due to their diverse phenolic profile possess strong antimicrobial and antioxidant potential, they open new doors of research to develop less hazardous food preservatives and drugs. These films and coatings improve nutritional and sensory attributes of packaged food. These films not only improve food quality but also overcome the burden of environmental pollution.

For citation: Saeed, K., Ali, Z. (2024). Biodegradable meat packaging: Microbial safety and control for environmental pollution. *Theory and Practice of Meat Processing,* 9(2), 169–179. https://doi.org/10.21323/2414-438X-2024-9-2-169-179

Graphical Abstract:



Introduction

Nowadays, with increasing awareness regarding the harmful impact of synthetic material both on health and environment, people started focusing their attention on usage of the natural and highly nutritious food with least synthetics involved [1]. However, there are certain perishable commodities which will deteriorate without use of chemical additives. One of the most spoilage prone food category is meat and meat products; as they have high percentage of fat content particularly unsaturated fatty acids.

Lipid oxidation is the major type of quality deteriorative change that occur in meat and meat products [2]. During lipid oxidation chemical reactions results in color and textural changes, off-odor, off-flavor, discoloration caused due to myoglobin oxidation occur, which greatly impacting consumer acceptance and choice [3]. In addition to organoleptic changes, certain toxic compounds like aldehyde and ketones are formed during lipid oxidation and some valuable nutrients are lost [4].

Deteriorative losses of meat and meat products cause immense economic losses in global industry i. e. up to 40% of total meat production [5]. In order to control these deteriorative losses, we need to explore and understand the chemical interactions in the process of deteriorative changes. Selection and designing of packing material crucial in terms of material and format to reduce quality losses. One of the most commonly adapted technique to prevent lipid oxidation is by reducing oxygen levels in packaging either by creating vacuum or by adding other gases like nitrogen [6]. Residual oxygen even at low concentration (0.05%) can still cause pigmentation and lipid oxidation [7]. In case of fresh red meat, it is usually packed in oxygen rich environment in order to maintain it pinkish red color due to oxymyoglobin but this often result in to undesirable lipid oxidative changes [8]. In order to deal with the abovementioned problems food industries and scientific community have been working to create new packaging systems that will extend meat shelf life and retain its desired quality characteristics [9].

Packaging system in food processing occupy prominent position, having dispensable use in distribution as well as commercialization in the market [10]. Food packaging is greatly influenced by emerging novel technologies and innovative materials like active packaging [11]. The increasing consumer demand of natural, unprocessed and chemical free food, can be fulfilled through sustainable packaging system [12]. The central idea behind these innovative technologies involves incorporation of active compounds in packaging designs like antioxidant packaging contains antioxidants either natural or synthetic with the purpose of lipid oxidation prevention, which will eliminate the need of adding antioxidants during meat processing [13]. The objective of the current review is to study the impact of biodegrade packaging material with plant extracts and essential oils on safety profile of highly perishable meat and meat products.

Objects and methods

Analysing the research of national and international scientist from around the globe on effect of synthetic meat packaging material on environmental pollution and exploration of safe and sustainable, natural biodegradeable packagaing materials was the prime objective of the study. Data collection was based on most recent advances and findings regarding biodegradable meat packing available on electronic sources like google scholar, ScienceDirect, Elsevier, Wiley, PubMed and eLibrary. English keywords like meat and meat products, antimicrobial, biodegradable, edible film were used. Citation links were used to explore thematically similar articles. While, the data that is irrelevant to the topic, uninformative, duplicated or from non-peer-reviewed sources has been excluded from the study.

Synthetic material used in meat packaging

Different packaging materials are used for packing of meat products: flexible plastic films as well as combination of flexible plastic packing material, carton boards, rigid containers are also used [14]. Mechanical strength provided by polymeric material is a necessity for proper protection of packaged food; particularly in the final application where packages are subjected to low storage and transportation temperature [15]. For packing of meat, prime wraps are usually the multilayer films made from polyvinylidene chloride (PVDC) polymers [16]. Facilitating consumption into the distant locations, meat packaging is one of the most complicated domains into food packaging, assigned for proper protection of goods, combination of list of materials and processes are developed [15]. In packaging structures, different polymer families are used which are polyethylene (PE), polypropylene (PP), polyvinylidene chloride (PVDC) and different copolymers [17].

Various features can be aggregated into few elements with the objective of protection are the following:

- Sealing for proper hermeticity;
- For food protection, providing barrier to keep the internal environment safe;
- To evade failures due to internal punctures caused by mechanical impact from cured and bone meat pieces [15].

By modern processes such as co-extrusion, different elements are assorted into united mixtures [18]. To achieve the objective, all packaging materials together, adhesive lamination or co-extrusion coatings are used. Co-extrusions coatings are being used for the few recent years because it simplifies production process of packaging films, that complies to the performance requirements by direct extrusion. The responsibility of packaging designers is to reduce the packaging impact on broadened product life, minimizing footprint from packaging as well as product and providing proper information of product life, materials usage, reusing, disposing and recycling within the extended process in order to select the appropriate process [19].

Environmental impact of synthetic material used in food packaging

The need to provide ordinary plastic materials has been a subject of paramount interest, considering their unsustainable nature and maintenance of sheen appearance. In the modern culture, these materials are found all over. They are among the top used materials primarily because of their principle attributes: they are adaptable, simple to measure and control, naturally non-reactive, and can be acquired at low expenses [20].

All of the abovementioned properties have strongly promoted using of plastics for various applications, from cell phones, 3D printing to the food business [21,22]. These days, plastic pollution is found all over the globe, including soil, seas, drinking water resources, in living bodies being found in residual form [23]. The deposition of plastics in environment will increase by twofold over the course of the following 20 years, outperforming to a disturbing degree of the current waste administration and recycling capacities. It appears to be that plastic contamination has become the greatest ecological concern of present days. To deal with the problem, various organizations are joining their efforts with the assistance of non-administrative associations and common society, through various projects that address the current issue, provide assistance for accessing the circumstances and develop remedial measures [24]. Indeed, in 2018, the European Union, reclamation programs planned to create systems to reduce the utilization of plastics in order to conserve the climate [24]. There are two issues that should be addressed; the first one is the monetary evaluation: it is estimated that only 5% of the plastic materials is kept up in the economy, the rest being lost after the primary use, which brings about yearly financial losses of 70–105 million Euros; and the second one: due to their diminished quality over excessively significant period of time that it take, non-reused plastic requires a long time to decay, unlike other materials like glass, paper, or metals. In this way, loads of non-degradable materials came into existence as a savior of environment. Among all the plastic pollution cases, the most disturbing results are gigantic sea depositions ranging from 5 to 13 million tons/year [25].

It is assessed that burning plastics produces around 400 million tons of additional CO_2 each year. The methodology adapted by European pioneers revolves around the concept of bioeconomy, with ultimate objective of protecting the planet and its residents from the havocs of non-degradable plastics. As indicated by these plans, by 2030, packaging material used in European markets will be recyclable and the utilization of plastics packaging will be diminished. Various organization like European bioplastics are focused on decreasing plastic waste, preventing mass stockpiling, and putting resources into nature restoration, and developing new materials [26]. Reevaluating and improving this framework require participation and due endeavors from all players of the field, from plastic producers to recyclers, retailers and its buyers. Without the dynamic contribution of every prime-level entity, a definitive objective cannot be accomplished i. e. to create revolutionized plastic economy. The plan is to create plastic that can be reused, this activity will relieve environmental constrains because of plastics and its unfavorable effects on individuals and the overall environment. Figure 1 shows the environmental impact of synthetic material used in food packaging [27].

