



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

# *THEORY AND PRACTICE OF MEAT PROCESSING*

2025, vol.I0, no.I



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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](http://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

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Free price

Frequency — 4 issues a year

Signed print 28.03.2025

Released from press 31.03.2025

Circulation — 300 copies. Order № 20.

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16+

ISSN 2414-438X (Print)

ISSN 2414-441X (Online)

DOI-prefix: 10.21323/2414-438X

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# PREVALENCE AND SEROTYPING OF *SALMONELLA* IN BEEF CARCASSES SOLD IN MARKETS OF HAMA CITY, SYRIA

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**Keywords:** *Salmonella*, meat, serotyping, cow, public health

## Abstract

*Salmonella* is one the most important pathogenic bacteria, which causes food poisoning in human consumers worldwide. This study aimed to detect the prevalence and serovars of *Salmonella* in beef in markets of Hama city, Syria. The study was carried out on 200 beef samples from 20 retail shops in markets of Hama city, Syria. Investigation was conducted using bacterial cultures and serotyping. Bacterial cultures showed that 11 samples out of 200 samples from different retail shops were positive, with an overall prevalence of 5.5% (95% CI: 2.34–8.66). The isolated serotypes were *S. Typhimurium* (36.36%), *S. Enteritidis* (27.27%), *S. Reading* (9.09%), *S. Bredeney* (9.09%), *S. Chester* (9.09%), and *S. Anatum* (9.09%). Significantly higher ( $p < 0.05$ ) prevalence was revealed in the east district (8%, 4/50) compared to other districts, in informal shops (7.5%, 6/80) than in other outlets, in female animals (10%, 4/40) compared to male animals, in slaughtered animals at the age of more than 12 months (7.5%; 6/80), in summer (12%, 6/50) compared to other seasons, in unclean shops (7.38%, 9/122), and in liver (7.5%, 6/80) compared to other meat types. These results are considered an important threat to public health and indicate food contamination.

**For citation:** Faour, O., Alkouljah, Kh. (2025). Prevalence and serotyping of *Salmonella* in beef carcasses sold in markets of Hama city, Syria. *Theory and Practice of Meat Processing*, 10(1), 4–10. <https://doi.org/10.21323/2414-438X-2024-10-1-4-10>

## Funding:

The research was funded by the University of Hama as well as the Animal Health Directorate of the Ministry of Agriculture and Agrarian Reform in Syria.

## Acknowledgments:

The authors express their sincere gratitude to the Animal Health Directorate of the Ministry of Agriculture and Agrarian Reform of Syria, Damascus, Syria, the Department of Animal Diseases, and Department of Public Health and Preventive Medicine, College of Veterinary Medicine, University of Hama, Hama, Syria, for supporting this work in all possible ways.

## Introduction

Beef is one of the animal products that are widely consumed by humans and is one of the foods that are most exposed to bacterial contamination as it contains many nutrients that bacteria need to multiply [1,2]. Cattle carcasses consist of parts that are edible for human consumers and others that are not edible. Among the edible parts are the carcass meat, liver, heart, kidneys, tongue and brain. Despite the high nutritional value of beef, it is a potential nutritional material for the transmission of foodborne pathogens, as it contains a high level of protein and a low percentage of carbohydrates in addition to a moderate acidity with sufficient water, which helps in the growth and survival of pathogenic bacteria [3,4,5]. The storage conditions of beef, which include many factors such as humidity and temperature of meat storage, especially with regard to poor cooling, are conducive to the growth and proliferation of bacteria, including bacteria that cause zoonotic diseases in humans [6]. The most important sources of beef contamination are the skin of animals (soil attached to the skin) as well as the intestines (feces) of animals processed for human consumption. Microbial contamination occurs

especially when the entrails of slaughtered animals are removed in slaughterhouses [7,8]. Contaminated tools used in slaughtering animals and tools for cutting and cleaning carcasses such as knives and cleavers are potential sources of bacterial contamination of this meat [9].

Another potential source of beef contamination are vehicles used to transport meat from slaughterhouses to retail outlets due to poor hygiene, not to mention the poor hygiene of the stores where this meat is displayed [8].

Bacteria, including *Salmonella*, *Shigella*, *Escherichia coli*, *Campylobacter*, and *Staphylococcus aureus*, are among the most important foodborne causative agents and are very common in beef, in addition to other pathogens such as viruses and parasites that cause zoonotic diseases in humans who consume this meat [10,11].

*Salmonella* is a genus of bacteria that is a leading cause of foodborne illness globally, responsible for millions of infections each year [12]. The *Salmonella* genus is currently comprised of two species *Salmonella enterica* and *Salmonella bongori*, with *Salmonella enterica* being the most clinically significant. Within *S. enterica*, over 2,500 serotypes have been identified, distinguished by their unique combi-



nations of surface antigens, specifically O (somatic) and H (flagellar) antigens [13]. This classification system is known as serotyping and is vital for epidemiological surveillance, outbreak investigations, and understanding the transmission dynamics of *Salmonella* infections.

Serotyping plays a crucial role in identifying specific serotypes associated with particular sources of infection. For example, *Salmonella* Typhimurium and *Salmonella* Enteritidis are frequently linked to poultry and egg products, while *Salmonella* Typhi is associated with typhoid fever and human carriers [14]. Understanding the diversity of *Salmonella* serotypes is essential for developing targeted public health interventions and improving food safety practices.

Recent advancements in molecular techniques, such as whole-genome sequencing (WGS), have enhanced our ability to characterize *Salmonella* strains more precisely. However, traditional serotyping methods remain fundamental due to their cost-effectiveness and ease of use in routine diagnostics [15]. The combination of traditional serotyping and modern genomic approaches provides a comprehensive framework for tracking transmission pathways and identifying contamination sources.

There are several serotypes of *Salmonella* detected in contaminated beef sold in local markets in many areas that have been investigated in several previous studies. These studies reported that the most prevalent serotypes of *Salmonella* are *Salmonella* Typhimurium and *Salmonella* Enteritidis in addition to other serotypes identified in meat [16].

*Salmonella* are facultatively anaerobic, gram-negative, oxidase negative, catalase positive, nonspore forming rods. Almost all *Salmonella* serotypes are motile via peritrichous flagella except *S. Pullorum* and *S. Gallinarum* [17]. The optimal growth temperature of *Salmonella* is 37°C; however, growth has been recorded between 2 and 4°C and as high as 45°C, *Salmonella* can live in a wide pH range from as low as pH 3.8 to as high as pH 9.5 with an optimum pH of 6.5–7.5 [18].

It can ferment glucose, mannitol, arabinose, maltose, dulcitol and sorbitol, forming acid and gas except for *S. Typhi*, *S. Gallinarum* and rare aerogenic variants in other subtypes form only acid and no gas. Generally, *Salmonella* does not ferment lactose, sucrose, salicin or adonitol. It is indole negative, Methyl Red positive (MR), Voges Proskauer negative (VP), and citrate positive (IMViC – + – +) except for *S. Typhi* and *S. Paratyphi A*, which are citrate negative as they need tryptophan as the growth factor. Hydrogen sulfide is produced except for *S. Paratyphi A*, *S. Choleraesuis*, *S. Typhisuis* and *S. Sendai*. Urease is not hydrolyzed by *Salmonella* [19].

*Salmonella* is a significant foodborne pathogen that poses a serious public health risk worldwide, particularly in relation to the consumption of contaminated beef. According to the Centers for Disease Control and Prevention (CDC), *Salmonella* is one of the leading causes of bacterial food poisoning in the United States, contributing to approximately 1.35 million infections annually [20]. Beef, as a widely consumed protein source, has been identified as a common

vehicle for *Salmonella* transmission, with outbreaks often linked to undercooked or improperly handled meat [21].

The contamination of beef with *Salmonella* can occur at various stages of the production chain, including slaughter, processing, and distribution [22]. Factors such as inadequate hygiene practices, cross-contamination, and improper cooking temperatures contribute to the persistence of *Salmonella* in beef products [15].

In addition to direct contamination from livestock, environmental factors and feed sources also play crucial roles in the prevalence of *Salmonella* in beef [23]. The emergence of antibiotic-resistant strains of *Salmonella* further complicates the issue, as these strains pose challenges for treatment and control measures [24].

Given the public health implications associated with beef contaminated with *Salmonella*, it is essential to implement rigorous food safety practices throughout the meat production and supply chain. This includes monitoring and controlling *Salmonella* levels in cattle herds, enhancing hygiene practices during processing, and educating consumers on safe cooking methods [12].

The control of foodborne pathogens such as *Salmonella enterica* is difficult because of their ability to survive during food production, processing, storage and improper cooking. Therefore, it is important to understand the ecology of *Salmonella enterica* and the genetic variation of different strains in order to design specific management practices to reduce risks associated with this pathogen. Several molecular typing methods are used to differentiate *Salmonella enterica* isolates, including multilocus variable-number tandem-repeat analysis, multilocus sequence typing or multiplex-PCR-based methods and whole genome sequencing [25].

Meat production is central to livelihoods in many countries, with meat from livestock and poultry being a key protein source in subsistence communities [26]. In many low-resource settings, industrialization, urbanization, and the shift from planned to market economies are leading to rapid changes in the way that food is produced, distributed, sold, and consumed [27]. Such market-driven changes within agricultural production towards wider distribution networks, centralized processing, larger-scale and more intensive systems, have been linked to the emergence of zoonotic diseases [28].

Therefore, it was necessary to investigate *Salmonella* and its serotypes in beef carcasses sold in markets of Hama city, Syria.

## Materials and methods

### Samples

Beef samples were taken from four different districts for bacteriological examination. A total of 200 beef samples were randomly taken from 20 retail shops, during a period from October 2023 to September 2024. Samples were used to detect *Salmonella*.

### *Sample collection*

Samples of beef were collected in sterile plastic bags and kept in them at a temperature of 4–8°C for the period of transference to the research laboratory for microbiological analysis.

### *Epidemiological data collection*

Epidemiological data on the studied beef and retail shops were collected using special questionnaires based on previous studies, which involved information about retail shops such as shop name, district, age of animals, sex of animals, outlet, and season as well as cleanness of retail shops. Questionnaires were filled out during visits to the studied retail shops.

### *Microbiological analysis of Salmonella*

Twenty five grams of the examined samples were weighed aseptically into sterile blender container and thoroughly homogenized with 225 ml of sterile lactose broth. The homogenate was incubated at 37°C for 24 hours. In order to detect *Salmonella* using the traditional method, the following procedure was used [29].

**Enrichment in a selective liquid medium:** In this stage, Tetrathionate broth manufactured by HiMedia® was used, with potassium iodide solution added to it. Amounts of 0.1 ml and 1 ml of the incubated pre-enrichment homogenate were transferred to 10 ml Tetrathionate broth as selective enrichment. Tetrathionate broth was incubated at 42°C for 24–48 hours.

**Isolation and growth in selective solid medium:** XLD (Xylose Lysine Deoxycholate) agar (HiMedia®) was used, which was prepared and poured into petri dishes according to the manufacturer's instructions. Following primary enrichment, 20 µl from the TTB culture was streaked onto XLD medium. The dishes were incubated in the incubator at 37°C for 24–48 hours. After incubation, the cultural properties of the growing colonies were studied. Small, round colonies with a smooth surface and a black center with a metallic sheen or brown, green or gray colonies appeared were considered to be *Salmonella* colonies.

Several biochemical tests were performed to identify *Salmonella*. The biochemical tests performed were: catalase — oxidase — indole — methyl red — Voges-Proskauer — citrate — urease.

### *Serotyping of Salmonella*

Biochemically confirmed *Salmonella* sp. isolates were further serologically identified using a series of slide agglutination specific for O and H antigens (White — Kauffmann — Le Minor scheme) [30]. These tests were performed at the Animal Health Directorate of the Ministry of Agriculture and Agrarian Reform of Syria, Damascus, Syria.

### *Statistical analysis*

Field and laboratory recorded data were entered into a Microsoft Excel 2010 spreadsheet (Los Angeles, CA, USA). Then the data was checked to detect errors and the data

was coded in preparation for statistical analysis, which was done using the statistical program SPSS version 22 (IBM Inc., Chicago, IL, USA), after exporting the data to it.

### *Descriptive statistics for recorded data*

Absolute frequency and relative frequency were calculated for the studied variables with a categorical pattern. The value of the prevalence of *Salmonella* in beef was also calculated based on laboratory results using bacterial culture for each of the categorical variables studied. For the recorded prevalence, 95% confidence intervals (CI) were calculated.

### *Analytical statistics of recorded data*

Analytical statistics were conducted for each of the categorical variables studied and included in the questionnaires, which are: districts (4), outlets (3), sex of animals (2), type of meat (3), age of animals (4), seasons (4), and cleanness of retail shops (2).

The association between the prevalence of *Salmonella* and the variables (risk factors) under consideration was studied using chi-square method.  $P < 0.05$  was statistically considered significant.

### *Ethical Approval*

Those responsible for taking meat samples from retail shops adhered to ethical principles and general rules. The shop owners agreed with collection of samples from their shops.

## **Results**

### *Distribution of the studied samples*

As can be seen from Table 1, the number of the samples taken from four districts and during four seasons was equal and made up 25% for each category of these variables. As regards other variables and categories, most samples were taken from male animals (80%), animals at the age of 7–12 months (40%), liver (40%), supermarkets and butchery shops (80%), and unclean retail shops (61%).

### *Prevalence*

The study recorded an overall prevalence of *Salmonella* of 5.5% (11/200; 95% CI: 2.34–8.66%) in retail shops according to bacterial cultures. The highest prevalence was revealed in the east district (8%; 95% CI: 4.24–11.76%); in informal shops (7.5%; 95% CI: 3.85–11.15%); in liver (7.5%; 95% CI: 3.85–11.15%); in summer (12%, 95% CI: 7.5–16.5%); in unclean shops (7.38%; 95% CI: 3.75–11%); in female animals (10%; 95% CI: 5.84–14.16%); and in animals at the age of 13–24 months (7.5%; 95% CI: 3.85–11.15%) as shown in Table 1.

### *Distribution of Salmonella serovars in beef in retail shops*

Only six *Salmonella* serovars were identified in beef samples: *S. Typhimurium* (36.36%), *S. Enteritidis* (27.27%), *S. Reading* (9.09%), *S. Bredeney* (9.09%), *S. Chester* (9.09%), and *S. Anatum* (9.09%) (Table 2).



Table 1. Prevalence of *Salmonella* in beef meat in markets of Hama city, Syria and its association with categories of studied variables

Variable	Category	N	positive	%	95% CI		p-value
					lower	upper	
Districts	East	50	4	8.00	4.24	11.76	0.00
	West	50	3	6.00	2.71	9.29	
	North	50	1	2.00	0.06	3.94	
	South	50	3	6.00	2.71	9.29	
Outlets	Informal shops	80	6	7.50	3.85	11.15	0.00
	Butchery shops	80	4	5.00	1.98	8.02	
	Supermarkets	40	1	2.50	0.34	4.66	
Type of meat	Thigh	60	3	5.00	1.98	8.02	0.00
	Shoulder	60	2	3.33	0.85	5.82	
	Liver	80	6	7.50	3.85	11.15	
Seasons	Winter	50	1	2.00	0.06	3.94	0.00
	Spring	50	2	4.00	1.28	6.72	
	Summer	50	6	12.00	7.50	16.50	
	Autumn	50	2	4.00	1.28	6.72	
Cleanness of shops	Clean	78	2	2.56	0.37	4.75	0.00
	Unclean	122	9	7.38	3.75	11.00	
Sex of animals	Male	160	7	4.38	1.54	7.21	0.00
	Female	40	4	10.00	5.84	14.16	
Age of animals	0–6 months	40	1	2.50	0.34	4.66	0.00
	7–12 months	80	4	5.00	1.98	8.02	
	13–24 months	40	3	7.50	3.85	11.15	
	Above 24 months	40	3	7.50	3.85	11.15	

Table 2. Distribution of *Salmonella* serovars in beef in retail shops in markets of Hama city, Syria

Salmonella serovars	n	%
S. Typhimurium	4	36.36
S. Enteritidis	3	27.27
S. Reading	1	9.09
S. Bredeney	1	9.09
S. Chester	1	9.09
S. Anatum	1	9.09
TOTAL	11	100%

#### Study of variables associated with the prevalence of *Salmonella*

The study showed a relationship between the prevalence of *Salmonella* in beef and several studied variables that were considered risk factors for this prevalence in the studied retail shops, as shown in Table 1. A statistically significant relationship was observed for each of the following variables: district ( $P < 0.001$ ), outlet ( $P < 0.001$ ), type of meat ( $P < 0.001$ ), season ( $P < 0.001$ ), cleanness of shop ( $P < 0.001$ ), sex of animals ( $P < 0.001$ ), and age of animals ( $P < 0.001$ ).

#### Discussion

The study is one of the quantitative epidemiological studies carried out for the first time in Syria on the prevalence of *Salmonella* in markets of Hama city, which included different retail shops.

Twenty different retail shops were studied in markets of Hama city, Syria, where beef is sold, and 200 beef samples were collected from markets to study the prevalence of *Salmonella* in beef. The study showed that prevalence of

*Salmonella* was 5.5% of the total beef samples examined according to the scientific methodology.

*Salmonella* contamination in beef remains a critical public health concern, particularly given its association with foodborne illnesses. The present research indicates that approximately 5% of beef samples were positive for *Salmonella*, highlighting the need for effective monitoring and control measures throughout the beef supply chain.

The presence of *Salmonella* in beef can be attributed to several factors, including animal husbandry practices, processing conditions, and environmental factors. Cattle can harbor *Salmonella* in their gastrointestinal tracts without showing clinical signs of illness, making it challenging to detect and manage [31]. During slaughter and processing, improper handling and cross-contamination can facilitate the transfer of the pathogen to beef products.

Consumer handling also plays a crucial role in the risk of *Salmonella* infection. According to [22], improper cooking and cross-contamination in home kitchens contribute significantly to foodborne illness outbreaks associated with beef. The recommended cooking temperature for ground beef is 71°C, which is effective in killing *Salmonella*; however, many consumers do not adhere to these guidelines [32]. This gap in consumer knowledge and practice can exacerbate the risks associated with even low levels of contamination.

Moreover, the emergence of antibiotic-resistant strains of *Salmonella* poses additional challenges for public health. Studies have shown that certain strains found in beef have developed resistance to commonly used antibiotics,

complicating treatment options for infected individuals [24]. This highlights the importance of implementing robust surveillance systems and improving biosecurity measures on farms to reduce the prevalence of *Salmonella* in cattle.

The results of our study are consistent with the studies on the prevalence and risk factors for contamination by *Salmonella* that were conducted in Namibia, where the prevalence of *Salmonella* in beef carcass in markets was 2.67% [33], and in Istanbul, Turkey, where the prevalence of *Salmonella* in ground beef was 0.98% [34].

On the other hand, the prevalence of *Salmonella* in beef meat in markets of Hama city was less than what was stated by Hassanein et al. [23] in Egypt, where the prevalence of *Salmonella* in beef retail supermarkets was 20%. This percent is also similar to previous epidemiological studies conducted by researchers [35,36] in separate areas of the western Asian continent such as Tehran, Iran (20.2%) and Malaysia (15.4%). The study conducted in Vietnam by Van et al. [37] revealed the presence of *Salmonella* in retail beef samples at a level of 62%, which was much higher than the level of *Salmonella* positive beef samples (48.6%) recorded by Phan et al. [38] in the same country.

This varying prevalence of *Salmonella* in retail shops may be attributed to many reasons, including the differences in the show conditions of beef in retail shops, differences in breeding systems, differences in methods of diagnosing the bacteria.

The present study recorded that the highest prevalence (8%) of the contamination was in the east district in the Hama city, Syria, compared to the other districts in the city ( $P < 0.00$ ). This may be attributed to the fact that the east district contains a higher number of retail shops than the other regions, and is an open area for other districts, which helps in the entry of illegal meat into it.

The study showed that the prevalence of *Salmonella* in informal shops is higher compared to other outlets ( $P < 0.00$ ), which is consistent with the findings of Shafini et al. [36]. This is due to the lack of proper sanitary conditions for selling beef in informal shops.

Contamination by *Salmonella* was more common in carcasses of female animals compared to males ( $P < 0.00$ ) in this study, which is consistent with [39,40,41]. Apparently, this result was obtained because females are more exposed to pathogens than males.

The study recorded a higher prevalence of *Salmonella* in beef from animals more than 12 months old compared to other age groups, with significant differences ( $P < 0.05$ ). This can be attributed to previous infections in older animals [42].

The current study showed that liver had higher prevalence of *Salmonella* compared to other types of meat ( $P < 0.00$ ). This result confirms that the liver is more contaminated by *Salmonella* due to its closeness to intestines and is consistent with the results of [43]. Intestinal perforation may occur during opening the abdominal cavity of the carcass.

The current study also confirmed that the prevalence of *Salmonella* is more in the summer compared to other seasons ( $P < 0.00$ ). This contradicts the findings of Brichta-Harhay et al. [44].

The study recorded a higher prevalence of *Salmonella* in beef in unclean shops compared to others, with significant differences ( $P < 0.05$ ), which may be due to cross contamination with existing pathogens in the shop [45].

In our study, *Salmonella* Typhimurium and *Salmonella* Enteritidis were found to be among the most common serotypes in contaminated beef, which is consistent with several previous studies [46,47,48].

## Conclusions

Contamination of beef by *Salmonella* in retail shops in Hama city, Syria, is considered an important health problem as it may be a cause of food poisoning in human consumers. There are several predisposing factors to contamination by *Salmonella*, such as the district of retail shops, sex and age of slaughtered animals and type of outlets, in addition to the season and cleanness of shops. We propose improving health practices in places where beef is sold, and adhering to the high hygienic conditions of selling and trading beef in markets of Hama city, Syria.

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The authors declare no conflict of interest.



# FAST FOOD CONSUMPTION HAS A GREAT IMPACT ON THE AGING PROCESS — A REVIEW

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**Keywords:** fast food, aging process, nutritional quality, metabolic diseases, obesity

## Abstract

*This review examines the impact of fast food consumption on health and its role in the development of chronic diseases that accelerate the aging process. A comprehensive literature review was conducted to explore the relationship between fast food intake and the onset of cardiovascular diseases, diabetes, cancer, and obesity, all of which are associated with premature aging. The findings indicate that fast foods high in fat and sugar contribute to chronic inflammation — a phenomenon referred to as “inflammaging”, which damages the vascular system and heightens the risk of cardiovascular diseases, including atherosclerosis and heart failure. Additionally, metabolic disorders such as insulin resistance and dyslipidemia disrupt glucose homeostasis, further exacerbating chronic inflammation and promoting accelerated aging. Moreover, fast food consumption is linked to an increased risk of cancer, largely due to the presence of carcinogenic compounds in processed meats and the obesity epidemic, which amplifies aging through mechanisms involving chronic inflammation, oxidative stress, and hormonal imbalances. Collectively, these factors impair immune function and elevate the risk of neurodegenerative diseases. Consequently, fast food consumption significantly contributes to premature aging by fostering chronic inflammation, metabolic disorders, and oxidative stress. Urgent public health interventions are necessary to mitigate these adverse effects and promote healthier dietary patterns, to enhance quality of life and longevity.*

**For citation:** Awlqadr, F. H., Altemimi, A. B., Qadir, S. A., Alkanan, Z. T., Faraj, A. M., ALKaisy, Q. H. et al. (2025). Fast food consumption has a great impact on the aging process — A review. *Theory and Practice of Meat Processing*, 10(1), 11–31. <https://doi.org/10.21323/2414-438X-2024-10-1-11-31>

## Introduction

These days fast food does not seem only as a dietary pattern in the USA but also as a diet used globally. Fast foods are convenient, affordable, and have the flavor most people like. However, they are energy-dense and fatty. Such foods have high levels of trans fats, sugars, and sodium, but contain low levels of essential nutrients, such as vitamins and minerals, as well as fiber. Thus, the fast food eating pattern has concerning public health outcomes, especially regarding the long-term effects of growing old. High calorie content, unhealthy trans fats from fast food, and insufficient amounts of essential nutrients may be a cause of many diseases. Nutrients are necessary for the body for providing energy, building and repairing tissues, regulating metabolism and maintaining homeostasis [1,2]. But excessive calorie intake can lead to obesity and other metabolic malfunctions that are key factors for the speed of aging among people [3].

The surge in obesity rates is the major consequence of fast food eating. Obesity is primarily a recognized risk factor for several chronic diseases, including CVD, diabetes

type 2, and certain types of cancer. As a result, it tends to decrease life expectancy and speed up the aging process [4,5]. Moreover, the caloric content of overly consumed fast foods equals weight gain and obesity, leading to chronic inflammation, oxidative stress, and hormonal imbalances that promote accelerated aging [6,7]. The accumulation of bad fats and too much sodium in fast foods harms heart function [8].

Saturated and trans fats raise the level of LDL cholesterol, causing atherosclerosis, a hardening that may even advance to arterial narrowing. In turn, these are critical causes of heart attacks, strokes, and other important cardiovascular events that are leading causes of morbidity and mortality in older adults. These cardiovascular diseases are critical to the aging process because they impair the body's various organs and systems from functioning at total efficiency [9,10]. Moreover, fast food is highly associated with growing metabolic diseases, especially metabolic syndrome and diabetes type 2 [1,11]. Fast foods have a high glycemic load, leading to a quick elevation of blood sugar, and therefore insulin resistance increases

over time [12,13]. More important is the fact that insulin resistance is a determinant of type 2 diabetes and, thus, related to severe complications, such as kidney failure, neuropathy, and cardiovascular diseases — all of which serve to reduce life expectancy and accelerate aging [14]. In addition, fast food usually contains ingredients with pro-inflammatory and oxidative properties. Unhealthy fats and sugar-rich diets increase pro-inflammatory cytokines and reactive oxygen species (ROS) after cellular damage and chronic inflammation. Numerous age-related diseases, such as Parkinson and Alzheimer disease, among other neurodegenerative conditions, are associated with these factors [15,16]. In addition, other emerging reports indicate a negative association between fast food consumption, cognitive function, and mental health [17]. Unhealthy fat and high-sugar diets are associated with reduced memory, cognitive flexibility, and an increase in dementia risk. The neuroinflammatory and oxidative effects of such diets can accelerate cognitive decline, an essential aspect of the aging process [18]. In other words, the overall impact of fast food consumption is general enough to reach most aspects of the aging phenomenon, including both physical and cognitive health [19]. The purpose of this review is to underscore the tremendous impact that fast food has on public health and, further, on aging phenomena. We offer another piece of evidence to raise serious discussion on nutrition education for public intervention and policy changes to shift the balance toward healthy eating and increase quality of life and healthy aging.

### **Objects and methods**

The sources of information were the following scientific databases: ScienceDirect, PubMed, Scopus, ResearchGate, and Google Scholar. The search strategy included the following keywords: fast food, aging process, nutritional quality, metabolic diseases, obesity. The following acceptance criteria for research characterization were considered: the role of fast food consumption in the development of aging processes. The parameters of the publications were as follows: publication from 1977 until 2024 (178 references were selected for this review); language: English. Exclusion criteria: no access to the full text articles. The published and selected research results were analyzed, systematized, summarized, after which conclusions were drawn by sections and a general conclusion.

### ***Types of fast food***

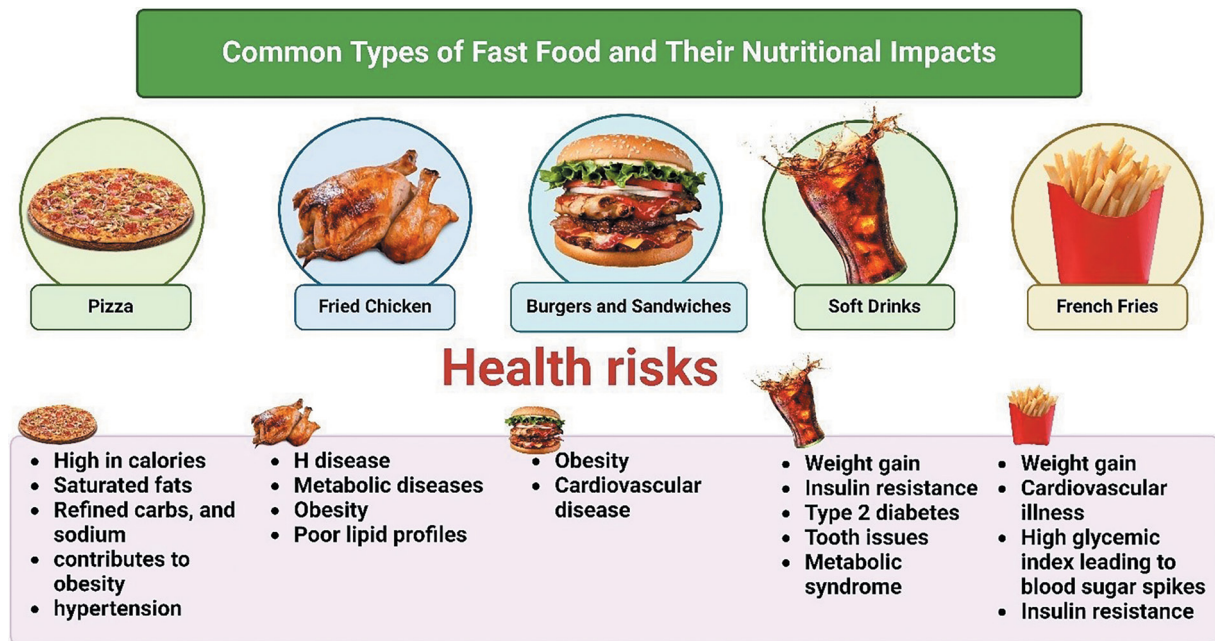
Modern diets include fast food, which is prepared and served rapidly in restaurants or for takeaway. These foods are heavy in energy and low in nutrients, frequently containing harmful fats, sugars, and sodium. This section discusses common fast foods, their nutritional value, and their appeal across demographics. The first type of fast food are burgers and sandwiches, which are popular worldwide because of their convenience, price, and taste. A burger often has a beef patty, buns, condiments, cheese, lettuce, and tomatoes. Chicken, fish, and vegetarian sandwiches are available. These

foods are unhealthy due to their high caloric content, saturated fats, and sodium [20]. Due to their high fat and calorie content, such meals can raise obesity and cardiovascular disease risk [21]. The second type is fried chicken, which is popular among Asian and young adult population. Chicken chunks are battered and deep-fried for a crispy, delicious exterior. Fried chicken is tasty but heavy in trans fats and cholesterol, which can cause heart disease and other metabolic diseases. KFC's brand influence contributes to its widespread consumption [22]. Fried chicken is linked to obesity and poor lipid profiles, increasing cardiovascular disease risk [23]. In the West, the popularity of the third type of fast food, pizza, is huge. Teens and young adults eat it often because of its convenience and diversity of toppings [24]. A common pizza has a bread base, tomato sauce, cheese, pepperoni, vegetables, and other ingredients. Pizza has calcium and protein from cheese, but also saturated fats, refined carbs, and sodium. The high calorie and sodium content of pizza may contribute to obesity and hypertension [25]. The fourth type are soft drinks, commonly served with fast food, which drastically increase sugar intake. These drinks are high in added sugars and calories but low in nutrients. Sugary drinks can cause insulin resistance, diabetes type 2, and weight gain [26]. Regular consumption of these drinks can also cause tooth issues and metabolic syndrome [27]. The fifth type are French fries, which are a fast food staple commonly served with burgers and sandwiches. They contain harmful fats and sodium from deep-fried potatoes. Due to their high trans fat content, French fries can cause weight gain and cardiovascular illness [21]. The high glycemic index of potatoes causes blood sugar increases and insulin resistance [27]. Figure 1 shows fast food types and their impact on health.

### ***Overview of fast food consumption***

Fast-food consumption has become a pervasive aspect of modern diets worldwide, beginning from the mid-20th century and continuing to expand through 2024. Initially popularized in the United States by chains, such as McDonald's and Burger King in the 1950s, fast food quickly became entrenched in the diets of many people due to its convenience, affordability, and palatability. The trend gained momentum in the early 2000s, with significant growth in developing countries experiencing rapid urbanization and economic changes. For instance, the fast-food market in India was projected to be worth 27.57 billion dollars by 2020, highlighting its widespread popularity [28]. Recent studies show that fast food consumption remains high, with 36.5% of U.S. adults consuming fast food on any given day in 2017–2018, and over 55% of young adolescents in low- and middle-income countries (LMICs) consuming fast food at least once a week [29]. Younger individuals, particularly those under 30, and lower-income households are the most frequent consumers, driven by the affordability and accessibility of fast food. Fast food is characterized by high caloric content, unhealthy fats, sugars, and sodium, which contribute to various adverse health outcomes. Frequent





**Figure 1.** Common types of fast food and their associated health risks

consumption is associated with elevated calorie intake as well as a low-quality diet that contains higher amounts of total fat, saturated fat, sodium, and sugars, which are the essential factors in the onset of obesity and related metabolic disorders [30]. Research studies have continuously revealed that repetitive eating of fast foods can lead to a major increase in weight and insulin resistance. For instance, people who frequently eat fast food take up more kilograms and have more probability of developing insulin resistance as compared to those who do not consume it that often [31]. The level of obesity among people tends to correlate positively with the number of fast-food restaurant visits [32]. In addition, the consumption of fast food endangers cardiovascular health as the existence of trans fats and cholesterol are high leading to risks of increased heart disease and metabolic diseases. Frequent consumption of fried dishes, which is popular in fast foods, has been proven to be connected with bad cholesterol and higher chances of having heart diseases [33]. Fast food is really popular, but its unfavorable health consequences oblige people to continue making efforts to replace their unhealthy eating habits with healthier ones. These efforts may include introducing or extending a range of healthy food alternatives at fast-food restaurants and making nutritional information more understandable to people. However, the efficacy of these interventions is not all that clear. Notwithstanding the willingness of a significant percentage of the populace who claim to prefer healthy alternatives, the data from the questionnaire show that only a small number consult nutrition information [32]. As a result, tackling the increase in fast-food eating and related health issues is the most dominant public health problem, which asks for various measures promoting the production of healthier foods and minimizing the risk of obesity and metabolic diseases. Table 1 provides a detailed overview of global trends in fast food consumption.

### *Nutrient composition of fast foods*

The nutritional profile of fast food has been examined thoroughly, and it was found that fast food is capable of producing a lot of adverse health effects. This is the result of hypercaloric burgers, which contain unhealthy fats (including trans fats), high levels of sodium as well as sugars and low levels of nutrients such as fiber, vitamins, and minerals, which are vital for the body. Saunders and Middleton [41] studied fatty levels, along with the trans-fatty acids and salt, in popular fast foods that are taken out and consumed in the most deprived urban community in the UK. Most of these products were found to exceed the recommended daily allowance for at least one studied parameter, more than 30% and 27% of products exceeded the recommended levels for total fat or SFA and salt, respectively, which means their poor quality. A study conducted by K k  an and G k ay in Turkey [42] examined the nutritional characteristics of commercially available food products for infants and toddlers. The study revealed that a significant number of these products did not meet the recommended nutrition requirements, especially in terms of their sugar and sodium content. This underscores the necessity for more stringent rules to guarantee healthier food choices for children, which might also encompass fast food consumed by older demographics. Jindarattanaporn et al. [43] assessed the nutritional profile of popular menu items available through online food delivery applications in Bangkok, Thailand. The study found that most items were unhealthy, with high sodium and sugar content, suggesting that the convenience of fast food delivery may exacerbate poor dietary choices and related health issues. Bernstein et al. [44] compared the nutritional compositions of foods in the Canadian Community Health Survey to a representative database of branded food products. This comparison highlighted significant differences in the nutrient content, particularly concerning saturated fats and

**Table 1. Global trends and opportunities, demographic effects, and the changes in the fast food consumption patterns**

Category	Trends and opportunities	Demographic impacts	Changing patterns	References
<b>Age</b>				
Children [2–19 years]	Fast food consumption has increased.	Global fast food consumption among children is increasing, especially in urban areas.	The USA and Cebu consume more fast food, while China and Russia consume less.	[34]
Adolescents [12–17 years]	Weekly consumption is high.	Adolescents frequently consume fast food and carbonated drinks.	There is significant variability by region, with higher consumption in Latin America and high-income countries.	[35]
Young adults [18–29 years]	The fast food industry is growing.	Convenience and busy lifestyles drive high consumption among young adults.	Urbanization and increased disposable income are major factors.	[22]
Adults [30–50 years]	Increasing trends	Fast food consumption is increasing due to lifestyle changes and time constraints.	The preference for fast food varies by age and increases with urbanization.	[36]
<b>Gender</b>				
Male	Consumption frequency is higher.	Men consume fast food more frequently than women.	Fast food advertising and marketing have a greater influence.	[37]
Female	Increasing trends	Women are increasingly consuming fast food, driven by urban lifestyles and work commitments.	Consuming fast food has an impact on diet and nutrition.	[38]
<b>Types of fast food</b>				
Burgers and sandwiches	Popular globally	All age groups widely consume it, particularly in urban areas.	Consumers are driven by increased availability and convenience.	[20]
Fried chicken	High consumption	It is particularly popular in Asian countries and among young adults.	Brand influence (e. g., KFC, McDonald's) plays a significant role.	[39]
Pizza	Increasing popularity	It is consumed frequently among teens and young adults, especially in Western countries.	Fast delivery and online ordering increase consumption.	
Soft drinks	Commonly paired with fast food	Fast food intake rises with high consumption, particularly in adolescents.	Marketing and availability in fast food outlets drive consumption.	

fiber, reflecting the nutritional inadequacies of many fast food products. Marshellina et al. [45] conducted a study on medical students in Indonesia and discovered that the consumption of fast food was widespread. This was mostly due to the convenience and high stress levels experienced by the students. As a result, their protein intake was low while their fat consumption was high. This study emphasizes the necessity for improved nutritional instruction to alleviate

the adverse health effects of fast food. Lastly, the study by Rodríguez-Martín et al. [46] compared the nutrient profiles of plant-based and animal-based foods in Spain. They found that plant-based foods, while generally healthier, still posed certain nutritional challenges, such as lower protein content compared to their animal-based counterparts.

Table 2 summarizes research findings on the nutrient composition of fast foods.

**Table 2. Nutrient composition of fast foods**

Study title	Key findings	Year	Reference
Energy, sodium, sugar, and saturated fat content of New Zealand fast-food products and meal combos in 2020.	Fast food diet increased bile-tolerant microbial genera and decreased fiber-fermenting bacteria, altering gut microbiome and metabolites.	2020	[47]
Availability and nutrient composition of vegetarian items at US fast-food restaurants	Menu items that are low-calorie, vegetarian, and free of gluten typically have healthier nutrient profiles	2021	[48]
Evaluation of the nutritional quality of ultra-processed foods (ready to eat + fast food): Fatty acid composition.	Fast-food products in NZ provided more energy, saturated fat, sugars, and sodium than recommended.	2021	[49]
Nutrient intake and dietary quality among children and adolescents by fast food consumption status: What we eat in America, NHANES2013–2016.	Vegetarian fast-food items generally lower in calories, saturated fat, protein, and sodium, but higher in sugar and non-sugar carbohydrates than non-vegetarian items.	2021	[50]
Nutritional composition of breakfast in children and adolescents with and without celiac disease in Spain-Role of gluten-free commercial products	Ultra-processed foods, including fast food, were high in saturated and monounsaturated fats, with seafood being an exception.	2021	[51]
Nutrient profile of commercially packaged food products in Türkiye	Regular consumption of fast food among children and adolescents is linked to worse diet quality and increased consumption of harmful nutrients.	2020	[52]
Level of knowledge on the effect of fast foods on health among young hypertensive patients in Bangladesh	Gluten-free breakfast products often had less protein and saturated fat but more salt compared to gluten-containing counterparts.	2023	[53]
Fast food and its effects among teenagers in the Municipal of Cachoeiro De Itapemirim-Espirito Santo, Brazil	Snacks had the highest energy and saturated fat, while beverages had the lowest energy, fat, and protein. Confectionaries were high in carbohydrates and sugars.	2023	[54]

### *Health impacts of fast food consumption*

The use of fast food has been increasingly associated with a variety of negative health effects, especially metabolic problems. Studies offer a thorough examination of the diverse effects of fast food on health. A study conducted by Taniim et al. [53] reveals that young hypertensive individuals in Bangladesh possess an inadequate understanding of the adverse health consequences associated with fast food consumption. This underscores the necessity for health education initiatives aimed at enhancing awareness. Parvin et al. [55] demonstrated that there is a correlation between fast food intake and higher BMI among nursing and public health students in London. The study also reveals that females are more likely to be attracted to the flavor of fast food, while males are more inclined towards its convenience. Nyangoya and Attoni [54] found that fast food consumption among teenagers in Brazil is linked to obesity, depression, diabetes, heart disease, and reproductive health issues, highlighting the broad spectrum of health risks associated with fast food. Marshellina et al. [45] identified that medical students at Tanjungpura University have low protein intake and high-fat consumption due to frequent fast food intake, necessitating better nutritional education. Baskati and Pareek [56] discuss the shift towards high-calorie fast foods in India, which has contributed to rising obesity, coronary artery disease, and diabetes mellitus. Wijaya et al. [57] emphasize that junk food, including fast foods, significantly impacts body weight and is a key factor in the global obesity pandemic. Ramadani and Jannah [58] examined the relationship between fast food consumption and obesity among high school students in Indonesia, finding no significant relationship, but noting the need for further education on healthy eating. El-hasry et al. [59] assessed the perception of mothers regarding the effect of fast food on preschool children's health, finding poor knowledge and practices among mothers, highlighting the need for targeted health education programs. Pratheepkumar et al. [60] found a high prevalence of fast food consumption among university students, with significant associations between fast food intake and obesity, emphasizing the influence of peer pressure and convenience. AlTamimi et al. [61] noted that fast food intake is prevalent among middle-aged men in Saudi Arabia, with significant associations with nationality and obesity. Fitrianti et al. [62] identified key factors influencing fast food consumption among adolescents in Jakarta, including knowledge, body image, and promotional influences. Abrahamsson et al. [63] found that exposure to fast food restaurants during childhood and adolescence increases BMI and negatively impacts cognitive ability. Alanazi et al. [64] reviewed the impact of social media on fast food consumption, finding that social media significantly influences poor nutritional habits, particularly among children and adolescents. Pushkar et al. [65] found a high prevalence of fast food consumption among medical students, with significant associations between

consumption frequency and BMI. Kasmarini et al. [66] noted that frequent fast food consumption among adolescents is linked to poor sleep quality and higher rates of overweight and obesity. Lestari et al. [67] found a significant relationship between fast food consumption and obesity among adolescents in Kendari, Indonesia. Mendonca discusses how modern food habits, including increased fast food consumption, have led to a rise in health issues such as obesity, diabetes, and cardiovascular diseases [68]. Kagathara et al. [69] found that dietary practices, including frequent consumption of fast food, are associated with mental health issues such as stress, depression, and anxiety among medical students. Finally, Saragih et al. [70] showed that nutritional education using animation media can effectively reduce fast food consumption habits and obesity rates among adolescents. Figure 2 summarizes some diseases linked to fast food consumption.