Biodegradable and edible packaging

The introduction of entirely biodegradable and edible materials derived from bio-based polymers to the environment can be a real and authentic solution for eradication of pollution. The polymeric materials derived from renewable raw materials are known as bio-based polymers [28]. Some noteworthy properties of these packaging material includes its biodegradability, ability to be recycled, having low cost of development, naturally abundancy, non-toxicity and biocompatibility, these are the reasons behind their possible extensive applications in order to generate novel materials [29]. In 2011, production of bio-based polymers

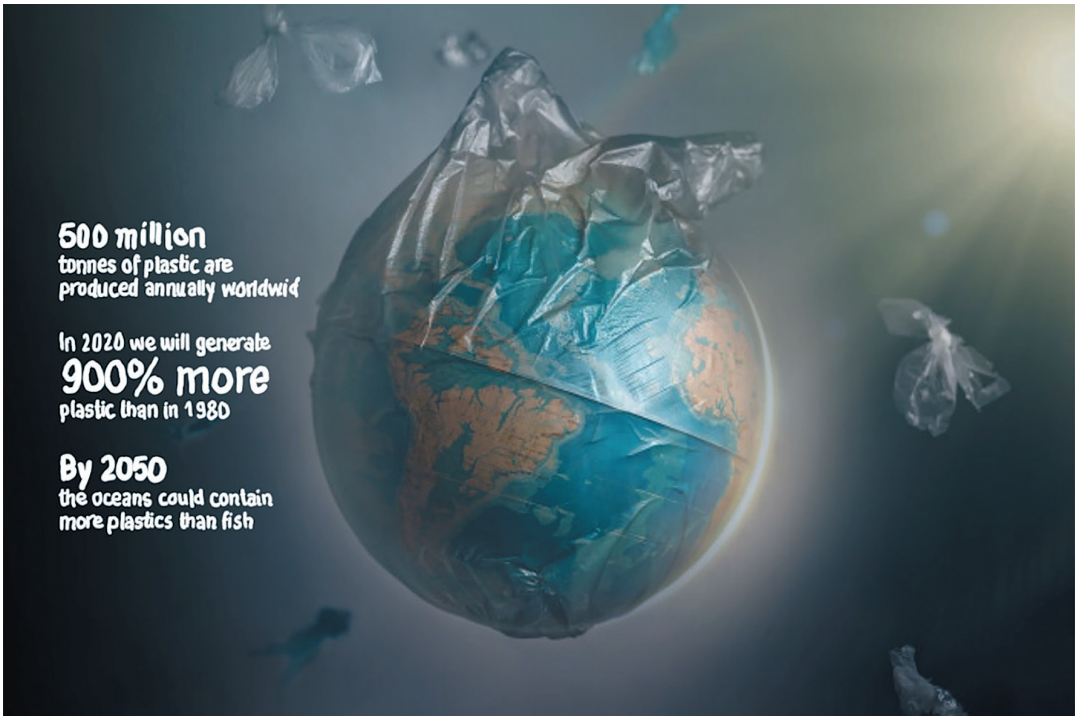


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Figure 1. Artistic representation of the environmental impact of synthetic material used in food packaging

was around 3.2 billion tonnes and it reach to 12 billion by 2020 [30]. Biomass can be used to generate bio-based polymers in the food industry, which can be either synthesized by microorganisms or by bio-derivative monomers. Bio-based polymers used in the food industry can be obtained from bio-monomers derived from biomass, produced by microorganisms. While choosing these polymers, the requirements of the coating material which has to fulfilled is taken into keen consideration. The consumers' interest in minimally processed foods has been increased by application these coatings or films [31].

Even though biodegradable packaging seems to be a cut-the-edge technological solution, there are historic evidences of its application since 16th century [32]. Covering the meat pieces or carcasses in lipid-based coatings have been used since prehistoric times [33]. Emulsion derived from waxes and oils in water is the most used and common method of applying edible coatings to the meat. In case of food material, it is sprayed directly on to the surface, which helps in improving its sensory properties like glossiness, appearance, color, fineness [34]. These coatings also control the browning process and loss of water from the tissue cells. Numerous polysaccharide-based coatings and films are used to enhance the quality and storage of meat including pectin, alginates, cellulose and starch derivatives etc [35].

Edible packaging is defined as packaging technique in which the packing material i. e., films, coatings etc. are a part of that product and is consumed together with the food [36]. Films are independent materials that cover the surface of food materials while coatings are applied to the surface directly. Edible properties of these coatings can only be acceptable for food applications only if the ingredients used in the packing materials are food graded and the methods of their processing and obtaining are approved by the food safety regulating authorities. Product quality and freshness of the food material depends largely upon the correct selection of materials and the packaging technologies used [37].

Even though the edible films and coatings can be regarded as part of food, but it cannot be considered as the finished product. Edible coatings are not classified within food class as they do not provide the calculated nutritional value [38]. Material used for generation of edible films and coatings should have properties such as (a) decreased permeability (b) water tightness (c) strong barrier properties and mechanical efficiency (d) high sensory quality [39] (e) ability of endure low pressure processing conditions (f) non-toxic and non-hazardous for consumer's health [40] (g) decreased viscosity (h) non-polluting (i) physiochemical, microbiological and biochemical stability (j) producible through low cost and simple development technology (k) easily emulsifiable and non-sticky (l) should not produce excessive CO_2 (m) made of cheap plant materials (n) does not interfere with the food quality (o) has no taste or smell which is detectable at the time of consumption (p) mildly transparent but not like glass (q) have decreased

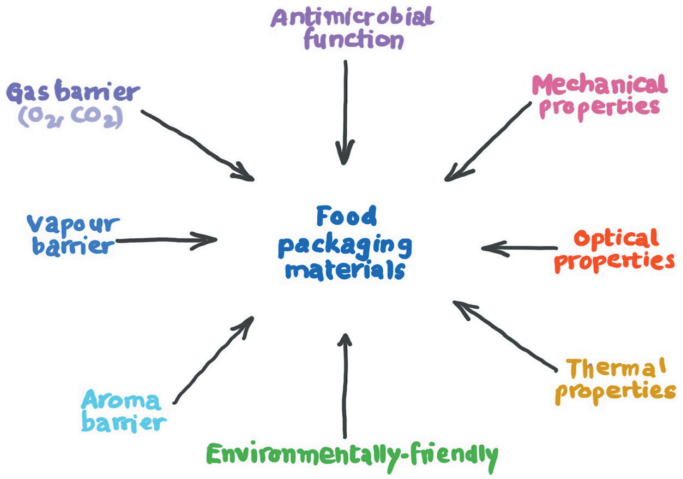

Figure 2. List of the functions of food packaging material

viscosity. Refer to Figure 2 as it enlists the functions of food packaging material [41].

Despite of the edible packaging with properties similar to the plastics, on the basis of functional performance the edible food packaging competes really well with the plastics, especially due to strong barrier properties against loss of vapors and solutions, but usage of these material requires some additional packaging support to sustain hygiene and handling operations. The efficiency of bio-based polymeric coatings and films can be augmented by additions of natural substances which improve the physicochemical, mechanical and microbiological characteristics of the packaging material [42]. Due to consumers' demand for natural products this trend is flourishing. To fulfil this consumer requirement; dyes, antioxidants, essential oils and flavoring compounds are being added to the food packaging [11]. Research trials are also being conducted for development of smart nano-material packaging which have features far more superior then the conventional films [43].

Renewable source derived polymeric materials are regarded as biopolymers. These materials have numerous food applications. These biopolymers have three major classifications on the basis of their origin and method of production. First, biomass-derived polymers. For example, protein-based polymers of casein and gluten, polysaccharide-based polymers of chitin, cellulose, chitosan and starch. Figure 3 shows the biopolymers which can be used as meat packaging raw material. Second category includes renewable monomeric biomass; polymers derived from classical chemical synthesis e. g., polylactic acid, a lactic acid monomer made via bio-polyester polymerization. The third category includes polymers produced by microorganisms and genetically modified bacteria e. *g.*, polyhydroxy alkenoates [6].

Commonly in retail trade the plastic trays, used in order to pack fresh poultry, mutton, beef or fish, are destroyed during storage due to dripping of juices, that makes the packaging unappealing to the consumer [44]. Bio-based meat coating have ability to retain natural meat juices which help to reduce dripping, and eliminate the need of absorbent pads beneath meat cuts in the trays, thus, improving

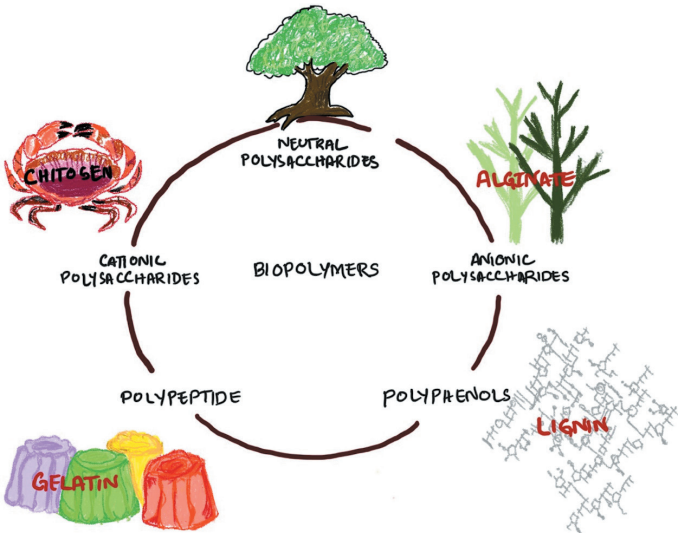

Figure 3. Biopolymers which can be used as the raw materials for meat packaging

the product presentation [45]. Bio-based coatings also prevent meat browning caused by myoglobin oxidation, prevent rancidity due to low oxygen permeability, reduce deteriorative activity of proteolytic enzymes, reduce microbial load in the packaged meat; these packaging materials also restrict loss of volatile flavors, and prevent absorbtion of foreign odors by poultry meat and seafood [6]. These bio-based packaging contains natural antimicrobials and antioxidant compounds, thus can be used for direct application or covering onto the meat surface, resulting in color retention, delayed rancidity and decreased microbial load [46]. These coatings if applied prior to procedures like battering, breading or frying, preserve product nutritional profile by restricting oil uptake during cooking procedure [47].

Biodegradable meat packaging materials

Polysaccharide films in meat packaging

The polysaccharide derived films are made from materials like cellulose, starch, carrageenan, pectin, ether, alginate, chitosan and etc. [35]. The peculiar properties of these materials include compactness, rigidity, tackiness, viscosity, thickening and structure in aggregate forming the capability necessary to generate packaging films. Gas permeability of polysaccharide film is higher in comparison to lipids films which create an anaerobic environment within food package; making polysaccharide film is more suitable for creating modified atmosphere-permeable packaging to enhance product shelf life without creating anaerobic conditions. These films also prevent rancidity, dehydration and surface browning issues of the packaged food. The polymeric network of polysaccharide films, restrict gaseous exchange across the film but being hydrophilic in nature this material is a weak barrier against water vapors [35]. In Japan for years these polysaccharide films have been used to wrap the meat, when it is subjected to steam smoke. During processing these films become integrated to the meat surface, resulting in improved texture, moisture retention, better structural characteristics [48].

Seaweed derived alginates have film forming properties; these films are usually created in combination with cations like Ca, Mg, Fe or Al to improve gelling characteristics. According to the findings of a study conducted by Gutt and Amariei [37] sodium alginate base creates eco-friendly meat packing material, and reduces the use of plastic material. During this study it was found that alginates, in particular sodium alginate films, are tear-resistant, flexible, glossy, tasteless, odorless, feature low oxygen permeability thus effectively prevent color and taste degradation in meat.

Starch is composed by complex network of amylose and amylopectin, has physical properties comparable to synthetic plastics but is tasteless, colorless, odorless, non-toxic biodegradable, it exhibits strong oxygen barrier and has semi-permeability for carbon dioxide [49]. These films not only protect meat quality during storage but also become part of it during cooking. These films control microbial growth by lowering water activity, reducing drip losses and ensure moisture retention [50]. According to a study conducted by Zhao et al. [51] starch films created by combination of chitosan and caravol have been found effective in controlling *Listeria monocytogens* and ham meat microbiota.

Mixture of various polysaccharides like carrageenan, in combination with bioactive compounds like gallic and ascorbic acid, improve microbial stability of boneless meat. Farhan and Hani [52] created Kappa-carrageenan active edible biofilms to prevent oxidation and to retain color of packaged chicken breast. The study proved that bio-based film improved antioxidant activity and controlled microbial load on chicken breast during 7 days storage study. Cellulose, non-digestible fiber, is resistant to fats and oils permeation, it is flexible and water soluble. Cellulose-based films exhibit characteristics like mechanical strength, serve as oxygen- and oil-resistant barrier, making it perfect to store boneless meat. Pirsa and Shamu [53] created an active packaging made from cellulose-polypyrrole-ZnO to improve the shelf life of chicken thigh meat and found that this film reduced the microbial load of stored meat, increased its shelf life and improved rheological stability of chicken thigh because of enhanced antimicrobial and antioxidant activity.

Pectin, a plant-derived polysaccharide, has stable physical structure but it is poor moisture barrier. But there are numerous studies about effective pectinate gel packaging like Xiong et al. [54] prepared edible pectin coating containing nano-emulsion of oregano oil and resveratrol for preservation of pork loin, and found effective results at refrigeration temperature for 15 days. Similarly, Sani et al. [55], prepared potato starch, apple peel film with ZnO_2 nanoparticles and with microencapsulation containing *Zataria multiflora* essential oil for quail meat packaging and found that packaging film enhanced meat shelf life and retained its physiochemical properties. Agar is a seaweed-derived polysaccharide, having strong gel forming characteristics,

it is also used for meat packaging. Chitosan, a biodegradable polymer derived from arthropod exoskeletal compound chitin, demonstrate antimicrobial and antioxidant properties along with considerable tensile strength [56]. Souza et al. [57] created ecofriendly ZnO-chitosan packaging films for fresh poultry meat packaging and demonstrated its strong antimicrobial characteristics. Incorporation of nanoparticles enhanced antioxidant characteristics of the packaging film. Similarly, Arkoun et al. [58], created activated chitosan-based nanofiber meat packaging and tested it against *Salmonella enterica serovar Typhimurium*, *Staphylococcus aureus*, *Escherichia coli* and *Listeria innocua* strains that are blamed in quality deterioration of boneless meat, and found that film prevented meat contamination and extended meat self-life up to 1 week.

Lipid films in meat packaging

Fats in foods have been used as oxygen and moisture barrier in order to prevent shrinkage of food material. Lipid films, particularly wax-based have flexibility, feature better coating characteristics and improve cooking procedures by preventing sticking to utensils. These coatings prevent food moisture loss, thus retaining the meat flavors. Edible wax coating easily withstands rough market handling, lessen surface dehydration and preserve meat color [59]. Song et al. [34] incorporated sunflower oil into edible coating for pork meat hamburger, making it possible to control oxygen levels as well as modulating water vapors, thus preventing undesirable deteriorative reactions in meat. Despite the above-mentioned benefits at higher storage temperatures lipid-based films exhibit lower gas permeability particularly for ethylene, oxygen and carbon dioxide, creating anaerobic conditions leading to food safety issues. These films also have poor sticking properties as the hydrophilic surfaces lack structural integrity and are prone to oxidation, flaking, cracking, retain off flavors and have bitter aftertaste [60].

Protein films in meat packaging

Casein, gelatin/collagen, whey protein, fibrinogen, wheat gluten, corn zein, egg albumen and soy protein have been processed to produce biodegradable edible films [61]. These protein-based films cannot resist water diffusion but have ability to adhere to hydrophilic surfaces and provide barriers for oxygen and carbon dioxide [62]. Commercially milk proteins, casein and whey have been used in the manufacturing of these films. These proteins are desirable for films formation not only because of their packaging properties (excellent mechanical strenght, barrier properties, water solubility, emulsification properties) but also because of their nutritional value. For years research has been conducted on milk protein films for packaging of fruits, vegetables and other dairy foods and boneless meat [63].

Catarino et al. [64] prepared active whey protein coating enhanced with *Origanum virens* essential oil to increase the shelf life of processed meat. The coated sample — Portuguese sausage — was regularly monitored for 4 months long for total microbial load and physicochemical characteristics, and found an approximate 20 days extension in its shelf life. During storage the study considered color retention, the reduced lipid peroxidation was also observed. Similarly, Furcellaran and whey protein isolates were used to prepare biodegradable packaging material with *Borago officinalis* extracts to preserve ham. A 21-day storage study was conducted and the samples were tested every 7 day and found satisfactory results of oxidation product build-up, microbiological load and organoleptic characteristics [65].

Sanches et al. [66] prepared starch-based active packaging containing sweet whey and red cabbage extract for packaging of ground beef and found that films have strong antioxidant potential, good machine-processing strength, low permeability for water vapors, making it a suitable material for packaging of meat and meat products like ground beef. This film improves the shelf life due to presence of anthocyanins. Song et al. [34] utilized whey isolates-coated multi-layer films as a gas barrier in order to minimize qualitative changes in frozen and marinated meat loaves and the results demonstrated that these films preserve physicochemical and organoleptic characteristics of meat loaves during 6-month storage study, proving the potential of whey isolate-coated film for commercial application.

Edible meat packaging (films and coatings)

An edible coating/film is any material with an average thickness of less than 0.3 mm and which can be produced from combination of biopolymers and various additives dispersed in aqueous media. In literature authors use the terms of edible coating and edible films interchangeably; however, some others consider that coating and films have distinction in terms of the techniques used to incorporate them into the food product. The edible coating is formed directly on the food, whereas the edible film is as first prepared separately and then applied onto the product [67].

Microbial growth in meat, fish and derived products result in the quality decay, which can be prevented by adding or enhancing the antimicrobial activity of the packaging films and coatings, making it an interesting strategy to extend the food shelf life [50]**.** Essential oils, ethanolic, aqueous extracts of plants provide a diverse range of natural preservatives [68]. The key reason of encouraging the application of these natural substances in food preservation is linked to their compliance with desired characteristics of biodegradability, bioactivity and edibility of these bio-based edible film and coatings.