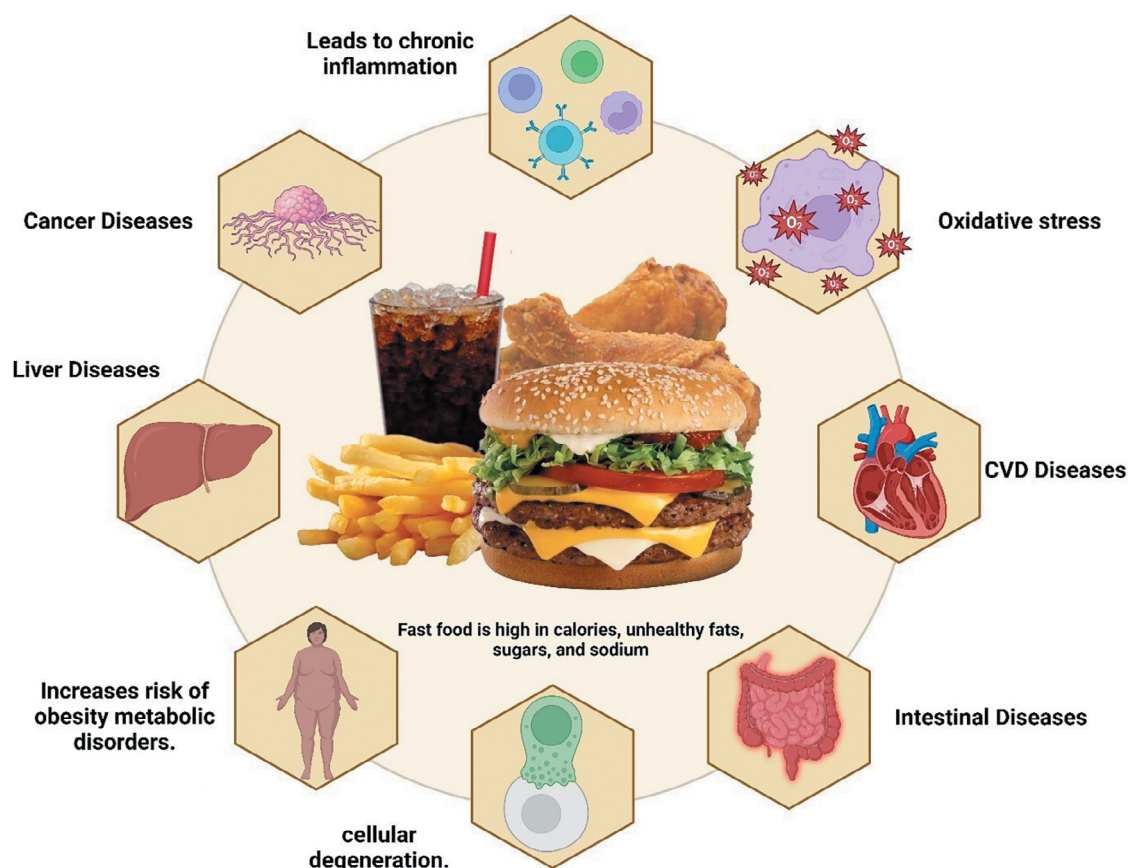
### *Obesity and weight gain*

There has been a link between the intake of fast foods and obesity, and many studies have pointed to the impact of fast food on weight gain and obesity. This section of the paper discusses how high intakes of fast food cause obesity and analyses the mechanisms through which this occurs and associated health effects. One of the many reasons why fast foods cause obesity is their trend toward high-calorie content. The average fast food meal, for example, hamburgers, fries, and drinks, among others, has more than 1200 calories, which is over 50% of what an ordinary adult requires in a day [69,70].

Such caloric excess easily translates into weight gain if consumed regularly [71]. Furthermore, fast food is typically high in unhealthy fats, particularly saturated and trans fats, and sugars. They are energy-dense nutrients that provide many calories in a small volume of food. Consumption of such fats and sugars contributes to weight gain not only by increasing calories but also by causing metabolic imbalances. For instance, consuming snacks that are high in trans-fatty acids increases abdominal fat, a risk factor for metabolic syndrome and cardiovascular diseases [22,72]. The composition of fast food may also result in low satiety for consumers. Fast food lacks dietary fiber and protein, which contribute to heightened satiety and reduced inter-meal food intake. This way, one will likely go beyond the required intake and eventually gain weight [73]. The combination of fat with a high content of sugar and salt in fast foods makes them highly palatable and, therefore, increases appetite, compelling people to overeat. Furthermore, due to accessibility and ease of use of fast foods, a significant number of people frequently opt for fast food in today's hectic lives. Studies have indicated that eating fast food two or more times each week significantly increases the risk of gaining a substantial amount of weight and becoming obese [14,74]. Furthermore, fast food companies' widespread marketing tools, such as advertising and promotion, encourage consumption, even among children and adolescents [75,76].



## Health Impacts of Fast-food Consumption



**Figure 2.** Health impacts of fast food consumption

Simply put, the trend of fast-food consumption correlates with the prevalence of obesity epidemics. Obesity is one of the leading risk factors for several chronic diseases, such as type 2 diabetes, cardiovascular diseases, and specific cancers. The presence of excess body fat, particularly abdominal body mass, has been associated with insulin resistance, dyslipidemia, and hypertension, each of which is related to the pathogenesis of the metabolic syndrome per se [77,78]. Obesity, therefore, poses a severe health threat leading to a reduction in quality and quantity of life expectancy [79]. The CARDIA study showed that frequent fast food consumption is associated with significant weight gain and increased insulin resistance over 15 years, increasing the risk of obesity and type 2 diabetes [51]. In addition, found that fast food consumption in teenagers was associated with a higher BMI and body fat percentage, leading to increased odds of obesity [71]. Moreover, an analysis of U.S. adults showed that fast food consumption results in a diet high in energy density and low in essential micronutrient density, contributing to overweight and obesity [72]. Fast food consumption among children leads to higher caloric intake, more total fat and added sugars, and poorer diet quality, which increases obesity risk [73]. Longitudinal research suggests that higher intake of fast food and skipping breakfast during youth are indicators of weight growth in adulthood, which contributes to obesity [83]. Table 3 summarizes some studies on the effects of fast food on obesity and weight gain.

### *Mechanisms of obesity and weight gain on aging*

Obesity and weight growth have a substantial impact on the aging process due to many biological factors. They lead to faster aging, higher rates of illness, and a shorter lifetime. Chronic inflammation is a significant pathway, in which adipose tissue in obese individuals releases pro-inflammatory cytokines such as TNF-alpha, IL-6, and CRP. This leads to systemic inflammation, which speeds up cellular aging and contributes to age-related illnesses such as cardiovascular disease, type 2 diabetes, and Alzheimer's disease [84]. Another important mechanism is oxidative stress. Increased adiposity causes an elevated production of reactive oxygen species (ROS) and a decrease in antioxidant defenses, leading to cellular damage and senescence [85]. Furthermore, obesity is closely linked to insulin resistance, causing metabolic dysfunction that elevates blood glucose levels and insulin production, ultimately leading to type 2 diabetes and its associated complications, which hasten aging [86]. Hormonal imbalances, such as leptin resistance, disrupt energy homeostasis and exacerbate weight gain, while also affecting reproductive health and accelerating aging [87]. Mitochondrial dysfunction, a hallmark of aging, is exacerbated by obesity, leading to decreased cellular energy production and increased ROS, further accelerating cellular aging [88]. Additionally, obesity accelerates telomere shortening, which limits cellular replication and longevity, contributing to premature aging and higher disease risk. Obesity also impairs immune

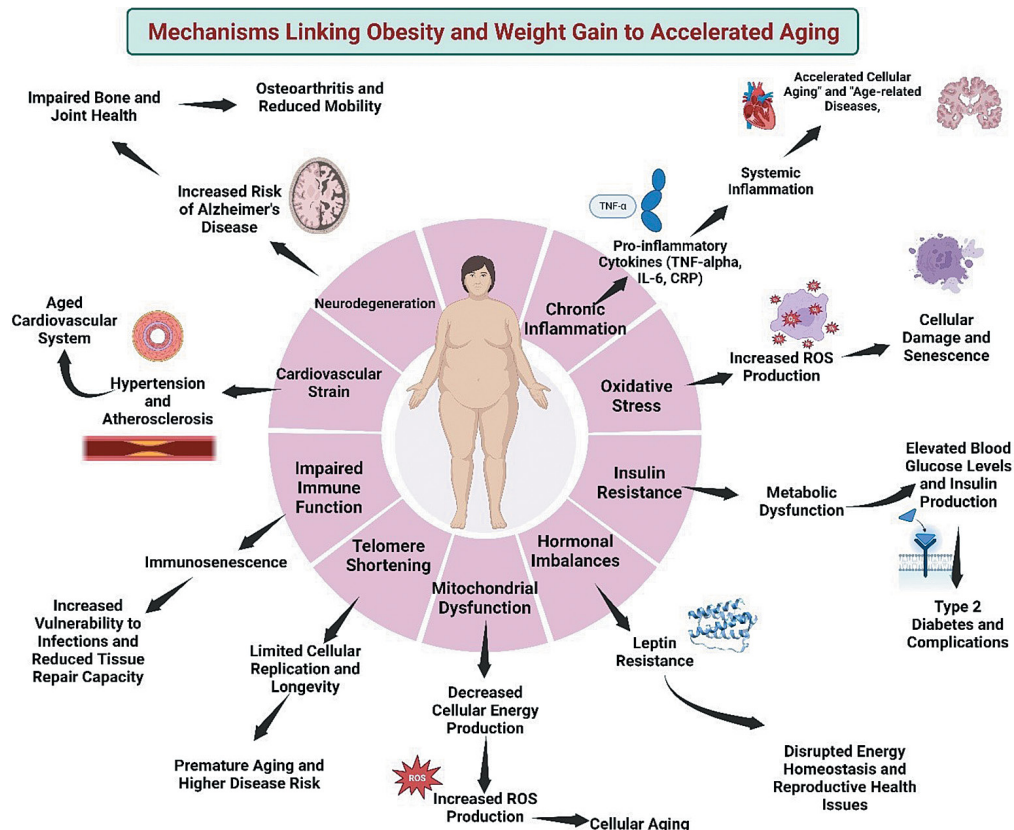
**Table 3.** Some studies on the effects of fast food on the obesity and weight gain

Key findings	Sample size	Human/ Animal models	Reference
Consuming fast food is a major factor in the development of weight gain and obesity	16 studies	Human	[74]
Regularly consuming fast food is linked to weight gain and heightened insulin resistance	3,031	Human	[31]
Fast food consumption in teenagers is linked to higher BMI and body fat percentage	Not specified	Human	[75]
Consuming fast food leads to a diet that is rich in energy but lacks important micronutrients	At least 1,000	Human	[72]
Fast food consumption in children leads to higher caloric intake and poorer diet quality.	6,212	Human	[76]
Heightened consumption of fast food and the habit of missing breakfast are indicative of weight increase from adolescent to adulthood.	9,919	Human	[77]
Aging reduces appetite and energy intake, impacting weight management	3,574 (older adults)	Human	[78]
Regular fast food consumption is linked to weight gain and adverse metabolic outcomes	3,643	Human	[79]
Fast food consumption in children is linked to increased caloric intake and poorer diet quality	National survey	Human	[80]
Periodic and intermittent fasting are helpful to health, since they combat age-related illnesses and obesity	Not specified	Animal (rats and mice)	[81]
The consumption of probiotic yogurt alongside a Western diet prevents age-related weight gain and alters the profiles of pro-inflammatory immune cells	Not specified	Animal (mice)	[82]
Obese mice showed increased body fat and weight gain compared to lean mice, indicating metabolic differences	Not specified	Animal (mice)	[83]

function, leading to immunosenescence, a diminished immune response that increases vulnerability to infections and reduces tissue repair capacity [89]. Cardiovascular strain from excess weight causes hypertension and atherosclerosis, further aging the cardiovascular system [90]. Lastly, the metabolic and inflammatory stresses induced by obesity can lead to neurodegeneration, increasing the risk of diseases such as Alzheimer's disease and impairing bone and joint health, leading to conditions such as osteoarthritis and reduced mobility, which are critical aspects of aging [91]. Figure 3 shows the mechanism of action on aging.

### Cardiovascular health

Regular consumption of fast food extends its effects on heart health. This section elaborates on how fast foods always have unhealthy fats, which therefore enhance the development of most heart diseases. To begin with, fast foods contain unhealthy fats such as trans fat and saturated fat. Saturated fats raise the level of low-density lipoprotein (LDL) in the blood, the so-called "bad" cholesterol. This is critical for the atherosclerosis process. Trans fats, which have a long shelf life in fast food items, are still more harmful. Not only do they raise serum LDL cholesterol, but they

**Figure 3.** Mechanisms linking obesity and weight gain with aging

also reduce the HDL cholesterol that removes LDL cholesterol from the blood [9]. Another extreme health concern is that the rate, at which the consumption of fast food increases the level of sodium in the body, is alarming. High sodium intake has been recorded as a substantial contributing factor to the increase in high blood pressure, which is the bedrock of most chronic illnesses, especially heart attacks and strokes [92]. In a simple meal, most fast foods exceed the limit on daily sodium consumption. For instance, fast food with a hamburger and fries can easily contain over 1,500 milligrams of sodium — close to the 2,300 milligrams per day recommendation from the American Heart Association [93,94]. Also, most fast food is high in sugar and refined carbohydrates, particularly beverages and desserts. Overconsumption of sugar leads to weight gain and obesity prevalence, both of which are associated with numerous cardiovascular disorders. Carbohydrates also cause the rapid elevation of blood glucose levels, leading to insulin resistance and type 2 diabetes — conditions closely related to cardiovascular health issues [26]. Furthermore, there are many studies showing how unhealthy fats and sugars in fast food raise levels of oxidative stress and inflammation, which are found in most cardiovascular diseases. Essentially, oxidative stress is a state that renders an individual susceptible to an imbalance between free radicals and antioxidant defense that leads to cellular and tissue damage. Inflammation is meant to be the body's defense against harmful stimuli, but it damages the arteries and, in that regard makes atherosclerosis worse once it becomes a chronic process [1]. It also leads to chronic blood lipid abnormalities among regular fast food consumers. High LDL cholesterol and triglycerides, along with a fall in HDL cholesterol, are expected consequences of fast-food consumption. These lipid abnormalities are significant contributors to the development of coronary artery disease and other cardiovascular diseases [1]. Several studies have found links between fast food consumption and adverse cardiovascular outcomes. For instance, revealed a significantly elevated risk for coronary heart disease in individuals who included fast foods in their daily meal plan. This study also found that the frequency of fast food consumption increased the risk [95]. High levels of CRP in the system indicate a high risk for cardiovascular events such as heart attack and stroke, independently of other risk factors [96,97]. In addition, a study found that regular fast food consumption led to significantly increased BMI, blood sugar levels, and lipid profiles, indicating higher cardiovascular disease risk [98]. A study conducted in Bangladesh showed that young individuals with hypertension who excessively consume fast food face an elevated susceptibility to obesity and cardiovascular complications. Implementing awareness programs is crucial to mitigate the consumption of fast food [53]. Ramadani and Jannah revealed a significant prevalence of fast food consumption among students, resulting in elevated obesity rates and possible cardiovascular hazards [58]. Vercammen et al. discovered

that there is a substantial increase in the likelihood of developing cardiovascular disorders when individuals experience food hardship. Individuals classified as adults with severe food insecurity had a greater likelihood of having a 10-year cardiovascular disease risk of at least 20%. This emphasizes the necessity for initiatives aimed at enhancing food security and diminishing reliance on fast food [99]. Bahadoran et al. [1] conducted a comprehensive analysis of the effects of fast food on cardiometabolic disorders, such as obesity, insulin resistance, and cardiovascular diseases. The study emphasizes that regularly consuming fast food is linked to higher calorie intake, lower diet quality, and an elevated risk of metabolic syndrome. Nadeem et al. [100] carried out a study to examine the correlation between the consumption of fast food and the occurrence of coronary heart disease in males residing in Peshawar. The results demonstrated that regular intake of fast food dramatically elevates the likelihood of acquiring coronary heart disease [100]. Duffey et al. [79] investigated the effects of consuming fast food on the quality of one's diet and metabolic outcomes. The results showed that a higher intake of fast food is linked to weight gain, insulin resistance, and dyslipidemia in young adults. Bowman et al. [76] studied the dietary habits of children and found that fast food consumption leads to higher caloric intake, increased fat and sugar consumption, and reduced dietary quality, contributing to obesity risk. Whitton et al. [101] revealed that a healthy dietary pattern is inversely associated with cardiovascular risk factors such as BMI, LDL cholesterol, and fasting triglycerides, highlighting the benefits of reducing fast food intake.

Fraser et al. [75] performed a spatial study that demonstrated a correlation between fast food intake and elevated BMI and body fat percentage in UK adolescents. This suggests a significant connection between the availability of fast food and obesity. Ferrara et al. [102] compared the impact of fast food versus slow food on hypertension control, showing that fast food significantly worsens blood pressure and metabolic profiles, while slow food, particularly Mediterranean diets, offers protective benefits. Basu et al. [103] highlighted that frequent fast food consumption is associated with lower nutrient adequacy, particularly in meeting Dietary Reference Intakes (DRIs) for essential nutrients, while increasing sodium and sugar intake. Bahadoran et al. [104] found that increased fast food consumption among Iranian adults is linked to higher intakes of unhealthy nutrients and poor cardiovascular health metrics, such as increased BMI and serum triglycerides. Odegaard et al. [105] demonstrated that frequent intake of Western-style fast food significantly increases the risk of type 2 diabetes and coronary heart disease mortality among Chinese Singaporeans. Sohoulou et al. [106] revealed that fast food consumption is linked to adverse lipid profiles and increased obesity rates among patients with diabetic nephropathy, exacerbating cardiovascular risk factors. Finally, Schmidt et al. [107] showed that fast food consump-



tion among black and white adolescent girls is associated with higher intake of calories, fat, and sodium, leading to poorer diet quality and increased cardiovascular risk. In conclusion, the effects of eating fast foods on the cardiovascular system are pretty pronounced and shocking. High amounts of fat clog the arteries. Moreover, fast food contains massive sodium and sugar levels and has relatively very low nutritional value. Public health interventions or individual lifestyle changes can assume dietary patterns to reduce the harm fast food may do to cardiovascular health.

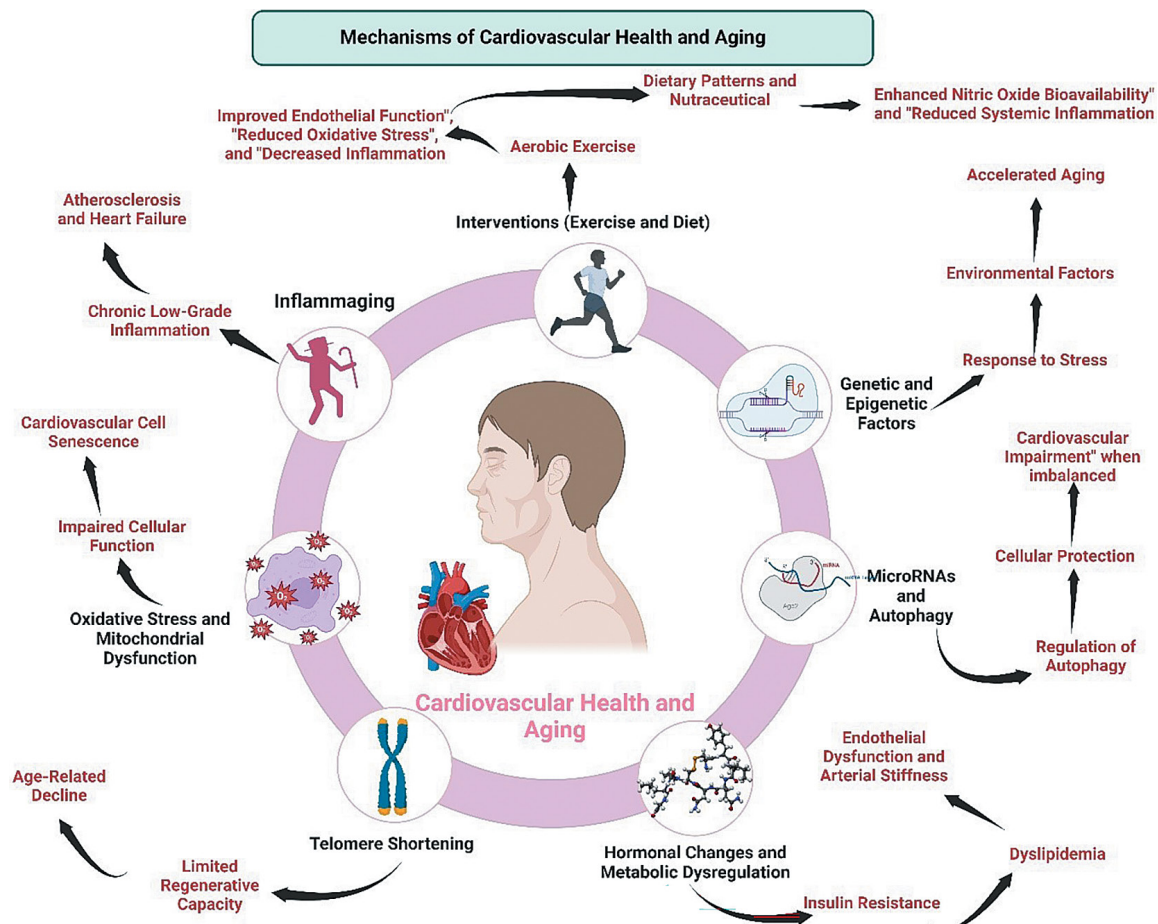
#### *Mechanisms linking cardiovascular health with aging*

Cardiovascular health plays a crucial role in the aging process, influencing the onset and progression of age-related diseases. The key aspect is the occurrence of inflammaging, in which there is a low-level systemic inflammatory state that speeds up vascular aging and contributes to cardiovascular diseases (CVD), such as atherosclerosis and heart failure [108]. Such an inflammatory process is mostly the result of oxidative stress and mitochondrial disarray, which is associated with the loss of cellular function and the appearance of senescence in cardiovascular cells. Telomere loss, a more common symptom of aging, will prevent cardiovascular tissues from being repaired and will thus worsen age-related conditions [109]. Furthermore, these also include hormonal changes and metabolic dysregulation, such as insulin resistance and dyslipidemia, that result in cardiovascular aging via the promotion of endothelial

dysfunction and arterial stiffness, respectively [110]. It is also important to examine the role microRNA is playing in the regulation of autophagy and other cellular protection. There are various control mechanisms and microRNA are among them being crucial for a cell to survive. The accurate regulation of this process is imperative when a person goes through the COVID-19 pandemic, otherwise, excessive cellular degradation and cardiovascular impairment can occur [111]. Furthermore, hereditary and epigenetic influences are the main factors that impact cardiovascular health in a significant manner, leading to the need to study how dietary interventions (for example, intermittent fasting) can affect the aging cardiovascular system [112]. Exercise and diet must be an integral part of person's efforts to keep a healthy heart and avoid heart diseases as they become older. Regular exercise improves endothelial function, reduces oxidative stress, and decreases inflammation, thereby mitigating age-related cardiovascular decline [113]. Similarly, certain dietary patterns and nutraceuticals can enhance cardiovascular health by targeting the fundamental mechanisms of aging, such as enhancing nitric oxide bioavailability and reducing systemic inflammation [114]. Figure 4 shows the mechanism of CVD and aging.

#### *Metabolic disorders*

A vast array of metabolic disorders, including clusters of conditions that increase the risk of heart disease, stroke, and type 2 diabetes, have closely linked fast food



**Figure 4.** Mechanisms of cardiovascular health and aging

consumption to their development [1]. This section focuses on how the average nutritional profile of fast food contributes to metabolic disorders, while also addressing broader health concerns. First, fast foods are high in refined carbohydrates and sugars, which bear a high glycemic load, i. e., they are foods with a high glycemic load. As a result, they can cause sharp, consecutive peaks in blood sugar, which then necessitate increased insulin production. This leads to cells becoming resistant to insulin, the hormone responsible for controlling blood sugar levels. This phenomenon is a classic characteristic of metabolic syndrome and type 2 diabetes [27,115]. For instance, studies have directly linked the development of insulin resistance to high consumption of sugar-sweetened beverages, a standard component of fast food meals. In addition to high caloric content and poor nutritional quality, fast food intake directly leads to weight gain and, subsequently, obesity — all crucial predictors of metabolic disorders. Obesity, especially abdominal obesity, assumes a central role in metabolic syndrome. This syndrome is characterized by a cluster of symptoms, which include elevated blood pressure, high blood sugar, excessive abdominal fat, and abnormal levels of cholesterol or triglycerides [116,117]. These conditions tend to occur together, thus raising the risk of heart disease, stroke, and diabetes. Fast foods, once again, contain high levels of unhealthy fats, such as saturated and trans fats, which negatively impact lipid profiles. Dyslipidemia is one of the most common outcomes associated with regular fast food consumption. It is characterized by high levels of LDL cholesterol and triglycerides, accompanied by significantly low HDL cholesterol. Such lipid abnormalities certainly contribute to the development of atherosclerosis, a condition that dramatically increases cardiovascular disease risk and is one of the metabolic syndrome components [9,118]. In addition, fast food causes chronic inflammation and oxidative stress, which contribute to metabolic disorders. Fast foods are usually rich in unhealthy fats and refined sugars, which can lead to obesity. A frequent consequence of obesity is metabolic syndrome associated with the pro-inflammatory states [119]. The synthesis of pro-inflammatory cytokines and reactive species of oxygen in the body is stimulated, thus causing cellular damage and developing chronic diseases. Chronic inflammation is a central player in insulin resistance and metabolic syndrome development in several cases. Furthermore, recent scientific studies have focused on diet and gut microbiota, a population of microorganisms living in the intestinal tract [120]. Fast food consumption can cause adverse change in gut microbiota, favoring the development of harmful bacteria while inhibiting good bacteria. This dysbiosis may, as a consequence of that, become an inflammatory, insulin-resistant and metabolic disorder [121]. It is important to mention that many studies have been focused on this close association of fast food consumption and metabolic disorders. Pereira et al. observed the presence of insulin resistance and type 2 diabetes for those who eat fast foods at least thrice or even

four times a week, which was the worst diet [13]. Fast food has been a major contributing factor to the development of inflammatory and oxidative stress markers, that eventually can lead to the development of metabolic syndrome [122]. The effect of fast food on metabolic diseases is not the only one but also the very sophisticated one. People must be serious in dealing with dietary problems that junk foods might cause. In particular, the intake of fast foods that are high in calories, and have unhealthy fats such as trans fats, refined carbohydrates, and sugars, constitute the major part of these problems. The fast food industry presents us with fast food as the primary cause of obesity, insulin resistance, dyslipidemia, inflammation, and gut microbiota imbalance, as well as a bad way of eating. Therefore, all these would consequently make metabolic syndrome and type 2 diabetes complicated ways. Even in the case of severe health complications, there should always be a rule on the public level of no junk food consumption, followed by alterations in dietary habits, to avoid these risks for good health. Furthermore, dietary patterns that incorporate high consumption of processed and fast foods have been linked to significant increases in abdominal adiposity and metabolic-associated fatty liver disease (MAFLD). Longitudinal studies have shown that higher average fast food intake over decades correlates with increased visceral adipose tissue and liver fat levels, indicating the long-term metabolic risks associated with fast food diets [123]. The relationship between fast food consumption and metabolic disorders is further complicated by its role in exacerbating inflammation and oxidative stress, key drivers of metabolic dysfunction. Fast food diets, rich in fats and sugars, contribute to chronic low-grade inflammation and oxidative stress, promoting insulin resistance and metabolic dysregulation [124]. One critical mechanism by which fast food exacerbates metabolic disorders is through its impact on gut microbiota and bile acid metabolism. The investigation has demonstrated that a single fast-food binge can change gut microbiota composition, which may lead to an increase in bile acids and lead to liver function and inflammation alterations [125]. This modification in the gut-liver axis is an original metabolic process keeping in touch fast food consumption with the birth of metabolic disorders.

#### *Mechanisms linking metabolic disorders with aging*

The aging process is significantly influenced by metabolic disorders through different pathways. One of the principal ways is the chronic inflammation called “inflammageing”, which is the case where the metabolic disorder is persistent low-grade inflammation. Chronic inflammation accelerates cellular aging and along the way, it is also a contributor to age-related diseases, such as cardiovascular disease and type 2 diabetes [126,127]. Besides this, mitochondrial disorder the problem with mitochondria, which are the powerhouses of the cells, is one of the prominent signs of old age and metabolism-related diseases. Throughout an individual’s lifespan, the efficiency of mitochondria drops,

which in turn, along with the higher production of reactive oxygen species (ROS), poses the risk of occurrence of oxidative stress later on, which in its sense can be extremely harmful to cell structures and lead to aging. Insulin resistance, a common feature of metabolic disorders, disrupts glucose homeostasis and exacerbates aging by impairing cellular metabolism and increasing the risk of developing age-related diseases [128]. Furthermore, metabolic slowdown, which involves a gradual reduction in metabolic rate and efficiency, contributes to the hallmark features of aging such as weight gain, basal inflammation, and insulin resistance [129]. Autophagy, the cellular process that removes damaged organelles and proteins, declines with age and in conditions of overnutrition, leading to the accumulation of cellular damage and further metabolic derangements [130]. Moreover, metabolic disorders, such as obesity and type 2 diabetes, induce changes in cellular energy metabolism, including decreased insulin sensitivity and altered mitochondrial function, which are significant contributors to aging [131]. The hypothalamus, which plays a central role in regulating metabolic physiology, also undergoes functional decline with age, further exacerbating metabolic dysregulation and accelerating the aging process [132]. The interplay between metabolic disorders and aging is also evident in the regulation of proteostasis, where metabolic imbalance affects protein homeostasis, leading to the accumulation of misfolded proteins and cellular stress [133]. Additionally, metabolic disorders exacerbate neurodegenerative diseases, with mechanisms such as insulin resistance and neuroinflammation playing critical roles [134].

### **Cancer risks**

The starved generation has been mentioned for a long time. If one is consuming a diet of unhealthy food for a long time and is not active, he/she is likely to get metabolic disease specifically diabetes, stroke, kidney disease, and heart disease. The greater the obesity, the higher the likelihood of developing the irremediable problem. This section discusses how the nutritional elements of fast food result in cancer and delves deeper into broader health concerns. First, fast foods are often highly processed and include meats such as bacon, sausage, and hot dogs, which fall into the category of Group 1 carcinogens, according to the International Agency for Research on Cancer [135]. Smoking, curing, salting, or adding chemical preservatives preserves these meats, but introduces carcinogenic compounds like nitrates and nitrites. These compounds, upon ingestion into the body, form N-nitroso compounds (NOCs), which are carcinogenic and known to cause DNA damage and cancer, such as colorectal cancer [136]. Many fast food items, especially fried ones, contain acrylamide, a chemical formed during the high-temperature cooking of starchy foods. The IARC has classified this chemical as a probable human carcinogen. Frequent consumption of deep-fried food items, like French fries and deep-fried

chicken, raises the intake of acrylamide [137], linked to a higher risk of cancers such as ovarian, endometrial, and renal cell cancer. Additionally, most fast food-related meals serve high-sugar drinks, which significantly contribute to the daily caloric intake and further promote obesity. Obesity is known to be an essential risk factor for many cancers, including those of the colorectum, endometrium, postmenopausal breast, kidney, and pancreas. Excess body fat capacitively increases levels of insulin and insulin-like growth factors, leading to increased cell proliferation and decreased apoptosis; it also produces and circulates estrogen. Furthermore, adipose tissue is linked to hormone-related cancers. Moreover, the high fat content in most fast foods, which is incredibly saturated with trans fats, is a worrying factor. Researchers have linked these types of fats to an increased risk of contracting cancer. For example, a high intake of saturated fats has been associated with breast cancer. Risk factors include maintaining a state of chronic inflammation, insulin resistance, and changes in cell membrane structures that can potentiate cancer cell differentiation and metastasis [117,138]. Fast foods are also generally low in essential nutrients and antioxidants that protect cells from oxidative damage. Antioxidants, primarily present in fruits, vegetables, and whole grains, neutralize free radicals that often cause DNA damage and increase the risk of cancer. A diet high in fast food and low in nutrient-dense foods might predispose one to cancer due to the insufficient levels of compounds with protective effects [118,139]. Therefore, the impact of fast food consumption on cancer risk is vast and multi-fold. Fast food consumption significantly increases the risk of various cancers due to its high content of processed meats, the presence of acrylamide in fried foods, the consumption of sugary beverages that lead to being overweight, and the high-fat content with low nutrient density. Thus, the public health intervention that would change these dietary habits and thus provide a balanced diet with high amounts of fruits such as vegetables and whole grains instead of fast food would be the best solution to lower the cancer risk. It certainly would. A study by Huybrechts et al. [140] found that ultra-processed foods (UPFs), including fast foods, were associated with an increased risk of breast cancer in young women in Latin America. Papadimitriou et al. [141] reviewed diet and cancer risk, finding strong evidence linking alcohol and red meat consumption to increased cancer risks, whereas fast food consumption was implicitly connected to these risk factors. Similarly, Jafari et al. [142] demonstrated a positive association between UPF consumption, including fast foods, and colorectal cancer risk in Tehran, Iran. Farvid et al. [143] identified processed meat, often found in fast foods, as a significant risk factor for various cancers, including colorectal, lung, and breast cancers. Wang et al. [144] found that high consumption of ultra-processed foods was associated with increased colorectal cancer risk, with significant gender differences in risk profiles. Bevel et al. [145] highlighted the association



between living in food swamps, characterized by high fast food availability, and elevated obesity-related cancer mortality rates in the US. Zhong et al. [146] found that deep-fried foods, common in fast food diets, were linked to a reduced risk of pancreatic cancer, though further research is needed to confirm these findings. Arya [147] studied the link between fast food consumption and anthropometric risk factors among college students, indirectly highlighting cancer risks through obesity-related mechanisms. Wang et al. [144] quantified the obesity-related cancer burden associated with UPF consumption, indicating significant contributions to new cancer cases. Khong et al. [148] found a correlation between high fasting blood glucose levels and increased cancer risk, implicating dietary habits including fast food consumption. Wu et al. [149] discussed the rising fast food consumption in Asia and its implications for obesity and cancer risk, calling for regulatory measures. Li et al. [150] compared fast food consumption across 54 low- and middle-income countries, highlighting the prevalence and associated health risks, including cancer. Bohlouli et al. [151] reviewed the impact of fast food consumption on COVID-19 severity and long-term complications, including increased cancer risks through chronic inflammation. Papier et al. [152] examined the association between meat consumption, common in fast foods, and various health conditions, highlighting increased cancer risks. Additionally, Kim et al. [153] investigated the link between fasting blood glucose levels and pancreatic cancer, underscoring the role of dietary habits in cancer risk. Brandhorst [154] discussed how dietary interventions, including fasting, can augment cancer treatment, indirectly implicating the role of fast food in cancer progression. Furthermore, Aveta et al. [155] reviewed the impact of meat intake on bladder cancer, emphasizing the carcinogenic potential of red and processed meats found in fast foods. Givens [156] summarized evidence linking dairy consumption to cancer risk, indirectly relating to fast food dietary patterns. Finally, Wijaya et al. [57] explored the impact of junk food, including fast foods, on body weight and associated cancer risks, emphasizing the role of dietary habits in health outcomes.

#### *Mechanisms linking cancer risk with aging*

Cancer risk increases significantly with aging due to various biological mechanisms that interlink aging and cancer development. One major mechanism is chronic inflammation, also known as "inflammageing," where persistent low-grade inflammation contributes to genomic instability and carcinogenesis. Aging-related changes in the immune system, such as immunosenescence, reduce the body's ability to detect and eliminate cancer cells, thereby increasing cancer risk [157]. Additionally, the accumulation of DNA damage over time, coupled with decreased DNA repair efficiency, leads to mutations that drive cancer progression [158]. Mitochondrial dysfunction, common in both aging and cancer, results in increased production of reactive oxygen species (ROS), which further

damages DNA and cellular components, promoting oncogenesis [159]. Epigenetic alterations, such as DNA methylation and histone modification, also play a crucial role in linking aging to cancer by modifying gene expression in a way that favors tumorigenesis [160]. Furthermore, cellular senescence, a state where cells stop dividing but do not die, is a double-edged sword in aging and cancer. While it initially acts as a barrier to cancer by halting the proliferation of damaged cells, the accumulation of senescent cells contributes to the pro-inflammatory environment and tissue dysfunction, creating a conducive environment for cancer development [161]. Metabolic reprogramming in aging, characterized by altered nutrient sensing and energy production, supports cancer cell survival and growth [162]. The declining efficiency of proteostasis, the process by which cells maintain protein balance, leads to the accumulation of misfolded proteins and cellular stress, which are implicated in both aging and cancer [163]. Besides this, cell damage caused by telomere shortening, a part of the natural aging process, hinders the cell's ability to divide and preserve tissue integrity, and it also causes the genomic instability that has the starting power for the development of cancer [164]. To summarize, the interaction between aging and cancer is considerably complicated and is rooted in chronic inflammation, the malfunctioning immune system, DNA damage, mitochondrial dysfunction, epigenetic changes, cellular senescence, metabolic reproduction, impaired proteostasis, and telomere shortening. These pathways collectively result in the augmentation of cancer susceptibility linked to old age.

#### *Liver disease*

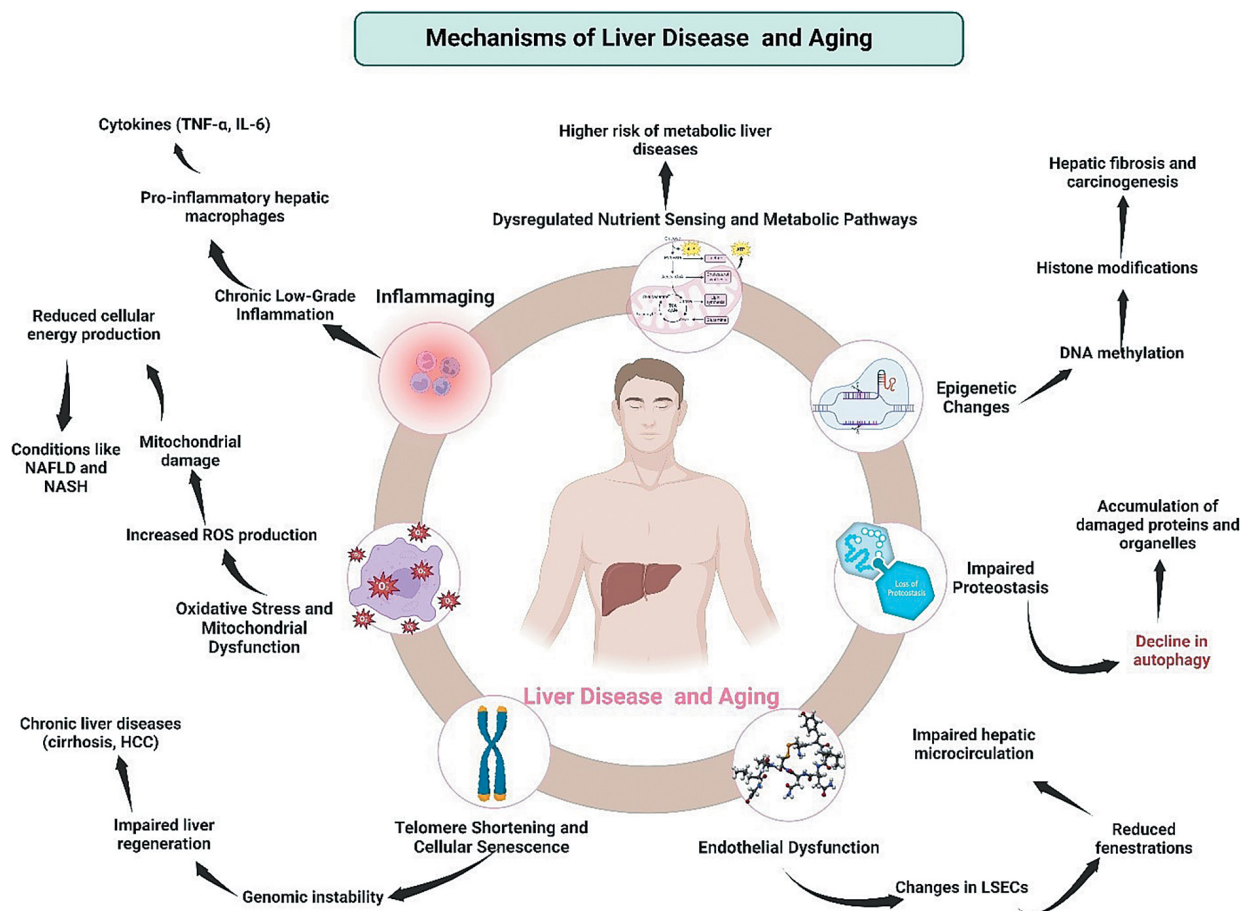
A lot of discussions have been going over recent months on the bad impact of fast food on people's health. The increasing prevalence of fast food meals has been associated with one of the primary concerns called non-alcoholic fatty liver disease (NAFLD). Studies have indicated that lower fat and lower calorie intake cause a significant decrease in liver disease. Moreover, the research indicates that the causes of the disease are the high caloric and unhealthy fat content typical of fast foods. The given collection of studies aims to supply a comprehensive overview of the vital link between fast food consumption and the development and progression of NAFLD. The relationship between fast food and liver disease, particularly non-alcoholic fatty liver disease (NAFLD), has been investigated extensively. Tavakoli et al. [165] discovered that NAFLD risk significantly grows in men who consume fast foods incessantly. Moreover, Mohammadi et al. [166] specified that the consumption of different kinds of fast foods, especially pizza, was the factor that made the risk of NAFLD higher, and pizza had the highest risk association. The study by Mohammadi et al. [166] eventually led to the conclusion that the intake of fast food in the patients with NAFLD was by far most pronounced among the reasons for the diseases as compared to the normal healthy individuals, hence, drawing

the researcher's pointed call for both the medical community and public to look into the future driver of the turn in liver health due to fast food intake. This conclusion is similar to that made by Marchesini et al. [167] who talked about the likely damage to the liver by fast food, namely the metabolic syndrome and liver dysfunction in the case of over-consumption of fast food. Furthermore, Mager et al. [168] discovered that consuming a meal rich in saturated fat resulted in an extended period of elevated levels of fat in the blood after eating, increased insulin levels, and changed expression of lipoproteins in obese children. These findings establish a connection between these factors and non-alcoholic fatty liver disease (NAFLD). Figge et al [125] showed that even a single fast food binge can induce significant metabolic changes and liver injury markers, suggesting a robust gut-liver axis response. Odegaard et al. [104] found a significant correlation between regular intake of fast food over a period of 25 years and the occurrence of NAFLD. This correlation remained significant even after accounting for other characteristics related to diet and lifestyle. In addition, Odegaard et al. [123] investigated the correlation between the consumption of fast food and the accumulation of fat in the liver. They found that higher levels of fat in the abdominal area and an increased likelihood of developing metabolic-related fatty liver disease (MAFLD) were associated with a larger intake of fast food. Khatatbeh et al. [169] observed that fast food consumption was associated with significant weight gain and altered liver enzyme levels among university students, suggesting potential long-term liver health implications. Takahashi et al. [170] highlighted a gender difference in the association between eating speed and NAFLD in type 2 diabetes patients, with fast eating significantly increasing NAFLD risk in men. Tamargo et al. [171] demonstrated that food insecurity, often leading to higher fast food intake, exacerbated the risk of NAFLD and liver fibrosis, particularly among low-income individuals. Charlton et al. [172] developed a mouse model demonstrating that a fast food-based diet induced progressive fibrosis and steatohepatitis, mirroring human NAFLD. Moreover, Bayol et al. [173] showed that a maternal junk food diet during pregnancy and lactation predisposed offspring to NAFLD, highlighting the long-term effects of early dietary exposure. Kalafati et al. [174] found that a fast food-type dietary pattern significantly increased the odds of NAFLD in a Greek population, with associated higher levels of C-reactive protein and uric acid. In the same vein, Uchiyama et al. [175] conducted a pilot study showing that a traditional Japanese dietary pattern inversely related to liver fat indices, suggesting that diet quality impacts liver health.; Delzenne et al. [176] showed that fasting followed by a high carbohydrate-fat-free diet in rats led to significant liver lipid accumulation and steatosis, offering insights into dietary impacts on liver health. Ouyang et al. [177] found that patients with NAFLD had significantly higher fructose consumption, with fructose metabolism contributing to liver fat accumulation. Finally,

Henney et al. [178] demonstrated a dose-response relationship between ultra-processed food intake and NAFLD, suggesting that public health measures to reduce such consumption are crucial.