Films with incorporated active components like herb extracts are rich in phenolic compounds and terpenoids that prevent growth and propagation of microbial flora in meat products. Essential oils contain bioactive compounds like carvacrol, thymol, menthol, eugenol and etc. Isopropyl phenols like carvacrol are hydrophobic compound with capability to accumulate in the microbial cell membrane,

where they induce conformational modification, that ultimately leads to microbial cell death. Monoterpenes like thymol and menthol cause lipid fraction perturbation in microbial cell membrane, re-arrange its permeability thus resulting in leakage of microbial cell content. Similarly, oregano essential oil transmutes microbial membrane permeability thus resulting in leakage of essential nutrients like phosphates, potassium and protons. These bioactive agents also upgrade film barrier properties due to chemical interactions between film matrix and polyphenolic compounds [69]. Figure 4 explains the possible mechanisms of antimicrobial activity of bioactive compounds.

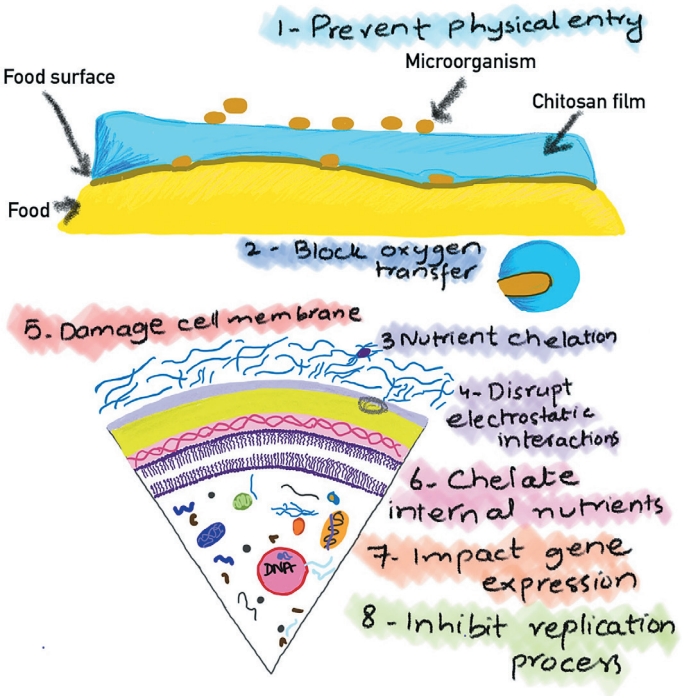

Figure 4. Visual explanation of the possible mechanisms of antimicrobial activity in bioactive compounds

Edible coating made of immiscible biopolymer composite increases shelf life fresh meat cuts by reducing drip losses, lipid oxidation, metmyoglobin formation and retains natural volatile meat flavors [48]. Gheorghita et al. [70] developed edible material film using stevia, sodium alginate and agar for study of storage of ham slices and cheese for 5 months at refrigerated conditions and after the expiry of this period evaluated color, waters activity index and peroxide index. The results made it evident that the film does not support the growth of existing meat and cheese microflora proving that this biopolymer can be a promising substitute for unsuitable commercial plastics.

Giatrakou et al. [71] conducted a study on antimicrobial impact of chitosan coating in a 4 weeks storage study on pastrami, and reported one log of CFU/g reduction in aerobic plate count. Fattahian et al. [72] prepared edible chitosan coating to create modified atmospheric packaging for meat. *Caminum cyminum* essential oil was incorporated in the film because of its antimicrobial and antioxidant properties. Bazargani-Gilani et al. [73] reported another interesting outcome of chitosan film with *Zataria multiflora* essential oil. This experiment was run on chicken breast, kept at 4 °C for a period of 20 days, revealed a significant inhibition of both total viable and psychotropic microflora. Another trial on microbial growth of selected species (*Pseudomonas spp., Enterobacteriacea*, yeasts and molds) in untreated meat, packed in chitosan coating with grape seed extract, exhibited significant reduction of microbial growth in chicken breast meat [74]. Incorporation of oleoresin extracted from kaffir lime leaves into cassava starch coating improved the microbial stability of beef samples during storage [75]. The study indicated a remarkable reduction in the growth of microorganism in 2 weeks storage study. Similar outcomes were reported by Dharmalingam et al. [76] for beef fillets packaged in cassava starch coating enhanced with clove and cinnamon essential oils.

There are numerous studies that highlight meat and fish preservation against target microorganisms primarily responsible for meat spoilage (*Escherichia coli, Listeria monocytogenes* and *Staphylococcus aureus*). For instance, in one study there was tested the antimicrobial activity of thyme and oregano essential oil in soy protein coating at a concentration of 3% for its capability to inhibit the growth of *Escherichia coli, Listeria monocytogenes* and *Staphylococcus aureus* in refrigerated beef fillets kept for two weeks. The researchers monitored the samples periodically and found that the growth of all microorganisms was drastically inhibited throughout the storage period [77]. In another study of the wrapped flounder fillets, the protein hydrolysate of agar film with incorporated clove essential oil inhibited the growth of food spoiling microorganisms under refrigerated storage conditions, thus, improving the shelf life from 10 to 15 days [78].

Due to high protein content beef is more prone to microbial and enzymatic spoilage. *Pseudomonas* spp., lactic acid bacteria, *Enterobacteriaceae*, yeasts and molds are the major spoilage causing agent. In order to prevent the oxidative reactions and microbial growth during a study of 20 days of storage, chitosan edible film enhanced with *Zataria multiflora Boiss* oil and sumac extract was prepared to maintain a modified atmosphere condition for beef packaging and demonstrated encouraging results proving its suitability as meat edible packaging for commercial applications [79].

Dalvandi et al. [80] created an edible poultry packaging by dipping meat into solution of carboxy methyl cellulose with added extracts of turmeric and black pepper seeds, vacuum-packed the meat and placed it under refrigerated conditions for a storage study, and found that this edible packaging can considerably enhance the durability of breast fillets of chicken. In another study, edible composite film of gelatin enriched with polyphenolic nano-emulsions was used to create packaging for chicken meat and showed that water stability and moisture of the film reduced with increasing nano-emulsion concentration. This film has strong antioxidant potential due to curcumin extracts in nano-emulsions, which also prevented growth of *Salmonella typhimurium* and *Escherichia coli* in packaged meat, thus, extending the

shelf life of meat up to 17 days [81]. Interestingly, in another study the antimicrobial characteristics of both sodium alginate-carboxymethylcellulose film and coating infused with *Ziziphora clinopodioides* essential oil, apple peel extract and zinc oxide nanoparticles were prepared, and the results of meat storage study exhibited the considerable improvement in preservation of meat quality [82].

Conclusion

Current alarming levels of environmental pollution are driving the researcher to develop the alternatives for non-biodegradable packaging materials that naturally disintegrate without causing damage to the environment. These packaging materials are edible and are derived from renewable and sustainable agricultural products. The edible films enhanced with natural plant extracts have antimicrobial and antioxidant properties that prolongs the shelf life and safety of foods by preventing the growth of pathogenic and spoiling microorganisms. In meat industry, microorganisms may cause severe food safety issues linked with health problems, such as foodborne diseases. However, development of the new bio-based films and coatings is urgently required to respond to the problems of environmental pollution and provision of safe food.

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The authors declare no conflict of interest.

DOI: https://doi.org/10.21323/2414-438X-2024-9-2-180-187
Received 28.03.2024
Accepted in revised 22.06.2024
Accepted for publication 27.06.2024

Available online at https://www.meatjournal.ru/jour
Original scientific article
Open Access

EFFECT OF ELECTROLYZED WATER ON PHYSICO-CHEMICAL AND SENSORY QUALITIES OF BEEF

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Keywords: *efficacy, electrolyzed water, proximate composition, meat, characteristics*

Abstract
During beef processing, contamination by microorganisms from diverse sources poses a significant risk to its quality and safety. This contamination can lead to reduced shelf life, compromised meat quality, and increased health hazards. In recent years, electrolyzed water (EW) has emerged as a promising solution for sanitizing and cleaning beef. The purpose of this study was to evaluate the physicochemical and sensory qualities of beef that had been treated with EW. In this experiment, there were three replications with a factorial Randomized Complete Block Design (RCBD). Factor-A: consisted of six (06) treatments concentrations: T_0 = Control sample (fresh water); T_1 = 10 ppm electrolyzed water; T_2 = 20 ppm electrolyzed water; T_3 = 30 ppm electrolyzed water; T_4 = 40 ppm electrolyzed water; T_5 = 50 ppm electrolyzed water; Factor-B: consisted of three (03) durations: TM_1 = 5 minutes; TM_2 = 10 minutes; TM_3 = 15 minutes. The findings showed that the moisture content (%), crude protein (%), ether extract (%) and ash content (%) of beef samples ranged from 72.31 ± 0.29 to 73.93 ± 0.30, 19.95 ± 0.16 to 21.91 ± 0.19, 4.28 ± 0.09 to 5.06 ± 0.09, 1.29 ± 0.09 to 1.76 ± 0.07 respectively. Beef's proximate composition (moisture, crude protein, ether extract, dry matter, and ash) and physical analyses (cooking yield, cooking loss, and pH) were not significantly affected by the EW treatments ($p > 0.05$). However, drip loss and beef color showed substantial significant effects ($p < 0.05$). Findings suggest that EW treatments with concentration up to 50 ppm can effectively decontaminate beef while maintaining its nutritional and sensory properties.

For citation: Biswas, G., Islam, S., Rahman, S.M.M., Al Mamun, S.M.A. (2024). Effect of electrolyzed water on physico-chemical and sensory qualities of beef. *Theory and Practice of Meat Processing,* 9(2), 180–187. https://doi.org/10.21323/2414-438X-2024-9-2-180-187

Introduction

Red meat and poultry stand out as the primary sources of high-quality protein in the human diet. In addition, meat products are the concentrated sources of vitamins of B group, notably vitamin B_{12}, which is lacking in plant meals, and the meat is a reasonably rich source of iron that is easily absorbed. Pork makes about 40% of the world's meat consumption, with beef and poultry coming in close behind at roughly 25% and 30%, respectively [1].

Fresh beef is easily contaminated by naturally occurring microorganisms from a variety of sources during processing of all edible carcass tissues [2]. This might lead to a decrease in the quality and shelf life of beef during its storage, and increase health risks. Therefore, it is necessary to develop an effective preservation method that can prolong the shelf life of fresh beef during its storage. Recently, various sanitizing processes have been adopted to improve the safety and quality of fresh meat and meat products before refrigeration [3,4]. The most widely used chemical decontaminants in the meat and poultry industries are organic acid solutions [5,6]. While chlorine rinses are generally used during processing of poultry for pathogens reduction [7], other various processes have been proposed as alternatives to eliminate or substantially decrease bacterial population on poultry carcasses. However, most of these processes are not completely acceptable due to the chemical residues, discoloration of chicken carcasses, high costs, or limited effectiveness. Chlorine solution was usually used for fresh meat refrigeration. But the excessive use of chlorine (Cl_2) can lead to several environmental problems [8]. Furthermore, the consumers are concerned about the use of these chemicals because they may potentially provide undesirable effects on human health. Therefore, most studies on the decontamination of fresh meat or vegetables have focused on sanitizing agent alternative to chlorine [9,10]. Electrolyzed water with a pH of 5.0–6.5 is well recognized as an alternative sanitizer, containing a high concentration of hypochlorous acid [11]. It is produced by the electrolysis of diluted salt (sodium chloride) solution. Compared to conventional disinfectants, the use of EW offers a number of benefits, including cost effectiveness, simplicity of application, efficient disinfection, on-the-spot production, and environmental and human safety. A few studies on electrolyzed water for decontamination and shelf life extension of beef are currently being carried out [2]. The study mostly focused on the reduction of microbial population and did not consider physicochemical and sensory

properties of beef. Tango et al. [2] evaluated the changes in color, odor and texture of the EW-treated fresh beef compared to the control sample of beef by the sensory index (SI) and pH. Rahman et al. [12] showed that there was no adverse effect of slightly acidic electrolyzed water (pH 5–6.5) on the poultry meat. Notably, the use of EW on raw meat in Bangladesh has never previously been the subject of a above-specified research.

This study aimed to evaluate the effectiveness of electrolyzed water on physicochemical (moisture, crude protein, ether extract, ash, pH, drip loss, cooking loss), and sensory qualities of fresh beef.

Materials and methods

Collection of raw materials

Beef sample was obtained from a retail meat shop in Gollamari market, Khulna, Bangladesh. The sample was placed in a sterile polythene bag and promptly transported to the laboratory for analysis within one hour.

Experimental design

All of the beef samples were measured and divided into five experimental groups and one control group. The studies were conducted in a factorial RCBD with three replications. Factor-A: consisted of six (06) treatments concentrations: T_0 = Control sample (fresh water); T_1 = 10 ppm electrolyzed water; T_2 = 20 ppm electrolyzed water; T_3 = 30 ppm electrolyzed water; T_4 = 40 ppm electrolyzed water; T_5 = 50 ppm electrolyzed water; Factor-B: consisted of three (03) durations: TM_1 = 5 minutes; TM2 = 10 minutes; TM_3 = 15 minutes. The effect of EW on the nutritional quality (proximate analysis, pH, drip loss, cooking yield, cooking loss and sensory analysis) of beef meat was studied.

Preparation of electrolyzed water (EW) solution

Electrolyzed water with pH of 2.3, available chlorine concentration (ACC) of 70ppm, and oxidation reduction potential (ORP) of 1100 mV was used for the preparation of EW (electrolyzed water) solution of varying concentrations like 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm. Electrolyzed water was diluted with distilled water of varying volume and the pH, ORP and ACC of each solution was measured. The pH and ORP were measured using a dual scale pH/ORP meter CON60 (Trans-Wiggens, Singapore). The ACC was determined using a digital chlorine test system RC-2Z (Kasahara Chemical Instruments Co., Saitama, Japan). The final pH of the solution was 5.8, 5.3 5.0, 4.2, 3.5 for 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50ppm of EW solution respectively.

Determination of proximate composition

Using the method of [13], the proximate composition of beef was evaluated in terms of moisture, crude protein, ether extract, dry matter, and ash content.

Moisture content

Drying oven (SH Scientific, Korea) was used to determine the moisture content. For this, 50 g of ground meat samples from each treatment concentration mode were oven dried at 75 °C for 24 hours till a constant weight was obtained and thus calculated:

$$\text{Moisture (\%)} = \frac{(W_1 - W_2)}{W_1} \times 100 \quad (1)$$

$$\text{Dry matter (\%)} = \frac{W_2}{W_1} \times 100 \quad (2)$$

Where,

W_1 = Initial weight of sample (g);

W_2 = Final weight of sample (g).

Crude protein

Semiautomatic digestion and distillation unit (VELP Scientifica, Italy) was used to determine the crude protein of meat samples by Kjedahl method. The actual crude protein values of the meat samples were obtained by converting nitrogen content of meat with constant 6.25.

$$\text{Crude protein (\%)} = (6.25 \times N\%) \quad (3)$$

Ether extracts (Fat)

Semiautomatic solvent extractor (VELP Scientifica, Italy) was used to determine the ether extracts by Soxhlet method.

$$\text{Ether extract (\%)} = \frac{W_2}{W_1} \times 100 \quad (4)$$

Where,

W_1 = Weight of sample (g);

W_2 = Weight of fat (g).

Ash content

Ash content was determined by igniting 3g of ground meat samples in a muffle furnace SH-FU-5MGE (SH Scientific, Korea) at 600 °C for 5 hours until ashes were formed.

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

Where,

W_1 = Weight of sample (g);

W_2 = Weight of ash (g).

Measurement of pH

The pH was determined with a digital pH-meter (Seven Easy pH, Mettler-Toledo GmbH, Switzerland). For this, the beef sample was homogenized with a Polytron blender (Brinkman Instrument, New York) at 1000 rpm for 30 seconds. A 10 g of homogenized sample was weighed into a beaker along with 50ml of distilled water. Next, the sample was added to 5 different beakers containing 10, 20, 30, 40, and 50 ppm EW solution. A control experiment was also conducted without the addition of EW solution. A stir bar was placed in the homogenized solution and pH was measured while stirring.

Determination of cooking yield and cooking loss

The cooking loss was calculated as the percentage difference between the weights before and after cooking, as described in the reference [14]. Initially, fresh samples were sliced and weighed to obtain their initial weight and then cooked using a dry heat method. Briefly, the meat was placed on a water bath with a beaker containing the meat extending above the water surface. Traditional cooking times were adhered to determining cooking loss and cooking yield. It is important to note that it took 20 minutes to reach a temperature of 100 °C. Subsequently, the meat was removed from the beaker, dried, and weighed. Cooking loss was measured in duplicate. Following this, the meat sample was cooked for an additional 10 minutes at 100 °C (totaling 30 minutes), surface dried, and weighed. Finally, the meat sample was cooked for another 10 minutes at 100 °C (totaling 40 minutes, the traditional cooking time for beef in Bangladesh), surface dried, and weighed again. Cooking loss was then determined in duplicate.

$$\text{Cooking loss (\%)} = \frac{W_1 - W_2}{(W_1)} \times 100 \quad (6)$$

$$\text{Cooking yield (\%)} = \frac{(W_2)}{(W_1)} \times 100 \quad (7)$$

Where

Weight of sample before cooking = W_1;
Weight of sample after cooking = W_2.