#### *Mechanisms linking liver disease with aging*

Liver disease significantly impacts the aging process through a variety of complex mechanisms that exacerbate age-related physiological decline. Chronic inflammation, known as "inflammaging" plays a central role in aging and liver disease, where persistent low-grade inflammation accelerates liver dysfunction and fibrosis. This chronic inflammation is often driven by age-related changes in the immune system, particularly the accumulation of pro-inflammatory hepatic macrophages, which secrete cytokines such as TNF- $\alpha$  and IL-6, leading to tissue damage and impaired liver function [179]. Fuel molecules, such as glucose or fatty acids are burnt during the process of respiration in the cells to produce ATP. There are other important pathways to aging including the above mentioned ones, but the oxidative stress abbreviation comprising liver disease emerges on top. Age-related ROS rise is the primary cause of mitochondrial damage in the liver, which results in the imbalance of energy that eventually becomes NASH and NAFLD. Thus, the aforementioned process of living takes its toll and then we feel old, wondering how on earth it has happened [180]. In addition to this, liver mitochondria malfunction stops the liver from breaking down fats, something that already exists. Besides this, in elderly people or patients with NAFLD, the condition is significantly progressive, and the prognosis is even worse than in younger people [181]. Liver aging gets more help from telomere shortening and cellular senescence rather than anything else. Telomere shortening and senescence are the two main mechanisms that function in the aging of hepatocytes. As the hepatocytes get older, their telomeres are diminished, which causes a series of events resulting in genomic instability and cellular senescence that prevents liver regeneration and causes chronic liver diseases such as cirrhosis and hepatocellular carcinoma (HCC) [182]. Moreover, the sequestration of the cell implies that the inflammatory and fibrogenic substances are increasing that is making liver fibrosis and hepatic dysfunction worse [183]. What's more, aging -induced liver injury goes on a separate but important course and that has to do with deteriorating endothelial function of the liver. Changes over time in the liver sinusoidal endothelial cells (LSECs) such as a decrease of fenestration and an increase of oxidative stress are the main factors that affect hepatic microcirculation and, thus, cause liver fibrosis and inflammation [184]. Additionally, dysregulated nutrient sensing and altered metabolic pathways in the aging liver lead to a higher risk of metabolic liver diseases, further complicating the aging process [185]. Epigenetic changes and impaired proteostasis also play vital roles in liver aging. Age-related epigenetic modifications, such as DNA methylation and histone



**Figure 5.** Mechanisms linking liver disease and aging

modifications, alter gene expression patterns, promoting hepatic fibrosis and carcinogenesis [186]. Additionally, the decline in autophagy and proteostasis with age leads to the accumulation of damaged proteins and organelles in liver cells, exacerbating liver disease progression and impairing liver function [187]. Figure 5 shows mechanisms linking liver disease and aging.

### Conclusions

In conclusion, fast food consumption has profound effects on the aging process through multiple biological

mechanisms. Chronic inflammation, oxidative stress, and mitochondrial dysfunction are central to the accelerated aging associated with cardiovascular diseases, metabolic disorders, cancer risks, liver disease and obesity. Public health strategies focused on reducing fast food consumption and promoting healthier dietary choices are essential to mitigate these adverse effects and enhance the quality of life and longevity in aging populations. Comprehensive nutrition education and policy changes are imperative to shift dietary patterns and reduce the global burden of fast food-related health issues.

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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.



# GREEN PROCESSING TECHNOLOGY OF MEAT AND MEAT PRODUCTS: A REVIEW

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**Keywords:** *eco-friendly meat production, high-pressure processing, cold plasma technology, pulsed electric field, meat processing sustainability*

## Abstract

Green processing technologies are revolutionizing the meat industry by addressing the environmental and health challenges associated with traditional meat processing methods. This review explores several novel green technologies, including high-pressure processing (HPP), cold plasma, ultrasound, pulsed electric field (PEF) processing, and fermentation. These technologies offer significant improvements in terms of energy efficiency, waste reduction, and reduction of chemical additives. This review examines their operational principles, current research findings, and emerging applications. Additionally, the review highlights the integration of these technologies, their environmental impact, economic feasibility, and regulatory landscape. The findings suggest that while green technologies hold substantial promise for enhancing sustainability in meat processing, further research and industry adoption are necessary to fully realize their potential.

**For citation:** El-tahlawy, A. S. (2025). Green processing technology of meat and meat products: A review. *Theory and Practice of Meat Processing*, 10(1), 32–44. <https://doi.org/10.21323/2414-438X-2024-10-1-32-44>

## Acknowledgments:

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

## Introduction

Green processing technology refers to a suite of innovative and sustainable methods aimed at minimizing the environmental impact of food production, including meat processing [1]. These technologies are designed to reduce energy consumption, decrease waste, and limit the use of harmful chemical additives while maintaining or even enhancing the quality and safety of food products [2]. In the meat industry, which is often scrutinized for its environmental footprint and resource intensity, green processing technologies are becoming crucial as the sector seeks to align with broader sustainability goals [3].

Traditional meat processing methods such as curing, smoking, and the use of synthetic preservatives have long been the mainstay of the industry [4]. These techniques serve to ensure food safety, extend shelf life, and enhance the flavor of meat products [5]. However, they come with significant drawbacks. The reliance on chemical preservatives not only poses potential health risks to consumers, such as increased exposure to carcinogenic compounds and allergens, but also contributes to substantial environmental challenges [6]. Traditional methods often require high energy inputs, leading to increased greenhouse gas emissions and considerable waste generation [7]. These environmental impacts contribute to climate change and environmental degradation, underscoring the need for more sustainable alternatives.

The push towards green processing technologies is driven by the dual goals of mitigating environmental impact and responding to consumer demand for safer, healthier, and more sustainable food options [8]. Consumers are increasingly aware of and concerned about the environmental and health implications of their food choices, prompting a shift towards products and practices that align with sustainability and health-conscious principles [9]. In this context, green processing technologies offer promising solutions to the challenges faced by traditional meat processing methods [10].

Emerging green technologies such as high-pressure processing (HPP), cold plasma, ultrasound, and pulsed electric fields (PEF) are at the forefront of this shift [11]. High-pressure processing is known for its ability to extend the shelf life of meat products while preserving their nutritional quality and sensory attributes [12]. Cold plasma technology offers an innovative approach to decontaminating meat surfaces, reducing microbial load without the need for chemical agents [13]. Ultrasound technology enhances meat tenderness and marination, while pulsed electric fields improve microbial safety and reduce energy consumption [14,15]. Additionally, fermentation represents a significant advancement in green processing, leveraging natural microbial processes to improve food safety and extend shelf life without relying on synthetic additives [16].

The integration of these technologies into meat processing not only addresses environmental and health concerns but also aligns with evolving industry standards and consumer expectations [17]. By incorporating green processing technologies, the meat industry can reduce its ecological footprint, enhance product safety, and offer more sustainable options to consumers [18]. This shift is not merely a trend but a fundamental change driven by both regulatory pressures and market demands.

The aim of this review is to provide a comprehensive examination of these novel green technologies in meat processing. By evaluating the mechanisms, benefits, and limitations of each technology, this review seeks to highlight their potential to offer sustainable alternatives to traditional meat processing methods. The review will also explore the role of fermentation as an emerging technology, emphasizing its contributions to sustainability and health in the meat industry. Through this detailed analysis, the review aims to outline the current state of green meat processing technologies, assess their impact on food safety, nutritional quality, and environmental sustainability, and identify future directions for research and development in this evolving field.

### Objects and methods

This review aims to provide a comprehensive analysis of green processing technologies in the meat industry, specifically focusing on HPP, cold plasma, ultrasound, PEF, and fermentation. The goal is to evaluate an impact of these technologies on sustainability, energy efficiency, waste reduction, and their effectiveness in enhancing meat quality and safety. A systematic literature review was conducted using academic databases such as PubMed, ScienceDirect, and Google Scholar to identify relevant research articles, reviews, and case studies published within the past 14 years. Data were collected on operational principles, applications, energy efficiency, environmental impact, economic feasibility, and regulatory considerations of each technology. Subsequently, a comparative analysis was performed, examining the environmental benefits, cost-effectiveness, and meat quality and safety outcomes associated with each technology.

Inclusion criteria were as follows:

1. Published literature: peer-reviewed articles, conference proceedings, and reviews published within the last 14 years.
2. Relevance to green processing: studies directly discussing HPP, cold plasma, ultrasound, PEF processing, and fermentation in the context of meat processing.
3. Sustainability and environmental impact: research articles evaluating environmental, economic, and health impacts of these technologies.
4. Language: studies published in English.

Exclusion criteria were as follows:

1. Non-relevant processing methods: studies focused on conventional processing methods without integrating green technologies.

2. Irrelevant product types: studies not focused on meat and meat products (e. g., studies on dairy or plant-based products).
3. Insufficient data on sustainability: studies lacking substantial discussion on sustainability metrics or environmental impact.
4. Publications that are purely theoretical or do not include experimental data with practical application

Data sources and geographic information:

The data for this review were primarily sourced from peer-reviewed journals, industry reports, and case studies. Governmental and non-governmental reports on food processing technologies and sustainability, published by organizations such as the FAO, WHO, and Codex Alimentarius Commission, were also included to provide broader insights and regulatory context.

Geographically, the review encompasses studies and data from multiple regions, with a primary focus on research conducted in North America, Europe, and Asia, where green processing technologies have seen significant development and application. Studies from the United States, Canada, South Korea, and China were particularly emphasized due to their advancements in food technology and regulatory frameworks promoting sustainable practices. Where available, comparative data from emerging economies in South America and Africa were also examined to provide a global perspective on feasibility, application, and challenges of green processing technologies.

Research subjects and analysis techniques:

The reviewed studies cover various meat types, including poultry, beef, and fish, treated with green processing technologies. Key parameters include consumer safety, meat quality, and sustainability metrics. The analysis techniques encompass several domains, including environmental impact assessment, which uses methods such as Life Cycle Assessment (LCA) to evaluate energy consumption, emissions, and waste reduction; quality assessment through texture analysis, microbial analysis, and chemical profiling to determine meat quality and safety post-treatment; economic feasibility analysis, involving cost-benefit assessments and case studies of green technology implementation in industrial contexts; and regulatory analysis, evaluating the current regulatory frameworks surrounding these technologies based on guidelines from organizations such as the FDA, EFSA, and Codex Alimentarius Commission. This approach aims to determine the efficacy and potential for industry adoption of each technology.

### Overview of traditional meat processing techniques

Traditional meat processing techniques, such as curing, smoking, and the use of chemical preservatives, have long been employed to extend the shelf life of meat products, enhance flavor, and ensure food safety [19,20]. These methods have been effective in preventing spoilage and controlling pathogenic microorganisms, but they come with significant environmental and public health concerns [21,22].

Curing involves treating meat with a combination of salt, nitrates, nitrites, sugar, and spices to preserve it [23]. This process inhibits the growth of spoilage bacteria and pathogens, such as *Clostridium botulinum*, by reducing water activity and introducing chemical preservatives [24]. However, curing relies heavily on nitrates and nitrites, which can react with amines in meat to form nitrosamines — compounds that have been linked to an increased risk of cancer [25]. Additionally, the production and transportation of these curing agents contribute to environmental degradation, including water pollution and greenhouse gas emissions [26].

Smoking is another traditional method that involves exposing meat to smoke from burning wood or other plant materials [27,28]. The smoke imparts flavor, reduces water activity, and introduces antimicrobial compounds, making it an effective preservation technique [29]. However, smoking meat releases polycyclic aromatic hydrocarbons (PAHs), which are known to be carcinogenic [30]. Furthermore, the energy required for smoking, along with the consumption of wood, contributes to deforestation and air pollution, raising concerns about the sustainability of this practice [31].

The use of chemical preservatives, such as sodium benzoate, potassium sorbate, and sulfur dioxide, is common in the meat industry to inhibit microbial growth and prolong shelf life [32]. While these chemicals are effective, they can pose health risks, including allergic reactions and potential toxicity with long-term exposure [33]. There is also a growing consumer demand for clean-label products with fewer synthetic additives, challenging the meat industry to find safer and more natural alternatives.

Despite their effectiveness, traditional meat processing methods have several limitations, particularly concerning environmental sustainability [34]. Many of these methods rely on non-renewable resources and produce substantial waste, contributing to pollution and climate change [35]. For example, the use of chemical preservatives involves the production of synthetic chemicals, which generates harmful by-products and waste [36]. Smoking processes, requiring large amounts of wood and emitting significant amounts of smoke, lead to deforestation and increased carbon emissions, further exacerbating environmental damage [37].

Another major limitation is the high energy consumption associated with traditional processing techniques. Smoking meat requires constant heat, which consumes a significant amount of energy [38]. Curing processes often need refrigeration over extended periods, increasing energy demands [39]. This high energy usage not only elevates operational costs but also contributes to the overall carbon footprint of the meat industry [40], making it less sustainable in a world increasingly focused on reducing energy consumption and greenhouse gas emissions [41].

Therefore, while traditional meat processing methods have been essential for ensuring the safety and longevity of meat products, their environmental impact, high energy

consumption, and associated health risks underscore the need for more sustainable and health-conscious alternatives. The meat industry must explore and adopt novel green processing technologies to reduce its ecological footprint and meet evolving consumer demands.

### **Green processing technologies: a novel approach**

Green processing technologies in meat production refer to innovative methods that aim to minimize the environmental impact of processing while maintaining or enhancing the safety, quality, and nutritional value of meat products [42]. These technologies focus on reducing energy consumption, minimizing waste, and avoiding harmful chemical additives [43]. The goal of green processing is to create a more sustainable meat production system that aligns with the growing demand for environmentally friendly and health-conscious food products [44].

Energy efficiency is a core principle of green processing technologies [45]. Unlike traditional methods, which often require significant amounts of energy for processes such as heating, cooling, and drying, green technologies aim to use less energy through advanced methods and equipment [46]. For example, technologies such as HPP and PEF can achieve microbial inactivation and extend shelf life without the need for high temperatures, thus saving energy [47,48]. This reduction in energy use not only lowers the carbon footprint of meat production but also reduces operational costs, making it a more sustainable and economically viable option for the meat industry [49].

Waste minimization is another fundamental principle of green processing technologies [50]. Traditional meat processing often generates substantial waste, including organic by-products and packaging materials that contribute to environmental pollution [51]. Green processing technologies seek to minimize this waste through techniques that optimize resource use and reduce by-products [52]. For instance, membrane filtration technologies can recover valuable proteins and other components from processing wastewater, turning what was once waste into useful ingredients [53]. Additionally, the use of biodegradable or recyclable packaging materials further reduces the environmental impact of meat production, aligning with circular economy principles [54].

Reduction of chemical additives is a key objective in the application of green processing technologies [55]. Conventional methods often rely on chemical preservatives and additives to ensure product safety and extend shelf life, which can pose health risks to consumers and contribute to environmental pollution [56]. Green technologies aim to replace these synthetic chemicals with natural alternatives or physical processes that achieve the same goals without the associated risks [57]. For example, cold plasma treatment and ultraviolet (UV) light are non-thermal methods that can effectively inactivate pathogens on meat surfaces without the need for chemical additives [58]. By reducing reliance on chemicals, these technologies not only enhance



food safety but also meet consumer demand for "clean-label" products with fewer artificial ingredients.

Finally, green processing technologies offer a novel approach to meat production by prioritizing energy efficiency, waste minimization, and the reduction of chemical additives. These principles help create a more sustainable, health-conscious, and economically viable meat industry that is better aligned with environmental goals and consumer expectations.

### Emerging green processing technologies

Emerging green processing technologies are revolutionizing the meat industry by providing sustainable alternatives to traditional methods [59]. These technologies not only enhance food safety and quality but also reduce environmental impact and minimize the use of chemical additives [60]. Below is an overview of some of the most promising green processing technologies currently being explored in meat production (Table 1).

HPP is a non-thermal preservation method that inactivates microorganisms by applying extremely high pressure (up to 600 MPa) to meat products [61]. This process disrupts microbial cell membranes and proteins, effectively eliminating pathogens and spoilage organisms without the need for heat [62]. HPP is used for a variety of applications, including extending shelf life, maintaining fresh-like quality, and enhancing safety in ready-to-eat meat products [63].

One of the main benefits of HPP is its ability to retain nutrients, flavors, and sensory attributes of meat because it does not involve high temperatures, which can degrade heat-sensitive compounds [64]. Additionally, HPP reduces the need for chemical preservatives, aligning with consumer demand for cleaner labels and more natural products [65]. Recent research has focused on optimizing pressure levels and treatment times to maximize microbial inactivation while preserving the quality of meat [66,67]. Innovations include the development of HPP-compatible packaging materials and the integration of HPP with other mild preservation methods to further enhance safety and quality [68].

Cold plasma technology is an emerging non-thermal method that generates reactive gas species at low temperatures to decontaminate meat surfaces [69]. This technology works by exposing meat to ionized gas, which produces reactive oxygen and nitrogen species capable of inactivating a broad spectrum of microorganisms, including bacteria, viruses, and molds [70]. Cold plasma is particularly effective in reducing surface contamination without affecting the core temperature or quality of meat [71].

Compared to conventional decontamination methods, such as chemical washes or heat treatments, cold plasma offers several advantages. It requires no water or chemical additives, thus minimizing waste and avoiding chemical residues on meat products [72]. Moreover, the process is energy-efficient and can be applied in real-time during

meat processing, reducing the need for additional handling or storage [73]. Recent advancements in cold plasma technology have focused on developing scalable systems for commercial meat processing and optimizing the plasma parameters for different types of meat products [74].

Ultrasound technology uses high-frequency sound waves to create cavitation bubbles in liquid environments, which implode and generate localized high temperatures and pressures [75]. In meat processing, ultrasound is used to tenderize meat by breaking down muscle fibers and connective tissues and enhancing marination by increasing the penetration of marinades into meat [76]. This technology can significantly improve the texture and flavor of meat products without the need for extended marination times or mechanical tenderization [77].

Ultrasound technology also offers environmental benefits, such as reduced water and energy usage compared to traditional methods [78]. It can be integrated into existing processing lines with minimal modifications, making it a cost-effective option for meat processors [79]. Recent advancements in ultrasound technology include the development of low-frequency systems that minimize heat generation while maximizing the tenderizing effects, as well as combined ultrasound treatments with other non-thermal technologies to further enhance meat quality and safety [80,81].

PEF processing involves the application of short bursts of high-voltage electric fields to meat products, which disrupts cell membranes and inactivates microorganisms [82]. PEF is primarily used to enhance microbial safety in meat by effectively reducing the load of pathogens such as *Escherichia coli* (*E. coli*), *Listeria monocytogenes* (*L. monocytogenes*), and *Salmonella* species [83]. The technology can also improve the extraction of intracellular compounds, such as proteins and flavors, contributing to the enhancement of meat quality [84].

PEF processing has minimal effects on meat quality, as it operates at low temperatures, preserving the sensory and nutritional attributes of the product [85]. Additionally, PEF is energy-efficient, as it requires less energy compared to thermal pasteurization methods [86]. Current research is exploring the synergistic effects of PEF when combined with other preservation technologies, such as HPP and cold plasma, to enhance microbial inactivation while maintaining product quality [87,88].

Fermentation and bio-preservation utilize natural fermentative microbes and bio-preservatives, such as bacteriocins, to extend the shelf life and improve the safety of meat products [89]. Fermentative microbes, such as *Lactobacillus* species, are used to ferment meat, producing organic acids and antimicrobial peptides that inhibit the growth of spoilage and pathogenic microorganisms [90]. Bio-preservatives, such as nisin and pediocin, are naturally occurring antimicrobial peptides produced by certain bacteria that can be added to meat products to control microbial growth [91]. Innovations in this area include the

development of specialized starter cultures that are tailored to specific meat products, improving flavor, texture, and safety while reducing the need for chemical preservatives [92]. Research is also focused on the production of novel bacteriocins with broader antimicrobial spectra and enhanced stability under various processing conditions [93].

Irradiation and UV processing are technologies that use ionizing radiation and ultraviolet light, respectively, to inactivate pathogens and extend the shelf life of meat products [94]. Irradiation exposes meat to gamma rays, X-rays, or electron beams, which disrupt the DNA of microorganisms, effectively reducing the microbial load [95]. UV processing involves the use of UV-C light to penetrate the surface of meat and kill bacteria and viruses [96].

These technologies offer significant potential for pathogen control and shelf-life extension without the use of chemical additives or high temperatures. However, consumer perception and regulatory challenges remain obstacles to widespread adoption. Consumers often associate irradiation with negative connotations, such as "radiation" and "radioactivity," despite extensive evidence demonstrating its safety and efficacy [97,98]. Regulatory bodies in different countries have varying standards and approval processes for irradiation and UV treatments, further complicating their implementation in the global meat industry [99,100]. Ongoing research aims to improve effectiveness of these technologies while addressing consumer concerns through education and transparent communication about the benefits and safety of these methods.

**Table 1. Comparison of green processing technologies: energy consumption, waste reduction, cost, and microbial inactivation**

Technology	Energy consumption	Waste reduction	Cost	Microbial inactivation
HPP	Low	High	High	Very effective
PEF	Moderate	Moderate	Moderate	Effective
Cold plasma	Low	Moderate	High	Effective
Ultrasound	Moderate	Low	Moderate	Moderate

### Integration of novel green technologies in meat processing

The integration of novel green technologies in meat processing involves combining multiple methods to maximize their individual benefits and achieve superior product quality, safety, and sustainability (Table 2). Combination approaches leverage the synergistic effects of different green technologies to enhance microbial inactivation, preserve sensory and nutritional qualities, and reduce environmental impact [101]. For example, combining HPP with cold plasma can provide a dual mechanism of microbial inactivation, where HPP targets the internal pathogens while cold plasma efficiently decontaminates the meat surface [102]. This combination not only extends the shelf life of meat products but also minimizes the need for chemical preservatives and reduces energy consumption by lowering the required pressure levels and treatment times [103].

Another effective combination approach is using PEF processing with ultrasound technology [104]. While PEF disrupts microbial cell membranes to ensure food safety, ultrasound aids in tenderizing meat and enhancing marinade absorption, thereby improving texture and flavor [105]. This combined approach can significantly reduce the processing time and energy consumption compared to conventional methods, such as prolonged marination and heat treatments. By integrating these technologies, meat processors can achieve a more efficient and sustainable production process, meeting both industry standards and consumer demands for high-quality, minimally processed products.

### Environmental impact and sustainability

Green processing technologies offer a more sustainable alternative to traditional meat processing methods by significantly reducing their environmental impact [113]. Traditional meat processing methods, such as curing, smoking, and chemical preservation, often rely heavily on energy-intensive processes, high water usage, and the application of synthetic chemicals. These methods contribute to higher greenhouse gas emissions, increased water pollution from chemical runoff, and excessive energy consumption [114]. In contrast, green processing technologies, such as HPP, PEF, and cold plasma technology, are designed to minimize energy usage, reduce waste, and lower chemical inputs [11]. For example, PEF and ultrasound technologies require less energy compared to conventional thermal processing methods, as they operate at lower temperatures and reduce processing times [115]. Similarly, HPP and cold plasma do not produce harmful emissions or chemical residues, thereby reducing the environmental footprint of meat processing [102]. Overall, green technologies provide a cleaner, more efficient alternative that aligns with global sustainability goals and consumer demand for environmentally friendly products.

Recent LCAs of green processing technologies in the meat processing industry highlight their environmental advantages across several impact categories, including carbon footprint, water usage, and energy consumption [116]. Studies have shown that technologies such as HPP and PEF have a significantly lower carbon footprint compared to traditional heat treatments. For instance, an LCA study comparing HPP to conventional thermal pasteurization found that HPP resulted in a 30–40% reduction in greenhouse gas emissions due to lower energy consumption and the elimination of heat production [117]. Additionally, an assessment of cold plasma technology demonstrated its potential to reduce water usage by up to 50% compared to chemical-based decontamination methods, as it requires no water or chemical solvents [118]. These findings suggest that green processing technologies can substantially reduce the environmental impact of meat production throughout the entire product life cycle, from raw material processing to waste management.

Table 2. Case study comparison of green processing technologies: impacts on microbial reduction, shelf life extension, and quality attributes of meat and meat products

Processing method	Meat product	Microbial reduction	Shelf life extension	Impact on quality attributes	Specific microorganisms affected	Reference
HPP	Bratwurst sausages	Total microbial count reduced by up to 4.2 log CFU/g; LAB reduced from 2.4 to 1 log CFU/g	5–8 times longer than untreated	No alteration in color and texture of treated samples.	Significant reduction of <i>Pseudomonas</i> spp., <i>Enterobacteriaceae</i> , yeasts and molds, <i>Staphylococcus</i> spp., <i>Brochothrix thermosphacta</i> (below detection limits throughout storage period).	[106]
Cold plasma + modified atmosphere packaging	Meatballs	Initial microbial counts decreased by 1.02 to 1.19 log CFU/g with 6–9 min Ar-based treatment	Extended by 14 days	Slight increase in lipid oxidation values (Ar-6 min: 0.93 mg/kg; Ar-9 min: 0.92 mg/kg) compared to control (0.83 mg/kg); no significant change in TVB-N.	Reduction in relative abundance of unclassified- <i>Enterobacteriaceae</i> (19.04% to 12.54%) and <i>Acinetobacter</i> (30.88% to 2.25%) in the Ar-6 min group.	[107]
Cold plasma using argon, helium, nitrogen	Various meat surfaces	Decreased psychrotrophic and total bacteria by 2–3 log CFU/cm <sup>2</sup> (argon and helium, respectively)	—	No significant interaction with nitrogen for psychrotrophic bacteria; reduction in yeasts and molds by 1 log CFU/cm <sup>2</sup> after 10 min.	Decrease in psychrotrophic bacteria and total bacteria with argon and helium treatment; nitrogen treatment primarily affected yeasts and molds.	[108]
High-intensity ultrasound (HIU)	Semitendinosus beef muscle	Decreased counts of mesophilic, psychrophilic bacteria, <i>Staphylococcus</i> spp., and coliform bacteria	Extended during storage at 4°C	HIU decreased pH and color difference ( $\Delta E$ from 5.99 to 1.43); drip loss decreased but was similar to control at the end of 9 days; no difference in water-holding capacity and shear force compared to controls.	Reduction in mesophilic, psychrophilic bacteria, <i>Staphylococcus</i> spp., and coliform bacteria.	[109]
Moderate intensity pulsed electric field (MIPEF)	Chicken breast meat	Total mesophilic aerobic bacteria (TMAB) count exceeded 2 days later in 4.67 and 7 kV/cm groups; Almost 2 log reduction in total coliform bacteria.	Extended by 2 days compared to control.	pH, CIE L*, b*, C* color values unaffected; $\Delta E$ values showed maximum change in the control group	<i>Pseudomonas aeruginosa</i> count remained unchanged; <i>E. coli</i> and <i>C. jejuni</i> showed resistance at 4.67 kV/cm and 7 kV/cm, respectively; <i>L. monocytogenes</i> growth promoted by 4.67 kV/cm.	[110]
Bio-preservation by LAB	Sliced fresh beef	Reduction in <i>Enterobacteriaceae</i> , <i>Staphylococcus</i> , coliforms, <i>L. monocytogenes</i> and <i>Salmonella</i> Typhimurium	—	—	<i>Salmonella</i> Typhimurium and <i>L. monocytogenes</i>	[111]
Gamma radiation	Fish meat	Aerobic Plate Count (APC) reduced by 100% at 5 KGy; pathogenic bacteria counts were reduced dose-dependently	Significantly extended, especially at higher doses	No significant effect on proximate composition; carbohydrates, proteins, and lipids were not significantly affected by low and medium doses of radiation	High prevalence of <i>Staphylococcus aureus</i> among untreated samples; pathogenic bacteria completely eradicated at 5 KGy.	[112]

Green processing technologies also offer innovative solutions for waste management and by-product utilization in the meat processing industry [119]. Traditional methods often generate significant amounts of organic waste, including meat scraps, fat trimmings, and bones, which are typically discarded or used for low-value applications [120]. In contrast, green technologies facilitate the conversion of these by-products into valuable resources [121]. For example, technologies such as fermentation and bio-preservation can utilize meat scraps and trimmings to produce bioenergy, bioplastics, or high-value protein hydrolysates for use in animal feed or nutritional supplements [122]. Moreover, processes such as cold plasma and ultrasound can enhance the recovery of collagen and gelatin from bone and connective tissue, contributing to the production of functional ingredients for the food and pharmaceutical industries [123]. By effectively managing waste and utilizing by-products, green technologies not only reduce the environmental impact of meat processing but also create additional revenue streams and promote a circular economy within the industry.

### **Economic feasibility and market potential**

The economic feasibility of adopting green processing technologies at a commercial scale depends on several factors, including initial investment costs, operational expenses, and potential savings [124]. While the upfront costs for equipment such as HPP machines, PEF systems, and cold plasma generators can be substantial, these investments can lead to significant long-term savings [125]. Green technologies typically consume less energy and reduce water and chemical usage compared to traditional processing methods, leading to lower operational costs over time [126]. For instance, HPP systems, despite their high initial cost, can lower energy costs due to reduced processing times and the elimination of the need for high-temperature treatments [127]. Furthermore, the reduction in spoilage and waste, along with extended shelf life of products, can decrease overall production costs and increase profitability [128]. Additionally, government incentives and subsidies aimed at promoting sustainable practices can help offset the initial costs of adopting these green technologies. Companies that invest in these technologies may also gain a competitive advantage by differentiating their products in the marketplace as sustainable and environmentally friendly, potentially capturing a larger share of the growing market for green and clean-label products.

Market trends indicate a growing consumer demand for sustainably processed meat products, driven by increasing awareness of environmental issues and health concerns associated with traditional meat processing methods [44]. Consumers are becoming more conscious of the environmental impact of their food choices and are willing to pay a premium for products that are marketed as green, natural, or free from synthetic additives [129]. Surveys and market analyses show that there is a strong consumer preference

for meat products processed with novel green technologies that retain natural flavors and nutrients without compromising food safety [130]. However, consumer acceptance of these products is influenced by several factors, including education about the benefits of green technologies, transparency in labeling, and trust in the safety and quality of the final products [34]. Effective communication and marketing strategies are essential to educate consumers about the advantages of green processing technologies and dispel any misconceptions regarding the safety and efficacy of these methods [131]. Additionally, as the market for green-processed meat products continues to expand, retailers and food service providers are increasingly incorporating these items into their offerings, further driving consumer acceptance and market penetration [132]. With a favorable market outlook and growing consumer demand, the adoption of green processing technologies presents a promising opportunity for meat processors to align with sustainability goals and cater to evolving consumer preferences.

### **Regulatory landscape and challenges**

The regulatory landscape for green processing technologies in meat processing is evolving as governments and international organizations seek to address food safety, environmental sustainability, and public health concerns [133]. Currently, regulations on green technologies in meat processing vary significantly across regions, depending on the technology and its application. For example, in the United States, the Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA) regulate technologies such as HPP and PEF processing, requiring comprehensive safety assessments and validation studies before these methods can be used commercially [134]. In the European Union, the European Food Safety Authority (EFSA) plays a similar role, establishing guidelines and safety standards for novel processing technologies, including cold plasma and ultrasound, to ensure they meet stringent safety and quality requirements [135]. Additionally, regulations around labeling and marketing of green-processed products are in place to ensure transparency and protect consumer interests [136]. While these regulations aim to safeguard public health and promote food safety, they can also be restrictive, requiring substantial documentation and scientific evidence to demonstrate that new technologies are safe and effective.

The adoption of green processing technologies in the meat industry faces several regulatory challenges, including the lengthy and complex approval processes, the need for extensive scientific validation, and the lack of harmonized international standards [137]. For many emerging technologies, such as cold plasma and UV processing, the regulatory framework is still developing, creating uncertainty for companies looking to innovate [135]. This uncertainty can deter investment and slow the commercialization of these technologies. Moreover, the rigorous safety assessments and validation studies required for regulatory



approval can be costly and time-consuming, particularly for small and medium-sized enterprises (SMEs) [138]. Additionally, the lack of harmonization in regulations across different regions can pose challenges for global companies, as they must navigate multiple regulatory environments and adapt their technologies to meet diverse safety standards and requirements [139].

Despite these challenges, there are significant opportunities for advancing green processing technologies within the regulatory framework. Increased collaboration between industry stakeholders, regulatory bodies, and scientific communities can help develop more streamlined and flexible regulatory pathways. For example, establishing clear guidelines and protocols for validating the safety and efficacy of new technologies could accelerate their approval and adoption. Furthermore, as consumer demand for sustainable and minimally processed foods grows, there is a strong incentive for regulatory bodies to support innovations that enhance food safety and quality while reducing environmental impact. Developing a more supportive regulatory environment could encourage innovation, promote the adoption of green technologies, and ultimately lead to more sustainable and resilient food systems.

#### Future directions and research needs

The future of green processing technologies in the meat industry hinges on continued innovation and research. Key areas for future research include enhancing the efficiency and scalability of existing technologies and developing novel methods with broader applications. For instance, research could focus on improving the energy efficiency and cost-effectiveness of HPP and PEF systems, making them more accessible to smaller processors. Additionally, exploring the integration of green technologies with emerging smart processing systems, such as Internet of Things (IoT) and artificial intelligence (AI) for real-time monitoring and optimization, holds promise for advancing the industry. Innovations in materials and processes, such as biodegradable packaging and sustainable waste management solutions, are also crucial. By investing in these research areas, the meat processing industry can advance towards more sustainable practices, improve product quality, and meet the evolving demands of both regulators and consumers.

Encouraging the industry-wide adoption of green processing technologies involves addressing several challenges and implementing strategic initiatives. One key strategy is

to provide financial incentives and support to companies that invest in green technologies, such as subsidies, tax breaks, or grants. This can help offset the high initial costs and facilitate a smoother transition. Additionally, fostering partnerships between technology developers, industry stakeholders, and government agencies can promote the sharing of knowledge, resources, and best practices. Industry associations and consortia can play a crucial role in setting standards, providing training, and demonstrating the benefits of green technologies through pilot projects and case studies. Engaging in collaborative efforts and creating a supportive ecosystem can accelerate the adoption of green technologies across the meat processing sector and drive widespread industry transformation.

Educating consumers about the benefits of green processing technologies is essential for driving market demand and acceptance. Initiatives to increase consumer awareness can include targeted marketing campaigns that highlight the environmental and health benefits of green-processed meat products. Transparency in labeling, including clear information about the use of green technologies and their advantages, can help build consumer trust and confidence. Public education campaigns, in partnership with industry organizations, environmental groups, and academic institutions, can further enhance understanding and support for sustainable practices. Additionally, incorporating educational content into food safety and nutrition programs can raise awareness from an early age. By fostering a well-informed consumer base, the meat industry can encourage the adoption of green processing technologies and contribute to a more sustainable food system.

#### Conclusion

Green processing technologies offer transformative benefits for the meat industry, enhancing food safety, quality, and sustainability. These methods, such as high-pressure processing and cold plasma, reduce energy consumption, minimize chemical use, and improve waste management. They represent a significant step towards addressing environmental and health challenges associated with traditional meat processing. To maximize their potential, it is essential to advance research, develop supportive policies, and encourage industry adoption. Collaborative efforts, innovative solutions, and consumer education will drive the widespread implementation of these technologies, leading to a more sustainable and efficient meat production system.

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The author bears responsibility for the work and presented data.

The author declares no conflict of interest.



# PROTEOLYTIC ACTIVITY OF *SECHIMUM EDULE*, *COSMOS CAUDATUS*, *MEDICAGO SATIVA* IN MEAT TENDERIZATION

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**Keywords:** collagen, muscle tissue, perimysium, endomysium, metalloproteases, aspartic protease (AP), serine protease (SP)

## Abstract

The number of plant proteases that still not applied is the subject of this research. This study was to test the effectiveness of plant protease in *Sechium edule*, *Cosmos caudatus* Kunth, and *Medicago sativa* L. in meat tenderization. The research included goat meat and beef that was sprinkled with extracts (15% w/w) of chayote fruit (*Sechium edule*), kenikir leaf (*Cosmos caudatus* Kunth), and Alfalfa sprout extract (*Medicago sativa* L.). The process was carried out at 50°C, for 30 and 60 min. SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) analysis was performed to see the degradation zone, while SEM (scanning electron methods) analysis was performed to inspect the condition of the meat connective tissue. The findings of the study showed that all three plant proteases were able to tenderize beef and goat meat. *Cosmos caudatus* Kunth extract showed the highest effectiveness in degrading beef proteins in the zone of 10–22 kDa (small peptide — troponin I) and  $\alpha$  and  $\beta$  tropomyosin (33 kDa) with  $V_{Max} = 0.134 \mu\text{g}/\mu\text{L}/\text{min}$  and  $K_M = 17.05 \mu\text{g}/\mu\text{L}$ . In goat meat, the extract was only able to degrade the small peptide area and troponin C (10–17.5 kDa;  $V_{Max} = 0.087 \mu\text{g}/\mu\text{L}/\text{min}$ ;  $K_M = 7.23 \mu\text{g}/\mu\text{L}$ ). Conclusion: all three plant proteases proved to be effective in the process of beef and goat meat tenderization.

**For citation:** Budianto, B., Feri, Z.O., Suparmi, A. (2025). Proteolytic activity of *Sechium edule*, *Cosmos caudatus*, *Medicago sativa* in meat tenderization. *Theory and Practice of Meat Processing*, 10(1), 45–53. <https://doi.org/10.21323/2414-438X-2024-10-1-45-53>

## Introduction

Meat industry players continue to strive for an environmentally friendly and natural way of tenderizing meat. The damage caused by the tenderization process can be minimized when compared to cooking or heating. Plant protease is the main choice related to this problem.

Proteases in plants are highly abundant as they are involved in the plant life cycle viz: physiology and development [1,2]. The widely used and researched plant proteases (Figure 1) are: cysteine protease (CP), aspartic protease (AP), serine protease (SP), and metalloprotease (MP). Among these four groups, CPs prevail in usage and testing, namely papain and bromelain, although other types are insufficiently researched (capparin, asparagus protease, caricain, etc.).

Tantamacharik et al. [2] categorized the types of plant proteases that are often studied and those that studied a little. Plant proteases for the SP and AP groups are found in plant tissues such as tomato plant leaves [3], thistle flowers [4], cornpollen[5], potatoplantleavesandtubers[6], Arabidopsisthalianaseeds[7,8], cucumber [9], and flax seeds [10,11]. Different conditions in the SP (Asian pumpkin protease) and AP (Phytpsin) groups have not been widely researched.