Determination of drip loss

Drip loss was determined by the standard bag method [15]. Approximately 40 g of meat sample was utilized for assessing drip loss. The sample was then ensconced in netting and suspended within an inflated bag to prevent contact with the bag, or placed in a container on a supporting mesh, and sealed securely. Following a chilling period of 24 hours, the samples were reweighed. The same samples were used for subsequent drip loss measurements for up to 3 days, with the initial weight serving as the reference point per each experimental trial. When measured, samples were promptly removed from the containers, gently blotted dry, and then weighed.

$$\text{Drip loss (\%)} = \frac{W_1 - W_2}{(W_1)} \times 100 \quad (8)$$

Where

Weight of sample before thawing = W_1
Weight of sample after thawing = W_2

Sensory evaluation

The sensory evaluation study was conducted following the procedures [16]. Six panelists participated in the sensory evaluation which was carried out at Animal Husbandry Laboratory of Agrotechnology Discipline, Khulna University, Bangladesh. In order to minimize bias, three samples were coded before being evaluated by a sensory panel based on how similar the samples were in terms of appearance, texture, scent, and general acceptability. Panelists were served in their separate locations. A nine-point hedonic scale was used, with nine being the lowest score (dislike extremely) and one representing the greatest score (like extremely).

Color analysis

For color analysis of the samples, a CM (Minolta Chromometer CR-400, Osaka, Japan) with a 1 cm aperture, illuminant C and a2 viewing angle was used. A white calibration plate was used to calibrate the device prior to data collection. Evaluations were made of lightness (L*), redness (a*), and yellowness (b*). Readings were obtained close to each core's center. Color coordinates (L^*, a^* and b^*) were observed on the surface exposed by cutting. Coordinate a* ranged from red (+a*) to green (–a*) and coordinate b^* from yellow (+b^*) to blue (–b^*) [17]. Three readings of L^*, a^*, b^*values were obtained at different sites.

Statistical analysis

Data entry was conducted using Microsoft Excel, and subsequent analysis was performed using Statistix-10 software. The impact of electrolyzed water on the physicochemical and sensory qualities of beef was assessed through analysis of variance. The least significant difference (LSD) test was used to compare treatment means in cases where significant differences were identified, with $p < 0.001$ being considered statistically significant.

Results and discussion

Proximate composition of beef meat

The findings of the proximate composition are summarized in the Table 1. Moisture content gradually increased along with the increase of concentration of EW, whereas dry matter content, crude protein, ether extract and ash content gradually decreased. Crude protein content, ether extract and ash content were negatively correlated with moisture content [18]. Fat and moisture content are inversely proportional [19], as was observed in this study as well. There was no significant (NS) difference($p > 0.05$) among the treatments.

Drip loss and pH

The drip loss of beef samples was evaluated in 24 hours, 48 hours, and 72 hours, corresponding to day 1, day 2, and day 3, respectively. The results are listed in the Table 2. Drip loss is a critical aspect in the meat industry, particularly from a financial standpoint [20]. Generally, beef with high drip loss has an unattractive appearance. It also decreases meat tenderness and juiciness. Drip loss (%) was increased along with advancement of storage time. It was found that drip loss gradually decreased with the increase of concentration of EW. The treatments resulted in a significantly different drip loss of meat ($p < 0.001$).

Table 1. Proximate composition of beef treated with electrolyzed water

Treatment combination mode	Moisture (%)	Dry matter (%)	Crude protein (%)	Ether extract (%)	Ash (%)
$T_0 \times TM_1$	72.31 ± 0.29	27.68 ± 0.29	21.91 ± 0.19	4.55 ± 0.16	1.48 ± 0.16
$T_0 \times TM_2$	72.68 ± 0.28	27.32 ± 0.28	21.90 ± 0.31	4.65 ± 0.17	1.29 ± 0.09
$T_0 \times TM_3$	72.42 ± 0.30	27.58 ± 0.30	21.74 ± 0.16	4.86 ± 0.18	1.72 ± 0.17
$T_1 \times TM_1$	73.17 ± 0.36	26.82 ± 0.36	20.33 ± 0.34	5.06 ± 0.09	1.55 ± 0.08
$T_1 \times TM_2$	72.96 ± 0.35	27.04 ± 0.35	20.70 ± 0.35	4.72 ± 0.11	1.49 ± 0.17
$T_1 \times TM_3$	72.90 ± 0.36	27.11 ± 0.36	20.31 ± 0.27	5.0 ± 0.19	1.76 ± 0.07
$T_2 \times TM_1$	73.11 ± 0.30	26.89 ± 0.30	20.41 ± 0.27	4.69 ± 0.25	1.44 ± 0.08
$T_2 \times TM_2$	73.23 ± 0.32	26.77 ± 0.32	20.19 ± 0.19	4.60 ± 0.10	1.47 ± 0.03
$T_2 \times TM_3$	73.05 ± 0.36	26.94 ± 0.36	20.52 ± 0.16	4.54 ± 0.12	1.60 ± 0.14
$T_3 \times TM_1$	73.51 ± 0.30	26.49 ± 0.30	20.23 ± 0.27	4.51 ± 0.16	1.48 ± 0.10
$T_3 \times TM_2$	73.63 ± 0.32	26.37 ± 0.32	20.30 ± 0.18	4.33 ± 0.14	1.36 ± 0.13
$T_3 \times TM_3$	73.35 ± 0.35	26.65 ± 0.35	20.29 ± 0.19	4.46 ± 0.09	1.56 ± 0.08
$T_4 \times TM_1$	73.60 ± 0.30	26.39 ± 0.30	20.26 ± 0.14	4.37 ± 0.14	1.35 ± 0.04
$T_4 \times TM_2$	73.69 ± 0.31	26.30 ± 0.31	20.11 ± 0.15	4.27 ± 0.09	1.49 ± 0.10
$T_4 \times TM_3$	73.42 ± 0.35	26.58 ± 0.35	20.02 ± 0.16	4.34 ± 0.06	1.45 ± 0.04
$T_5 \times TM_1$	73.84 ± 0.30	26.16 ± 0.30	19.95 ± 0.16	4.29 ± 0.10	1.40 ± 0.04
$T_5 \times TM_2$	73.93 ± 0.30	25.96 ± 0.30	20.02 ± 0.15	4.28 ± 0.09	1.40 ± 0.05
$T_5 \times TM_3$	73.72 ± 0.35	26.28 ± 0.35	19.98 ± 0.15	4.31 ± 0.11	1.36 ± 0.06
Significant Level	NS	NS	NS	NS	NS

The pH of samples was analyzed within 24 hours, and the findings are presented in the Table 2. pH levels ranged from 5.42 to 6.11. As the concentration of EW increased, there was a gradual decrease in pH. However, no significant difference ($p > 0.05$) in pH was observed among the treatments.

Table 2. Drip loss and pH of the beef treated with electrolyzed water

Treatment combination mode	pH	Drip loss–day 1 (%)	Drip loss–day 2 (%)	Drip loss–day 3 (%)
$T_0 \times TM_1$	6.11 ± 0.007^{a}	3.31 ± 0.05^{a}	3.77 ± 0.05^{a}	4.92 ± 0.02^{a}
$T_0 \times TM_2$	6.10 ± 0.007^{a}	3.29 ± 0.08^{a}	3.54 ± 0.009^{b}	4.76 ± 0.02^{b}
$T_0 \times TM_3$	6.10 ± 0.006^{a}	3.28 ± 0.03^{a}	3.48 ± 0.007^{c}	4.67 ± 0.06^{c}
$T_1 \times TM_1$	5.84 ± 0.003^{b}	3.18 ± 0.02^{b}	3.44 ± 0.003^{cd}	4.13 ± 0.03^{d}
$T_1 \times TM_2$	5.80 ± 0.007^{c}	3.14 ± 0.006^{bc}	3.41 ± 0.04^{d}	3.91 ± 0.02^{e}
$T_1 \times TM_3$	5.75 ± 0.01^{d}	3.10 ± 0.04^{cd}	3.40 ± 0.02^{d}	3.85 ± 0.04^{f}
$T_2 \times TM_1$	5.72 ± 0.007^{e}	3.06 ± 0.02^{d}	3.23 ± 0.009^{e}	3.77 ± 0.06^{g}
$T_2 \times TM_2$	5.69 ± 0.006^{f}	3.05 ± 0.03^{de}	3.16 ± 0.03^{f}	3.70 ± 0.03^{hi}
$T_2 \times TM_3$	5.69 ± 0.009^{f}	3.06 ± 0.05^{d}	3.11 ± 0.003^{f}	3.69 ± 0.006^{hi}
$T_3 \times TM_1$	5.66 ± 0.009^{g}	2.98 ± 0.04^{ef}	3.11 ± 0.009^{f}	3.67 ± 0.006^{i}
$T_3 \times TM_2$	5.64 ± 0.003^{h}	2.90 ± 0.06^{fg}	3.01 ± 0.007^{g}	3.74 ± 0.05^{gh}
$T_3 \times TM_3$	5.63 ± 0.007^{h}	2.89 ± 0.01^{g}	3.03 ± 0.02^{g}	3.70 ± 0.06^{hi}
$T_4 \times TM_1$	5.60 ± 0.003^{i}	2.86 ± 0.07^{g}	2.90 ± 0.01^{hi}	3.11 ± 0.01^{j}
$T_4 \times TM_2$	5.58 ± 0.009^{i}	2.88 ± 0.09^{g}	2.94 ± 0.01^{h}	3.05 ± 0.05^{k}
$T_4 \times TM_3$	5.56 ± 0.007^{j}	2.84 ± 0.04^{g}	2.85 ± 0.03^{i}	2.98 ± 0.006^{l}
$T_5 \times TM_1$	5.56 ± 0.003^{j}	2.47 ± 0.006^{h}	2.51 ± 0.02^{j}	2.63 ± 0.006^{m}
$T_5 \times TM_2$	5.53 ± 0.007^{k}	2.31 ± 0.02^{i}	2.43 ± 0.03^{k}	2.55 ± 0.02^{n}
$T_5 \times TM_3$	5.52 ± 0.006^{k}	2.27 ± 0.05^{i}	2.32 ± 0.009^{l}	2.49 ± 0.006^{o}
Significant level	***	*	***	***

Means with different superscripts within same column differ significantly; *** = Highly significant ($p < 0.001$), * = Significant ($p < 0.05$).

Cooking loss and cooking yield

The Table 3 presents the cooking yield and cooking loss of beef treated with varying concentrations of electrolyzed water. Cooking loss, measured at 20 minutes, 30 minutes, and 40 minutes, represents the reduction in weight of beef during cooking [21]. Due to the possibility of moisture loss and soluble nutrients loss, this parameter is crucial for the meat processing sector [22]. The percentage of cooking loss varied from 33.86% to 35.82% [14]. The cooking loss that we observed in our results, which varied between 38.70% and 47.51%, was slightly greater than what was previously reported [14]. Cooking loss (%) decreased gradually with the increase of concentration of EW but yet increased along with advancement of cooking time. Meat's pH value has a significant impact on cooking loss; if the meat's pH is higher or lower than its isoelectric point (5.0–5.1), the amount of cooking loss will be decreased [23]. Elevated cooking temperatures may be linked to increased cooking loss because they denaturize collagen and actin protein (over 60 °C), which shrinks muscle fibers parallel to their axis and extracts water from the space between them [24]. No significant (NS) difference was observed among the treatments.

Sensory evaluation

Table 4 summarizes the results of the tasting panelists' evaluation of the beef's appearance, aroma and texture among other quality criteria. The highest values were found in the trial $T_2 \times TM_2$ for appearance and aroma. Beef samples treated with 20 ppm EW solution was chosen as the most desirable appearance and aroma. As the addition of 50 ppm EW solution treated with meat, appearance got worse but texture improved. No significant difference (NS) was observed among the treatments in case of appearance, aroma and texture. The findings of our study showed that the meat

Table 3. Cooking loss and cooking yield of beef treated with electrolyzed water

Treatment combination mode	Cooking loss-20 minutes (%)	Cooking yield-20 minutes (%)	Cooking loss-30 minutes (%)	Cooking yield-30 minutes (%)	Cooking loss-40 minutes (%)	Cooking yield-40 minutes (%)
$T_0 \times TM_1$	43.27 ± 0.10	56.73 ± 0.10	45.79 ± 0.15	54.21 ± 0.15	47.51 ± 0.12	52.48 ± 0.12
$T_0 \times TM_2$	43.10 ± 0.01	56.89 ± 0.01	45.70 ± 0.16	54.54 ± 0.16	47.49 ± 0.11	52.50 ± 0.11
$T_0 \times TM_3$	43.07 ± 0.06	56.92 ± 0.06	45.60 ± 0.11	54.19 ± 0.11	47.48 ± 0.10	52.52 ± 0.10
$T_1 \times TM_1$	42.73 ± 0.19	57.26 ± 0.19	43.91 ± 0.03	56.08 ± 0.03	45.12 ± 0.10	54.88 ± 0.10
$T_1 \times TM_2$	42.71 ± 0.18	57.28 ± 0.18	43.74 ± 0.15	56.25 ± 0.15	45.20 ± 0.28	54.80 ± 0.28
$T_1 \times TM_3$	42.72 ± 0.20	57.28 ± 0.20	43.68 ± 0.08	56.32 ± 0.08	45.03 ± 0.14	54.96 ± 0.14
$T_2 \times TM_1$	41.59 ± 0.17	58.40 ± 0.17	42.67 ± 0.16	57.32 ± 0.16	44.04 ± 0.14	55.96 ± 0.14
$T_2 \times TM_2$	41.59 ± 0.14	58.40 ± 0.14	42.55 ± 0.15	57.44 ± 0.15	44.03 ± 0.16	55.99 ± 0.16
$T_2 \times TM_3$	41.54 ± 0.12	58.45 ± 0.12	42.52 ± 0.12	57.47 ± 0.12	43.91 ± 0.16	56.09 ± 0.16
$T_3 \times TM_1$	40.89 ± 0.02	59.11 ± 0.02	42.34 ± 0.04	57.62 ± 0.04	43.18 ± 0.16	56.81 ± 0.16
$T_3 \times TM_2$	40.79 ± 0.04	59.17 ± 0.04	42.34 ± 0.05	57.66 ± 0.05	43.10 ± 0.14	56.89 ± 0.14
$T_3 \times TM_3$	40.69 ± 0.08	59.31 ± 0.08	42.19 ± 0.03	57.74 ± 0.03	43.0 ± 0.10	56.99 ± 0.10
$T_4 \times TM_1$	40.11 ± 0.13	59.88 ± 0.13	42.05 ± 0.07	57.95 ± 0.07	42.93 ± 0.11	57.06 ± 0.11
$T_4 \times TM_2$	40.09 ± 0.10	59.90 ± 0.10	41.91 ± 0.14	58.09 ± 0.14	42.90 ± 0.11	57.09 ± 0.11
$T_4 \times TM_3$	40.01 ± 0.05	59.99 ± 0.05	41.83 ± 0.04	58.17 ± 0.04	42.80 ± 0.10	57.19 ± 0.10
$T_5 \times TM_1$	38.97 ± 0.07	61.02 ± 0.07	40.22 ± 0.03	59.78 ± 0.03	41.71 ± 0.09	58.28 ± 0.09
$T_5 \times TM_2$	38.77 ± 0.13	61.22 ± 0.13	40.11 ± 0.03	59.88 ± 0.03	41.69 ± 0.10	58.31 ± 0.10
$T_5 \times TM_3$	38.70 ± 0.09	61.29 ± 0.09	40.10 ± 0.03	59.60 ± 0.03	41.55 ± 0.05	58.45 ± 0.05
Significant Level	NS		NS	NS	NS	NS

treated with EW had better sensory score than the untreated sample. Rahman et al. [1] reported that EW treated samples had better sensory scores than the untreated samples, as observed in this study as well. These results indicate that EW treatment can improve sensory qualities and extend shelf life of meat during storage at 5 °C. EWs contained OH and HOCl that have strong antimicrobial activity and antioxidant effect. Furthermore, residual NaCl content of EW provides meat samples with freshness color and keeps meat aroma as well.

Table 4. Sensory evaluation of beef treated with electrolyzed water

Treatment combination mode	Appearance	Aroma	Texture
$T_0 \times TM_1$	2.67 ± 0.006	2.65 ± 0.007	2.33 ± 0.01
$T_0 \times TM_2$	2.66 ± 0.009	2.67 ± 0.007	2.33 ± 0.02
$T_0 \times TM_3$	2.67 ± 0.007	2.64 ± 0.01	2.33 ± 0.02
$T_1 \times TM_1$	2.67 ± 0.02	3.31 ± 0.03	2.0 ± 0.04
$T_1 \times TM_2$	2.67 ± 0.009	3.33 ± 0.02	2.02 ± 0.02
$T_1 \times TM_3$	2.65 ± 0.03	3.33 ± 0.02	2.01 ± 0.02
$T_2 \times TM_1$	3.64 ± 0.03	4.66 ± 0.03	3.33 ± 0.01
$T_2 \times TM_2$	3.67 ± 0.03	4.67 ± 0.01	3.32 ± 0.02
$T_2 \times TM_3$	3.65 ± 0.03	4.64 ± 0.02	3.33 ± 0.03
$T_3 \times TM_1$	2.3 ± 0.007	4.33 ± 0.01	4.29 ± 0.04
$T_3 \times TM_2$	2.33 ± 0.007	4.33 ± 0.009	4.31 ± 0.01
$T_3 \times TM_3$	2.33 ± 0.03	4.30 ± 0.01	4.32 ± 0.007
$T_4 \times TM_1$	2.66 ± 0.007	4.33 ± 0.01	4.33 ± 0.01
$T_4 \times TM_2$	2.65 ± 0.007	4.32 ± 0.009	4.33 ± 0.02
$T_4 \times TM_3$	2.66 ± 0.007	4.32 ± 0.01	4.33 ± 0.01
$T_5 \times TM_1$	3.31 ± 0.009	4.66 ± 0.03	4.65 ± 0.009
$T_5 \times TM_2$	3.32 ± 0.007	4.64 ± 0.009	4.66 ± 0.03
$T_5 \times TM_3$	3.30 ± 0.09	4.64 ± 0.01	4.63 ± 0.009
Significant Level	NS	NS	NS