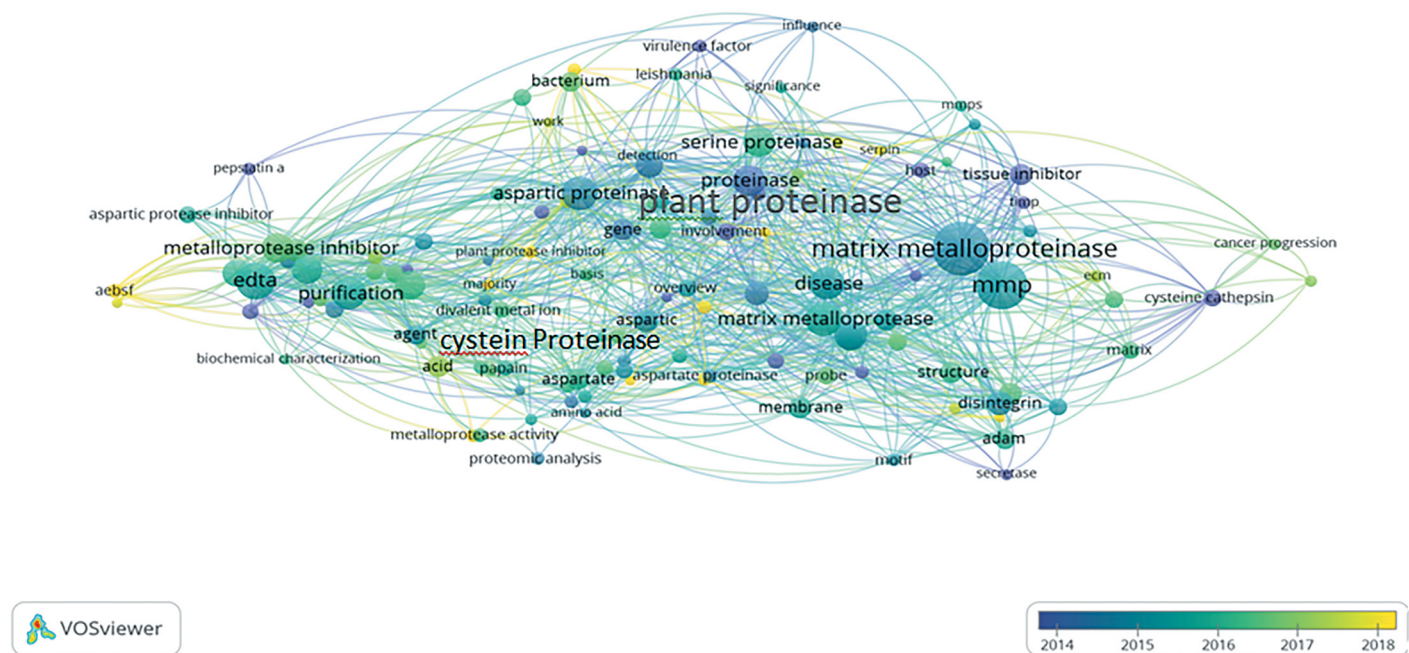
In the MP group, only 2 types are known, namely metzincin and cotinifolin. It is possible that there are some plants that have not been identified. Among plant proteases, metalloproteases (MP) are the least characterized [12]. However,

MP proteolytic activity was detected in several sources such as *Arabidopsis thaliana* [13], sorghum [14], soybean leaves [15], sugarcane [16], germinated corn [17], pea seeds [18], buckwheat seeds [19], and wheat [20]. This is inversely proportional to the genus *Medicago*. The lack of information about the genus is interesting for our research object.

On the other hand, research related to protease enzymes continues to grow. Figure 1 provides an overview of the lack of research on the latest protease sources. The search for protease sources is not something interesting. We collected 1000 journal articles from 2010 to 2023, then we mapped them using vosviewer. From the collected journals, we searched for the keyword "new plant protease", and found 120 out of 1000 papers (0.12%). This motivates us to use protease plants that have not been researched or were researched just a little.

In this study, plant protease (which has not been widely studied) was assigned to be used for tenderizing meat. We selected three groups that were slightly researched, namely SP (Asian pumpkin protease) in *Sechium edule*, aspartic protease (phytpsin) in *Cosmos caudatus* leaves and MP group in *Medicago sativa* L. The Siamese pumpkin (*Sechium edule*) was detected to contain asian pumpkin serine protease [21] but their use for tenderizing meat has not been studied until now. *Cosmos caudatus* is assumed to contain the enzyme phytpsin AP, while phytpsin is abundant in plants of the Asteraceae family [22]. *Medicago sativa* L. was proved to contain MP enzymes based on the metalloprotease





**Figure 1.** The development of research on plant proteases within 2010–2023

matrix genome sequence [12]. These three samples have the opportunity to be used in meat processing.

The aim of this study is to ascertain the efficacy of plant proteases in the three samples (*Sechium edule*, *Cosmos caudatus* Kunth, and *Medicago sativa* L.), which is supported by the preceding description. We tested its effectiveness based on: (i) enzyme kinetics through maximal speed ( $V_{\max}$ ) and Michaelis Menten constant ( $K_m$ ) of protein degradation; (ii) the effects of plant proteases on to the areas of protein degradation in goat meat and beef; (iii) the impact of plant protease on collagen, perimysium, endomysium, and muscle connective tissue in beef and goat meat.

## Objects and methods

### Sample preparation

The materials used in this study included beef, goat meat, and extracts from chayote (*Sechium edule*), kenikir leaves (*Cosmos caudatus* Kunth), and alfalfa sprouts (*Medicago sativa* L.). The beef and goat meat were obtained fresh from a local market and stored at 4°C until use. Meanwhile, the plant materials were sourced from local farms, thoroughly washed with distilled water, and sun-dried for 48 hours. After drying, the plant materials were ground into a fine powder using a high-speed blender Cosmos CB-801 (Star Cosmos, Indonesia) before undergoing the extraction process. This procedure was conducted, referring to our previous research [23]. We extracted 150 g of dry powder with 100 mL of distilled water for 72 h to get a thick extract. After that, the final extract concentration used in the analysis was equal to 20% (20 mg/mL), which volume we achieved by filtering and evaporating it in an evaporator DLAB RE100-PRO (Wahana Hilab, Indonesia) for 1 h.

Thin slices of beef and goat meat (4 cm × 4 cm × 2 mm) were cut. 20% extract was applied to the beef, with the ratio of extract weight to meat weight. We allowed the meat to

rest for 30 min before storing it for 60 min at 30–35 °C to avoid temperature fluctuations. Untreated beef (B) and untreated goat meat (GM) were used as control samples in this study. While the protein degradation kinetics analysis (SDS PAGE) was carried out at 30 and 60 min, the SEM test was undertaken at 60 min.

The sample codes are defined as follows:

**B (Beef):** Untreated beef (control).

**B-C:** Beef treated with *Cosmos caudatus* Kunth extract.

**B-M:** Beef treated with *Medicago sativa* L. extract.

**B-S:** Beef treated with *Sechium edule* extract.

**GM (Goat Meat):** Untreated goat meat (control).

**GM-C:** Goat meat treated with *Cosmos caudatus* Kunth extract.

**GM-M:** Goat meat treated with *Medicago sativa* L. extract.

**GM-S:** Goat meat treated with *Sechium edule* extract.

## Protein level

Determination of protein content was conducted in accordance with Manzoor et al. [24]. Analysis was done using the biuret method with a UV-Vis spectrophotometer (Spectronic 200, Thermo Fisher Scientific, USA) at wavelength 595 nm. Biuret reagent used was 0.2 g  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and 0.6 g potassium tartrate mixed together in 50 mL of distilled water and 40 mL of 15% NaOH was added. The amount of protein content was determined via the absorption of UV light.

Maximum speed ( $V_{max}$ ) and Michaelis Menten constant ( $K_m$ )

The maximum speed ( $V_{\max}$ ) and substrate efficiency (KM) were calculated by the correlation between reaction rate (V) and substrate concentration (S). The Lineweaver-Burk equation was also used by [25].

$$\frac{1}{V} = \frac{K_m}{V_{\max}} \cdot \frac{1}{S} + \frac{1}{V_{\max}} \quad (1)$$

Given that  $Y = bx + a$  and that the  $y$ - and  $x$ -axes on the graph are  $1/V$  and  $1/S$ , respectively,  $V_{\max} = 1/a$  and  $K_m = V_{\max} \cdot b$

#### SDS-PAGE (sodium dodecyl sulfate — polyacrylamide gel electrophoresis) analysis

The procedure refers to the one of Association of Official Analytical Chemists [26]. Acrylamide gel electrophoresis was used in the analytical process. The concentration of the top (stacking gel) and bottom (separating gel) was 5% for the stacking gel and 12% for the separating gel. 200 V voltage, 15 mA/gel, and 60 min were used for the electrophoresis using a Mini-PROTEAN Tetra Cell-BIO-RAD.

After electrophoresis, 0.05% (w/v) coomassie blue R-250 was added to 15% (v/v) methanol and 5% (v/v) acetic acid to stain the gel. It was then microwave-heated for 30 seconds and allowed to incubate for 60 min. After the gel was submerged in a solution containing 30% methanol and 10% acetic acid, it was incubated for 2 h in a waterbath (Julabo TW12, Julabo GmbH, Germany).

#### SEM Analysis (Scanning Electron Methods)

This procedure is described by Koga et al. [27]. Meat structure was analyzed using SEM (ZEISS EVOMA10, Carl Zeiss Microscopy GmbH, Germany). The dried specimens were examined using a scanning electron microscope (S-4100; Hitachi, Japan) and placed on an aluminum platform that had been ion-sputter coated with platinum-palladium (EI030; Hitachi, Japan). The images were viewed with SE (Secondary Electron) detector, Working Distance 9.0 mm and EHT 16.00 kV at 500 x magnification (ZEISS EVOMA10, Carl Zeiss Microscopy GmbH, Germany).

#### Statistical test

IBM SPSS Statistics software of version 26 (SPSS Inc., Chicago, IL, USA) was used for the analysis. To see the variations in protein levels over time, we used an ANOVA test with Tukey HSD\_ post hoc ( $x$ ,  $y$ , and  $z$ ). Changes in protein levels in each sample were observed using  $a$ ,  $b$ ,  $c$ , and  $d$ , respectively. The mean  $\pm$  SD of the results was presented.  $p < 0.05$  was used as the threshold for statistical significance.

## Results and discussion

### Enzyme kinetics

In Table 1, it can be seen that the change in protein content for between goat meat and beef did not show a significant difference ( $p > 0.05$ ) at 0 min. Within 30 and 60 min, there was a significant effect ( $p < 0.05$ ) on protein content and there was a significant difference in the three sample extracts. There was drastic degradation at 60 min, and mild degradation at 30 min. The protein content comparison scale to see the type of degradation (mild, moderate, and complete) can be seen in Figure 2.

Although there was a significant difference in the change of protein content at 30 and 60 min (Table 1), the researcher

Table 1. The change in protein content for goat meat and beef meat

Sample	0 min	30 min	60 min
B	17.35 $\pm$ 0.28 <sup>ax</sup>	17.34 $\pm$ 0.21 <sup>dx</sup>	17.35 $\pm$ 0.24 <sup>dx</sup>
B-C	17.28 $\pm$ 0.22 <sup>az</sup>	13.05 $\pm$ 0.25 <sup>ay</sup>	2.59 $\pm$ 0.21 <sup>ax</sup>
B-M	17.34 $\pm$ 0.23 <sup>az</sup>	16.1 $\pm$ 0.26 <sup>cy</sup>	8.02 $\pm$ 0.3 <sup>cx</sup>
B-S	17.34 $\pm$ 0.22 <sup>az</sup>	15.03 $\pm$ 0.23 <sup>by</sup>	2.59 $\pm$ 0.21 <sup>ax</sup>
GM	8.56 $\pm$ 0.23 <sup>ax</sup>	8.54 $\pm$ 0.21 <sup>cx</sup>	8.54 $\pm$ 0.26 <sup>dx</sup>
GM-C	8.55 $\pm$ 0.11 <sup>az</sup>	6.23 $\pm$ 0.32 <sup>ay</sup>	2.43 $\pm$ 0.17 <sup>ax</sup>
GM-M	8.56 $\pm$ 0.3 <sup>ay</sup>	8.01 $\pm$ 0.13 <sup>cy</sup>	4.78 $\pm$ 0.17 <sup>cx</sup>
GM-S	8.56 $\pm$ 0.12 <sup>az</sup>	7.5 $\pm$ 0.25 <sup>by</sup>	3.59 $\pm$ 0.28 <sup>bx</sup>

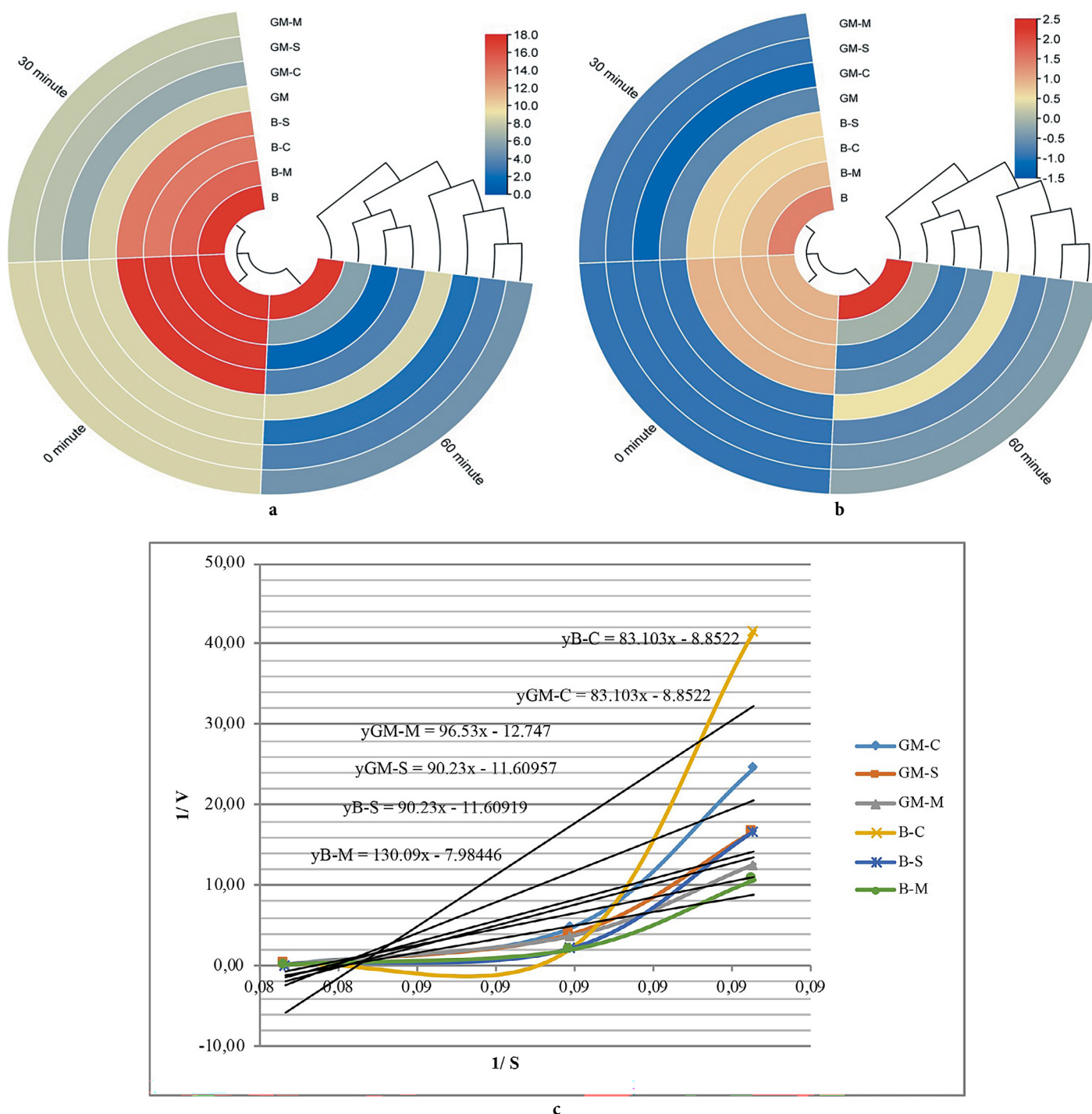
Difference of sample extracts =  $a$ ,  $b$ ,  $c$ , and  $d$ ; difference of time =  $x$ ,  $y$ , and  $z$ . Significant level ( $p < 0.05$ ).

also tried to represent the difference in the form of color variations to easily classify the type of degradation. The representation of color variations in Figure 2a. was not clearly visible at 30 min. The significant range of color variations occurred at 60 min. If to represent the Figure 2a as a scale, the color variation is more clearly visible at 30 min (mild and moderate degradation) and 60 min (moderate and complete degradation).

The time variable provided a significant effect ( $p < 0.05$ ) on reducing meat protein content. Although the protease activity of *Medicago sativa* L. was the lowest, optimization can be done by increasing the time duration and extract concentration ( $> 15\%$  w/w). Extract concentration and time duration significantly affected the degradation of meat protein in the tenderization process. This seems to be the general consensus among the researchers [28,29].

The  $V_{\max}$  of protein degradation of *Cosmos caudatus* Kunth leaf extract was the highest (0.134  $\mu\text{g}/\mu\text{L}/\text{min}$ ) with a substrate  $K_m$  of 17.05  $\mu\text{g}/\mu\text{L}$  in on beef. Goat meat also showed the highest value (0.087  $\mu\text{g}/\mu\text{L}/\text{min}$ ). In goat meat, there was almost no difference in the  $V_{\max}$  of protein degradation of the three sample extracts. The difference in  $V_{\max}$  was seen in beef but the substrate  $K_m$  in the three samples were almost the same. *Medicago sativa* L. sprout extract gave the lowest  $V_{\max}$  in beef (0.125  $\mu\text{g}/\mu\text{L}/\text{min}$ ) and goat meat (0.078  $\mu\text{g}/\mu\text{L}/\text{min}$ ). The effectiveness of this kinetics was seen from the use of the lowest substrate ( $K_m$ ) but was able to provide a greater  $V_{\max}$ . The term 'lowest substrate' refers to the ability of the system to reach  $V_{\max}$  with a lower substrate concentration, which is indicated by a lower  $K_m$ . A lower  $K_m$  suggests a higher enzyme-substrate affinity, meaning that less substrate is needed to achieve high reaction velocity. In this study, the effectiveness of the kinetics was evaluated based on achieving high  $V_{\max}$  with a relatively low  $K_m$ . In this study, the effectiveness of *Cosmos caudatus* Kunth leaf extract was demonstrated in goat meat but not in beef (Figure 2c and Table 2).

The above series of analysis proved that the extracts of *Cosmos caudatus* Kunth leaves, *Sechium edule* fruit, and *Medicago sativa* L. sprouts can be used in meat processing. Although the  $V_{\max}$  was relatively low compared to commonly used plant proteases, i. e. bromelain, obtained from *Ananas comosus* has 3,969 U/min (79.38  $\mu\text{g}/\mu\text{L}/\text{min}$ ) [28]. Papain enzyme from *Carica papaya* leaves has a  $K_m$  value of 1.47–8.70 mg/mL



**Figure 2.** The comparison of the effect of plant protease on protein content of beef and goat meat. Changes in protein content in  $\mu\text{g}/\mu\text{L}$  (a). Scale of changes in protein content (b). Degradation reaction kinetics due to plant proteases (c)

**Table 2.** Degradation reaction kinetics due to plant proteases in a meat of goat and beef

Sample	Item	<i>Medicago sativa</i> L.	<i>Secchium edule</i>	<i>Cosmos caudatus</i> Kunth
Goat meat (GM)	$y$	$96.53x - 12.747$	$y = 90.23x - 11.609$	$y = 83.103x - 8.8522$
	$R^2$	0.9598	0.9996	0.9984
	$V_{\max}$	0.078 $\mu\text{g}/\mu\text{L}/\text{min}$	0.086 $\mu\text{g}/\mu\text{L}/\text{min}$	0.087 $\mu\text{g}/\mu\text{L}/\text{min}$
	$K_m$	7.53 $\mu\text{g}/\mu\text{L}$	7.76 $\mu\text{g}/\mu\text{L}$	7.23 $\mu\text{g}/\mu\text{L}$
Beef (B)	$y$	$130.09x - 7.9844$	$128.8x - 7.7046$	$127.2x - 7.4566$
	$R^2$	0.9896	0.9983	0.9984
	$V_{\max}$	0.125 $\mu\text{g}/\mu\text{L}/\text{min}$	0.129 $\mu\text{g}/\mu\text{L}/\text{min}$	0.134 $\mu\text{g}/\mu\text{L}/\text{min}$
	$K_m$	16.29 $\mu\text{g}/\mu\text{L}$	16.61 $\mu\text{g}/\mu\text{L}$	17.05 $\mu\text{g}/\mu\text{L}$



and a  $V_{\max}$  value of 0.42–0.4167  $\mu\text{mol/mL/min}$  [30]. In addition, for hydrogen peroxide substrate, ficin has been reported to have a  $V_{\max}$  of 4.69  $\mu\text{g/mL}$  at 0.35 mMol [31]. However, discovering plant proteases in under-researched plants is equally important.

### The impact of plant protease on the degradation of proteins

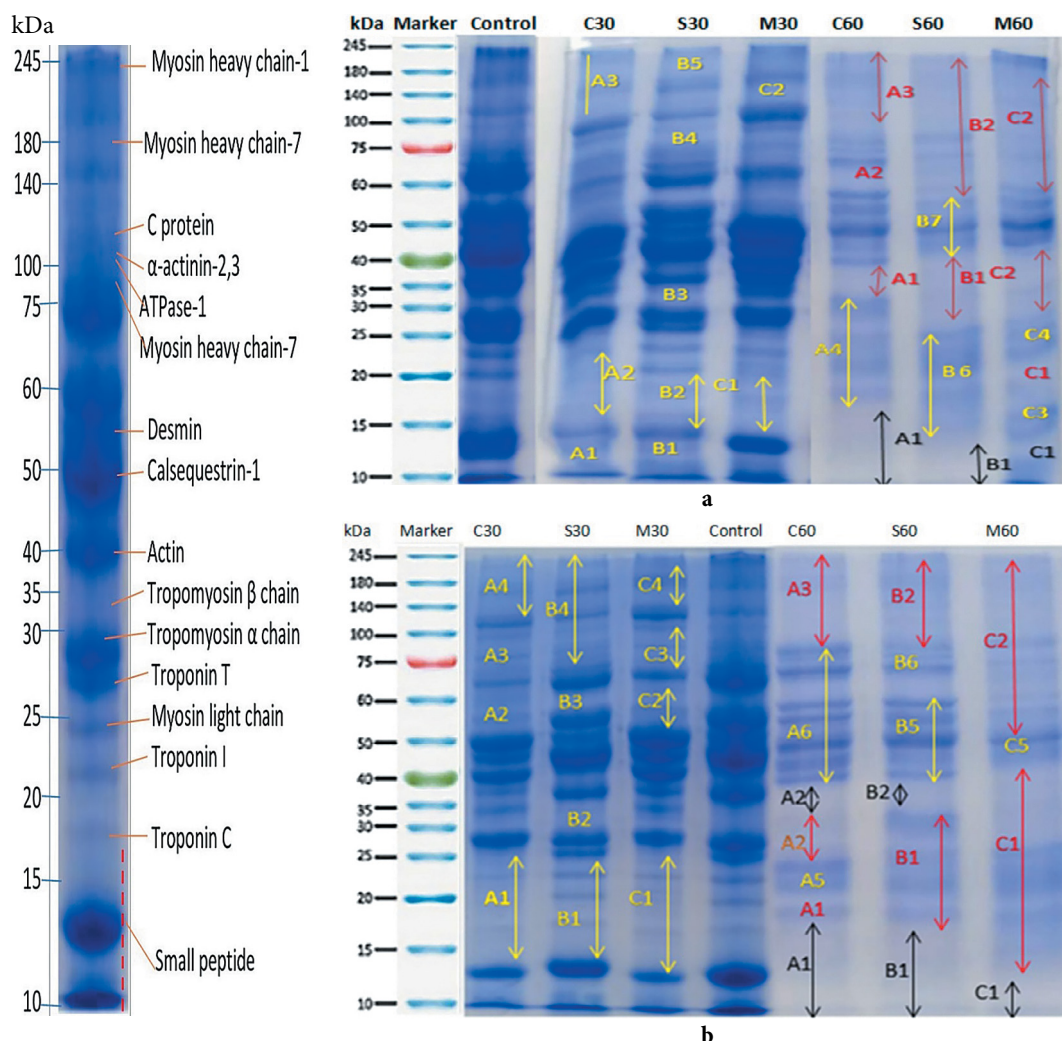
The results of SDS-PAGE analysis are shown in Figure 3. The effect of plant protease caused degradation of goat meat protein (Figure 3a). All sample extracts were only capable of mild degradation at 30 min, moderate degradation and complete degradation at 60 min. *Cosmos caudatus* Kunth was able to degrade completely in the area of small peptide and troponin C (10–17.5 kDa). Intermediate degradation occurred in the area of  $\alpha$  and  $\beta$  tropomyosin (33 kDa) and  $\alpha$  actinin to myosin heavy chain (103–223 kDa).

*Sechium edule* degrades completely (Figure 3a) in the area of small peptide (10–15 kDa) and intermediate scale degradation in the zone of myosin light chain, troponin T,  $\alpha$  and  $\beta$  tropomyosin and actin (25–42 kDa). *Medicago sativa* L. was only able to degrade in the narrower area of

small peptide (12–14 kDa) but was able to intermediate scale degradation in a fairly wide area of 17–21 kDa, 26–38 kDa and 53–223 kDa).

Degradation of beef protein (Figure 3b) using all three sample extracts for 30 min resulted in only minor degradation. The 60-min aging time also caused minor degradation in the 40–75 kDa area. *Cosmos caudatus* Kunth was able to completely degrade proteins in the area of small peptides (10–17 kDa), troponin C (17.8 kDa), troponin I (22 kDa), and in the narrow area of  $\alpha$  and  $\beta$  tropomyosin (33 kDa). There was intermediate-scale degradation in the narrow area of troponin C (17.8 kDa), the broad area including myosin light chain (25 kDa), troponin T (30 kDa),  $\alpha$  and  $\beta$  tropomyosin (33 kDa) and the broad area of 75–200 kDa.

*Sechium edule* degrades completely in the small peptide area (10–17 kDa) and in the narrow area of  $\alpha$  and  $\beta$  tropomyosin (33 kDa). Intermediate scale degradation was seen in the 20–33 kDa area (troponin I, myosin light chain, troponin T,  $\alpha$  and  $\beta$  tropomyosin) and the 75–223 kDa area ( $\alpha$  actinin, protein C and myosin heavy chain). Small-scale degradation was formed in the coverage area of 40–75 kDa (Figure 3b).



**Figure 3.** Meat protein degradation zone based on SDS-PAGE analysis. Protein degradation zones of goat meat (a) and beef (b) using *Cosmos caudatus* Kunth extract for 30 and 60 min (C30, C60), with *Medicago sativa* L. extract for 30 and 60 min (M30, M60), with *Sechium edule* extract for 30 and 60 min (M30, M60). The effects of *Cosmos caudatus* Kunth (A), *Sechium edule* (B) and *Medicago sativa* L. (C) extracts resulted in mild (yellow), moderate (red) and complete (black) degradation

*Medicago sativa* L. degrades completely (Figure 3b) in the area of 10–12 kDa (small peptide). Medium-scale extensive degradation areas were seen in the 13–40 kDa (small peptide, troponin C, troponin I, myosin light chain, troponin T, and  $\alpha$  and  $\beta$  tropomyosin) and 75–223 kDa areas ( $\alpha$  actinin, protein C and myosin heavy chain).

Different results of *Sechium edule*, shown by Darmawati et al. [32], with degradation observed in buffalo meat (10–26 kDa), beef (10–43 kDa), and goat meat (10–17 kDa). Unfortunately, we found no information on *Cosmos caudatus* and *Medicago sativa* L. used directly in meat tenderization.

### The effects of plant proteases on meat connective tissue

In muscle tissue, collagen will form the *perrymisium* and *endomysium*. Collagen is the most abundant component in muscle tissue. *Perrymisium* will separate muscle fibers, and *endomysium* serves as a coating on muscle fibers. The tissue (*Perrymisium* and *endomysium*) will be visible if there is a tear in the meat fiber.

#### Goat meat

Goat meat's connective tissue was exposed to plant protease treatment (Figure 4), with untreated meat serving as the control sample (Figure 4a). The muscle tissue was still tightly packed and collagen dominated in appearance. *Endomysium* tissue was also found (*endomysium* may be formed due to cutting factors).

The protease in *Cosmos caudatus* Kunth affected the goat meat tissue (Figure 4b). The muscle tissue was separated when compared to the control sample of meat (Figure 4a).

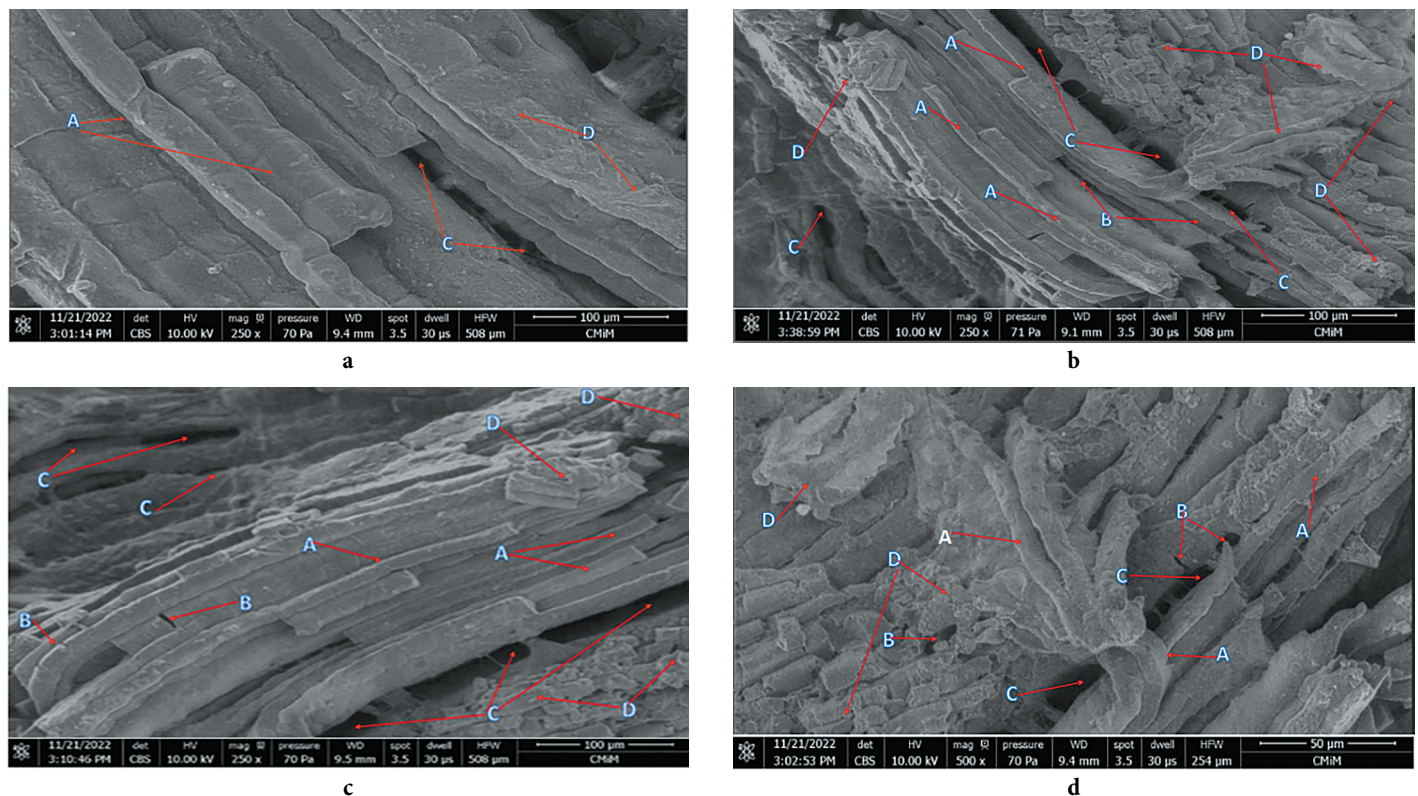
The dominance of collagen was reduced as the *perrymisium* and *endomysium* tissues increased. *Endomysium* dominates which is characterized by broken cross-links so that tears are formed in the meat fibers and *perrymisium* tissue is also visible at some points but does not dominate. The number of broken cross-links will affect the tenderness of the meat. In Figure 4b, the tear in the meat is long and wide and small cracks are visible at some points.

The protease in *Sechium edule* also showed its effect on meat connective tissue (Figure 4c). The changes in connective tissue were similar to those of *Cosmos caudatus* Kunth extract, where muscle tissue was seen to have separated at some points. Collagen content in tissue was reduced. *Endomysium* tissue dominated in the meat and *perrymisium* tissue was seen at some points.

Changes in the meat connective tissue due to the effect of proteases in *Medicago sativa* L. (Figure 4d) did not show long cracks or tears like the extracts of *Cosmos caudatus* Kunth and *Sechium edule* did. Muscle tissue appeared to have separated, while collagen bundles were also reduced along with the prevalence of *endomysium* bundles. *Perrymisium* tissue was visible at some points.

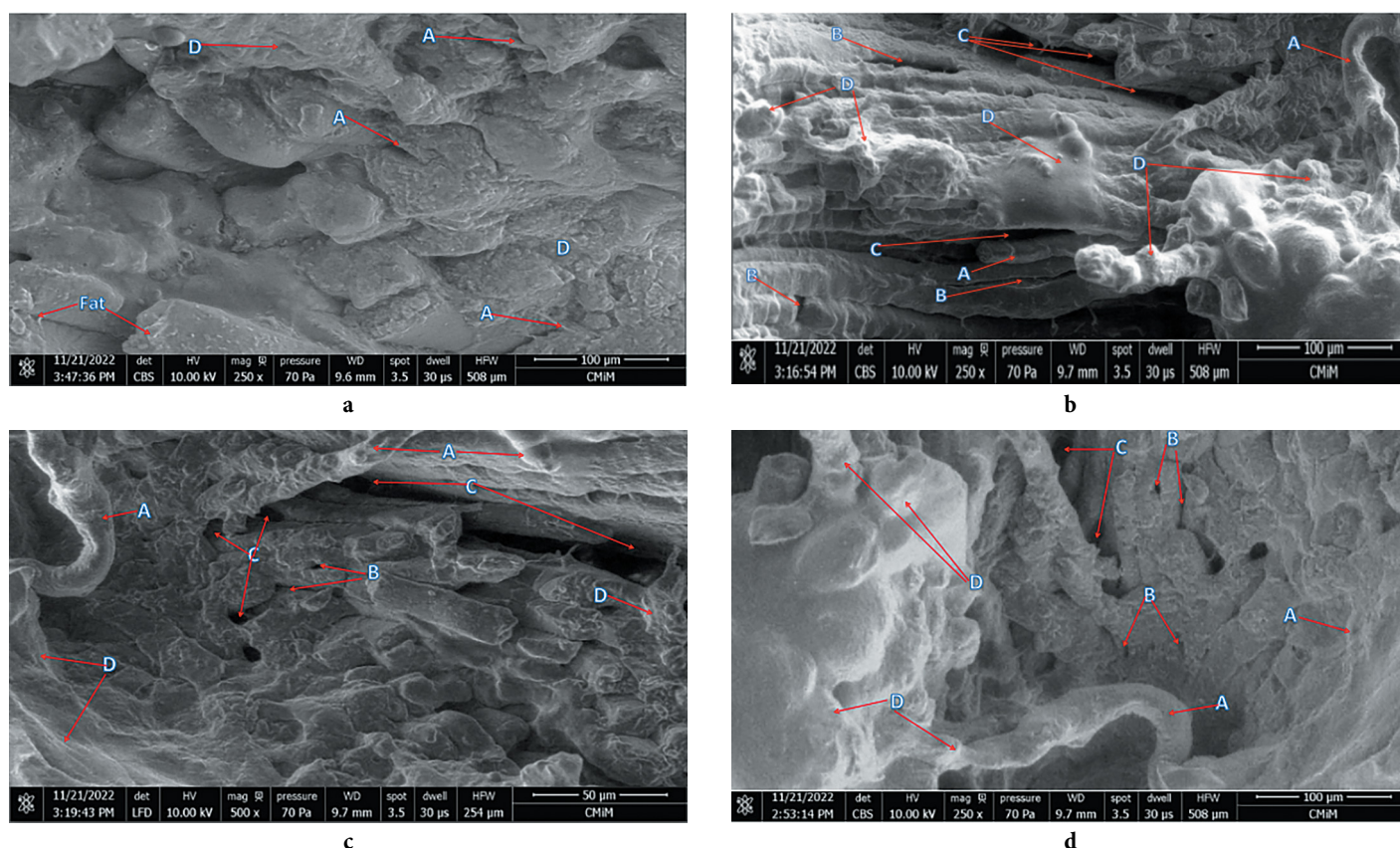
#### Beef

The impact of plant protease on cattle connective tissue is shown here (Figure 5). and control sample of meat (Figure 5a). The muscle tissue was still tight and collagen dominates in appearance. No *endomysium* and *perrymisium* tissues were found. This is because the cross-linking tissue is still strong. In the control sample, fat was found, which is almost similar to the appearance of collagen.



**Figure 4.** The effects of plant proteases on connective tissue of goat meat. The shape of connective tissue in control sample of meat (a) degraded by *Cosmos caudatus* Kunth leaf extract (b), *Sechium edule* fruit extract (c), and *Medicago sativa* L. sprout extract (d) will result in changes in muscle tissue (A), perimysium (B), endomysium (C), and collagen (D)





**Figure 5.** The effects of plant proteases on beef connective tissue. The shape of connective tissue in control sample of meat (5a) degraded by *Cosmos caudatus* Kunth leaf extract (5b), *Sechium edule* fruit extract (5c), and *Medicago sativa* L. sprout extract (5d) will result in changes in muscle tissue (A), perimysium (B), endomysium (C), and collagen (D)

The protease in *Cosmos caudatus* Kunth affected the beef tissue (Figure 5b). Muscle tissue appeared separated when compared to control sample of meat (Figure 5a). Collagen did not prevail as perimysium and endomysium tissues increased. Endomysium prevails, which is characterized by the breaking of cross-links resulting in tears in the meat and perimysium tissue is also visible at some points. In Figure 5b, the tears are narrow and short but evenly distributed on all sides.

The protease in *Sechium edule* showed its effect on meat connective tissue (Figure 5c). Muscle tissue was seen as having separated at some points. Collagen tissue was reduced, the dominance of perimysium endomysium bonds was seen as clustered in the center or unevenly distributed on all sides of the meat. Meat fibers tears are shorter but deeper.

The effect of proteases in *Medicago sativa* L. on beef connective tissue (Figure 5d) did not show long cracks or tears like the extracts of *Cosmos caudatus* Kunth and *Sechium edule* did. Muscle tissue appeared to have separated; collagen bundles were also reduced with the dominance of endomysium bundles. Perimysium tissue was visible at some points.

Protein degradation kinetics shows the speed at which collagen molecules change from helical to coiled structure and fibrous collagen changes to fibrillar. The breaking of hydrogen bonds will be followed by the shrinkage of collagen fibers (becoming short). During the degradation process, collagen contraction will occur; the more often it contracts, the

lower its mechanical strength. This is in line with Astruc [33] statement about collagen damage due to thermal factors.

The three plant proteases are also able to separate myofibers from the perimysium. Perimysium tissue is the most susceptible tissue to damage. Before the degradation process sometimes the damage can be caused by meat cutting factors. Intramuscular isometric tension can decrease due to storage conditions as well [34]. Plant proteases (*Cosmos caudatus* Kunth, *Sechium edule*, and *Medicago sativa* L.) in this study were able to activate perimysium and separate muscle fibers in muscle connective tissue. Perimysium is a large circular fascicle that has an order based on the size of the diameter, namely primary, secondary and tertiary fascicles [33].

The prevalence of *endomysium* appearance (Figure 4 and Figure 5) in goat meat and beef due to the degradation of plant protease (*Cosmos caudatus* Kunth, *Sechium edule*, and *Medicago sativa* L.) is the first step of meat tenderization. This condition indicates that the endomysium has been detached from the sarcomere. *Endomysium* surrounds muscle fibers which include basal lamina, proteoglycans, collagen and laminin [35], so *endomysium* will be visible if a tear is formed in the meat fiber.

## Conclusion

The extracts of *Cosmos caudatus* Kunth leaves, *Sechium edule* fruit, and *Medicago sativa* L sprouts demonstrated their ability to degrade meat proteins, so their using in meat processing should be considered. Based on protease enzyme



kinetics, *Cosmos caudatus* Kunth leaf extract showed higher effectiveness on goat meat ( $V_{\max} = 0.087 \mu\text{g}/\mu\text{L}/\text{min}$ ) and beef ( $V_{\max} = 0.134 \mu\text{g}/\mu\text{L}/\text{min}$ ) compared to *Sechium edule* fruit and *Medicago sativa* L sprouts, while the lowest  $V_{\max}$  was shown by *Medicago sativa* L sprouts extract.

In goat meat, *Sechium edule* fruit extract and *Medicago sativa* L sprouts were only able to degrade in the small peptide zone area (10–15 kDa). Meanwhile, *Cosmos caudatus* Kunth leaf extract could only affect the area of small peptide (10–17 kDa), and troponin C (17.8 kDa). In beef, the affected area of *Cosmos caudatus* Kunth and *Sechium edule*

degradation covers a wider area in the range of 10–33 kDa. While *Medicago sativa* L was only able to degrade in the small peptide zone (10–15 kDa).

The prevalence of endomysium content in mutton and beef due to the degradation of plant protease (*Cosmos caudatus* Kunth, *Sechium edule*, and *Medicago sativa* L.) is the first step of meat tenderization. The three plant proteases were able to separate the myofibers from the perimysium, change the collagen molecules from a helical state to a circular structure, and transform fibrous collagen into fibrillar collagen.

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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.



# ASSESSMENT OF THE CONSUMERS' ATTITUDE TO THE ALTERNATIVE MEAT. REVIEW

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**Keywords:** meat substitutes (analogues), cultivated meat, alternative proteins, consumers' behavior, food neophobia

## Abstract

Cultivated meat technology is a new and pretty controversial food technology presented as a method of meat production without dependence on large-scale farming of industrial livestock. It is based on the principles of people's humanistic attitude to animals and environmental care. The article summarizes the results of the "life cycle" assessment of the cultivated meat and the possible environmental effect of its production technology on global warming. The presented review is aimed at assessing consumer perception of cultivated meat by analyzing and systematizing the results of previous studies that examined the consumers' attitudes to the risks and benefits of the alternative meat. Systematized research data allowed identifying key factors that influence onto the consumers' perception of the food products. For example, one of the main reasons for a negative attitude towards cultivated meat is food neophobia. In addition, differences in this product acceptance by various consumers groups were analyzed. Generalization of the results allowed systematizing the motivators and barriers that may affect the mass consumption of the cultivated meat in the future, taking into account the innovations in the new food technologies development. Acceptance rates of the cultivated meat vary in relevance with demographics, socio-cultural, religious, ethical perceptions and traditions. However, it should be noted that there is no consensus on the perceived advantages, disadvantages, threats and opportunities of the consumers' acceptance of the cultivated meat. This review notes that a number of studies show a relatively high level of consumer/population willingness to try the cultivated meat. However, the acceptance rates of the cultivated meat are generally lower than for other alternative proteins (e. g. legumes, plant-based meat). The main negative factor in the acceptance of the cultivated meat is that consumers perceive the cultivated meat as the unnatural one.

The study therefore examines various issues related to the formation of both positive and negative attitudes towards the cultivated meat. It also helps to better understanding the consumers' psychology and allows more accurate prediction of their behavior.

**For citation:** Gorbunova, N. A. (2025). Assessment of the consumers' attitude to the alternative meat. Review. *Theory and Practice of Meat Processing*, 10(1), 54–66. <https://doi.org/10.21323/2414-438X-2024-10-1-54-66>

## Funding:

The article was published as part of the research topic No. FGUS-2024–0001 of the state assignment of the V. M. Gorbatov Federal Research Center for Food Systems of RAS.

## Introduction

Diet and environmental sustainability are closely linked. Food choices, eating habits and consumption patterns affect climate change, biodiversity and the way of using the energy, water and land. Although consumers are generally unaware that their dietary patterns and eating behaviors are part of a broader concept of environmental sustainability, the scientists dispute that prevailing dietary principles are having a threatening effect on the planet's ecological environment. In this context, the livestock sector became the focus point of the heightened attention of the scientific community due to its impact on climate change, including methane emission from the decomposition of organic waste, as well as ethical issues and impacts on human health [1,2,3,4]. The livestock industry is estimated to account for 14.5% of anthropogenic greenhouse gas emissions [5].

In recent years meat processing plants, engaged in production of organic meat, have been exposed to increasing pressure due to heightened attention to the role of corpo-

rate actors and their responsibility for the effect on climate change [6]. The meat industry has been criticized for its economic inefficiency, environmental costs, and its negative impact on human health [4,7].

More and more studies highlight the consumers' concerns about the environmental, human health and animal wellbeing impacts of meat consumption. Additionally, taking the COVID-19 pandemic into consideration, there is a growing awareness that meat production may cause the zoonotic diseases [8]. In addition, there is a concept known as the "meat paradox", which is the contradiction between love and respect for animal life on the one hand, and the pleasure experienced from eating meat, on the other hand [9]. These arguments are used by some market players to promote meat analogues or meat alternatives from various protein sources. All of the above, together with forecasts of global population growth and increasing demand for protein products, set the preconditions for development of the alternative proteins [1].



Researchers have analyzed the various options of using the existing resources pursuing the target to improve the sustainability of food production. The attention is focused on minimizing the using additional agricultural land, water, and other natural resources in order to reduce the load on the environment. Key measures include changing diets to be healthier and more plant-based ones, improving manufacturing technologies and management practices, and reducing food loss and waste volume [10,11].

New food products like meat substitutes, including insect protein, plant proteins and the cultivated meat [12], are ultimately intended to replace traditional meat partially.

One of the available meat analogues, the cultivated meat, is considered as a promising solution to meet the consumers' demand for meat products. Its production is aimed at reducing the negative impact on the environment, solving problems of antibiotic resistance, and ensuring humanistic attitude to the animals. In recent years, there has been a surge of interest from investors and the media to the cultivated meat production technology. At the end of 2022, there were more than 156 publicly announced companies worldwide that produce the cultivated meat [13].

However, despite the potential benefits of *in vitro* meat, further research is needed into its environmental benefits, nutritional characteristics, production ethics and the safety of products made from it [14,15,16].

The cultivated meat (also known as cellular, the cultivated, clean, slaughter-free, *in vitro*, lab-grown, and nanopastured meat) has recently gained popularity. The cultivated meat does not require large-scale farming methods and is produced by culturing animal cells *in vitro* without raising animals [17]. Unlike plant-based meat, which imitates the taste and texture of traditional meat, the cultivated meat is derived from animal muscle tissue [1].

In addition, the results of a preliminary life cycle analysis of *in vitro* meat production by Tuomisto and de Matos [18] showed that using, for example, cyanobacterial biomass as a nutrient source could reduce energy consumption and land use by 99%, water consumption by 90%, and energy consumption by 40%. If this reduction in resources using were implemented, it would lead to a significant reduction in greenhouse gas emissions and an improved environmental situation.

Currently, the above-specified calculations are contradictory and not comprehensive enough, given the following arguments: 1) the various life cycle assessments of the alternative meat, that are currently available, are based on hypothetical data, and do not provide an accurate assessment (since the cultivated meat is not yet produced in industrial volumes); 2) a comparison based solely on quantitative data (based on CO<sub>2</sub> equivalent only) is meaningless, since it is necessary to take into account, for example, the differences between methane CH<sub>4</sub> and carbon dioxide CO<sub>2</sub> [15].

For example, in their study, Lynch, J. and R. Pierrehumbert [19] compared the potential climate impacts of the cultivated meat and cattle production using a simple cli-

mate model that simulates the behavior of carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O), rather than relying solely on CO<sub>2</sub> equivalents. Cattle production systems cause the emissions of all three of these greenhouse gases, including significant emissions of CH<sub>4</sub>, while emissions from the cultivated meat production are almost entirely limited to CO<sub>2</sub>. However, emissions of short-life gases like methane behave very differently in comparison with CO<sub>2</sub>. Lynch, J. and R. Pierrehumbert [19] concluded that, in the short term, global warming will be less associated with the cultivated meat production rather than with the cattle meat production. However, in the long term, the impact of the cultivated meat production will be more significant because short-life gases such as CH<sub>4</sub> build-up in the atmosphere in fewer quantities compared to CO<sub>2</sub>. It can therefore be assumed that the warming impact of livestock farming will decrease and stabilize the environment over the years, while the warming due to the long-life CO<sub>2</sub> gas emitted by the production of the cultivated meat will remain. That is, the potential advantage of the cultivated meat over cattle meat in terms of global greenhouse gas emissions is not fully proven [19].

The recent life cycle assessment of the cultivated meat demonstrates that it will be the most environmentally friendly meat product if produced using sustainable energy [20]. Accordingly, the widespread introduction of the cultivated meat into human diets could improve the sustainability of the global food system [21].

For a new meat substitute to be widely adopted, it must imitate closely or, even better, recreate all the properties of traditional meat, including appearance, texture, flavor and taste. If successfully developed, it could be considered a meat equivalent without derogatory terms [17]. Proponents of alternative meat argue that its production would require significantly fewer or no farm animals, which in turn could help reducing the environmental concerns related to the high carbon and water footprint of the traditional livestock farming [18,22]. Since the cultivated meat is "real meat", it is expected to have the same or even improved properties compared to conventional meat. Given that the cultivated meat is nearly identical to conventional meat at a molecular level, it is likely to have similar organoleptic characteristics, including taste, flavor, texture and appearance, and could therefore be a viable substitute for traditional meat [23]. *In vitro* meat culturing could promote the development of new products with improved or specialized properties. For example, the biochemical composition of meat could be altered in a way to improve its nutritional quality by adding more polyunsaturated fatty acids or vitamins, which could be achieved by altering culturing conditions [17,24].

The technology of meat culturing is still under research, and different production methods (e. g. cyanobacterial-based culturing media and plant-based culturing media for tissue growing) are being studied to improve its characteristics and organoleptic properties [18].

Industrial production the cultivated meat is still in its early stages of development [25]. Companies developing the cultivated meat are looking for ways to improve their efficiency and reduce costs in order to bring their products to a competitive market, given that there are certain social and ethical limitations, and a number of technological issues (effective culturing conditions, etc.) still need to be addressed. However, the most important step towards commercialization of the cultivated meat is its acceptance by the consumers. Researchers have already established that consumers' attitudes play a key role in the acceptance of new food technologies [2,5,7,14,26–29].

Consumers' experience plays an important role in ensuring the sustainable competitive advantage of a product [30]. Consumers' experience is defined as the sum of customers' perceptions during consumption, purchase, use, and feelings from their interaction with a product or the goods [31]. Although there is no consensus on the definition and concept of consumers' experience, most scholars agree that this experience is formed during the decision-making process. It covers the entire consumption chain, which includes a series of interactions with the various objects. These interactions effect the cognitive, affective, sensory, and behavioral reactions. As a result, the total sum of feelings, perceptions and attitudes are formed, which constitute the consumers' experience [31].

Researches on food consumption show that food consumption experiences include sensory perceptions such as taste, flavor, smell and appearance. They play an important role in shaping consumers' hedonistic and emotional reactions of the consumers to the food. Moreover, the consumers look for the food products with some novel ingredients that contribute to the sustainability of food production systems and improve the health [32]. In other words, the perceived food attributes such as tenderness, juiciness, flavor and taste can enhance positive food experience [33] and contribute to consumers' behavioral intentions such as repeated purchase. For these reasons, food producers should take into account the changes in food preferences and choices to improve food quality and to understand better the consumers' behavior.

## Materials and methods

The purpose of this article is to provide the review of interdisciplinary literature on the potential benefits and risks of the cultivated meat, considered from an environmental care and healthcare perspective.

This review is based on the scientific articles published in English and Russian from January, 2005 to July, 2024. The publications were selected from the databases of Scopus, Google Scholar, Science Direct and eLibrary. These articles examine data targeted to analyzing and summarizing the evidence base for the consumers' acceptance of the cultivated meat as an alternative for the natural meat. Particular attention is paid to the perception of these technologies by various population groups, and society as a whole.

The extensive literature search methodology, used to conduct the study, consisted of two stages. The first stage involved a literature search to collect the representative studies. The second stage involved selection of source based on the analysis of the title and abstract of each publication. The selection was conducted via using keywords and phrases such as: "meat substitutes", "alternative proteins", "the cultivated meat", "*in vitro meat*", "cellular agriculture", as well as terms related to "sustainability", "food system", "consumers' eating behavior", "consumers' acceptability", "willingness to reduce animal protein intake", "motivation to consume the cultivated meat" and "health". The documents related to the analysis tools of the consumers' perception for the cultivated meat were selected. Motivators and barriers that could influence its mass consumption in the future, including the acceptance of food innovations, were also examined. Key risks that prevent the population's mass acceptance of the cultivated meat were then defined. Among them are safety and nutrition issues, the feeling of unnaturalness of the product, mistrust, disgust and food neophobia. At the same time, economic and ethical issues are highlighted, as well as two uncertainties that will significantly influence consumers' perception in the long term: price and taste. The review concludes with a discussion of the main strategies aimed at defining the ways of increasing the acceptability of the cultivated meat.

Inclusion criteria:

- results of quantitative studies of the cultivated meat perception, conducted among the adult population in the various focus groups;
- results of studies on the consumers' willingness to reduce their consumption of animal protein and the study of the consumers' behavior in this context;
- research into the consumers' behavior and assessment of the level of public acceptance of the new protein sources introduced to replace animal protein from the meat;
- assessment of the factors that positively and/or negatively influence the consumers' perception of the cultivated meat;
- possible strategies for the introduction of the cultivated meat and meat products into the diet.

Exclusion criteria:

- research not related to the consumers' behavior;
- research in the vegetarianism and veganism field;
- scientific works limited to the meat consumption analysis without taking into account changes in the food consumption structure;
- publications focused on the physiological aspects of meat consumption;
- studies on the consumption of the organic meat compared to the conventional meat;
- scientific publications targeted to analyzing the consumers' perceptions of alternative proteins — plant-based, including algae and legumes, and insect proteins.

The initial selection by the terms presented 793 articles, with the largest number of publications (97.7%) taking place within the period 2015–2024. 670 articles were excluded

because they did not meet the inclusion criteria. In particular, these articles were not related to consumers' behavior or their perception of the cultivated meat. As a result, 123 full-text articles were selected, 36 of which were excluded. For the final selection and selection of articles, the "snowball" method was used — a non-probability (chain) sampling method and the inclusion and exclusion criteria were considered. Thus, the reference list of the analyzed articles was used to identify additional publications. Only those documents were taken into account that contained a detailed analysis aimed at studying the perception of the cultivated meat by consumers, as well as the factors that determine and influence consumers' attitudes towards it. The literature search and "snowball" build-up were conducted until new correlations and information ceased to be found. As a result, 87 articles were included into the systematic review. Duplicates of articles were screened out and were not considered.

### **Alternative meat substitutes (protein sources)**

Meat is an essential source of protein, of fat, iron and many other nutrients crucially essential to humans. Meat is a food that has significant cultural and social significance, as its consumption is associated with hedonism, satiety and celebratory moments. However, environmental, nutritional, social and moral issues associated with its production, processing and consumption are gradually stimulating demand for alternative proteins [34].

Market trends contribute significantly to the high demand for meat, including poultry, as the alternative protein segment accounts for less than 4% of the total global protein share. At the same time, the accelerated growth of the alternative protein industry (its compound annual growth rate is 2–3 times higher than that of meat, including poultry) and its market penetration, especially among the fast-growing sector of the flexitarians [35,36], facilitate the search for new ways of protein producing. These methods should ensure food security for the growing global population, while promoting environmental protection and animal wellbeing. Since the industrial revolution and changing eating habits, people's need for meat has increased many times over. There is an interesting phenomenon related to economic stability and meat consumption. Meat consumption is higher in the developed countries of the world, and its consumption keeps gradually increasing as the number of middle-income people increases worldwide. Taking this trend into consideration, it is feasible to develop an efficient meat production system to satisfy the future meat demand [37].

Pathways to reducing natural meat production may include reducing meat consumption in favor of unprocessed plant-based sources, developing various "meat alternatives" based on plant proteins, fermentation proteins, invertebrate proteins, or lab-grown proteins, based on farm animal cells [7,38].

Alternative protein sources are used to substitute the protein-rich animal products, and are an integral part of sustainable food systems that satisfy human protein needs,

which are predicted to nearly double by 2050. It was noted that there are two opposing trends in protein consumption: low-income populations are shifting from plant-based to animal-based protein sources, while high-income populations are seeking to substitute animal-based protein sources with alternatives [39].

The studies conducted have systematized groups of the products that are alternatives to animal/fish proteins [39,40]:

- 1) using the substitutes. This option provides for the using of readily available substitute of the target compound, like available vegetarian diet options;
- 2) modification of existing non-animal/non-fish protein sources. This option provides for the modifying the available non-animal/non-fish protein sources in order to replace the target compound with, for example, insects-derived protein;
- 3) creation of alternative sources of proteins. This option is innovative and offers the greatest potential for solving the most complex problems. It involves the use of new technological processes for the production of proteins, such as 3D bio-printing, cell culture products, precise fermentation, etc. in terms of product characteristics / in the context of creating a new product / in terms of product production;

The most important groups of alternative proteins are insect-derived proteins, as well as plant proteins including algae and legumes, and the cultivated meat [41,42]. However, the potential of the cultivated meat, algae and insects as an important part of the future diet is considered to depend on nutrient bioavailability and digestibility, food safety, production costs and the consumers' acceptance [43].

Meat substitutes made entirely from plant components are increasingly present on the market, and their share is gradually increasing. Most products are based on soy proteins, milk proteins, wheat proteins or on mycoprotein. Although texturing technologies of improving the sensory perception and taste of these products are constantly being improved, it is quite difficult to accurately imitate meat when using plant proteins, carbohydrates and fats. Therefore, plant-based meat substitutes are mainly used in processed meats such as hamburgers, sausages or other types of minced meat products [17].

Insects are another source of alternative proteins. As food the insects are generally considered a healthy, nutritious alternative to conventional meat products such as chicken, pork, and beef. At all stages of their life cycle, they contain significant amounts of protein (40% to 70% of dry matter), minerals such as calcium, iron, zinc, and vitamins. Their amino acid composition is similar to amino acid composition of beef and soy. The content of unsaturated fats is 10–30% of dry matter" [44,45].

In addition, insect proteins in average are digestible better (76–98%) than plant proteins, such plant proteins from peanuts and lentils (52%). The digestibility of insect proteins is only slightly lower compared to animal proteins in beef and egg whites (100%) [45,46].



However, it should be noted that persistent negative social attitudes towards insect consumption hinder the expansion of the global food market and limit the use of insects as a food option. This may be due to the fact that people are skeptical about new products due to neophobic tendencies, as they consider some products to be exotic, “disgusting” and alien to European food culture [45,47]. In studies of attitudes towards insect consumption among people with different dietary styles (omnivores, vegans and non-vegan vegetarians), it was found that vegans have the highest neophobia scores not because they express disgust towards insects, but because of their ethical objections to eating animals or animal products in general. Much more favorable attitudes were observed among non-vegan (non-strict) vegetarians, who are more concerned with environmental sustainability than animal rights and who believe that insects are not “proper” animals and that’s why can be eaten [11].

One of the alternatives to animal proteins is the actively developing sphere of biotechnology — meat production by *in vitro* cell culture, or production of the cultivated meat, which will provide the population with a sufficient amount of meat by creating a complex structure of muscles of the farm animals without deteriorating the taste qualities. The introduction of this product will reduce dependence on traditional animal husbandry, but it should be noted that there are technical challenges in meat tissue reproducing [48,49].

*In vitro* meat production is a potential viable alternative to the natural meat that could provide consumers with a product that is indistinguishable from the original, with very similar nutritional and culinary value. That is, the cultivated meat should either be similar in taste, aroma, appearance (including color, texture, tenderness) and nutritional value, or should even be superior to “animal” meat. Given that currently available alternative products often do not have comparable properties to their animal-based analogues, still there is a long way to go before reaching the industrial production of the cultivated meat. Important issues to consider include scalability of the production process, quality control of mammalian cell/tissue cultures, maintaining sterility in culture, preventing contamination or diseases development, and controlled breeding of stem-cell-donor animals [17]. In addition, there is also the challenge of the product marketing, which arises due to the certain features of society’s perception of the cultivated meat. According to research [50], it is impossible to predict precisely the attitudes towards a product since it has not yet been fully introduced to the market.

Growing meat in labs and factories will likely change the meat industry. It will take time, will take a lot of researches and developments, and a gradual change of the negative perception of alternative meat among the consumers [17].

No doubts the meat industry of the future will be more complex than the meat industry of today, with a greater number of meat products or meat substitutes on the market obtained from different sources or processes. For suc-

cessful marketing of the new products, the new products should be a commercially viable alternative to conventional meat production. The success of the cultivated meat as an alternative option, as substitute or as supplement to conventional meat will play an important role, because the consumers will highly likely turn to the products with similar market positioning [26].

It should be noted that early studies suggested the cultivated meat’s potential to reduce land use by 99%, water consumption by 96%, and energy consumption down to 45% [51]. Subsequent studies have shown that as the cultivated meat production has smaller ecological footprint than beef production and lower greenhouse gas emissions than poultry, pork, and beef production, it requires more energy than poultry and pork production and yet is comparable with beef production. A controlled production environment, in which the cultivated meat is produced, could provide conditions for improved public health and safety, reducing the risk of diseases [19,26]. However, a number of authors have noted that large-scale cell culture production cannot be perfectly controlled and that unexpected biological mechanisms, such as cancer cell proliferation, may arise during the production process, which is a health concern for the consumers [26].

Alternative protein sources such as legumes, algae, insects, plant-based meat alternatives and the cultivated meat [52,53] are generally considered to be healthier and more environmentally friendly than the traditional animal proteins. However, the advantages of producing alternative proteins to meat still have not been fully scientifically proven, especially with regard to benefits for the environment. For example, it is still not clear whether the cultivated meat will be produced in a more sustainable manner than conventional meat. For example, analysis shows that high-tech and potentially destructive innovations require high degree of societal coordination to make them viable. At the same time, the potential sustainability benefits of these technologies may be limited by necessity of intensive processing that includes significant energy consumption and significant losses during the conversion of the raw material into final products. Thus, the priority given to meat alternatives with limited environmental potential is not only an issue of technological optimization of the production systems, but are also the second-order problems related to formulating the necessary tasks, creating control networks, evaluating innovative solutions and economic-technological representation [41].

All of the above meat alternatives are being researched and implemented, but so far no specific strategy has proven to be perfect or a completely implementable solution [7,17]. Furthermore, the researchers acknowledge that meat alternatives are currently embedded in “very different socio-legal regimes.” In practice, this means that regulatory ambiguities and barriers are relevant for more innovative types of alternative proteins [41,54,55].

### **Paradox of pantophagy and food neophobia**

The food industry is constantly encountering the necessity of finding the new concepts in order to meet increasingly specific demands of the consumers. However, innovative food products, such as meat alternatives, do not always become part of consumers' habits nor create a market. One of the main sources of resistance to nutrition novelty is the consumers' attitude, who in some cases treats a new product with suspicion or hostility due to specific ideologies, excessive adherence to traditions or due to neophobia.

In addition to availability and economic factors, all other factors that determine food choice can be divided into biological (genetically determined), cultural, or individual (psychological) factors. These three categories can be applied to human universal food preferences, to differences between cultures, and to individual differences within one culture [56].

The paradox of pantophagy, first described by the psychologist Paul Rozin, is the tension and fear, that people experience when choosing food. These feelings arise from the conflict between the desire to vary the diet and try new foods, on the one hand, and the fear of unfamiliar foods or possible disgust to them due to safety concerns, on the other.

Thus, a person's attitude to food is characterized by duality, expressed in fluctuations between food neophobia (distrust to the new products) and food neophilia (curiosity and attraction to some food novelty). However, as scientific literature analysis shows, it is not always easy to understand when the consumers' resistance can be overridden by improving the product [57], and when it is explained by personal opinion and thus cannot be quickly eliminated.

Paul Rozin, who first described food neophobia, suggested that it has an adaptive and evolutionary function. As omnivores, humans must follow the strategy to avoid toxic foods and to prefer foods that are beneficial to their health and growth [56]. Evolutionarily, this is facilitated by neophobia from the moment a child begins to move independently of his/her parents. Aversion to bitterness, for example, due to innate hedonic neurobiological mechanisms, helps a child avoid eating potentially toxic plants and may last as long as adulthood [11].

A number of researchers have identified disgust as a major concern with the cultivated meat [27,58]. Wilks et al. [28] measured sensitivity of disgust, which is an individual's predisposition to experience disgust when stimulated by various stimuli, which is thought to be a predictor of food choice behavior and disgust reactions. The results of the study showed that food neophobia was the highest predictor of willingness to try the cultivated meat and perceiving the health benefits of the cultivated meat. Various factors are responsible for the development of food disgust, with cultural and social norms leading to deeply ingrained perceptions of disgust. Disgust sensitivity has been used to determine acceptability of novel foods, including novel animal products and novel food technologies [28].

The food neophobia scale reflects attitudes or emotions associated with food, so a better understanding of the values specific to a particular culture may be more efficient in collecting knowledge about whether such new products match the consumers' profile [58].

Consumers believe that food safety is an essential requirement for product quality [59], as consuming unsafe food can cause harm to human health. Indeed, studies have proven that fear of harmful effects is one of the main factors in the consumers' refusal to try new food products. It is suggested that consumers' perception of food safety risks contributes to the food neophobia development [5].

Since neophobia puts obstacles to the desire to try new foods, while neophilia promotes it, addressing both poles of the paradox of pantophagy is a promising approach to better understanding the consumers' perceptions of the cultivated meat [5].

### **Consumers' reactions to the cultivated meat**

Researches of the consumers' acceptance of the cultivated meat have become numerous in recent years and have identified a consistent set of motivators and barriers to its future large-scale consumption. Although the consumers in general acknowledge the animal and environmental safety benefits of the cultivated meat, many of the yet have concerns about taste, price, and safety, as well as ethical, cultural, and religious issues.

Results from various studies show that the consumers' perceptions of the cultivated meat have mixed nature [2,29,60,61].

People's sensitivity to the sufferings of the farm animals has contributed to the rise of vegetarianism popularity. However, this has not reduced the desire to eat meat, especially among the consumers with higher incomes, who nevertheless also state that they do not want to contribute to animals' sufferings. From this perspective, the cultivated meat is an excellent compromise option for the animals' wellbeing and for addressing the ethical concerns of meat consumers [11].

Although the cultivated meat is unlikely to enter the market at the nearest future, potential producing companies are already studying the profiles of potentially interested consumers. Providing information, especially about the environmental benefits, is important to create a positive opinion among the potential consumers. Lack of awareness about the new technologies has been referred to as a cause of mistrust, uncertainty, and concerns about potential long-term negative impacts [27,50].

Many studies have shown that while most consumers were willing to try the cultivated meat, only few were willing to buy it, especially at a higher price [50,62]. Although many consumers supported the idea of the cultivated meat production, they chose not to consume it, considering the product beneficial to society but potentially dangerous to themselves in particular. This attitude covers the cultivated meat to a greater extent than any other alternative proteins [29].

It's interesting that the results of all surveys show that meat eaters are potentially more interested in the cultivated meat than the vegetarians and the vegans. However, the boundaries between these two groups of the consumers are not definitely clear, and the majority of the consumers, who are interested in meat alternatives, are mainly meat eaters, while the vegans/vegetarians still remain a minority [9]. Vegetarians and vegans, despite being in favor of any alternative to intensive animal farming, seem to have no desire to try and consume a product that in any case is derived from animal raw materials [25].

The similar results, where the vegans and vegetarians are more positive about the cultivated meat but are less interested in tasting it in comparison with the meat eaters, have also been found in the studies conducted in the United States [63]. The explanation for this apparently contradictory behavior is that these categories of the consumers do not object to the production of the cultivated meat, but at the same time are not interested in eating it. In this regard, it is necessary to conduct a research of the people's motives when they choose the food products, since these motives are likely to be driven by strong internal logic, even if at first glance these motives seem contradictory. Positive consumers' perception of new products should not be interpreted as a sign of commercial success [63].

Paradoxically, the vegetarians who were not interested in tasting the cultivated meat, had higher expectations of its taste than the meat eaters, who were actually interested in purchasing it. It has been noted that consumers with the greatest interest in purchasing are predominantly young, well-educated, and knowledgeable about the cultivated meat [11,14,63].

Another group of the consumers to consider are those who are not ready to refuse from eating meat but who have already reduced their consumption or are considering doing so. They are known as meat reducers or the flexitarians. Unlike the vegans and the vegetarians, who have been the subject of research for decades, meat reducers have received little attention, and few studies have analyzed their motivations for reducing meat consumption [1,64].

Willingness to buy and consume the cultivated meat depends on a number of demographic and sociocultural factors: men (compared to women), liberals (compared to conservatives), and low-income respondents (compared to high-income respondents) were significantly more ready to try the cultivated meat [65]. A number of studies show a strong correlation between the political orientation and attitudes toward the cultivated meat [13,66]. Liberals were seen as more tolerant than conservatives and linked the consumption of the cultivated meat to other agendas of the animals' wellbeing and environment protection [66]. Right-wing political parties were more likely to support the basics of loyalty, power, and purity, while left-wing politicians were more prone to focus on concepts of harm-minimizing and care-maximizing. This may indicate a link between the attitudes toward the cultivated meat and approval of certain moral principles [13].

Gomez-Luciano et al. found that although the cultivated meat is perceived as more delicious than insect-based or plant-based meat in some markets, across the countries it is generally considered the least healthy, least nutritious, and unsafe alternative to animal proteins. Ideas of perceived healthiness and nutritional value of the cultivated meat took place among the most important predictors of willingness to pay for the cultivated meat across all countries studied [12].

Zhang et al. [62] examined the consumers' awareness, acceptance, and their willingness to pay for the cultivated meat. Their approach is different because they examined the consumers' perceptions before and after being provided with information about the cultivated meat. Before learning about the cultivated meat, most consumers were either against the cultivated meat or were neutral towards it. After receiving the additional information, the percentage of consumers who were against the cultivated meat decreased from 22% down to 12%. Most respondents were willing to try (85%) or even buy (78%) the cultivated meat after receiving the information.

The summary assessment of the valence of the consumers' perception of the cultivated meat showed that social and cultural benefits (minimal risks) were identified as driving forces and turned to be stronger motivators than health and safety benefits (minimal risks), which were classified as relatively strong driving forces. On the other hand, the concerns of the cultivated meat quality (minimal benefits) were defined as those causing strong disgust [67–69].

#### **Public opinion about the cultivated meat as an unnatural product and about the ethical aspects of its production**

People who are concerned about the naturalness of food products are less likely to accept the cultivated meat. Here the term "naturalness" refers to the extent at which this product is perceived as the product of natural origin (e. g. produced by conventional agriculture), as opposed to a technological process by which the product is produced "artificially" [70].

The first important ethical and legal question concerns the nature of the product, since it must be determined whether it is meat or not. According to the definition of the American Meat Science Association, not only for biological or technological reasons, but also for semantic and commercial reasons, "the cultivated meat" is not meat actually [15]. Indeed, meat is defined as "edible tissues of an animal, consumed as food" and "to be considered meat, *in vitro* meat must be originally derived from an animal cell, tested and found safe for human consumption, and be comparable in composition and organoleptic characteristics to the meat, naturally obtained from the animals," according to Woerner and Boler [71]. Consequently, the authors of this article consider that the use of the term "meat" has created an ambiguity that is beneficial to the proponents of the cultivated meat. They strive for elimination



of the negative aspects related to natural meat (environmental degradation, animals' sufferings) while focusing on the positive properties of meat for the consumers, like strength, vitality and a healthy lifestyle. In this way, start-ups could successfully introduce the name "meat" for these cultivated muscle fibers into everyday language. Indeed, the main keywords used in media articles are "meat" and, to a lesser extent, "food" [15]. It is therefore necessary to ensure that meat substitute products are correctly labeled (so as not to mislead the consumers) and that their nutritional value is comparable to the products they are intended to substitute.

There are many issues related to the cultivated meat industry that need to be addressed through appropriate legislation and regulations. Food adulteration is a major concern in the regulation of the cultivated meat, where the cultivated meat may be marketed as conventional meat or vice versa [21].

Although the environmental benefits of the cultivated meat may play a key role in changing the potential consumers' attitude, yet there is a general distrust due to its "unnaturalness" and concerns about the possible health consequences caused by new technologies [72]. Recent research has shown that the perceived unnaturalness of pure meat and concerns about its safety are two key psychological barriers to the acceptance of pure meat. While some people are reluctant to accept the cultivated meat due to its assumed unnaturalness, the others believe that naturalness is unimportant to their eating decisions. Similarly, while some people experience strong discomfort and fear in relation with new food technologies, the others are confident that new technologies are generally safe and scientifically based [73]. In other words, people vary greatly in their assessment of the foods naturalness (i. e., the importance of foods naturalness, given the degree of fear of new food technologies) [74].

It is a common belief that everything natural is healthy, while everything unnatural (artificial) is harmful to eat. This is just an assumption that has nothing to do with reality. In ancient times, there was no intensive animal husbandry, meaning that animal breeding became itself an unnatural process. Thus, the terms "natural" and "unnatural" are very ambiguous, especially in relation to the cultivated meat production [75]. Even though the cultivated meat is grown artificially in a laboratory, the product is similar to the original (regular meat) and does not pose a health risk [37]. Since meat is grown in the controlled environment, the chance of generating the harmful by-products, excessive fat and pathogens is reduced [19].

The biggest challenge to the general acceptance of the cultivated meat still lies in the consumers' acceptance, while researches found the varying levels of acceptance and purchase intentions across the cultures [7,29,75]. Qualitative investigations of the reasons for this uneven acceptance assumes that it is related to unnaturalness as it is perceived by the consumers, lack of trust in the technology and companies producing it, public health risks, and taste/price issues. It has been declared that "natural"

meat excites emotions, wakes up nostalgia for traditions at home, and the cultivated meat is associated with phrases such as "messing with nature" and "playing God" [58].

Idiomatic expressions such as "playing God" and "messing with nature" described the participants' ideas on the unnaturalness of the cultivated meat and were used to reject the technology or express doubts about its purported benefits, particularly in relation to nutritional value and health [27]. This reaction matches the findings of de Barcellos et al. [76], who found that consumers perceive new beef production technologies like shock wave processing as "messing with their food" and they prefer less invasive (and more familiar) technologies.

The researchers suggested that consumers' assessment of the cultivated meat as unnatural was, to some extent, an emotional reaction, as it was closely linked to feelings of disgust towards this new product. In the context of food, the term "natural" often possesses emotional appeal [77], and indeed, it can be argued that "natural" can evoke nostalgia and adherence to culinary traditions, identity, childhood memories or the home comfort.

Specific cultural and religious issues make the situation more challenging. There is disagreement among the religious communities, including Jews, Muslims, and Hindus, about the cultivated meat due to its uncertain status. In the consumers' survey on the cultivated meat among 3,030 participants, including Jews, Muslims, and Hindus, the majority of the participants responded that they would be willing to eat the cultivated meat [72]. However, both Muslim and Jewish authorities still debate whether the cultivated meat of any origin can be classified as halal or kosher, and in Hinduism there are also food restrictions on eating beef that require discussion [78].

It should be noted that some people ask question on the ethical status of the cultivated meat. The cultivated meat requires fetal bovine serum (FBS) as a nutrient medium, which is an animal product made from blood taken from cattle fetal through a closed blood collection system at a slaughterhouse. This raises serious ethical questions about the potential suffering of a living organism. The use of such a nutrient medium should be gradually abandoned, and therefore various alternative media are being sought. For example, a serum-free medium was developed that supported the propagation of satellite cells of turkey in nutrient culture [79,80]. Moreover, there are various serum substitutes that are a good alternative to fetal bovine serum. The example is Ultrosor G, one of many commercially available substitutes that contains all the nutrients necessary for the growth of eukaryotic cells (growth factors, binding proteins, adhesion factors, vitamins, hormones, and mineral trace elements) [21]. A serum-free medium made from maitake mushroom extract was also successfully used, with the growth rate in it being higher than with fetal bovine serum [79].

In addition to the culture media, some scientists fear that widespread use of the cultivated meat will encourage cannibalism, because once this technology is developed,

any type of meat can be grown in the laboratory using a cell line. This is also a serious ethical issue that requires proper legislation regarding production of meat worldwide [37].

### **Role of information in the optimization and acceptability of the cultivated meat**

Positive factors do not always increase consumers' acceptance. For example, Escribano et al. [81] found out that the aspects such as regional and local production, sustainability, environmental concerns, consumers' health, and product quality were not sufficient to increase the acceptance of the cultivated meat. Asioli et al. [82] reported that consumers, interested in new food products, would pay less for the cultivated meat labeled "no antibiotics ever" (i. e., with a human health claim) than for a product without such a label. Both studies were conducted online and provided the participants with technical information about the cultivated meat production. The availability of such technical information resulted in significantly lower preference for the cultivated meat. In comparison with the conventional meat [34,81].

And vice versa, the conventional meat labeled as the cultivated was preferred over the conventional meat labeled as the conventional before and after tasting, provided that the participants were adequately informed about the personal, social, or tasting benefits [83]. In this case the personal benefits gave rise to significantly higher positive expectations, followed by social and tasting benefits. Therefore, when promoting this new product, positive information, especially about its health benefits, may facilitate its acceptance by the society, in contrast to the technical or "anti-traditional" data [29,34,52].

Well-presented information can even override the sensory appeal of the product [52]. For example, when the consumers received positive information on the cultivated meat [83], their attitude towards a regular burger, presented as "the cultivated", remained unchanged after its tasting because it provided the same sensory experience as the conventional meat. This sensory similarity is critical to attracting meat eaters, who are more likely to choose the cultivated meat over the plant-based alternatives [52].

Moreover, the information about personal benefits leads to a significantly greater increase in acceptance of the cultivated meat than other information conditions, suggesting that messages aimed at persuading the consumers to eat the cultivated meat should focus primarily on the benefits for the consumers (rather than the benefits to the society, the environment, or the animals). Verbeke, W. et al. [27] noted that the latter is usually initially more obvious to the participants of the survey.

The researches assessing the impact of positive information on the perception of the cultivated meat have shown that information about the safety and nutritional properties of the product significantly influences the consumers' willingness to purchase it and to try. However, information about the taste of the cultivated meat, on the contrary, does not contribute to the formation of positive perceptions. The results also showed that providing positive informa-

tion increases the willingness to buy the cultivated meat, but does not affect the willingness to try it. It is clear that willingness to try depends on additional incentives that involve a more in-depth analysis of the nutritional profile and food preferences of the particular consumers' group.

Research has shown that women showed a higher willingness to replace conventional meat with the cultivated meat if they were informed about its safety for human health. Young adults (under 30), who are likely to be potential consumers, showed a greater preference for the cultivated meat if they were provided with information related to animal wellbeing and human safety [84]. This information was less efficient among the older respondents, which may indicate their preference for established habits and, therefore, their more cautious attitude towards the cultivated meat. Other categories that were less affected by the information were those who do not eat meat, those who do not intend to reduce their meat consumption, and the people with lower levels of education [25]. The observation of the latter group is consistent with previous studies reporting that people with higher education are more likely to make decision on the basis of analytical rather than an emotional approach, which possibly makes them more open for new dietary scenarios than less educated consumers.

Nomenclature and terminology as an information factor are also important. Bryant et al. [85] found that different product names provide a significant impact on the rates of acceptance. For example, the use of the term "pure meat" led to significantly higher acceptance than "lab-grown meat," while "the cultivated meat" and "animal-free meat" fall somewhere in between. In addition, it was found that the difference between the groups perception was explained by the positivity of the associations that respondents made. This suggests that the nomenclature affects the acceptance is through mechanism of association with the concepts that are more or less attractive for the consumers.

Another condition that influences the acceptance of the cultivated meat is the form of information presentation (framing). Thus, the use of frames that emphasize the social advantages of the cultivated meat or its similarity to the conventional meat lead to significantly higher rates of acceptance in comparison with the frames that emphasized the advanced scientific aspect of its production technology [29].

### **Possible strategies for promoting the alternative meat**

To increase the acceptability of the cultivated meat, it is important to inform and educate the consumers about new foods and methods of production.

Strategies to support the cultivated meat can use various approaches. Consumers' perception of the cultivated meat can be improved through different content strategies depending on specific consumers' preferences [66].

To cope with the criticism that the cultivated meat is unnatural, its proponents should focus on the benefits that the technology may bring [66,86]. Marketers can take advan-

tage of the moral ambiguity related to the conventional animal agriculture by drawing attention to an ethical issue that many meat eaters do not typically consider, and by presenting the cultivated meat as a transparent and credible option. However, the producers should be careful when moralizing this issue, as this approach may turn off not only the consumers but also the conventional meat producers whose investments may be vital to their success [86].

Another approach is to highlight the environmental benefits of the cultivated meat, although some evidence suggests that arguments based on self-interest (such as improved health and food safety) are likely to be the most persuasive [83]. In particular, the prevention of antibiotic resistance development and zoonotic pandemics favorably show up the cultivated meat in comparison with the conventional animal agriculture, which is often criticized for these disadvantages [87].

The long-term success of the cultivated meat will depend on its ability to compete with the conventional meat in terms of price and taste. Experts agree that the cultivated meat is unlikely to compete on price with the conventional meat in the near future. This is considered a significant obstacle to widespread acceptance, and some experts believe that the cultivated meat will either occupy a luxury niche or will be associated with health benefits for the consumers to justify its higher cost [29]. As with any technology, it is likely that the price of the cultivated meat will reduce over time as the producers compete and production methods become more efficient.

Recent researches show that most consumers find the cultivated meat to be less tasty, as well as inferior in texture and appearance. This pessimistic approach to the quality of the cultivated meat can be seen as an opportunity: the cultivated meat companies can convincingly imitate the taste and texture of hamburger patties in order to exceed the consumers' expectations. Indeed, the high possibility of testing the cultivated meat compared to other technological innovations allows consumers experiencing the key aspects by themselves without much effort. Experts in the sphere consider it a priority to create a product that imitates not only the taste but also the texture and smell of conventional meat [29].

Using structural equations modeling method, Lin-Hi et al. [20] investigated the role of so far ignored organizational factors of the producing company (trustworthiness, reliability, corporate social responsibility, and external motivations) as preconditions for the consumers' acceptance of the cultivated meat, given its status as a radical innovation. The authors find that a key characteristic of the radical innovations is a high level of uncertainty regarding the consequences of their use, for example in terms of the lack of reliable knowledge about the potential functional shortcomings and social disadvantages of the product. The results showed that organizational factors matter for the consumers' acceptance of the cultivated meat, as perceived organizational reliability of the producing company signals the benevolence, honesty, and competence of the product

manufacturer or the seller, especially when the product's characteristics are perceived as ambiguous [20].

The authors note that the study has some limitations, taking into consideration that the acceptance of the cultivated meat is taken at an intentional rather than behavioral level. However, since the cultivated meat is currently not available to most consumers it is still not possible to measure the consumers' reactions to the cultivated meat in terms of actual purchasing behavior. While the cultivated meat is still in the process of its development, future studies should apply multiple methods to examine the consumers' perceptions from various angles. This will help set the foundation for analyzing actual purchasing behavior when such a product finally comes to the supermarket shelves [20].

### Conclusion

In modern society, where the alternative food products are available, people develop their own food identity by defining their eating behavior (whether they consider themselves as health-conscious, environmentalists, animal rights activists, or traditional omnivores, etc.). Therefore, future research should experimentally assess how these factors and benefits affect the consumers' acceptance of new food products.

The issues outlined in this review may form the basis for efforts to formulate a standard description and set of measures that can be used in future studies to obtain more commensurate and comprehensive data on the perceptions of various consumers' groups towards the cultivated meat and on assessment the actual consumers' behavior. In particular, future research should examine the most effective ways to handle the concerns about the "naturalness" of food products, given the central role of naturalness in the perception of safety and acceptance of new food technologies in general. The consumers' concerns about the unnaturalness of the cultivated meat should be solved to encourage them to become more familiar with the product and change their attitudes towards it. One way to do this may be using less technical terminology and product labelling. Information about the production (benefits and risks) of the cultivated meat should be as accessible and transparent as possible.

Moreover, it should be taken into consideration that cross-cultural and ethical directions in the consumers' perception researches are directly related to researches of understanding the food identity profile of the members of the focus group being investigated, and may be important for the formation of future marketing or regulatory strategies.