Color analysis

The Table 5 summarizes the findings of the evaluation of the color of beef based on numerous flame qualities, including brightness, redness, and yellowness of beef treated with varying quantities of electrolyzed water. Consumer tastes and the quality of beef products are significantly influenced by beef color. Customers' opinions of the quality of meat are significantly influenced by the redness of the beef [25]. Numerous factors, such as the type of animal,

Table 5. Color analysis of beef treated with electrolyzed water

Treatment combination mode	Lightness (L*)	Redness (a*)	Yellowness (b*)
$T_0 \times TM_1$	42.89 ± 0.16^{n}	11.93 ± 0.18^{p}	9.70 ± 0.11^{a}
$T_0 \times TM_2$	43.11 ± 0.08^{m}	12.30 ± 0.06^{o}	9.67 ± 0.03^{a}
$T_0 \times TM_3$	43.23 ± 0.07^{m}	12.39 ± 0.07^{no}	9.51 ± 0.1^{b}
$T_1 \times TM_1$	43.98 ± 0.06^{l}	13.11 ± 0.06^{m}	8.52 ± 0.07^{c}
$T_1 \times TM_2$	43.83 ± 0.20^{l}	12.47 ± 0.12^{n}	8.57 ± 0.02^{c}
$T_1 \times TM_3$	44.06 ± 0.22^{k}	13.39 ± 0.18^{l}	8.44 ± 0.03^{d}
$T_2 \times TM_1$	45.01 ± 0.21^{j}	14.18 ± 0.06^{k}	7.71 ± 0.02^{f}
$T_2 \times TM_2$	45.09 ± 0.16^{j}	15.29 ± 0.18^{i}	7.79 ± 0.18^{e}
$T_2 \times TM_3$	45.35 ± 0.09^{i}	15.19 ± 0.15^{j}	7.63 ± 0.10^{g}
$T_3 \times TM_1$	46.69 ± 0.19^{g}	16.32 ± 0.08^{g}	6.28 ± 0.05^{j}
$T_3 \times TM_2$	46.52 ± 0.14^{h}	16.12 ± 0.17^{h}	6.62 ± 0.09^{h}
$T_3 \times TM_3$	46.97 ± 0.07^{f}	16.83 ± 0.13^{f}	6.51 ± 0.15^{i}
$T_4 \times TM_1$	47.32 ± 0.11^{e}	17.75 ± 0.11^{e}	5.45 ± 0.13^{l}
$T_4 \times TM_2$	47.75 ± 0.14^{d}	18.08 ± 0.08^{d}	5.71 ± 0.12^{k}
$T_4 \times TM_3$	47.96 ± 0.11^{c}	18.39 ± 0.12^{c}	5.44 ± 0.12^{l}
$T_5 \times TM_1$	49.02 ± 0.11^{b}	19.25 ± 0.06^{b}	4.35 ± 0.01^{n}
$T_5 \times TM_2$	49.16 ± 0.16^{b}	19.43 ± 0.15^{a}	4.52 ± 0.10^{m}
$T_5 \times TM_3$	49.42 ± 0.15^{a}	19.51 ± 0.09^{a}	4.59 ± 0.16^{m}
Significant Level	***	***	***

Means with different superscripts within same column differ significantly; *** = Highly significant ($p < 0.001$).

age at slaughter, muscle position anatomically, fat level, and cooking method, can affect the color of beef [26].

There was a highly significant difference in color attributes such as lightness ($p < 0.001$), redness ($p < 0.001$) and yellowness ($p < 0.001$) among the treatments.

Correlation matrix of selected nutritional properties of beef

The results of Pearson's correlation coefficients in correlation analysis among selected physical and nutritional properties of beef are presented in the Table 6. There was nonsignificant (NS) correlation among the parameters. We found nonsignificant positive correlation between dry matter and crude protein (r = 0.8669); dry matter and ether extract (r = 0.6609); crude protein and ether extract (r = 0.3422); dry matter and ash (r = 0.5230); crude protein and ash (r = 0.1904); ether extract and ash (0.6763). We also found nonsignificant negative correlation between moisture and dry matter (r = –0.9448); moisture and crude protein (r = –0.8258); moisture and ether extract (r = –0.6845); moisture and ash (r = –0.4744)

Table 6. Pearson's correlation coefficients among selected nutritional properties of beef

Parameters	1	2	3	4
1. Moisture				
2. Dry matter	–0.9448^{NS}			
3. Crude protein	–0.8258^{NS}	0.8669^{NS}		
4. Ether extract	–0.6845^{NS}	0.6609^{NS}	0.3422^{NS}	
5. Ash	–0.4744^{NS}	0.5230^{NS}	0.1904^{NS}	0.6763^{NS}

Correlation matrix of selected physical properties of beef

The results of Pearson's correlation coefficients correlation analysis among selected physical and nutritional properties of beef are presented in the Table 7. There was nonsignificant (NS) correlation among the parameters. We found nonsignificant positive correlation between pH and drip loss after 24 hours (r = 0.7984); pH and drip loss after 48 hours (r = 0.8600); pH and drip loss after 72 hours (r = 0.9326); pH and cooking loss after 20 minutes (r = 0.8774); pH and cooking loss after 30 minutes (r = 0.9547); pH and cooking loss after 40 minutes (r = 0.9814) drip loss after 24 hours and drip loss after 48 hours (r = 0.9735); drip loss after 24 hours and drip loss after 72 hours (r = 0.9213); drip loss after 24 hours and cooking loss after 20 minutes (r = 0.9462); drip loss after 48 hours and cooking loss after 20 minutes (r = 0.9783); drip loss after 72 hours and cooking loss after 20 minutes (r = 0.9481); drip loss after 48 hours and cooking loss after 30 minutes (r = 0.9558); cooking loss after 20 minutes and cooking loss after 30 minutes (r = 0.9531); cooking loss after 30 minutes and cooking loss after 40 minutes (r = 0.9862).

Conclusion

The experiment was aimed to investigate the effect of electrolyzed water on the nutritional composition and sensory characteristics of beef. Electrolyzed water, specifically electrolytically generated hypochlorous acid, has been used as an effective sterilizing agent for raw meat for several decades. It was imperative to determine an acceptable level of electrolyzed water concentration and duration of exposure that would not compromise the nutritional quality of the meat. The study comprehensively analyzed physicochemical parameters such as proximate analysis, pH, drip loss, cooking yield, and cooking loss, as well as sensory attributes including appearance, aroma, texture, and color changes. The treatment with electrolyzed water did not induce significant changes in the proximate composition, pH, or cooking characteristics of the beef samples. However, a concentration of up to 50 ppm of electrolyzed water can be used for beef preservation to achieve desirable results, including enhanced nutritional and textural properties, as well as increased shelf life. The introduction of this practice offers the potential to reduce contamination and extend shelf life without compromising the sensory properties of the meat. These findings suggest that electrolyzed water can serve as a promising sanitizer for beef, thus contributing to improved food safety and consumers' confidence in meat products quality.

Table 7. Pearson correlation coefficients among selected physical properties of beef

Parameters	1	2	3	4	5	6
1. pH						
2. Drip loss after 24 h	0.7984^{NS}					
3. Drip loss after 48 h	0.8600^{NS}	0.9735^{NS}				
4. Drip loss after 72 h	0.9326^{NS}	0.9213^{NS}	0.9545^{NS}			
5. Cooking loss after 20 minutes	0.8774^{NS}	0.9462^{NS}	0.9783^{NS}	0.9481^{NS}		
6. Cooking loss after 30 minutes	0.9547^{NS}	0.9240^{NS}	0.9558^{NS}	0.9695^{NS}	0.9531^{NS}	
7. Cooking loss after 40 minutes	0.9814^{NS}	0.8739^{NS}	0.9207^{NS}	0.9492^{NS}	0.9379^{NS}	0.9862^{NS}

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All authors bear responsibility for the work and presented data.

All authors made an equal contribution to the work.

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

The authors declare no conflict of interest.