The consumers' perceptions of the cultivated meat will continue changing in the coming years as the technology becomes commercialized. The better awareness of the new product, including legal regulation and commercial availability, media coverage, and opportunities to try the product samples, along with the development of strategies to build positive attitudes towards food innovation, are all factors that are likely to facilitate the consumers' acceptance of the cultivated meat.



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Completely prepared the manuscript and is responsible for plagiarism.

The author declares no conflict of interest.





# EFFECT OF REPLACING NITRITE WITH GINGER POWDER IN BRINE SOLUTION ON THE QUALITY OF CURED BEEF

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**Keywords:** brine, cured beef, ginger powder, nitrite, quality

## Abstract

The purpose of this study was to evaluate the effect of replacing nitrite with ginger powder in brine on the quality of cured beef. Five kilograms of fresh beef from a mature White Fulani bull was purchased and used for this study. The excessive fat and connective tissues were trimmed off the meat, and the meat slab was chilled at 4 °C for 24 hours before further processing. Nitrite, salt, dextrose and ginger powder were purchased, measured out to prepare brine solution of five concentration levels each level constituted a mode of treatment, where  $T_0$  served as control reference sample with nitrite, which nitrite was replaced with ginger powder in the following concentrations:  $T_1 = 10\%$ ,  $T_2 = 15\%$ ,  $T_3 = 20\%$  and  $T_4 = 25\%$ . The beef was cured with brine injecting and immersing the samples into brine solution for 72 hours, and refrigerated at 4 °C. The cured beef samples were taken out of the brine, rinsed, wrapped into foil paper and baked in the oven at 204 °C for 20 mins and cooled to room temperature (27 °C). Cured beef samples data were analysed with the help of analysis of variance (ANOVA) following the procedures of SAS (2002) with means significance determined at  $p < 0.05$ . There were significant differences ( $p < 0.05$ ) in the physical properties, proximate analysis, vitamin and mineral contents, microbial loads and organoleptic characteristics of beef cured with ginger powder in brine, in  $T_3$  featuring the best-quality and highest ( $p < 0.05$ ) overall acceptability. In conclusion, ginger powder used in this study as replacement of nitrite enhanced the overall quality of cured beef without detrimental consequences for the consumers, especially in  $T_3$  which concentration was therefore recommended.

**For citation:** Apata, E. S., Lasisi, D. T., Olaleye, O. O., Apata, O. C., Okolosi, J. E., Uthman-Akinhanmi, Y. O. et al. (2025). Effect of replacing nitrite with ginger powder in brine solution on the quality of cured beef. *Theory and Practice of Meat Processing*, 10(1), 67–74. <https://doi.org/10.21323/2414-438X-2024-10-1-67-74>

## Introduction

Meat is the most valuable livestock product it serves as the first-choice source of protein for human population. However, it is an ideal medium for many micro-organisms for its being nutritious for them, as it provides a suitable environment for proliferation of spoilage bacteria and other food-borne pathogens [1]. Processing of meat products was borne out of the need to preserve meat for its later consumption and to make it available over a long period [2]. Meat preservation in brine has been practiced from immemorial time till modern time. The meat industries worldwide use the methods such as immersion into brine and injection of brine to improve the quality, colour. Moreover, the advanced applications like high pressure pulse vacuum and ultrasound treatments are currently being applied in meat brining for the purpose of improving the texture, colour, sensory characteristics and overall quality of meat [3]. Brining is a method of curing meat and the main ingredients or components of brine used in curing meat are water, salt (NaCl) nitrate or

nitrite and phosphates in mixture [4]. Nitrate and nitrite play important role on the safety and quality of cured meat products, and sodium or potassium nitrite are the most widely used as curing agents because it inhibits the growth and formation of neurotoxin *Clostridium botulinum*, hinders the development of oxidative rancidity, develops the peculiar flavour of cured meat and reacts with myoglobin to stabilize the red meat colour [5,6]. However, concerns over the safety of consuming nitrate or nitrite have arisen in modern times. The research [7] stated that the inhibitory effect on nutrient absorption in the intestine is caused by the adverse effects of nitrites and nitrates. The work [8] as well as [9] reported that nitrite in the acidic conditions of the stomach causes formation of nitrosamine which is carcinogenic compound. A study by [10] showed that application of nitrite and nitrate to preserve meat products increase the risk of gastric cancer development. This is defined by the activities of the bacteria naturally present in the meat or by addition of bacteria possessing a nitrate reductase activity which

include staphylococci, micrococci and lactic acid bacteria [11,12]. There is also the challenge of sodium chloride super doses presence in cured meat. This is because most brine-enhanced meat products contain high salt concentration of 200 mg to 500 mg of sodium per 100 g of meat product [13] and this could be dangerous, as excessive sodium intake has serious implications for human health especially the development of hypertension [14,15]. As a result, public health and regulatory authorities as well as meat processing industries are developing strategies to reduce sodium intake and to research into alternative substitute for salt, nitrate and nitrite for their application in meat products preservation [16]. Such substitutes are available in most of the plants spices such as ginger. It is an edible root rhizome or root part of the plant *Zingiber officinale* that belongs to the family *zingiberaceae* which has spicy and aromatic taste and smell due to phenolic compounds and volatile and non-volatile essential oils such as shogaols and gingerols. Ginger root is calories free and serves as good source of essential vitamins and other nutrients good for human health [17]. Ginger is well reported [18] as a spice used as food seasoning due to its sweet aroma, pungent taste and for having antioxidant activity that prevents oxidation of lipid as well as provides antimicrobial capacity to serve as effective alternative for either nitrate or nitrite in the prevention of meat deterioration and enhancement of meat quality [19].

This study was therefore carried out to investigate the effect of replacing nitrite with ginger powder in brine on the quality of cured meat to fill the gap in the literature.

## Materials and methods

This study was carried out in the Meat Science laboratory, Department of Animal Production, Olabisi Onabanjo University, Ayetoro Campus, Ogun State, Nigeria.

### Experimental materials

Five kilograms of beef from mature White Fulani bull was purchased from a reputable slaughter at Ayetoro city in Ogun State, Nigeria. The excessive fat and connective tissues were trimmed off the meat and was chilled at 4°C for 24 hours before its further processing. 1 kg of fresh beef was allotted to each of the 5 treatments, which piece was further cut into 5 replicates of 200 g per one replicate. Ginger power, salt, nitrite and dextrose were purchased from local market within the study area.

**Table 1. Percentage of the ingredients of brine solution**

Ingredients (%)	Treatments				
	$T_0$ (N)	$T_1$ (GG) 10%	$T_2$ (GG) 15%	$T_3$ (GG) 20%	$T_4$ (GG) 25%
Distilled water	82.00	77.00	72.00	67.00	62.00
Salt (NaCl <sub>2</sub> )	10.00	10.00	10.00	10.00	10.00
Sodium nitrite	0.5.00	—	—	—	—
Ginger	—	10.00	15.00	20.00	25.00
Dextrose	03.00	03.00	03.00	03.00	03.00
Total	100.00	100.00	100.00	100.00	100.00

N = Nitrite, GG = Ginger.

### Measurement of experimental materials

A digital sensitive scale Model WT-3003N (WANT Balance Instrument Co., Ltd, China) was used to measure out ginger powder, salt (NaCl), nitrite and dextrose for conducting research in the Meat Science, Laboratory in the Department of Animal Production, Olabisi Onabanjo University, Ayetoro Campus, Ogun state, Nigeria.

### Experimental brine solution preparation

Brine solution was prepared following the procedures of [4] as shown in Table 1.

### Experimental design

Five levels of brine concentrations were prepared and each level constituted a treatment mode where  $T_0$  served as control reference sample with nitrite, which nitrite was replaced with ginger powder in the following concentrations:  $T_1 = 10\%$ ,  $T_2 = 15\%$ ,  $T_3 = 20\%$  and  $T_4 = 25\%$  respectively.

### Curing of beef

The curing of beef was carried out following the procedures described by [4], when brine solutions (20 mls) were manually injected into 200 g replicate of beef in each treatment, using syringes of 25 ml volume and the needles, one syringe and needle per one treatment. The injected beef samples were immersed into each of the brine concentration for 72 hours in a refrigerator at 4°C.

### Cooking of cured beef

The cured beef samples were taken out from the brine after 72 hours, rinsed, wrapped into foil paper and baked in a laboratory oven Model: LO-201G (Grieve Corporation, USA) at 204°C for 20 mins with its turning over with periodicity of 5 mins to avoid burning at internal temperature of 73°C [4]. The cured, cooked beef samples were taken out of the oven and cooled in a washed and cleansed desiccator to room temperature of 27°C and stored in a refrigerator at 4°C until conducting of laboratory analysis and measurements.

### Analytical measurements of cured, cooked beef

#### Physical characteristics

#### Cooking loss

Percentage of cooking loss of the cured beef was determined by recording the initial weight of the cured beef samples in each treatment and recording the final weight

of the cooked beef samples and calculating the percentage difference between the two measurements divided by initial weight and multiplied by 100. The result was recorded as the cooking loss which is represented mathematically according to [20] as follows:

$$\text{Cooking loss} = \frac{W_tCB_1 - W_tCCB_2}{W_tCB_1} \times 100, \quad (1)$$

where:

$W_tCB_1$  = initial weight of cured beef;

$W_tCCB_2$  = final weight of cured cooked beef.

#### *Thermal shrinking*

The thermal (heat) shrinking of cooked cured beef was determined following the procedures of [21]. The initial length of the cured beef minus the final length of the cooked cured beef was divided by the initial length and multiplied by 100. The result was recorded as the percentage of thermal shrinking as follows:

$$\text{Thermal shrinking \%} = \frac{CBL_1 - CCBL_2}{CBL_1} \times 100, \quad (2)$$

where:

$CBL_1$  — initial length of cured beef;

$CCBL_2$  — final length of cured cooked beef.

#### *Cooking yield*

The cooking yield of cured beef measurement was carried out following the procedures of [35] and [22], which was calculated as the final weight of cured cooked beef divided by initial weight of cured beef and multiplied by 100. Thus:

$$\text{Cooking yield \%} = \frac{W_tCCB}{W_tCB} \times 100, \quad (3)$$

where:

$W_tCCB$  = final weight of cured cooked beef;

$W_tCB$  = initial weight of cured beef.

#### *Water holding capacity (WHC) of cured cooked beef*

Water-holding capacity of the cured and cooked beef was determined following the procedures of [23] and [24]. This was determined by press method. An approximately 2 g of cured cooked beef sample was placed between Whatman filter papers (Caver Inc, Wabash, USA). The cured cooked beef was pressed between two 10.2 × 10.2 cm<sup>2</sup> plexiglasses at 2 kg/cm<sup>3</sup> absolute pressure for 1 minute with manual vice. It was calculated with the formula:

$$\text{WHC} = \frac{W_{twp} - W_{tdp}}{W_{tccb}} \times 100, \quad (4)$$

where:

$W_{twp}$  = weight of wet filter paper (g);

$W_{tdp}$  = weight of dry filter paper (g);

$W_{tccb}$  = weight of cured cooked beef (g).

#### *Chemical analysis of cured cooked beef*

The proximate analysis and pH analyses of cured cooked beef product were carried out following the procedure described by [25].

#### *Lipid oxidation*

The thiobarbituric acid reactive substance (TBARS) assay was used to determine the lipid oxidation of the cured cooked beef following the procedures of [26] while vitamins and minerals content of cured cooked beef were determined following the procedures described by [27].

#### *Microbiological analysis of cured cooked beef*

The microbial loads of cured cooked beef samples were determined following the procedures described by [28–30].

#### *Sensory evaluation of cured cooked beef*

The sensorial properties of cured and cooked beef were evaluated following the procedures described by [31]. The 10 panelists were involved from among the students and staff of Animal Production Department, Olabisi Onabanjo University, Ayetoro campus. They were instructed to the extent of content of the forms they would complete about the cured cooked beef, and were provided with unsalted biscuits and water for taste perception refreshing in between the cured cooked beef samples degustation. Samples from each treatment were given sequentially to the taste panelists. Samples were served on clean saucers and were evaluated independently. The panelists rated the cured cooked beef samples for its colour, flavour, tenderness, juiciness, texture and overall acceptability on a 9-point hedonic scale on which 1 = extremely dislike and 9-extremely liked.

#### *Statistical analysis*

Data collected from this study were subjected to analysis of variance (ANOVA) using [32], and the significant differences between means were separated with Duncan multiple range test of the same analytical tool at  $p < 0.05$ .

#### **Results**

The results of physical properties of cured cooked beef affected by replacement of nitrite in brine with ginger powder are shown in Table 2.

**Table 2. Physical properties of cured cooked beef as affected by nitrite replacement with ginger in brine**

Variable (%)	Treatments					SEM
	$T_0$ (control) (N)	$T_1$ (GG) (10%)	$T_2$ (GG) (15%)	$T_3$ (GG) (20%)	$T_4$ (GG) (25%)	
Cooking loss	17.50 <sup>a</sup>	18.00 <sup>a</sup>	17.24 <sup>a</sup>	15.60 <sup>b</sup>	18.05 <sup>a</sup>	1.05
Cooking yield	82.50 <sup>b</sup>	82.00 <sup>b</sup>	82.76 <sup>b</sup>	85.40 <sup>a</sup>	81.95 <sup>b</sup>	1.12
Thermal shrinking	15.39 <sup>a</sup>	17.00 <sup>a</sup>	13.85 <sup>c</sup>	11.44 <sup>d</sup>	17.10 <sup>a</sup>	0.88
WHC	66.50 <sup>b</sup>	63.80 <sup>c</sup>	67.07 <sup>b</sup>	68.75 <sup>a</sup>	63.26 <sup>c</sup>	1.08

a, b, c, d — means on the same row with different superscripts are statistically significant ( $p < 0.05$ );

N = nitrite, GG = ginger, WHC = water holding capacity, SEM-standard error of the means of proximate analysis, TBARS and pH.



### Cooking loss

There were significant differences ( $p < 0.05$ ) in the values of cooking loss beyond the levels of nitrite replacement with ginger powder in comparison between the control  $T_0$  and other treatments that featured higher cooking loss values than  $T_3$ .

### Cooking yield

Cooking yield of cured cooked beef values had similar patterns like cooking loss. Treatment 3 with 20% ginger powder featured the higher (85.4%,  $p < 0.05$ ) cooking yield than other treatments which showed less yield of cured cook beef.

### Thermal shrinking

Cured cooked beef in treatment 3 exhibited lowest value (11.44%,  $p < 0.05$ ) of thermal shrinking in cured beef during cooking (baking) while shrinking value was higher ( $p < 0.05$ ) in  $T_1$  and  $T_4$ , this was more than it was observed in  $T_0$ ,  $T_2$  and  $T_3$  respectively.

### Water-holding capacity

Cured and cooked beef in  $T_3$  had higher ( $p < 0.05$ ) water holding capacity than in other treatments with 68.75% value, while treatments 1 and 4 had the least ( $p < 0.05$ ) values of 63.26 and 63.80 respectively. Table 3 shows the results of proximate TBARS and pH analysis.

### Moisture content

The value of moisture content of cured cooked beef was lower ( $p < 0.05$ ) in  $T_0$  (59.23%) compared with other treatments, while the value of moisture was higher ( $p < 0.05$ ) in  $T_1$  (67.13%) and decreased down from  $T_2$  to  $T_4$  as the level of ginger powder inclusion in the brine solution increased.

### Crude protein

The crude protein value was lower ( $p < 0.05$ ) (16.73%) in  $T_0$  compared with treatments  $T_1$  and  $T_2$ , while  $T_3$  and  $T_4$  had higher ( $p < 0.03$ ) protein values, as the values of moisture decreased

### Fat content

The value of fat was higher ( $p < 0.03$ ) in  $T_0$ , and was lower in  $T_1$ ,  $T_2$  and  $T_3$ , while it was high in  $T_4$  with value similar to the values obtained in other treatments, except for  $T_3$ .

### Ash content

Cured cooked beef in control sample treatment ( $T_0$ ) had lower ( $p < 0.05$ ) ash content, while this value increased from  $T_1$  to  $T_4$  with the last two treatments featuring the highest ( $p < 0.03$ ) values of 3.85 and 3.90% respectively.

### Nitrogen free extract (NFE)

The control sample ( $T_0$ ) treatment had highest ( $p < 0.03$ ) nitrogen free extract (NFE) value of 26.26% followed by  $T_1$  and  $T_3$ , while  $T_2$  and  $T_4$  had the lowest ( $p < 0.05$ ) values.

### Thiobarbituric acid reactive substance (TBARS)

The TBARS value was higher ( $p < 0.05$ ) values in control sample ( $T_0$ ) and  $T_1$  with 0.05 mg/100g, and decreased down to 0.04 mg/100g in  $T_2$ , and further decreased to 0.03 mg/100g in  $T_3$  and  $T_4$  as the level of ginger powder increased in the brine solution. The pH was higher ( $p < 0.05$ ) in  $T_1$  to  $T_4$ , and lower ( $p < 0.05$ ) in  $T_0$  with the value of 5.20 though the values still fell within same scale of alkalinity.

### Mineral and vitamins

The results of some minerals and vitamins composition of cured, cooked beef are presented in Table 4. The results on minerals showed that the values of the elements increased as the percentage of ginger inclusion in the brine solution increased, and was lowest ( $p < 0.03$ ) in  $T_0$  (N control) except for sodium, which content was highest ( $p < 0.05$ ) in  $T_0$ .

The results of all vitamins content observed in the cured cooked beef processed with ginger in brine solution instead of nitrite showed that the values of vitamins were lower ( $p < 0.05$ ) in  $T_0$  than in other treatments, and it increased across the treatments from  $T_1$  to  $T_4$  as the percentage of ginger inclusion in the brine solution increased.

### Microbial load

Table 5 presents the results of the microbial loads of cured and cooked beef processed with ginger in brine instead of nitrite solution.

All the microbial counts of thermophilic organisms and others were higher ( $p < 0.05$ ) in  $T_0$  than in treatments  $T_1$  to  $T_4$  and the organisms load also decreased as the percentage of ginger in the brine increased.

**Table 3. Proximate composition TBARS and pH of cured cooked beef as affected by nitrite replacement with ginger in brine**

	Variable Treatments					SEM
	$T_0$ (control) (N)	$T_1$ (GG) (10%)	$T_2$ (GG) (15%)	$T_3$ (GG) (20%)	$T_4$ (GG) (25%)	
Moisture (%)	59.23 <sup>e</sup>	67.13 <sup>a</sup>	65.3 <sup>b</sup>	64.26 <sup>c</sup>	62.24 <sup>d</sup>	0.86
Crude protein (%)	17.73 <sup>c</sup>	18.02 <sup>c</sup>	20.22 <sup>b</sup>	22.86 <sup>a</sup>	23.46 <sup>a</sup>	0.22
Ether Extract (fat)	5.47 <sup>a</sup>	5.45 <sup>a</sup>	5.32 <sup>a</sup>	4.20 <sup>b</sup>	4.10 <sup>b</sup>	0.12
Ash (%)	1.21 <sup>c</sup>	2.40 <sup>b</sup>	2.67 <sup>b</sup>	3.85 <sup>a</sup>	3.90 <sup>a</sup>	0.08
NFE (%)	26.26 <sup>a</sup>	7.00 <sup>b</sup>	5.45 <sup>c</sup>	6.03 <sup>b</sup>	4.98 <sup>c</sup>	0.13
TBARS (mg/100g)	0.05 <sup>a</sup>	0.05 <sup>a</sup>	0.04 <sup>b</sup>	0.03 <sup>c</sup>	0.03 <sup>c</sup>	0.05
pH	5.20 <sup>b</sup>	6.20 <sup>a</sup>	6.25 <sup>a</sup>	6.30 <sup>a</sup>	6.35 <sup>a</sup>	0.04

a, b, c, d, e — means on the same row with different superscripts are statistically significant ( $p < 0.05$ );

N = nitrite, GG = ginger, NFE = nitrogen free extract, TBARS = thiobarbituric acid reactive substances, SEM = standard error of the means with different superscripts are statistically significant ( $p < 0.05$ ).

**Table 4. Content of particular minerals and vitamins in cured beef as effected by replacing nitrite with ginger in brine**

Variable Treatments						
	$T_0$ (control) (N)	$T_1$ (GG) (10%)	$T_2$ (GG) (15%)	$T_3$ (GG) (20%)	$T_4$ (GG) (25%)	SEM
Minerals						
Calcium (mg/100 g)	8.20 <sup>d</sup>	10.26 <sup>c</sup>	12.05 <sup>b</sup>	13.67 <sup>a</sup>	13.84 <sup>a</sup>	0.28
Magnesium (mg/100 g)	0.25 <sup>c</sup>	0.28 <sup>b</sup>	0.30 <sup>b</sup>	0.43 <sup>a</sup>	0.45 <sup>a</sup>	0.07
Sodium (mg/100 g)	104.30 <sup>a</sup>	93.34 <sup>b</sup>	90.78 <sup>c</sup>	87.90 <sup>d</sup>	85.44 <sup>e</sup>	0.88
Phosphorus (mg/100 g)	106.00 <sup>d</sup>	123.66 <sup>c</sup>	126.05 <sup>b</sup>	133.08 <sup>d</sup>	132.60 <sup>a</sup>	1.04
Iron (mg/100 g)	1.52 <sup>d</sup>	2.87 <sup>c</sup>	4.67 <sup>b</sup>	6.55 <sup>d</sup>	6.52 <sup>a</sup>	0.26
Zinc (mg/100 g)	2.34 <sup>d</sup>	4.59 <sup>c</sup>	5.70 <sup>b</sup>	6.98 <sup>a</sup>	6.75 <sup>a</sup>	0.16
Vitamins						
Vit. C (mg/100 g)	15.20 <sup>d</sup>	22.43 <sup>c</sup>	25.55 <sup>b</sup>	31.67 <sup>a</sup>	32.50 <sup>a</sup>	0.55
B-carotene (ug/100 g)	0.10 <sup>d</sup>	0.81 <sup>c</sup>	0.95 <sup>b</sup>	3.10 <sup>a</sup>	3.15 <sup>a</sup>	0.21
Niacin (mg/100g)	10.70 <sup>d</sup>	13.13 <sup>c</sup>	16.25 <sup>b</sup>	19.37 <sup>a</sup>	19.60 <sup>a</sup>	0.52
Riboflavin (mg/100 g)	0.08 <sup>e</sup>	0.13 <sup>d</sup>	0.15 <sup>c</sup>	0.18 <sup>b</sup>	0.20 <sup>a</sup>	0.01
Thiamine (mg/100 g)	0.15 <sup>e</sup>	0.17 <sup>d</sup>	0.19 <sup>c</sup>	0.21 <sup>b</sup>	0.22 <sup>a</sup>	0.02

a, b, c, d, e — means on the same row.

**Table 5. Microbial load of cured beef as affected by replacing nitrite with ginger in brine**

	Variable Treatments					SEM
	$T_0$ (control) (N)	$T_1$ (GG) (10%)	$T_2$ (GG) (15%)	$T_3$ (GG) (20%)	$T_4$ (GG) (25%)	
TVC (CFU/ml)	4.50 <sup>a</sup>	3.90 <sup>b</sup>	3.60 <sup>b</sup>	2.80 <sup>c</sup>	2.50 <sup>c</sup>	0.01
TCC (CFU/ml)	5.70 <sup>a</sup>	4.50 <sup>b</sup>	3.30 <sup>c</sup>	3.00 <sup>c</sup>	2.40 <sup>d</sup>	0.02
TFC (CFU/ml)	4.60 <sup>a</sup>	3.20 <sup>b</sup>	3.00 <sup>b</sup>	2.00 <sup>c</sup>	1.10 <sup>c</sup>	0.06
TSC (CFU/ml)	3.40 <sup>a</sup>	3.05 <sup>a</sup>	2.10 <sup>b</sup>	2.07 <sup>b</sup>	1.05 <sup>c</sup>	0.02
TECC (CFU/m)	3.10 <sup>a</sup>	2.40 <sup>b</sup>	2.21 <sup>b</sup>	1.80 <sup>c</sup>	1.60 <sup>c</sup>	0.04

a, b, c, d — means on the same row with different superscripts are statistically significant ( $p < 0.05$ );

N = nitrite, GG = ginger, TVC = Total viable counts, TCC = Total Coliform Counts, TFC = Total Fungal Counts, TSC = Total Salmonella Counts, TECC = Total E. Coli Counts.

### Sensory properties

The results of sensorial properties of cured, cooked beef prepared with ginger in brine instead of nitrite are shown in Table 6.

#### Colour

The results of assessment of the colour of cured cooked beef showed that  $T_0$  had the lowest ( $p < 0.05$ ) score while  $T_3$  and  $T_4$  had the highest one, while similar colour values were recorded for  $T_1$  and  $T_2$ . Colour of the product was observed to have increased in intensity as the level of ginger in brine increased against nitrite.

#### Flavour

The scores of flavour for  $T_0$ ,  $T_1$  and  $T_4$  were similar and lower ( $p < 0.05$ ) than the scores of the product in  $T_2$  and  $T_3$ . Increase in flavour score was observed in cured cooked beef in correlation with increasing the level of ginger in brine solution.

### Tenderness

The cured, cooked beef in treatment 4 had highest ( $p < 0.05$ ) tenderness score followed by that in  $T_3$ , while  $T_0$  featured the lowest ( $p < 0.05$ ) score for tenderness. The trend in tenderness of the product showed that it increased as the percentage level of ginger increased in the brine solution.

### Juiciness

The scores for juiciness of cured, cooked beef product for  $T_0$ ,  $T_1$  and  $T_2$  were similar, but lower ( $p < 0.05$ ) than the scores for  $T_3$  and  $T_4$ . However, the score for juiciness was higher in  $T_3$  compared with  $T_4$ .

### Texture

The results for the texture of cured, cooked beef indicated that the score was lower ( $p < 0.05$ ) in  $T_0$  than in other treatments while the score was higher in  $T_3$  than in  $T_4$ . Also, the product texture scores were similar for  $T_1$ ,  $T_2$  and  $T_4$ , but lower than the textural score for the product of  $T_3$ .

**Table 6. Organoleptic profile of cured beef as affected by replacing nitrite with ginger**

	Variable Treatments					SEM
	$T_0$ (control) (N)	$T_1$ (GG) (10%)	$T_2$ (GG) (15%)	$T_3$ (GG) (20%)	$T_4$ (GG) (25%)	
Colour	4.00 <sup>c</sup>	5.00 <sup>b</sup>	5.00 <sup>b</sup>	6.00 <sup>a</sup>	6.00 <sup>a</sup>	0.05
Flavour	5.50 <sup>c</sup>	5.70 <sup>c</sup>	6.70 <sup>b</sup>	7.75 <sup>a</sup>	5.60 <sup>c</sup>	0.04
Tenderness	4.30 <sup>d</sup>	5.45 <sup>c</sup>	5.57 <sup>c</sup>	6.70 <sup>b</sup>	7.79 <sup>a</sup>	0.03
Juiciness	5.00 <sup>c</sup>	5.30 <sup>c</sup>	5.43 <sup>c</sup>	7.50 <sup>a</sup>	6.35 <sup>b</sup>	0.03
Texture	4.30 <sup>c</sup>	5.43 <sup>b</sup>	5.55 <sup>b</sup>	6.90 <sup>a</sup>	5.80 <sup>b</sup>	0.04
OA	5.20 <sup>c</sup>	6.50 <sup>b</sup>	6.55 <sup>b</sup>	7.65 <sup>a</sup>	5.50 <sup>c</sup>	0.02

a, b, c, d — means on the same row with different superscripts are statistically significant ( $p < 0.05$ );

N = nitrite, GG = ginger, OA = Overall Acceptability, SEM = Standard errors of the means.

### Overall acceptability

The score for overall acceptability of cured, cooked beef was higher ( $p < 0.05$ ) in  $T_3$  followed by scores in  $T_1$  and  $T_2$ , and the least score was recorded ( $p < 0.03$ ) in  $T_0$  and  $T_4$ .

### Discussion

Meat curing with plain salt has been used to preserve meat and meat from the immemorial time [33]. There are four methods of meat curing, which include dry curing, wet curing which is also called brine curing, combination of dry curing and sausage curing, and predominantly salt and nitrate/nitrite are used for the curing process. Meat curing could lead to change in the physical properties of the meat sample during cooking [34]. These include cooking loss, yield, thermal shrinking and water holding capacity.

The previous researchers opined that lower cooking loss and thermal shrinking contributed to higher cooking yield due to greater water-holding capacity and moisture [20–24,35]. Crude protein in cured, cooked beef was lower in  $T_0$  compared with other treatments with ginger inclusion. A inverse linear correlation was observed between moisture content and protein content in the cured, cooked beef such that as moisture content decreased protein content increased, which was high but similar in  $T_3$  and  $T_4$ . This could be due to addition of protein in ginger to the cured, cooked meat [36]. These results were in agreement with the findings of [4] who reported that crude protein increased in opposite to decrease in moisture content of cured turkey drumsticks. Inclusion of ginger as replacement of nitrite in the brine could be responsible for decrease in fat content of cured, cooked beef at higher levels of  $T_3$  and  $T_4$ . Also, the ash or mineral contents of cured, cooked beef increased, while the thio-barbituric acid reactive substances assay (TBARS) results revealed that lipid oxidation values decreased as the level of ginger inclusion in the curing solution increased, thus showing that ginger is potent enough to hinder lipolysis in the cured, cooked beef, especially in  $T_3$  and  $T_4$  respectively. These results were similar to the report of [37] on protein and lipid oxidation in meat, and [36] on the effect of ginger rhizome powder addition and storage time on the quality of pork. The pH of cured, cooked beef probably due to ginger inclusion in the curing solution was acidic in  $T_0$  showing the characteristic of nitrite, while the pH increased from  $T_1$  to  $T_4$  depicting the alkaline nature of ginger in the curing solution. This pH value in  $T_1$  to  $T_4$  could predispose the cured, cooked beef to microbial attack due to high water content as it was demonstrated with the value of moisture content of the product as reported by [38].

Vitamins and minerals are very important in human diets for their playing various roles in human metabolism, growth and maintenance [39]. The results of mineral and vitamins composition of cured, cooked beef revealed that those beef samples cured with ginger contained more minerals and vitamins. This could be explained by the fact that ginger is very rich in minerals and vitamins, and these might have been added to the inherent mineral and vitamin elements in beef thereby more enriching the product. The microbial load profile of cured, cooked beef indicated that the value of the microbes decreased as the level of ginger inclusion in the brine solution increased across the treatments, with the lowest record of total *Escherichia coli* and *Salmonella enteritidis* and the figures for total viable microbes count, coliform and fungal counts did not exceed the permissible and recommended levels in any meat products [12] which made the cured, cooked beef safe and wholesome for consumption. The organoleptic profile of cured, cooked beef processed with ginger in brine instead of nitrite showed that ginger improved the colour of cured, cooked beef as the score of colour assessment got increased as the level of ginger in the cured, cooked beef increased, and got to the peak at both treatments  $T_3$  and  $T_4$ . The cured, cooked beef tenderness had similar scores as the colour which increased as the level of ginger inclusion in the brine solution increased, with  $T_4$  reaching the highest score. However, treatment 3 had the highest scores for flavor, juiciness, texture and overall acceptability which made cured, cooked beef in  $T_3$  the best sort assessed by the sensory panelists in this study. The acceptability of any meat product greatly depends on colour, flavour, juiciness and texture which are influenced by water holding capacity of the meat product [4,40,41]. Therefore, as the above characteristics were very high in the cured, cooked beef processed with ginger in brine solution hence ensuring panelists' high acceptability of the product especially  $T_3$ .

### Conclusion

The application of ginger as replacement of sodium nitrite in brine solution to cure beef proved to be significantly effective, the cooking yield and water holding capacity were higher, while cooking loss and thermal shrinking of the product were relatively low; proximate analysis, TBARS and pH of the product were appropriate, the vitamins and minerals were not abysmally lost in the products, while the microbial load values were not above the recommended values; the organoleptic profile of cured, cooked beef featured high consuming qualities in the treatment 3 demonstrating the highest characteristics. Therefore it was recommended that ginger at 20% could be used to replace sodium nitrite in brine solution for curing beef without any detrimental effect on the consumers' health.



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The authors declare no conflict of interest.



# MEATBALL PROPERTIES AS AFFECTED BY SUBSTITUTION OF TAPIOCA WITH PURPLE SWEET POTATO

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**Keywords:** antioxidant, meatball, microstructure, physicochemical properties, purple sweet potato

## Abstract

This research investigated the effects of substituting tapioca flour with purple sweet potato flour (PSPF) on the physicochemical properties, antioxidant activity, and microstructure of chicken meatballs during cold storage. Purple sweet potato is rich in anthocyanins, which act as natural antioxidants and colorants, potentially enhancing the functional properties of meat products. Chicken meatballs were formulated with different levels of PSPF substitution (0%, 5%, 10%, and 15%) and evaluated for cooking loss, water holding capacity (WHC), gel strength, pH, color, total anthocyanin content, lipid oxidation, and scavenging activity over 15 days at 4°C. Results showed that PSPF substitution significantly influenced cooking loss, WHC, gel strength, and color. The addition of PSPF increased total anthocyanin content and antioxidant activity, contributing to enhanced oxidative stability by inhibiting lipid oxidation. Microstructural analysis revealed that PSPF substitution led to a more homogeneous and compact matrix, reducing the porous nature of the meatballs. However, excessive substitution ( $\geq 10\%$ ) resulted in a decline in WHC and gel strength. The findings suggest that incorporating PSPF in chicken meatballs at a substitution level of up to 10% improves antioxidant properties while maintaining acceptable texture and physicochemical characteristics. This approach could serve as a natural alternative to synthetic antioxidants like butylated hydroxytoluene (BHT), aligning with the growing demand for healthier and functional meat products.

**For citation:** Hajrawati, H., Maruddin, F., Hakim, M.R., Yulianti, Y., Rasak, A.N.M., Suharyanto, S. (2025). Meatball properties as affected by substitution of tapioca with purple sweet potato. *Theory and Practice of Meat Processing*, 10(1), 75–83. <https://doi.org/10.21323/2414-438X-2024-10-1-75-83>

## Funding:

The research was financially supported by Hasanuddin University through State University Operational Assistance (BOPTN) with a Basic Research Scheme by a contract number 915/UN4.22/PT.01.03/2021.

## Introduction

Minced meat-based food products such as burgers, sausages, and meatballs are very popular products at this time. In the manufacturing process, these products use non-meat ingredients as binders and fillers. Many non-meat ingredients are added to various meat products to increase the nutritional value and product quality. Many types of binders and fillers such as tapioca flour, potatoes, rice flour, barley flour, and corn flour [1], and sweet potato powder are used in sausages or meatballs production [2,3].

Traditionally, meatball formulations incorporate tapioca flour as a binder due to its ability to improve texture and water retention [4]. However, to enhance elasticity and cohesiveness, synthetic additives such as sodium tripolyphosphate (STPP) are frequently included [5]. Additionally, butylated hydroxytoluene (BHT) is commonly used as an antioxidant to prevent lipid oxidation and maintain product stability during storage. However, recently, a lot of studies have been directed to produce functional meat products by modifying binders and fillers, especially to produce healthier meat products. Much research has

been focused on the use of binders and fillers that function as antioxidants, such as corn flour [6] and sorghum [7].

This is in line with the consumers' demands who desire functional food, especially foods with health benefits [8]. The use of natural antioxidants can preserve essential characteristics of a product [9] and maintain shelf life. Long shelf life can be achieved by the addition of antioxidants to prevent rancidity due to oxidation of unsaturated fatty acids and to retain nutritional value. In addition, antioxidants are used not only as preservatives but also as compounds that have important effects on biochemical reactions in the human body, suppressing oxidation processes and preventing chronic diseases associated with oxidative stress [10].

One of the ingredients that can be used as a binder/filler and is rich in antioxidants is Purple Sweet Potato Flour (PSPF) [11]. Purple sweet potato has been greatly acknowledged for its health benefits for humans. It is rich in vitamins including A and C, dietary fiber and some minerals including manganese [12]. This kind of purple sweet potato cultivar (*Ipomoea batatas* (L.) Lam.) is high in the anthocyanin content ( $13.73 \pm 0.13$  mg/100 g), which contributes to the purple color and natural antioxidants [13].



Some studies indicate that when purple sweet potato is incorporated into confectionaries and yoghurt products, anthocyanins show surprising nutraceutical properties, such as antioxidant, antimicrobial, anticancer properties, and improve cardiovascular and sight health [14]. Chemical compositions of PSPF consist of 6.91% moisture, 5.82% protein, 0.39% fat, 88.15% carbohydrate, 3.07% ash in dry basis and 380 cal [15].

Substitution of tapioca flour with PSPF is expected to improve the quality of chicken meatballs. However, the substitution of fillers can affect the physical and sensory properties of sausages. Filler and binder also play an essential role in functional properties of meat processed products such as emulsification, and water-binding capacity, and textural properties [16]. In addition, many non-meat ingredients used as fillers can affect the appearance, taste, and texture of food products [17].

Based on this description, it is necessary to conduct research to determine an influence of the substitution of tapioca flour with purple sweet potato (*Ipomea batatas* L.) flour (PSPF) on the physical, chemical, microstructure, and sensory properties of chicken meatballs. The purpose of this study was to determine the effect of substitution of tapioca with PSPF on the physicochemical properties, antioxidant activity, and microstructure of chicken meatballs stored at a low temperature of  $4 \pm 1^\circ\text{C}$  for 15 days.

## Objects and methods

### Meatball preparation

Fresh chicken meat and other ingredients were purchased from a local market. The meat was transported to the laboratory in ice boxes. The purple sweet potato was purchased from Tangerang, Indonesia. To prepare meatballs, chicken meat was sliced into small pieces and excess fat and visible connective tissue were trimmed. Then, chicken meat was ground with a meat grinder (grinder-model MK-MG1300, Panasonic Manufacturing Malaysia Berhad), using an 8 mm plate, divided randomly into five

groups, and mixed with purple sweet potato used at different levels, tapioca, salt, fresh garlic, pepper, seasoning, and sodium tripolyphosphate (STPP) in Table 1. Each mixture from five treatment groups was chopped for 5 min. The batter was shaped into balls (approximately 11 g) and cooked in  $80^\circ\text{C}$  water for 15 min. The core temperature of sampled meatballs was checked using a digital thermometer — thermometer food grade  $-40$  to  $280^\circ\text{C}$ , Krisbow 10106736 (Krisbow, Indonesia). All samples were kept in the refrigerator ( $4 \pm 1^\circ\text{C}$ ) and analyzed on the 1st, 5th, 10th, and 15th day of storage.

### Proximate composition

The proximate composition was determined by referring to according to AOAC [18] and carbohydrate was calculated by difference.

### Cooking loss measurement

Cooking loss was calculated as the difference between the uncooked sample weight and cooking weight divided by uncooked weight. Cooking loss is expressed as a percentage.

### Water holding capacity (WHC)

The water holding capacity was determined according to Jung and Joo [19]. Briefly, 10 grams of minced samples were homogenized with 40 ml distilled water and then incubated in a water bath at  $30^\circ\text{C}$  for 30 min. The homogenized sample was centrifuged at 3000 rpm for 30 min. The supernatant formed was removed and then the mixture was re-incubated for 10 min and the supernatant was removed again. The WHC was calculated as follow:

$$\text{WHC}(\%) = \frac{A}{B} \times 100, \quad (1)$$

WHC is water holding capacity, A is weight of the sample after removing supernatant, and B is the weight of the sample mixed with distilled water.

### Gel strength measurement

The gel strength was evaluated following the method adapted from Yusof et al. [20] using a texture analyzer (TAXTplus Stable Micro System Texture Analyzer, Goldamig, Surrey, UK), equipped with a 5-kg load cell and a crosshead speed of 1 mm/s. The test was performed in triplicate using a flat-bottomed plunger with a diameter of 27 mm (0.5 inch) to ensure accuracy.

### Folding test

The folding test was determined according to Nurul et al. [21]. Briefly, a meatball was shaped into a 3-mm-thick piece and then was tested by folding a sample using the thumb and forefinger. The sample condition after folding was expressed on a numerical scale as follows: score 1 if the sample was broken by, score 2 if the sample cracked immediately when folding into half, score 3 if the sample cracked gradually when folding into half, score 4 indicated the sample without cracking after folding in half, and score 5 if the sample showed no cracks after folding twice.

**Table 1. Formulation of chicken meatballs prepared with BHT and substitution of tapioca flour with PSPF**

Ingredients, g	Treatment				
	$P_0$	$P_1$	$P_2$	$P_5$	$P_7$
Chicken meat	400	400	400	400	400
Tapioca	80	80	60	40	20
PSPF	0	0	20	40	60
Ice cube	120	120	120	120	120
Garlic fresh	4	4	4	4	4
Pepper	4	4	4	4	4
Salt	7.2	7.2	7.2	7.2	7.2
Seasoning	4	4	4	4	4
STPP	0.12	0.12	0.12	0.12	0.12
BHT 0.01%	—	0.04	—	—	—

BHT = butylated hydroxytoluene.

Treatments:  $P_0$ , tapioca flour 20%;  $P_1$ , tapioca flour 20% + 0.01% BHT;  $P_2$ , tapioca flour 15%; PSPF 5%;  $P_5$ , tapioca flour 10%; PSPF 10%;  $P_7$ , tapioca flour 5%; PSPF 15%.

### pH value

The pH was measured using a digital portable pH-meter (HI 99163, Hanna Instruments, Eibar, Spain) by injecting the probe into 15-g meatballs and for 10 s to obtain the pH value.

### Color determination

Color analysis of meatballs was carried out using a Tes-135A color meter (Test Electrical Electronic Corp, Taipei, Taiwan). The color was measured at room temperature ( $23 \pm 2^\circ\text{C}$ ) in triplicate. The color meter was calibrated with a standard plate before use.

### Total anthocyanin content (TAC)

The total anthocyanin content in chicken meatballs was quantified spectrophotometrically as monomeric anthocyanin by the pH differential method according to Lee et al. [22]. The extract of the sample was diluted with 25 mM NaCl buffer (pH 1) and another extract with 0.4 M sodium acetate buffer (pH 4.5) with a dilution factor of 1:4 for extract: buffer. The solution absorbance was measured at 700 and 516 nm wavelengths after 15-min equilibrium time. The TAC was calculated using equations 2 and 3.

$$A = (A_{516} - A_{700})_{\text{pH 1.0}} - (A_{516} - A_{700})_{\text{pH 4.5}} \quad (2)$$

Monomeric anthocyanin pigment

$$(\text{mg/L}) = (A \times MW \times DF \times 1000) / (\epsilon \times 1) \quad (3)$$

where *MW*: molecular weight (449.2), *DF*: dilution factor, and  $\epsilon$ : molar absorptivity (26,900), 1: diameter of the optical path (1 cm).

### Scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was done according to Hajrawati et al [23]. One gram of the meatball was extracted with 5 mL of methanol for 24 h at room temperature. After that, 400  $\mu\text{L}$  of the extract was reacted with 3.6 mL of 0.1  $\mu\text{M}$  DPPH, then homogenized and allowed to react for 30 minutes in a dark place. The percent inhibition against DPPH was calculated as the percentage reduction in absorbance at a wavelength of 517 nm.

### Lipid oxidation (TBARS assay)

The 2-thiobarbituric acid reactive substances (TBARS) assay was performed to evaluate lipid oxidation of meat-

balls, following the method described by Sørensen and Jørgensen [24]. The results were expressed as 2-thiobarbituric acid-reactive substances (TBARS) in malonaldehyde/kg samples. The concentrations were determined at 532 nm. A standard curve was prepared using 1,1,3,3-tetra ethoxy propane (TEP).

### Statistical analysis

The data were analyzed by ANOVA and the differences among treatment means were assessed using Tukey's test. Means were considered significantly different at  $p < 0.05$ . The results are presented as means  $\pm$  SD. Analysis was performed using SAS statistical software, NC, USA.)

## Results and discussion

### Proximate composition

The chemical or nutritional properties of the chicken meatballs in this study are shown in Table 2. In general, the moisture, protein, and fat contents of meatballs were not significantly different ( $P > 0.05$ ) among treatments with an average of 70.51%, 18.04%, and 2.14%, respectively. Meanwhile, the substitution of tapioca flour with PSPF had a significant effect ( $P < 0.05$ ) on the ash content and crude fiber of meatballs. The ash and crude fiber of the meatballs were higher with the greater proportion of PSPF. The comparison of tapioca flour with PSPF 10:10% ( $P_5$ ) and 5:15% ( $P_7$ ) resulted in meatballs with significantly higher ash content ( $P < 0.05$ ) compared to other treatments. The results indicated that at the proportion of 10:10 and 5:15, the ash content increased because purple sweet potato contains higher ash than tapioca flour. The addition of BHT in chicken meatballs did not affect the proximate composition of chicken meatballs ( $P > 0.05$ ). Sweet potatoes from various varieties contain ash levels ranging from 2.22% to 4.34% [25], whereas cassavas contain ash levels between 1.01% and 2.06% [26]. The inclusion of these ingredients will augment the overall ash content in the products.

Data in Table 2 also shows that PSPF contains higher crude fiber than tapioca flour. It can be seen that with a ratio of 15:5 ( $P_2$ ) to 5:15 ( $P_7$ ) chicken meatballs contain significantly different percent of crude fiber compared to meatballs without PSPF substitution. It was due to the

**Table 2. Proximate composition of chicken meatballs with addition of BHT and substitution of tapioca flour with PSPF**

Formula	Chemical composition, (%)				
	Moisture	Ash	Protein	Fat	Crude fiber
$P_0$	70.69 $\pm$ 0.91	1.65 $\pm$ 0.06 <sup>c</sup>	17.98 $\pm$ 0.74	2.17 $\pm$ 0.16	0.22 $\pm$ 0.04 <sup>d</sup>
$P_1$	70.65 $\pm$ 0.43	1.67 $\pm$ 0.06 <sup>c</sup>	17.94 $\pm$ 0.61	2.09 $\pm$ 9.15	0.26 $\pm$ 0.04 <sup>d</sup>
$P_2$	70.66 $\pm$ 0.77	1.74 $\pm$ 0.03 <sup>bc</sup>	18.15 $\pm$ 0.73	2.08 $\pm$ 0.24	0.41 $\pm$ 0.03 <sup>c</sup>
$P_5$	70.58 $\pm$ 1.06	1.82 $\pm$ 0.03 <sup>ab</sup>	18.05 $\pm$ 0.81	2.20 $\pm$ 0.12	0.61 $\pm$ 0.16 <sup>b</sup>
$P_7$	69.96 $\pm$ 0.21	1.93 $\pm$ 0.02 <sup>a</sup>	17.96 $\pm$ 1.00	2.15 $\pm$ 0.28	0.79 $\pm$ 0.10 <sup>a</sup>
Average	70.51 $\pm$ 0.31 <sup>ns</sup>	1.76 $\pm$ 0.11	18.04 $\pm$ 0.07 <sup>ns</sup>	2.14 $\pm$ 0.05 <sup>ns</sup>	0.46 $\pm$ 0.24

A different letter following the data in the same row or column indicates a significant difference ( $P < 0.05$ ); ns indicates a non-significant difference ( $P > 0.05$ ). Ratios between tapioca and PSPF were 20:0% ( $P_0$ ), 20:0% + 0.01% BHT ( $P_1$ ), 15:5% ( $P_2$ ), 10:10% ( $P_5$ ) and 5:15% ( $P_7$ ).

higher content of crude fiber in sweet potatoes compared to the crude fiber content in cassava. The crude fiber in sweet potatoes from various varieties ranges between 1.74% and 4.81% [25], whereas the crude fiber in cassava ranges from 0.15% and 0.37% [27]. The high content of ash and crude fiber in meatballs containing PSPF was associated with the levels of these two components in PSPF. The nutritional characteristics of the meatballs from this study were in agreement with the criteria of the Indonesian National Standard (SNI). SNI meatballs 3818–2014 states that the moisture content of meatballs is max. 70%, ash is 3% max, protein content is 11% max, and fat is a maximum of 10% [28].

#### *Cooking loss, gel strength, WHC, folding test*

The physical properties of chicken meatballs with or without substitution with PSPF are presented in Table 3. Substitution with a ratio of 5:15% ( $P_7$ ) caused a significantly higher cooking loss ( $P < 0.05$ ) compared to other treatments. This result is also in line with other physical properties, where the gel strength decreased at the substitution ratio of 10:10% ( $P_5$ ) and 5:15% ( $P_7$ ), WHC and the results of the folding test decreased at the substitution ratio of 5:15% ( $P_7$ ) ( $P < 0.05$ ). These data indicate that the substitution of tapioca with 10:10% PSPF ( $P_5$ ) causes the physical quality of chicken meatballs to decrease. However, 15:5% substitution led to the results with characteristics equivalent to those without substitution. Meanwhile, the addition of BHT in chicken meatballs did not affect the physical properties ( $P > 0.05$ ).

A decrease in the physical characteristics of the meatballs at  $P_5$  and  $P_7$  is most probably caused by several physical properties of the two types of flour. One of the important physical properties is the pasting properties of flour. These properties are closely related to the nature of the suspension during the cooking process, which is affected by the viscosity of the flour. Shittu et al [29] reported that the viscosity of sweet potato is lower than tapioca. It influences the cooking loss, gel strength, WHC, and folding test. Viscosity properties affecting the physical properties of the product are peak viscosity, breakdown viscosity, setback viscosity, and final viscosity. The peak viscosity is closely related to the maximum swelling and breakdown of starch granules at the equilibrium stage [30]. The low viscosity parameter causes the product to have a low cooking loss, gel strength, WHC, and folding test.

Another factor that may affect the physical properties of meatballs based on the flour used is the proportion of amylose and amylopectin in the starch [31]. Tapioca flour contains more amylopectin (about 87%) than other flour [31]. Meanwhile, sweet potatoes of various varieties contain amylopectin at a level of 76.2–78.1% [32,33]. Amylopectin has a high viscosity, which causes the starch in tapioca flour to be more sticky and viscous [32]. Amylose has properties that cause a product to become more solid or stiff [34]. This may have caused an increase in cooking loss and a decrease in gel strength, WHC, and folding test of meatballs that received a high proportion of purple sweet potatoes.

#### *pH value and color*

The pH values and color characteristics of chicken meatballs with and without substitution of tapioca flour with PSPF during cold storage for 15 days are presented in Table 4. The pH of meatballs was not affected by either substitution or storage time ( $P > 0.05$ ). The pH of the meatballs obtained was in the range of 6.06 to 6.11. The results of this study were in line with Al-Mamun et al. [6], who reported that the pH value of meatballs was not affected by the substitution of corn flour with tapioca. The similar pH value of all meatballs is probably because the pH value of tapioca flour and PSPF is also the same. The pH value of tapioca flour from several varieties and ages varied from 5.07 to 6.64 [35], and the pH value of PSPF from several varieties was 5.77–6.21 [36].

Each of the color characteristics of meatballs (lightness, redness, and yellowness) showed a different response (Table 4). The lightness and yellowness of the meatballs were affected by the tapioca substitution with PSPF and storage time, although there was no interaction between both ( $P > 0.05$ ). Meanwhile, the level of redness of the meatballs was influenced by the interaction between the substitution treatment and storage time ( $P < 0.05$ ).

Changes in the color characteristics of the meatballs are in sync with the increasing proportion of PSPF in the ingredients for making meatballs. The purple color in PSPF causes the redness level of the meatballs to increase and is accompanied by a decreased yellowness level. However, during storage, the redness and yellowness decreased. The brightness level of the meatballs began to decrease on the 10th day of storage. This is in line with the research by Jin et al. [37], who showed a decrease in the brightness level of sausages with additional PSPF. The purple color in PSPF changes after the meatball cooking process due to heating [38].

**Table 3. Physical properties of chicken meatballs with addition of BHT and substitution of tapioca flour with PSPF**

Formula	Cooking loss, %	Gel strength, g/cm <sup>2</sup>	WHC, %	Folding test
$P_0$	3.85 ± 0.94 <sup>b</sup>	1042.33 ± 82.57 <sup>a</sup>	31.34 ± 2.76 <sup>a</sup>	5.00 ± 0.00 <sup>a</sup>
$P_1$	3.92 ± 0.33 <sup>b</sup>	1009.55 ± 22.84 <sup>a</sup>	30.72 ± 0.75 <sup>a</sup>	5.00 ± 0.00 <sup>a</sup>
$P_2$	4.11 ± 0.34 <sup>b</sup>	1013.93 ± 55.68 <sup>a</sup>	30.66 ± 1.13 <sup>ab</sup>	5.00 ± 0.00 <sup>a</sup>
$P_5$	4.38 ± 0.46 <sup>b</sup>	899.53 ± 29.52 <sup>b</sup>	30.42 ± 1.99 <sup>ab</sup>	4.67 ± 0.58 <sup>ab</sup>
$P_7$	6.03 ± 0.31 <sup>a</sup>	797.21 ± 72.65 <sup>c</sup>	27.47 ± 1.47 <sup>b</sup>	4.00 ± 0.00 <sup>b</sup>

A different letter following the data in the same column indicates a significant difference ( $P < 0.05$ ). Ratios between tapioca and purple sweet potato flour were 20:0% ( $P_0$ ), 20:0% + 0.01% BHT ( $P_1$ ), 15:5% ( $P_2$ ), 10:10% ( $P_5$ ), and 5:15% ( $P_7$ ).



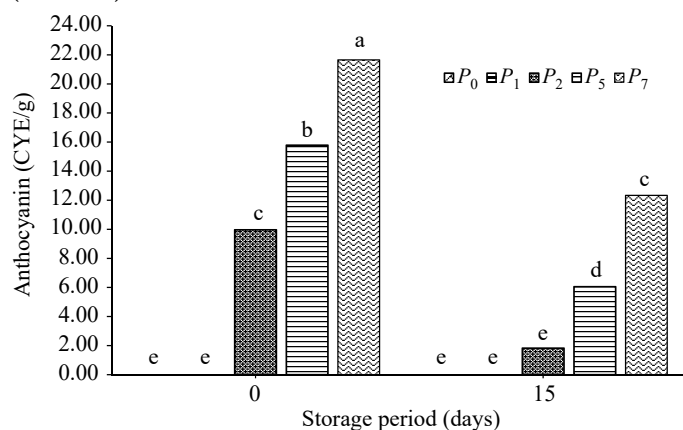
Table 4. Effect of BHT addition and substitution of tapioca flour with PSPF on the pH and color values of the chicken meatball

	Formula	Storage, day				Average
		0	5	10	15	
pH	P0	6.11 ± 0.05	6.09 ± 0.04	6.07 ± 0.07	6.09 ± 0.03	6.09 ± 0.02 <sup>ns</sup>
	P1	6.10 ± 0.03	6.10 ± 0.06	6.11 ± 0.05	6.11 ± 0.02	6.11 ± 0.00 <sup>ns</sup>
	P2	6.12 ± 0.03	6.08 ± 0.03	6.08 ± 0.02	6.09 ± 0.04	6.09 ± 0.02 <sup>ns</sup>
	P5	6.11 ± 0.02	6.08 ± 0.06	6.05 ± 0.06	6.05 ± 0.02	6.07 ± 0.03 <sup>ns</sup>
	P7	6.11 ± 0.06	6.09 ± 0.06	6.07 ± 0.11	6.09 ± 0.04	6.09 ± 0.02 <sup>ns</sup>
	Average	6.11 ± 0.01 <sup>ns</sup>	6.06 ± 0.01 <sup>ns</sup>	6.07 ± 0.02 <sup>ns</sup>	6.09 ± 0.02 <sup>ns</sup>	—
Ligthness	P0	73.57 ± 0.31	73.07 ± 0.06	72.17 ± 0.45	70.70 ± 0.66	72.38 ± 1.26 <sup>b</sup>
	P1	73.77 ± 0.57	73.43 ± 0.90	73.03 ± 1.01	72.53 ± 1.50	73.19 ± 0.53 <sup>a</sup>
	P2	59.13 ± 1.23	58.83 ± 1.38	58.43 ± 1.50	57.80 ± 0.95	58.55 ± 0.58 <sup>c</sup>
	P5	54.30 ± 0.96	53.83 ± 1.01	53.37 ± 1.16	53.30 ± 1.82	53.70 ± 0.47 <sup>d</sup>
	P7	51.30 ± 0.56	50.83 ± 0.45	50.20 ± 0.66	49.63 ± 0.84	50.49 ± 0.73 <sup>e</sup>
	Average	62.41 ± 10.6 <sup>a</sup>	62.00 ± 10.66 <sup>ab</sup>	61.44 ± 10.6 <sup>b</sup>	60.79 ± 10.3 <sup>c</sup>	—
Redness	P0	0.13 ± 0.06 <sup>i</sup>	0.17 ± 0.06 <sup>i</sup>	0.21 ± 0.40 <sup>i</sup>	0.40 ± 0.30 <sup>i</sup>	0.23 ± 0.12
	P1	0.13 ± 0.06 <sup>i</sup>	0.17 ± 0.06 <sup>i</sup>	0.23 ± 0.06 <sup>i</sup>	0.27 ± 0.10 <sup>i</sup>	0.20 ± 0.06
	P2	4.80 ± 0.10 <sup>g</sup>	4.30 ± 0.26 <sup>gh</sup>	4.20 ± 0.35 <sup>gh</sup>	3.87 ± 0.39 <sup>h</sup>	6.08 ± 0.41
	P5	6.53 ± 0.06 <sup>cd</sup>	6.27 ± 0.12 <sup>de</sup>	5.90 ± 0.30 <sup>ef</sup>	5.60 ± 0.50 <sup>f</sup>	7.20 ± 0.52
	P7	7.70 ± 0.10 <sup>a</sup>	7.50 ± 0.17 <sup>ab</sup>	7.07 ± 0.35 <sup>bc</sup>	6.53 ± 0.20 <sup>cd</sup>	3.30 ± 0.22
	Average	3.86 ± 3.55	3.68 ± 3.40	3.52 ± 3.18	3.33 ± 2.90	—
Yellownes	P0	13.70 ± 1.08	12.87 ± 1.10	12.67 ± 1.08	12.40 ± 1.23	12.80 ± 0.32 <sup>a</sup>
	P1	13.60 ± 1.13	13.33 ± 1.18	13.13 ± 1.10	12.87 ± 1.24	13.23 ± 0.31 <sup>a</sup>
	P2	7.07 ± 0.64	6.87 ± 0.72	6.67 ± 0.91	6.27 ± 0.76	6.69 ± 0.35 <sup>b</sup>
	P5	6.40 ± 0.20	6.00 ± 0.30	5.60 ± 0.31	5.47 ± 0.35	5.87 ± 0.42 <sup>c</sup>
	P7	6.33 ± 0.15	6.00 ± 0.46	5.73 ± 0.46	5.57 ± 0.51	5.91 ± 0.33 <sup>c</sup>
	Average	9.31 ± 3.73 <sup>a</sup>	9.01 ± 3.75 <sup>ab</sup>	8.74 ± 3.82 <sup>bc</sup>	8.51 ± 0.82 <sup>c</sup>	—

A different letter following the data in a row and column in the same variable indicates a significant difference ( $P < 0.05$ ). Ratios between tapioca and purple sweet potato flour were 20:0% ( $P_0$ ), 20:0% + 0.01% BHT ( $P_1$ ), 15:5% ( $P_2$ ), 10:10% ( $P_5$ ) and 5:15% ( $P_7$ ).

#### Anthocyanin content

Measurement of total anthocyanins was carried out on the 0th and 15th days of storage. The results for total anthocyanins in meatballs are presented in Figure 1. Figure 1 shows that  $P_0$  and  $P_1$  meatballs, both with the use of tapioca flour, did not contain anthocyanins. In the meatballs with PSPF substitution, the anthocyanin content was significantly different ( $P < 0.05$ ) and it was in line with an increase in the proportion of substitution with PSPF. However, during 15 days of storage, the total anthocyanin content in the meatballs decreased significantly ( $P < 0.01$ ).



**Figure 1.** Anthocyanin content of chicken meatballs with addition of BHT and PSPF substitution

A different letter following the bars indicates a significant difference ( $P < 0.05$ ). Ratios between tapioca and purple sweet potato flour were  $P_0$ ,  $P_1$ ,  $P_2$ ,  $P_5$ ,  $P_7$ .

The appearance of anthocyanins in the meatballs was due to the contribution of PSPF. Several studies have revealed that PSPF from various cultivars contains high levels of anthocyanins [11,12]. However, it is presumed that the total anthocyanin content in of meatballs decreased compared to the PSPF. This is because anthocyanins are less stable during heating [39]. However, a decrease in anthocyanins did not eliminate the anthocyanins in meatballs, so they continued playing a role in the color development and antioxidant properties in meatballs.

During storage, the anthocyanin content decreased. However, this decrease was accompanied by an increase in the color value since during storage anthocyanins were extensively polymerized [39]. A decrease in the anthocyanin content during storage could be influenced by several factors, such as enzyme residues or condensation reactions of anthocyanins with other phenolic compounds [40]. This led to a reduction of the total anthocyanin content on the 15th day of storage compared to the 0th day.

#### Scavenging activity

Scavenging activity in meatballs illustrates the ability of meatballs to scavenge free radicals, which in this case are DPPH radicals. The percentage of ability to scavenge DPPH radicals for each meatball is presented in Figure 2. Figure 2 shows that the scavenging activity of meatballs is influenced by the presence of antioxidants and storage time ( $P < 0.05$ ). The antioxidants here are BHT and PSPF.

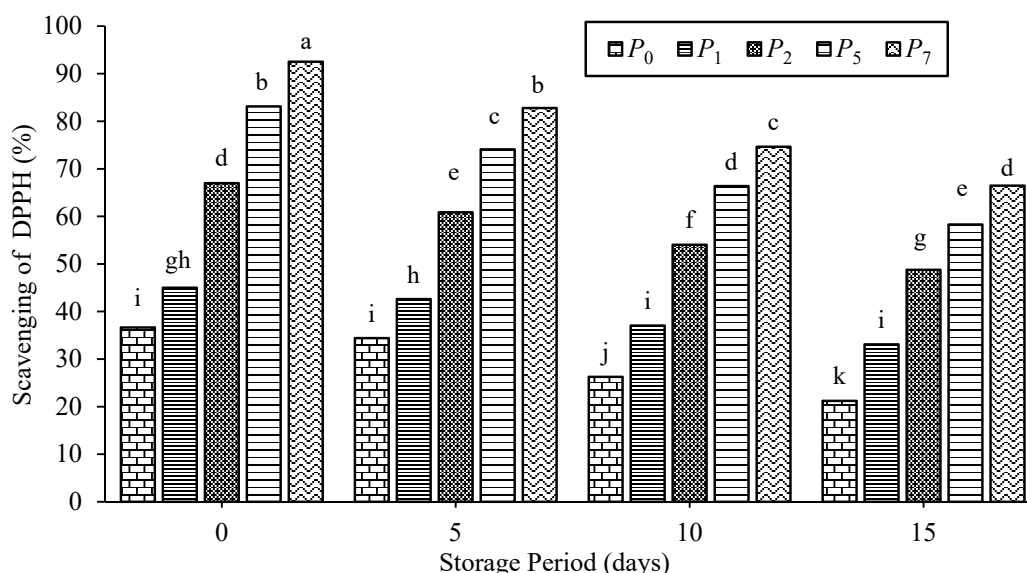
Meatballs that did not receive additional BHT and without substitution of tapioca flour with PSPF had the lowest scavenging activity. The interesting thing here was that PSPF substituting tapioca flour at 5–15% had a higher scavenging activity than tapioca with addition of BHT ( $P_1$ ). Figure 2 also indicates that the higher the substitution proportion, the higher its ability to scavenge DPPH radicals. The longer the storage, the lower the ability to scavenge free radicals.

The results of the study indicate that PSPF plays an essential role in contributing antioxidants to meatballs. The antioxidants given by PSPF were presumed to be due to the phenolic compounds in PSPF, mostly anthocyanins [11]. Anthocyanins in PSPF play an important role as compounds with the antioxidant, anti-inflammatory, and anticancer properties [12]. Their presence causes an increase in the scavenging activity of meatballs with a higher PSPF proportion.

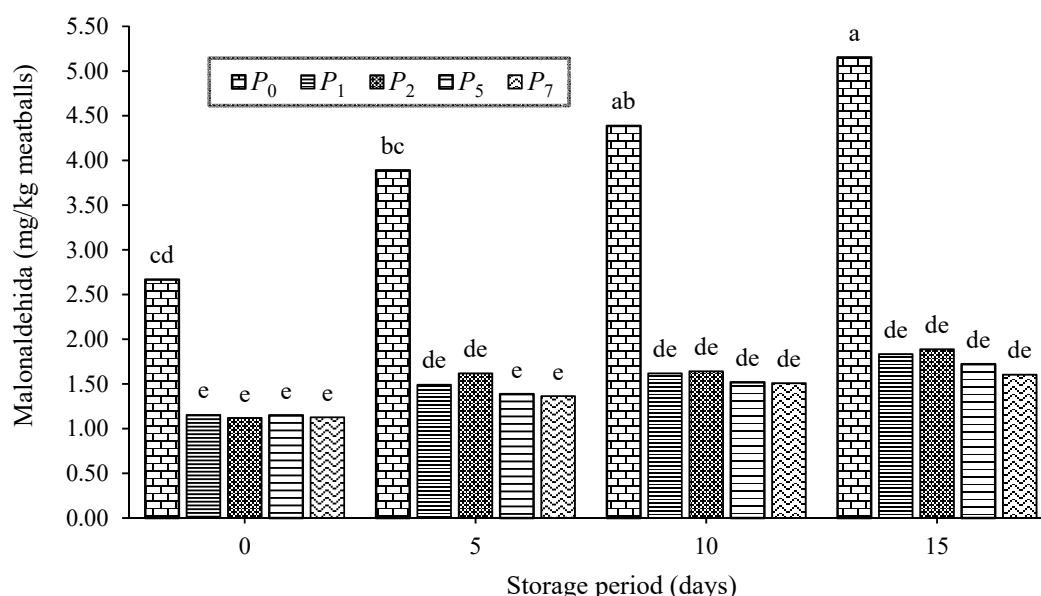
Figure 2 also shows that meatballs containing PSPF have a higher percentage of scavenging activity than meatballs containing BHT. It indicates that the anthocyanins in PSPF have a higher ability to scavenge DPPH radicals than BHT. This was in line with the results of the research by Jiao et al. [41], who stated that the scavenging ability of PSPF against DPPH radicals was higher than that of BHT. This is also indicated by the  $IC_{50}$  value of PSPF, which is lower than that of BHT [41].

#### Lipid oxidation (TBARS assay)

The TBARS value indicates the oxidation in meatballs expressed in mg malondialdehyde (MDA) per kg of meatballs. It can be seen from Figure 3 that meatballs without BHT and without replacement of tapioca flour with PSPF showed a significantly higher MDA level ( $P < 0.05$ ) than other meatballs. The levels of MDA were not significantly different in meatballs with BHT and substitution of PSPF in all proportions. The meatballs without the addition of



**Figure 2.** Scavenging activity against DPPH of chicken meatballs with addition of 0.01% BHT and PSPF substitution. A different letter following the bars indicates a significant difference ( $P < 0.05$ ). Ratios between tapioca and purple sweet potato flour were  $P_0$ ,  $P_1$ ,  $P_2$ ,  $P_5$ ,  $P_7$ .



**Figure 3.** The MDA of chicken meatballs with addition of BHT and PSPF substitution. A different letter following the bars indicates a significant difference ( $P < 0.05$ ). Ratios between tapioca and purple sweet potato flour were  $P_0$ ,  $P_1$ ,  $P_2$ ,  $P_5$ ,  $P_7$ .

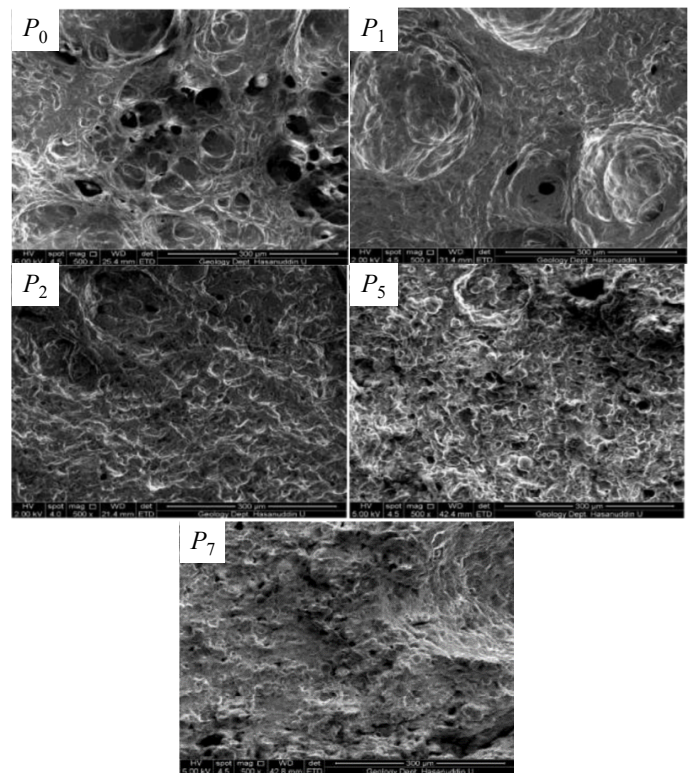
BHT and PSPF substitution showed a significant increase in the amount of MDA during storage for up to 15 days, while the amount of MDA in meatballs with BHT and PSPF substitution remained stable, suggesting that PSPF has the antioxidant properties. The antioxidant properties are also shown by its ability to scavenge DPPH radicals as shown in Figure 2. The replacement of tapioca flour with PSPF at ratios of 15:5, 10:10, and 5:15 led to an antioxidant capacity equivalent to 0.01% BHT by weight of meat.

The ability of PSPF to suppress the TBARS value of chicken meatballs was strongly anticipated because of the phenolic compounds in PSPF, especially anthocyanins. Anthocyanins in addition to acting as dyes or pigments in purple sweet potatoes, also have antioxidant properties [11,12]. In addition to anthocyanins, non-anthocyanin phenolic compounds in PSPF are also found and act as antioxidants [12]. The results of this study indicate that the substitution of tapioca flour with PSPF with a proportion of 15:5 exerted effects that were similar to the addition of 0.01% BHT in the manufacture of chicken meatballs.

#### *Meatballs microstructure*

Figure 4 shows the results of the descriptive analysis of the microstructural character of the meatballs upon substitution of tapioca flour with PSPF by comparing the structure visually using 500× magnification. The SEM results showed that meatballs with tapioca flour as a filler both with and without 0.01% BHT had large cavities and tended to be inhomogeneous ( $P_0$  and  $P_1$ ). Usually, tapioca granules are seen as solid granules that form aggregates with each other [42]. The combination of tapioca flour with PSPF resulted in homogeneous and dense-looking cavities ( $P_2$ ,  $P_5$ , and  $P_7$ ). The higher the proportion of substitution, the denser the texture as shown in Figure 3. ( $P_7$ , 5:15%). The denser the microstructure, the lower the water binding capacity and gel value. This value is in line with the resulting physical properties (Table 2). It can be seen in Table 2 that with a high proportion of tapioca flour substitution with PSPF, the physical characteristics of the meatballs decreased.

The denser and more compact meatballs with a high proportion of PSPF were probably obtained due to differences in the characteristics of the type of flour and the proportion of the type of starch. PSPF can produce a compact texture due to heating [43], and the process of making meatballs involves heating (cooking). Tapioca flour contains a lower proportion of amylose than PSPF [32,33],



**Figure 4.** Chicken meatball microstructure with filler combination of tapioca and PSPF. Ratios between tapioca and purple sweet potato flour were  $P_0$ ,  $P_1$ ,  $P_2$ ,  $P_5$ ,  $P_7$

and vice versa for amylopectin. High amylose makes starch easy to form starch-lipid conjugates and usually will easily undergo gelatinization [44]. The gelatinization process easily occurs when the amylose content increases or the amylopectin content decreases [45]. Therefore, the microstructure of food that is high in amylose is coarser than that of a product that contains little amylose [43]. The condition causes the meatballs from tapioca flour to have more and larger cavities than the meatballs with the addition of PSPF.

#### **Conclusion**

The substitution of tapioca flour with purple sweet potato flour in chicken meatballs causes changes in fiber content, cooking loss, WHC, gel strength and folding test and meatball color. In addition, the substitution of purple sweet potato flour significantly increased the anthocyanin content, scavenging activity and the ability to inhibit fat oxidation. The ability of purple sweet potato flour at a ratio of 10:10 to inhibit fat oxidation in chicken meatballs was equivalent to 0.01% BHT.

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The authors declare no conflict of interest.



## QUALITY EVALUATION OF FRESH CAMEL MEAT DIPPED IN EDIBLE CITRIC ACID

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**Keywords:** edible organic acids, antimicrobials, camel meat, shelf life

### Abstract

This study investigates the impact of dipping in food-grade citric acid on the shelf-life extension of fresh camel meat stored at refrigeration temperature. Fresh camel meat slices were treated with citric acid at concentrations of 0.5%, 1.0%, and 1.5%. The samples were then drip-dried packed into the vinyl acetate packs (VAP) and stored in a refrigerator at 5 °C for 15 days. The parameters studied included pH, water activity, Hunter color values, thiobarbituric acid reactive substances (TBARS) values, and total plate count (TPC). The pH of the treated samples decreased in comparison with the initial value of 5.30 (untreated sample) to a range within 4.20–4.47. The treated samples showed lower water activity values (0.95–0.99) than the control samples (0.97–0.987), especially the samples treated with 1.5% citric acid (0.95). The water activity ( $a_w$ ) values did not significantly differ among the treated samples. The control sample had an initial  $a^*$  ( $a^*$  — redness as per the colour chart) value of 16.4. Dipping the samples in citric acid significantly reduced the  $a^*$  values to a range of 7.3 to 11.6. The red colour values in the control sample decreased during its storage to a range of 12.8 to 14.3. On the first day of treatment, the control sample exhibited a TBARS value of 0.26 mg/kg MA, which was significantly higher than that of the treated samples. The samples dipped in a 1.00% acid solution demonstrated the lowest TBARS values at 0.12 mg/kg MA. The TBARS values levels for the samples treated with 0.5%, 1.0%, and 1.00% citric acid were generally low, peaking at 0.23 to 0.29 mg of malondialdehyde/kg after 15 days. The control sample exhibited a total plate count (TPC) of 5.3 CFU/g, with no significant difference observed between the control sample and the sample treated with 0.5% citric acid. No microorganisms were detected on the first day in camel meat samples treated with citric acid at concentrations of 1.0% and 1.5%. At the end of the storage period, the TPC levels in acid-treated samples remained below  $10^7$  CFU/g on days 5th, 10th, and 15th. The shelf life of the treated camel meat was extended to 15 days.

**For citation:** Abd Elgadir, M., Mariod, A.A., Alrumaih, N., Mohamed, S.H.S., Aladhadh, M.A., Alayouni, R.R. (2025). Quality evaluation of fresh camel meat dipped in edible citric acid. *Theory and Practice of Meat Processing*, 10(1), 84–90. <https://doi.org/10.21323/2414-438X-2024-10-1-84-90>

### Funding:

The research was financially supported by Hasanuddin University through State University Operational Assistance (BOPTN) with a Basic Research Scheme by a contract number 915/UN4.22/PT.01.03/2021.

### Introduction

Camel is a viable meat source in climate-affected areas, such as Saudi Arabia, when other animal production efficiency is reduced. The demand for camel derived products, particularly meat in various forms, is increasing among the majority of the population in the Kingdom of Saudi Arabia (KSA) due to the shifts in dietary habits and the population growth. Camel meat is rich in animal protein and is a popular source of meat in many African and Asian countries. In certain regions, especially in Arabian countries, camel meat is preferred over that of other animals, particularly for cooking traditional dishes, due to its perceived medicinal benefits. It is often considered a healthier alternative compared to other meats, as it contains low levels of fat and cholesterol. Furthermore, camel meat is an excellent source of essential minerals, vitamins, bioactive compounds, and important

fatty acids, including omega-3 fatty acids [1,2]. Camel can provide high-quality meat. Its meat provides significant therapeutic benefits due to its lower fat and cholesterol content compared to other livestock. It contains a higher level of polyunsaturated fatty acids. Consuming camel meat may help reduce the risk of various diseases in humans, including hypertension, pneumonia, hypersensitivity, and respiratory disease [3,4,5]. The demand for camel meat seems to be increasing due to health considerations, as camels produce carcasses with lower fat content, less cholesterol, and relatively higher levels of polyunsaturated fatty acids than other livestock according to Djenane and Aider [6]. Camel meat is highly susceptible to microbial contamination, which can result in spoilage and foodborne infections, thus leading to significant economic and health losses [7,8]. To prolong the shelf life period, antimicrobial compounds can be added to



fresh camel meat to inhibit the growth of pathogenic bacteria without reducing the quality of the product. Djenane et al. [9] investigated the impact of biopreservation treatment using *Olea europaea subsp. laperrinei* leaf extracts (laper.OLE) and nisin on the quality of camel steak during long-term refrigerated storage at  $1 \pm 1^\circ\text{C}$  in high  $\text{O}_2$  (80%) and low  $\text{CO}_2$  (20%) atmospheres. After 30 days of storage, the levels of psychrotrophic bacteria and *Pseudomonas* spp. were significantly lower in camel steaks treated with laper.OLE and nisin compared to untreated steaks. This treatment can extend the shelf life of the meat by up to 30 days. However, in the study conducted by Maqsood et al. [10], the addition of 200 mg/kg of tannic acid or catechin to camel meat reduced total mesophilic and psychrophilic bacterial counts down by one order of magnitude after 9 days of refrigeration. Consequently, the shelf life of the meat can be extended to 9 days. While artificial antimicrobials can be effective, there is a growing demand for natural preservatives. It was observed that herbs such as thyme, rosemary, and cinnamon significantly increased the shelf life of meat, with thyme exhibiting the most pronounced effect, extending the shelf life by up to 60 days [11]. Furthermore, it was found that mixtures of herbs had more effect on the storage stability of meat than individual herbs. It has been demonstrated that incorporating oregano essential oil into modified atmosphere packaging (MAP) extends the shelf life of various animal products, including beef and chicken [12]. Additionally, research has revealed that terpene and terpenoid compounds, such as menthol, geraniol, carvacrol, and thymol, exhibit strong antibacterial properties against *Enterobacter aerogenes*, *Listeria monocytogenes*, *Escherichia coli*, *Shigella flexneri*, *Shigella sonnei*, and species of *Aspergillus* [13,14]. Teshome et al. [15] reported that meat composition, processing techniques, and storage conditions are among the variables that influence the effectiveness of natural antibacterial agents in food industrial applications. They also reported that natural antimicrobials such as parsley, olive leaves, garlic, rosemary, pepper, thyme, sage and grape seeds are considered safe because they can reduce microbial resistance and align with consumer demand for healthier products. Many research works have been conducted to develop various preservation techniques to enhance the shelf life of fresh camel meat. Atika et al. [16] investigated the combined effect of a 2% lactic acid solution and refrigeration temperature on the shelf life of fresh camel meat. The meat samples were immersed in the acid solution and stored at a refrigeration temperature of  $4^\circ\text{C}$ . They found that the shelf life of the meat could be extended by up to nine days. Benyagoub et al. [17] asserted that using a traditional preservation method, specifically drying combined with salt covering, for camel meat could enhance consumer demand for products that promote health and environmental sustainability. This approach not only raises the potential for developing a camel meat drying industry but also encourages the promotion of camel breeding. On other research work it was found that storing fresh camel meat in a refrigerator at  $4^\circ\text{C}$  for 12 days extended the product's shelf life without negative affecting its sensory acceptability.

Moghimi et al. [18] applied 0.2% *Cuminum cyminum* L. essential oil as a natural preservative and stored it at  $4^\circ\text{C}$  for 15 days to extend the shelf life of fresh camel sausage. The results indicated that *Cuminum cyminum* L. essential oil can significantly prolong the shelf life of fresh camel sausage by 15 days. Tag et al. [19] found that treating fresh camel meat with gingerol at a concentration of 1.5% and nisin at a concentration of 2.5% can reduce the total microbial plate count (TPC) by 58.35% and 47.76%, respectively, while also enhancing the quality of the meat. Edible organic acids have the potential to prolong the shelf life of meat by controlling harmful bacteria and preventing oxidative spoilage, thus enhancing the overall quality of various types of meat. Bhagath and Manjula [20] reported that the application of organic acids in edible coatings for meat can reduce microbial counts in fresh meat. Similar findings were observed earlier by Siragusa and Dickson [21], who noted that the incorporation of edible organic acids, such as lactic acid at a concentration of 1.7% and acetic acid at 2%, into edible coatings can reduce microbial counts by 1.5 and 0.25 log units, respectively. However, incorporating 0.5% citric acid into an edible coating has proven its ability to enhance the shelf life of chicken meat, which should be stored at a refrigeration temperature of  $4 \pm 1^\circ\text{C}$  for 7 days [22]. To extend the shelf life of blood sausage, Diez et al. [23] conducted an independent investigation into the use of organic acids (L-potassium lactate, L-potassium lactate/sodium lactate, or L-potassium lactate/sodium acetate) and high-pressure treatments (300, 500, or 600 MPa for 10 minutes). The shelf life achieved was 15 days. Dipping fresh beef in 1 and 2% lactic acid, 1 and 2% acetic acid, 2.5 and 5% sodium lactate and 2.5 and 5% sodium acetate solutions then chilling at  $4^\circ\text{C}$  could extend the shelf life of fresh beef up to 21 days [24]. According to Teshome et al. [15], the effectiveness of natural antimicrobial compounds, such as edible organic acids, in meat covering applications is influenced by various factors, including food composition, processing methods, and storage conditions. However, Yu et al. [25] reported that to enhance the applicability of natural preservatives, several strategies should be implemented. These include combinations of various preservatives and food preservation methods, such as active packaging systems and encapsulation. Moreover, grapefruit seed extract, cinnamaldehyde, and nisin used in active packaging (AP) can delay lipid oxidation and protein deterioration in beef by up to 14 days. Edible organic acids and their salts are commonly used as food additives due to their safety for human consumption and are generally recognized as safe (GRAS) and can be safely applied in food [26]. The objective of this study is to investigate the use of citric acid to extend the shelf life of fresh camel meat.

## Materials and methods

### Materials

Fresh camel meat was purchased from a slaughterhouse in Buraidah, Al-Qassim region, Kingdom of Saudi Arabia (KSA), and transported immediately to the meat laboratory.

The meat was covered with crushed ice and placed in ethylene-vinyl acetate (EVA) bags and delivered to the laboratory within 30 minutes. All organic acids used in the experiment were the food-grade materials purchased from Sigma Chemical Company.

#### *Preparation of camel meat samples*

The fresh camel meat samples were sliced into pieces measuring roughly 15 cm by 10 cm by 1 cm (length × width × diameter), and each piece weighed about 200 g. Solutions of acetic acid, lactic acid, and citric acid at concentrations of 0.5%, 1.0%, and 1.5% were prepared using distilled water. The camel meat samples were dipped in these solutions, soaked for 5 minutes, drained, packaged in polyethylene bags, and stored in a refrigerator for 15 days. The samples were evaluated at 5-days intervals.

#### *pH value*

The pH was measured during storage periods (0 to 15 days) at a temperature of  $5 \pm 0.2^\circ\text{C}$  using pH meter, the model HI2211 — pH Meter (Hanna instruments, Germany) was used in the measurement.

#### *Water activity ( $a_w$ )*

The water activity was determined using the method of Abd Elgadir et al. [27]. AquaLab model 3TE, Pullman, WA, USA was used in the measurement. The device was warmed for 30 minutes, after which one gram of each sample was chopped and spread onto the plate. The samples were then placed in the drawer. The device measured the water activity of the samples in approximately 40 seconds at  $25^\circ\text{C}$ , recording three readings for each sample.

#### *Colour measurement*

HunterLab Ultrascan Sphere spectrophotometer Minolta Chroma Meter CR-300, Japan was used to measure colour. Three standard colour charts for  $L^*$  (lightness),  $a^*$  (redness), and  $b^*$  (yellowness) were employed to calibrate the device. Prior to measurement, each sample was placed separately in disposable Petri dishes. The average of three replicates was calculated and recorded as results for each color measurement value.

#### *Thiobarbituric acid (TBARS) value*

A 90% glacial acetic acid solution was utilized to prepare TBARS by diluting 0.2883 g of TBRS in 100 mL. Ten grams of meat were macerated with 50 mL of distilled water and combined with 2.5 mL of hydrochloric acid. The mixture was boiled for 10 minutes, and 50 mL of distillate was collected. Five mL of the distillate was mixed with TBRS and heated for 35 minutes. Absorbance was measured at 538 nm using a UV-spectrophotometer, model Novaspec 11, Biochrom Ltd, England. The malonaldehyde value was calculated in mg/kg, corresponding to  $7.8 \times D$ . Each sample was analyzed in triplicate.

#### *Total plate count (TPC)*

The anaerobic bacterial count of the samples was determined using the method described by Insausti et al. [28]. Sterilized peptone was prepared by diluting 15 g of peptone

in 1 L of distilled water and autoclaving the mixture at  $120^\circ\text{C}$  for 15 minutes. TPC agar was prepared with 22.5 g/L of agar in distilled water, then autoclaved, cooled, and poured into sterilized Petri dishes. Serial dilutions were performed, and 0.1 mL of each dilution was spread onto the agar substrate. The plates were incubated for 48 hours at  $37^\circ\text{C}$  in an anaerobic environment. Total counts were obtained from plates containing 30 to 300 colonies, analyzed in triplicate, and the results were reported as  $\log_{10}$  colony-forming units (CFU). The quantity of bacterial colonies is expressed in colony-forming units per milliliter (CFU/mL) and is calculated using the following equation:

$$\text{CFU/mL} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume Plated (mL)}}, \quad (1)$$

where:

*Number of colonies* = The count of visible colonies on the plate;

*Dilution factor* = The reciprocal of the dilution used;

*Volume plated* = The volume of diluted sample spread on the plate.

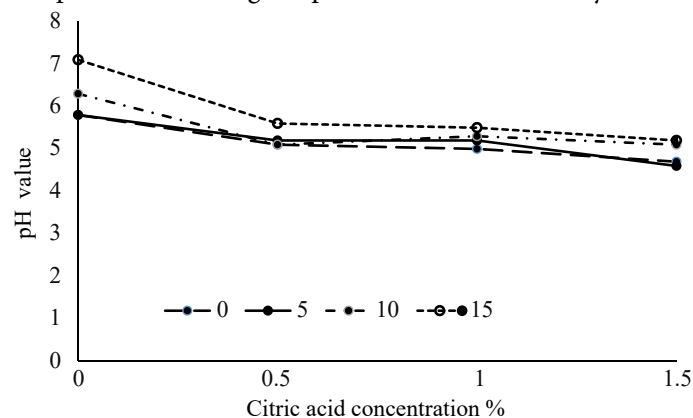
#### *Statistical analysis*

Two-way analysis of variance (ANOVA) was conducted using Minitab software version 17. The results are presented as mean  $\pm$  standard deviation (SD). Dunn's multiple range test was conducted to compare the samples' differences at a significance level of  $p \leq 0.05$ .

## **Results and discussion**

#### *pH value*

The pH values of the control sample and acid-treated samples are presented in Figure 1. All samples exhibited significant decreases in pH ( $p < 0.05$ ) when dipped in citric acid, with concentrations of 0.5%, 1.0%, and 1.5%. Specifically, the pH of the acid-treated samples decreased considerably ( $P < 0.05$ ) to 5.1, 5.0, and 4.7, respectively, while the pH of the control sample (0.00% citric acid) remained the highest at 5.8. Notable ( $p < 0.05$ ) reductions in pH were observed as the concentration of citric acid in the dipping solution increased [29]. All samples exhibited an increasing pH trend, reaching values of 5.6, 5.5, and 5.2 along with the addition of 0.5, 1.0, and 1.5 grams of citric acid, respectively. The control sample showed the highest pH value of 6.2 after 15 days. It was



**Figure 1.** pH values of control sample and citric acid treated camel meat during storage at  $5^\circ\text{C}$  for 15 days

reported that typically, the pH of fresh meat ranges from 5.5 to 6.5 [30]. However, when citric acid is applied to meat, it lowers the pH value. Edible citric acid, when applied to fresh camel meat, can influence its quality and preservation in several ways, mostly due to its acidic nature (pH ~2.2). The acidic environment caused by citric acid treatment can inhibit the growth of various bacteria and other pathogens, slowing down spoilage and thus extending the meat's shelf life. Citric acid disrupts microbial cell walls and interferes with essential metabolic processes, making it an effective preservative against bacteria like *E. coli* and *Salmonella*.

#### Water activity ( $a_w$ )

The water activity ( $a_w$ ) values for the samples presented in Figure 2 indicate that the treated samples (0.95–0.99) exhibited lower values than the control samples (0.97–0.987), with the most significant reduction observed at 1.5% citric acid (0.95). This might indicate that dipping samples into the citric acid in the above-stated concentration featured more rapid penetration in meat muscle compared with the others. The water activity ( $a_w$ ) values did not differ significantly ( $p > 0.05$ ) among the various concentrations of citric acid in the treated samples. At the end of the storage period, the  $a_w$  values of the samples gradually increased, ultimately reaching 0.99. It was reported that the addition of edible acids such as citric acid can reduce the water activity of fresh meats by binding water molecules more tightly, decreasing pH, and thereby controlling microbial growth [31,32]. This can enhance the meat's shelf life and food safety [33]. By adding citric acid, which is hygroscopic, water molecules may become more tightly bound to the acidic compounds [34]. This interaction reduces the overall free water in the meat, effectively lowering its  $a_w$ . Lower  $a_w$  helps inhibit microbial growth and spoilage [35]. It was reported earlier that there is a strong correlation between microbial growth and the water activity of the food [36]. It was also revealed that higher water activity values correspond to increased and accelerated microbial growth [37]. Therefore, the lower value of  $a_w$  in the acid-treated samples may have contributed to a decrease in microbial growth.

#### Colour

Table 1 illustrates the changes in colour values of acid-treated samples at various concentrations. The control sample exhibited reddish colour with an initial  $a^*$  value of 16.4. Dipping the samples into citric acid at various concentrations resulted in significant ( $p < 0.05$ ) decrease in the values of  $a^*$  values to the range between 7.3–11.6, which caused the camel meat samples to appear pale. The same finding is reported in the studies conducted by Awad et al. [38] and Osazuwa et al. [39]. The red colour also decreased significantly in the control sample during storage period to the values within the range of 12.8–14.3. Hunter  $L^*$  values increased significantly ( $p < 0.05$ ) after dipping in the acid in the first days (day 0) and after 5 days of storage in the control sample and the sample treated with citric acid in the concentration of 0.5%. However,  $L^*$  values decreased significantly ( $p < 0.05$ ) during the storage in the samples treated with acid at con-

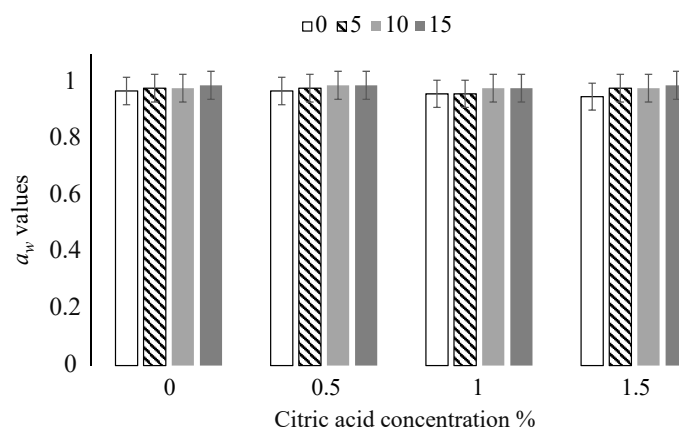


Figure 2. Water activity values of camel meat samples treated with citric acid during storage period

Table 1. Colour values of control sample and citric acid treated camel meat during storage at 5 °C for 15 days

	Storage period (days)	Control sample (0% citric acid)	Citric acid treated samples		
			0.5%	1.0%	1.5%
$L^*$	0	33.5 ± 0.3 <sup>Cd</sup>	37.3 ± 0.2 <sup>Bd</sup>	38.5 ± 0.3 <sup>Ab</sup>	38.7 ± 0.1 <sup>Ab</sup>
	5	37.9 ± 0.1 <sup>Db</sup>	47.4 ± 0.3 <sup>Ab</sup>	40.5 ± 0.1 <sup>Ca</sup>	42.2 ± 0.1 <sup>Ba</sup>
	10	46.3 ± 0.2 <sup>Ac</sup>	36.1 ± 0.1 <sup>Bc</sup>	34.7 ± 0.1 <sup>Dd</sup>	35.9 ± 0.1 <sup>Cd</sup>
	15	42.4 ± 0.1 <sup>Ba</sup>	48.1 ± 0.1 <sup>Aa</sup>	35.8 ± 0.1 <sup>Dc</sup>	37.6 ± 0.3 <sup>Cc</sup>
$a^*$	0	16.4 ± 0.3 <sup>Aa</sup>	11.6 ± 0.1 <sup>Ba</sup>	10.4 ± 0.1 <sup>Ca</sup>	8.8 ± 0.4 <sup>Da</sup>
	5	14.3 ± 0.1 <sup>Ab</sup>	10.4 ± 0.2 <sup>Bb</sup>	9.1 ± 0.2 <sup>Cb</sup>	8.3 ± 0.1 <sup>Da</sup>
	10	12.8 ± 0.1 <sup>Ad</sup>	8.2 ± 0.6 <sup>Bc</sup>	7.8 ± 0.3 <sup>Cc</sup>	7.6 ± 0.1 <sup>Cc</sup>
	15	13.8 ± 0.1 <sup>Ac</sup>	7.3 ± 0.3 <sup>Bd</sup>	7.8 ± 0.4 <sup>Bc</sup>	7.7 ± 0.1 <sup>Bc</sup>
$b^*$	0	11.1 ± 0.2 <sup>Dd</sup>	12.6 ± 0.1 <sup>Aa</sup>	14.4 ± 0.2 <sup>Bb</sup>	13.1 ± 0.1 <sup>Cd</sup>
	5	16.6 ± 0.3 <sup>Ab</sup>	16.3 ± 0.2 <sup>Ab</sup>	15.3 ± 0.3 <sup>Ba</sup>	12.6 ± 0.3 <sup>Ca</sup>
	10	15.4 ± 0.4 <sup>Ac</sup>	10.9 ± 0.2 <sup>Cd</sup>	11.3 ± 0.4 <sup>Bc</sup>	11.1 ± 0.1 <sup>Bc</sup>
	15	20.6 ± 0.1 <sup>Aa</sup>	14.3 ± 0.3 <sup>Bc</sup>	12.5 ± 0.1 <sup>Cd</sup>	12.1 ± 0.2 <sup>Cb</sup>

a, b, c, d Means with different lowercase superscripts within the rows are significantly different ( $p < 0.05$ ).

A, B, C, D Means with different uppercase superscripts within the column are significantly different ( $p < 0.05$ ).

centrations of 1.0% and 1.5%. The treated samples appeared lighter throughout the storage period. This finding agreed with those of Bernardez-Morales et al. [40]. The  $L^*$  value of the control sample was 33.5 at the first; this value increased significantly to 36.1–48.1, 34.7–40.5 and 35.9–42.2 when the meat was treated with the acid in the concentrations of 0.5%, 1.0% and 1.5%. A significant ( $P < 0.05$ ) decrease in  $a^*$  values ( $p > 0.05$ ) was observed. The  $a^*$  values in the control group ranged from 12.8 to 16.4 but significantly decreased to 7.3–11.6, 7.8–10.4, and 7.6–8.8 when treated with acid at concentrations of 0.5%, 1.0%, and 1.5%, respectively. A similar trend was noted in the  $b^*$  values. Treatment with acid at a concentration of 1.0% resulted in a significant ( $P < 0.05$ ) decrease in both  $a^*$  and  $b^*$  values.

#### Thiobarbituric Acid (TBARS) Value

Table 2 shows the various levels of TBARS in both fresh and treated samples during the studies along the storage period. On the first day of treatment, the control sample exhibited an initial TBARS value of 0.26 mg/kg MA, which was significantly higher ( $p < 0.05$ ) than that of the treated samples. Among the treated samples, those dipped in a 1.00% acid solution consistently showed the lowest TBARS values. Under



storage conditions, there was a significant decrease ( $p < 0.05$ ) in TBARS readings for camel meat that had been treated with organic acids. TBARS levels gradually increased during storage, with control samples exhibiting significantly higher levels ( $p < 0.05$ ) than the treated samples. In this study, the level of lipid oxidation in samples treated with 0.5%, 1.0%, and 1.00% citric acid was generally modest, peaking at 0.23–0.29 mg of malondialdehyde/kg after 15 days of storage. It was observed that a rancid flavor had been initially detected in meat with a TBARS value of 2.0 mg of malondialdehyde/kg [41]. Citric acid is expected to significantly reduce the development of rancidity in camel meat based on this cut-off value [42]. Citric acid can also act as antioxidant, it can donate hydrogen atoms to free radicals, thus stabilizing them and preventing them from interacting with fatty acids in animal products such as meat [43]. This slows down the oxidation chain reaction, reducing the development of malondialdehyde and other TBARS compounds [44, 45]. When used in combination with other antioxidants (like ascorbic acid), citric acid may exhibit synergistic effects, enhancing the overall antioxidant activity [46]. This can be particularly beneficial in fresh meats, where a blend of natural antioxidants can more effectively reduce TBARS and preserve meat quality. In summary, citric acid serves as an effective antioxidant in fresh camel meat by reducing lipid oxidation through chelation, acidification, and radical scavenging. This intervention can play an important role in extending the freshness and quality of camel meat, making it more desirable in the markets where meat quality preservation is essential.

**Table 2. TBARS values of control sample and citric acid treated camel meat during storage at 5 °C for 15 days**

Storage period (days)	Control sample (0% citric acid)	Citric acid treated samples		
		0.5%	1.0%	1.5%
0	0.26 ± 0.01 <sup>Ba</sup>	0.18 ± 0.03 <sup>Bb</sup>	0.13 ± 0.01 <sup>Bc</sup>	0.18 ± 0.01 <sup>Bb</sup>
5	0.19 ± 0.01 <sup>Cc</sup>	0.23 ± 0.01 <sup>Aa</sup>	0.29 ± 0.01 <sup>Ab</sup>	0.16 ± 0.01 <sup>Bd</sup>
10	0.22 ± 0.02 <sup>Ca</sup>	0.23 ± 0.01 <sup>Aa</sup>	0.12 ± 0.02 <sup>Bb</sup>	0.27 ± 0.02 <sup>Aa</sup>
15	0.36 ± 0.01 <sup>Aa</sup>	0.28 ± 0.03 <sup>Bb</sup>	0.23 ± 0.01 <sup>Bb</sup>	0.29 ± 0.01 <sup>Ac</sup>

a, b, c, d Means with different lowercase superscripts within the rows are significantly different ( $P < 0.05$ ).

A, B, C, D Means with different uppercase superscripts within the column are significantly different ( $P < 0.05$ ).

#### Total plate count (TPC)

The TPC values of the control sample and the treated samples during storage are shown in Table 3. The control had a TPC of 5.3 CFU/g. There was no significant difference between the control sample and the sample treated with 0.5% citric acid. However, no microorganisms were detected on

the first day when the camel samples were treated with citric acid in the concentrations of 1.0 and 1.5, respectively. Citric acid treatments at all concentrations (0.5, 1.0 and 1.5%) provided significantly lower ( $p < 0.05$ ) for citric acid treatments at all concentrations (0.5%, 1.0%, and 1.5%) compared to untreated samples over the same storage period. It is widely recognized that meat approaches deterioration and becomes unsalable at a level of  $10^7$  CFU/cm<sup>2</sup> [47,48]. Previously, it was proposed that the shelf life of fresh meat is influenced by time and the development of off-odor as well as storage temperature and the initial microbial load on the meat [49]. In this research, the samples treated with 1.0% and 1.5% citric acid were below the target count on the day 15<sup>th</sup>. On days 5<sup>th</sup> and 10<sup>th</sup>, the control sample and those treated with 0.5% citric acid exhibited microbial counts exceeding  $10^7$  CFU/g.

**Table 3. TPC values of fresh camel meat during storage at 5 °C for 15 days**

Storage period (days)	Control sample (0% citric acid)	Citric acid treated samples		
		0.5%	1.0%	1.5%
0	5.3 ± 0.1 <sup>Da</sup>	5.2 ± 0.1 <sup>Ca</sup>	ND	ND
5	6.2 ± 0.3 <sup>Ca</sup>	6.2 ± 0.1 <sup>Ba</sup>	6.5 ± 0.1 <sup>Aa</sup>	5.5 ± 0.2 <sup>Aa</sup>
10	8.4 ± 0.3 <sup>Ba</sup>	6.1 ± 0.1 <sup>Ba</sup>	6.7 ± 0.1 <sup>Aa</sup>	5.6 ± 0.3 <sup>Aa</sup>
15	9.5 ± 0.1 <sup>Aa</sup>	8.2 ± 0.2 <sup>Aa</sup>	6.8 ± 0.1 <sup>Aa</sup>	5.6 ± 0.2 <sup>Aa</sup>

a, b, c, d Means with different lowercase superscripts within the rows are significantly different ( $p < 0.05$ ).

A, B, C, D Means with different uppercase superscripts within the column are significantly different ( $p < 0.05$ ).

#### Conclusion

In conclusion, higher concentrations of citric acid significantly inhibited bacterial growth, thereby reducing spoilage and enhancing microbial safety of the camel meat. The citric acid treatment minimized lipid oxidation, preventing the formation of off-flavors and preserving the sensory qualities of the camel meat. Despite the acidic treatment, pH levels remained within the acceptable range, ensuring that the camel meat's natural texture and quality were maintained. These findings suggest that citric acid can serve as an effective natural preservative for extending the freshness of camel meat, thereby decreasing the reliance on the artificial preservatives. The quality of fresh camel meat can be preserved for a longer time when treated with citric acid. Higher concentrations of citric acid inhibit bacterial growth and reduce the rancidity values of the camel meat. The study found that pH levels remained within normal ranges, and the shelf life of the camel meat increased to 5, 10, and 15 days with citric acid concentrations of 0.5%, 1.0%, and 1.5%, respectively.

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All authors made an equal contribution to the work.

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

The authors declare no conflict of interest.





# ANTIOXIDANT ACTIVITY AND COLOR OF BEEF JERKY WITH KLUWEK

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**Keywords:** jerky, kluwek, antioxidant,  $L^*a^*b^*$ , cooking loss

## Abstract

Spoilage that often occurs in jerky is generally caused by the fat oxidation process, either during the manufacturing process, heating, or storage, and can lead to health hazards. This study was conducted to examine kluwak as a natural antioxidant that can reduce the oxidation process, by exploring an effect of the antioxidant activity and physical properties, namely  $L^*a^*b^*$  color, and cooking loss of jerky with the addition of kluwak. Kluwak originating from the community garden of Soppeng Regency, Makassar City, Indonesia, was used as an additional ingredient in making ground beef jerky. The part of meat taken was thigh from the slaughterhouse, and different levels of kluwak, namely 0%, 2%, 4%, and 6%, were added to it. The research results show that the addition of kluwek to jerky increases antioxidant activity, as indicated by the increased antioxidant activity test results using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, which signifies higher antioxidant capacity. In addition, the use of kluwak also showed a significant effect on the color parameters  $a^*$  and  $b^*$ , which indicated positive changes in the visual characteristics of the product. However, no significant effect was found for the color parameter  $L^*$ , which measures the brightness level, and no difference was observed in the shrinkage rate during the cooking process of jerky. In conclusion, kluwak can function as an effective natural antioxidant in reducing fat oxidation in jerky, while providing positive changes in product color without affecting the brightness level or shrinkage rate during cooking.

**For citation:** Febriana, A. R., Hajrawati., Hatta, W. (2025). Antioxidant activity and color of beef jerky with kluwek. *Theory and Practice of Meat Processing*, 10(1), 91–98. <https://doi.org/10.21323/2414-438X-2024-10-1-91-98>

## Introduction

Beef is a highly nutritious food commodity with an average content of water 77.65%, fat 14.7%, and protein 18.26%. It is usually used in a food menu in a ground form or in various processed forms [1]. The role of meat is very important, especially to meet the community's need for animal protein. Meat is a food product from livestock that is rich in nutrients, but is susceptible to spoilage (perishable food). Meat spoilage can be caused by physical, chemical, or biological contamination [2]. The high water and protein content in meat causes meat to be easily spoiled and reduces the usefulness and shelf life of meat. To overcome this, preservation or processing is carried out [3].

Preservation and processing of meat into various processed products aims to reduce quality degradation and extend shelf life while providing added value to the meat products produced [4]. Dendeng, Indonesian beef jerky, is one of the processed beef products that is quite well known to the public both domestically and abroad and is generally processed with different spice compositions, so the aroma and taste are also different [5]. Dendeng is produced using the drying technology to reduce the water content in food so that it is safe and the growth and reproduction of bacteria are suppressed [6]. Dendeng is an Intermediate Moisture Food (IMF) product, which gen-

erally has an  $a_w$  range of 0.60–0.90 and a water content of 10–50% [7]. The maximum water content limit for beef jerky is 12%. The use of sugar can reduce the water content of beef jerky and can inhibit the growth of microbes in jerky and extend the shelf life [3].

Through the drying process, jerky can be stored longer because its water content is reduced compared to fresh meat. Jerky is a sheet-shaped dish made from fresh or frozen meat that is cut or ground, then seasoned and dried [8]. In addition, the process of making dendeng also takes into account the homogenization of spices as one of the determining factors in increasing the shelf life of the product [9]. Ground beef jerky is a processed meat product made from ground meat that is seasoned, molded into thin sheets, and dried [4]. In the process of making ground beef jerky, the absorption of spices is much better compared to cut beef jerky. The grinding process can improve the taste and texture of the resulting beef jerky because the added spices will be absorbed more evenly throughout the surface of the beef jerky [10]. However, ground beef jerky is more likely to experience oxidation during the grinding process.

Processing meat into jerky will increase the intensity of malondialdehyde (MDA) formation as a secondary product of lipid oxidation [11]. Spoilage due to the fat oxidation

process causes a decrease in nutritional value and deterioration of taste of the resulting product [8]. Lipid oxidation can have a negative impact on meat quality, causing changes in sensory attributes and nutritional quality [12]. The oxidation process that occurs during processing and storage is influenced by the content of fat, myoglobin, oxidation enzymes, heat, light and water activity [13]. Oxidation of fat in meat produces unpleasant-smelling compounds, especially aldehydes [14]. In the oxidation process, metmyoglobin can also occur. Due to oxidation of the myoglobin pigment to metmyoglobin the color of meat changes to brown [15].

Free radicals formed during oxidation can damage meat molecules and accelerate decay. An imbalance between free radicals and antioxidants can cause oxidative stress, which can have an impact on health [15]. Free radicals produced during the processing of meat products oxidize fatty acids, especially polyunsaturated fatty acids through radical chain reactions [16]. The level of fat oxidation in jerky can be inhibited by adding ingredients or spices that contain antioxidants [8]. Antioxidants have an important role in the prevention and treatment of various chronic diseases caused by oxidative stress [17]. Antioxidant compounds found in fruit and vegetables are, for example, vitamins C and E,  $\beta$ -carotene, and polyphenols [18]. Natural antioxidant compounds in plants are generally phenolic or polyphenolic compounds, which can be flavonoids, cinnamic acid derivatives, coumarins, tocopherols and polyfunctional organic acids [19].

Antioxidants can be obtained from natural and synthetic materials. Several researchers have reported about bioactive components derived from plants as natural antioxidants. Some natural ingredients as sources of antioxidants include cloves, kluwak, cinnamon, cumin, and fennel seeds [20]. In addition to playing a role in preventing rancidity in products, natural antioxidants in food also have the potential to provide positive impacts for consumers.

One of the traditional spices that has the potential as an antioxidant is kluwak (*Pangium edule* reinw). Kluwak contains flavonoid compounds, vitamin C, iron ions and beta-carotene, which function as antioxidants [21]. Kluwak can replace synthetic dyes in addition to being a flavor enhancer. Color affects the quality and attractiveness of meat products, and reflects biochemical, physiological, and technological processes in the product [22]. Food colors that are pleasing to the eye are more likely to attract consumers. Therefore, color is an important factor in assessing the quality of a food product [23].

Kluwak fruit and seeds are used in Indonesia as ingredients in making *ise' pangi* vegetables, *lope' pangi* vegetables and cooking spices [24]. Kluwak is used as a cooking spice to produce a blackish brown color. It is best to choose the kluwak when it is ripe or old, which is indicated by the sound of the seed flesh when shaken [25]. Addition of kluwak to meat products such as beef sausage resulted in an increase in the antioxidant activity during storage [17]. So far, there has been no report on the use of kluwak seeds in making jerky. This is the background of the research on

jerky with the addition of kluwak (*Pangium edule* reinw) as an antioxidant and for improving physical properties of a product. Therefore, this study was designed to determine the antioxidant activity and physical properties, including color ( $L^*a^*b^*$ ) and cooking loss of jerky with the addition of kluwak at levels of 0%, 2%, 4%, and 6%.

### Objects and methods

The objects of the study were beef samples from the Manggala Slaughterhouse, Makassar City, South Sulawesi Province, Indonesia. Balinese beef thighs were immediately transferred to the laboratory in the Modena MD20 A Freezer (Modena, Italy) with a low temperature of  $4 \pm 1^\circ\text{C}$  in complete aseptic conditions. Before being processed, meat was thawed in the refrigerator for 24 hours.

This research was conducted in December 2023 — March 2024. Kluwak was obtained from community gardens (Figure 1) and brown sugar from traditional markets in Soppeng Regency, Makassar City, South Sulawesi province. The research samples were analyzed at the Meat and Egg Processing Technology Laboratory, Faculty of Animal Husbandry, Hasanuddin University, Makassar.

Other ingredients in this study such as coriander, salt, pepper, garlic, galangal, were from the tello market in Makassar City, South Sulawesi province, Indonesia. The materials tested were 0.01% butylated hydroxytoluene (BHT) synthetic antioxidant as a comparison solution, 1,1-diphenyl-2-picrylhydrazide (DPPH) and methanol.

The equipment and utensils used in making jerky included a grinder, jerky mold, analytical scales, bowls, spoons, spice blenders, knives, micropipettes, petri dishes, measuring cups, polypropylene plastic, and plastic gloves. All of them are available at the Meat and Egg Processing Technology Laboratory, Faculty of Animal Husbandry, Hasanuddin University, Makassar. For testing, the equipment used included TES-135A Color Meter Color Analyzer Portable (TES Electrical Electronic Corp, Taiwan), meat grinder Grinder Type Tc-12c (Gea Getra, China), food dehydrator (Getra, China), Freezer Modena MD20 A (Modena, Italy), Erlenmeyer flask (Pyrex, United States), 1000ml Kjeldahl flask (Iwaki, Japan), food processor (Braun, Germany), refrigerator Model MD10 W or refrigerator (Modena, Italy), and UV-VIS Spectrophotometer SHIMADZUUV-1800 (Shimadzu Corporation, Japan).



**Figure 1.** Kluwak (primary data, taken from the kluwak tree garden in Soppeng district)

This research was conducted experimentally using a Completely Randomized Design (CRD), four levels of kluwak treatment ( $P_0$ : 0%,  $P_1$ : 2%,  $P_2$ : 4%, and  $P_3$ : 6%) and addition of reference solution (BHT) ( $P_4$ ) with three replications for each treatment.  $P_0$  was used as a control and  $P_4$  as a comparison for synthetic antioxidants

To make fermented kluwak, kluwak seeds were washed first to remove dirt, then boiled for 1 hour, then dried. The kluwak seeds were left in the soil for 40 days [24]. After that, they were cleaned and brownish kluwak seeds were obtained. Kluwak seeds were broken, then the non-bitter kluwak fruit flesh was taken by prying off the fruit flesh attached to the shell and the kluwak was ready to use. After that, kluwak can be smeared on ground beef by mixing it with other spices.

The ground meat produced using a meat grinder Grinder Type Tc-12c (Gea Getra, China) with a plate hole size of 6 mm was then weighed and grouped based on its treatment. After that, fine spices were added to meat (250 g) according to each treatment, namely 3% salt (7.5 g), 34% brown sugar (85 g), 2.5% coriander (6.25 g), 1.5% garlic (3.75 g), 0.3% galangal (0.75 g), 0.3% pepper (0.75 g), tamarind (0.25 g) and kluwak 0%, 2%, 4%, 6% (0 g, 5 g, 10 g, 15 g) [15]. Beef jerky without the addition of kluwak (0%) with the addition of BHT at 0.01% of the meat weight (250 g). The mixture was then mixed evenly using a food processor (Braun, Germany) and then left for 24 hours in a refrigerator Model MD10 W (Modena, Italy) [27].

After that, the dough was molded using a 3 mm thick mold and the jerky was dried using a mold made from acrylic so that the thickness was as desired by consumers from the city of Makassar, Indonesia. Then, it was ovened using a food dehydrator (Getra, China) by an air drying method (at a temperature of 70 °C for 4 hours) so that the outer layer of the meat dried first. Heating was continued (at a temperature of 70 °C for 2 hours) by rolling the tray so that the heat could be evenly distributed [8,28,29]. The dried jerky was aired at room temperature in the oven and then analyzed.

#### *Antioxidant activity analysis*

Testing was carried out using the method used by [29]. The sample extraction ratio to methanol was 1:5 for homogenized and modified foods [30]. A total of 0.4 ml of beef jerky extract was reacted with 3.6 ml of DPPH (with a concentration of 0.1 mM). The mixture was then incubated at 37 °C for 30 minutes. Pure methanol was used as a reference material in the calibration of the SHIMADZU UV-1800 UV-VIS spectrophotometer (Shimadzu Corporation, Japan). The absorbance value of the solution was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm [29]. The amount of the antioxidant activity was calculated using the formula:

$$\text{DPPH inhibition (\%)} = \frac{\text{DPPH-Sample}}{\text{DPPH}} \times 100\%. \quad (1)$$

#### *L\*a\*b\* color of jerky*

The color testing of beef jerky was carried out using the CIE Lab method using a color reader or TES-135A Color Meter Color Analyzer Portable (TES Electrical Electronic Corp, Taiwan) and included L (brightness),  $a^*$  (redness) and  $b^*$  (yellowness) colors [31]. The “L” value indicates the brightness level from 0 to 100, with 0 indicating black and 100 indicating white. The “a” value reflects the red and green colors from –80 to 100. A negative “a” indicates green, a positive “a” indicates red. The “b” value indicates yellow and blue colors from –70 to +70. A negative “b” indicates yellow, a positive “a” indicates blue [31].

#### *Cooking loss*

Cooking loss is a major indicator of the nutritional value of meat and is related to the amount of water bound in the cells between muscle fibers [32]. To determine cooking loss of ground beef jerky cooked in the oven, meat samples were weighed before and after cooking. Cooking loss (CL) was calculated using the formula:

$$\text{CL (\%)} = \frac{W_1 - W_2}{W_1} \times 100\%, \quad (2)$$

where:  $W_1$  = Weight of sample before cooking;  $W_2$  = Weight of sample after cooking.

#### *Statistical analysis*

The data obtained from the research were analyzed by statistical data processing using the MS Excel and IBM SPSS Statistics 24 computer programs, the analysis of variance or ANOVA method. Analysis was continued with Tukey's advanced test with a 5% confidence interval or ( $P < 0.05$ ) [32].

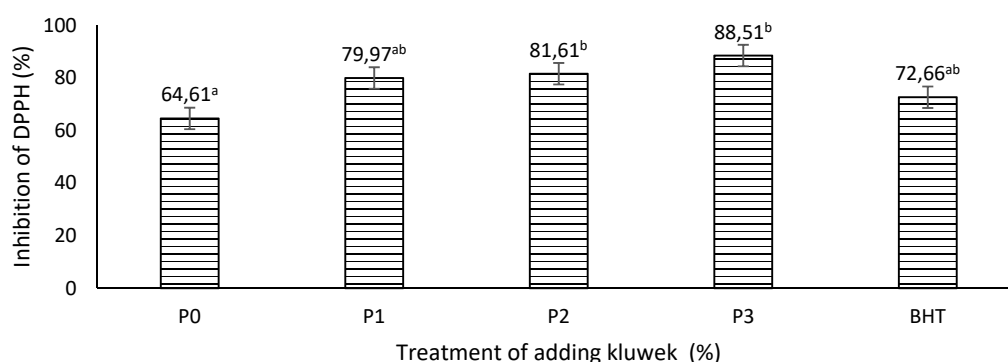
## **Results and discussion**

#### *Antioxidant activity*

As can be seen from the bar diagram above, the average antioxidant activity of beef jerky with the addition of kluwak (2%, 4% and 6%) was in a range from 79.97% to 88.51%, while it was 64.61% in beef jerky without the addition of kluwak (0%) and 72.66% in the samples with BHT. These results show that there was an increase in the antioxidant activity of 15.36–23.9%. This proves that kluwak can increase the antioxidant activity of beef jerky. The data above demonstrate that the antioxidant levels decreased in the following order:  $P_3$  (6% kluwak) >  $P_2$  (4% kluwak) >  $P_1$  (2% kluwak) >  $P_4$  (0.01% BHT) >  $P_0$  (without kluwak/control). Several research results also prove that the addition of kluwak can increase the antioxidant activity in meat products [17].

The results of the analysis of variance show that the addition of kluwak had a significant effect ( $P < 0.05$ ) on the antioxidant activity of jerky. Further Tukey test results showed differences in the antioxidant activity. This is thought to be due to the beta-carotene, flavonoids and vitamin C content in kluwak, which function as antioxidants [21]. Antioxidant compounds contained in spices inhibit lipid oxidation





**Figure 2.** Bar chart of the average antioxidant activity of ground beef jerky with the addition of kluwak ( $P_0$ : 0%,  $P_1$ : 2%,  $P_2$ : 4%, and  $P_3$ : 6%) and addition of BHT reference solution ( $P_4$ )

Note: Different superscripts in the figures indicate significant differences ( $P < 0.05$ ).

reactions, thereby inhibiting the formation of malondialdehyde [11]. The antioxidant compounds in kluwak have the ability to capture free radicals, so they can be used as an alternative to synthetic antioxidants [33]. Therefore, the treatment  $P_3$  (6% kluwak) gave the best effect compared to  $P_2$  (4% kluwak),  $P_1$  (2% kluwak),  $P_4$  (addition of BHT) and  $P_0$  (without kluwak/ control). This happens because kluwak contains antioxidants, which can reduce the negative impact of oxidants in the body by providing one electron to the oxidant compound, so that its activity is reduced [34]. Kluwak contains beta-carotene, which functions as an antioxidant by protecting and maintaining the integrity of cell membranes from free radicals, thus indirectly preventing lipid peroxidation in cell membranes [35].

Research on the antioxidant compound content in kluwak and processed meat products containing kluwak has been reported by several researchers. Kluwak contains alkaloids (2.69 ppm), tannins (16.0 ppm), flavonoids (1.23 ppm), and cyanide (122.7569 ppm) [36]. In addition, the antioxidant activity value of beef sausage with kluwak fermentation for 0 days and 40 days was  $32.43 \pm 8.14$  and  $34.39 \pm 6.94\%$  and the addition of kluwak at levels of 1%, 2%, and 3% could increase antioxidant activity [17]. Other studies also suggest that kluwak can be a natural antioxidant. For example, addition of 4% kluwak seeds can maintain the quality of patin fish after being stored at room temperature for 4 days [20].

The results of this study and several other studies that have been mentioned above show that processed meat products without the addition of kluwak still have the antioxidant activity that comes from the ingredients and spices used in making the product. In addition, kluwak, which is used as a spice in various dishes and traditional medicines, is also useful for maintaining immunity, maintaining

health, preventing cardiovascular disease, and functioning as a natural antioxidant [37].

### Color

#### Color $L^*$ (brightness)

The results of the measurement of the color of jerky (Table 1) showed that the addition of kluwak did not have a significant effect ( $P > 0.05$ ) on the color  $L^*$  (brightness) of jerky. The brightness value of jerky with the addition of kluwak ranged from 21.58% to 22.14%, while without the addition of kluwak (0%) it was 21.37%. This shows that the addition of kluwak reduces the brightness value of jerky and the color tends toward black. The low brightness of jerky with the addition of kluwak is caused by the presence of tannin compounds in kluwak, so that the resulting jerky is dark in color. This is supported by the opinion based on the results of phytochemical tests that water and ethanol extracts of kluwak with  $\text{FeCl}_3$  produce a blackish green color, because the reaction between tannin and  $\text{FeCl}_3$  forms a complex compound [22].

Additional ingredients in jerky also affect the color of jerky, such as brown sugar. The higher the sugar content in jerky, the lower its protein content. The Maillard reaction occurs (reaction between amino acids and ketone groups found in sugar), which produces brown color [38]. Jerky is generally brown or blackish in color due to the Maillard reaction during the jerky drying process [30]. The brown color that occurs can also be caused by tannin elements that have a yellowish to light brown color and when added to processed meat products can enhance the brown color so that it becomes darker and thicker [39].

#### Color $a^*$ (redness)

The results of the measurement of the  $a^*$  color value of dendeng (Table 1) show that the  $a^*$  color value of dendeng

**Table 1.** Color of ground beef jerky with the addition of kluwak

Parameter	0%	BHT	2%	4%	6%
$L^*$	$21.37 \pm 0.43$	$22.10 \pm 0.73$	$22.14 \pm 1.12$	$21.79 \pm 1.93$	$21.58 \pm 0.74$
$a^*$	$8.28 \pm 1.72ab$	$9.15 \pm 2.18b$	$7.33 \pm 0.77ab$	$5.86 \pm 0.89ab$	$4.89 \pm 0.57a$
$b^*$	$6.21 \pm 1.64b$	$5.27 \pm 0.39ab$	$5.348 \pm 0.26ab$	$3.98 \pm 0.93ab$	$3.29 \pm 0.48a$

Note: Different superscripts in the same column indicate significant differences ( $P < 0.05$ ).  $L^*$  (brightness) = 0 (black) — 100 (white);  $a^*$  (redness) ( $a = 0 - 80$  for red,  $a = 0 - (-80)$  for green);  $b^*$  (yellowness) ( $b = 0 - 70$  for yellow,  $b = 0 - (-70)$  for blue).

with the addition of kluwak ranged from 4.89 to 7.33, while without the addition of kluwak (0%) it was 8.28. These results indicate that the addition of kluwak has a significant effect ( $P < 0.05$ ) on the  $a^*$  color of dendeng. With increasing concentrations of kluwak added, the  $a^*$  color value tended to decrease but was still at the reddish level. This is due to the influence of flavonoid and tannin compounds in kluwak, where flavonoids give a reddish color while tannins give a blackish green color. In the extraction of dyes from kluwak, a red, yellow or orange color is formed on the amyl alcohol layer indicating the presence of flavonoid compounds and the formation of a dark blue or blackish green color indicates the presence of tannins [26,41].

Kluwak seeds contain tannin and flavonoid compounds as an alternative to synthetic dyes such as chocolate brown FH (referring to fashion products) and chocolate brown Htfood (referring to food products) [25]. Dendeng is generally light brown to dark brown in color due to the Maillard reaction, which is a reaction between the carbonyl group of reducing sugar and the amino group of protein and amino acids [39].

#### Color $b^*$ (yellowness)

The average  $b^*$  value of jerky color with and without the addition of kluwak (0%) ranged from 3.29 to 6.21. The addition of kluwak had a significant effect ( $P < 0.05$ ) on the  $b^*$  color of jerky (Table 1). The  $b^*$  color of jerky decreased with increasing concentration of added kluwak. This is because kluwak contains more tannin compounds than flavonoids, where tannins compound play a role in determining or changing the color to dark blue, while flavonoids compound change the color to yellow.

In general, jerky has a dark or dark brown color [40]. The yellow color of meat is caused by low levels of pigments, myoglobin, and hemoglobin. The content of marble fat in meat also affects the yellow color of stored meat due to the presence of beta-carotene [4].

#### Cooking loss

The addition of kluwak to jerky in amounts of 0–6% did not have a significant effect ( $P > 0.05$ ) on cooking loss, which ranged from 54.62% to 56.06% (Table 2). Cooking loss of jerky with the addition of 6% of kluwak tended to decrease. Low cooking loss in meat products can positively affect their quality. Meat with lower cooking loss has relatively better quality because there is less loss of nutrients during cooking [41,42].

**Table 2. Cooking loss of jerky with the addition of kluwak**

Treatment	Cooking loss (%)
0%	55.38 ± 2.92
2%	56.06 ± 0.96
4%	55.44 ± 1.31
6%	54.62 ± 0.53
BHT	55.38 ± 1.37



**Figure 3.** Ground beef jerky with added kluwak.

(Source: primary data (personal documentation, 2024))

Note: The color of jerky with the addition of kluwak ( $P_0$ : 0%,  $P_1$ : 2%,  $P_2$ : 4%,  $P_3$ : 6%, and  $P_4$ : addition of BHT comparison solution)

The non-significant effect is likely due to the method of storing meat during transport, which uses ice cubes to maintain the temperature of the meat [43].

Based on the research results, the relationship between cooking loss and beef pH is that the more acidic the meat condition, the lower the cooking loss of beef. Cooking loss refers to the loss of meat juice, which is a texture component that plays a role in determining meat tenderness, ranging from 15% to 40%, as well as the essence of meat during the cooking process, affecting its texture and tenderness [44]. Meat shrinkage can be influenced by several factors including muscle fiber, length of meat cut, weight of meat, and cooking time [45]. Meat that has a low cooking loss percentage, namely  $< 40\%$ , has better quality, because the loss of nutrients during the cooking process is less when compared to meat with a high cooking loss percentage [46].

#### Conclusion

This study generally identified that kluwak has oxidation resistance, functions as a natural antioxidant and is effective in inhibiting oxidation in ground beef jerky products, which have a high risk of oxidation that can lead to product spoilage and is potentially harmful to health if such a product is consumed. The addition of kluwak to jerky has been shown to increase antioxidant activity, as indicated by the increased antioxidant activity test results using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, which signifies higher antioxidant capacity. In addition, the use of kluwak also showed a significant effect on the color parameters  $a^*$  and  $b^*$ , indicating positive changes in the visual characteristics of the product. However, no significant effect was found for the color parameter  $L^*$ , which measures the level of brightness, and no differences were observed in the shrinkage rate during the jerky cooking process. Based on these findings, initiatives are needed to encourage the public to add natural antioxidants such as kluwak to jerky products in order to maintain product quality and consumer health.

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The authors declare no conflict of interest.