

UNIVERSITI TEKNOLOGI MARA

**ANTIOXIDANT ACTIVITY AND
NUTRIENT CONTENT OF MEAT
TENDERIZER DEVELOPED FROM
JACKFRUIT (*ARTOCARPUS
HETEROPHYLLUS*) LEAVES,
PINEAPPLES (*ANANAS COMOSUS*)
AND SELECTED HERBS**

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MSc

June 2026

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DZAHIR**

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CONFIRMATION BY PANEL OF EXAMINERS

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ABSTRACT

The increasing demand for natural and functional food products has encouraged the replacement of synthetic meat tenderizers with plant-based alternatives. Commercial meat tenderizers often contain synthetic additives, excessive sodium, or animal-derived ingredients that may not align with current consumer preferences. This study aimed to develop and evaluate natural meat tenderizer pastes formulated using jackfruit (*Artocarpus heterophyllus*) leaves, pineapple (*Ananas comosus*) and to compare their antioxidant, shelf life, nutritional, and sensory properties. Four formulations (Traditional, Fusion, Josapine, and MD2) were prepared and applied to meat samples. Tenderness was evaluated using shear force analysis. Antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, Total Phenolic Content (Folin–Ciocalteu method), Total Flavonoid Content (aluminium chloride colorimetric method), and antioxidant enzyme assays including Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione (GSH). Shelf-life stability including pH and moisture at room and chiller temperature and proximate analysis (moisture, protein, fat, and ash) were also assessed. Sensory evaluation was conducted to determine consumer acceptability. Statistical analysis was performed at $p < 0.05$. Results showed that all developed natural tenderizers significantly reduced shear force values compared to the commercial tenderizer ($p < 0.05$), with the MD2 formulation demonstrating the greatest reduction in meat hardness. DPPH radical scavenging activity revealed the meat tenderizers have poor DPPH inhibiting capability. Meat treated with the developed tenderizer exhibited significantly higher ($p < 0.05$) Total Phenolic Content, Total Flavonoid Content, and antioxidant enzyme GSH. The meat tenderizers themselves also have significantly higher CAT and SOD ($p < 0.05$) however this does not translate in cooked meat meaning these antioxidants are loss in the cooking process. The developed pastes remained microbiologically and physically stable for up to 20 days at room temperature significantly altering in pH ($p < 0.05$) after day 20 indicating spoilage. Proximate analysis indicated no undesirable alteration in nutritional composition. Sensory evaluation revealed significantly higher ($p < 0.05$) scores in texture, flavour, aroma, appearance, and overall acceptability for meat treated with natural tenderizers, with Josapine scoring highest for taste and overall acceptability, while MD2 excelled in tenderness and appearance. In conclusion, the developed natural plant-based meat tenderizers demonstrated superior tenderizing efficiency, enhanced antioxidant properties, maintained nutritional quality, and improved sensory acceptance compared to a commercial product, supporting their potential as functional alternatives in meat processing.

Keywords: Antioxidant, Jackfruit, Meat, Pineapple, Tenderizer

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LIST OF SYMBOLS

Symbols

α	Cronbach's alpha coefficient
β	Standardized path coefficient
χ^2	Chi-square statistic
f^2	Cohen's effect size
ρ	Correlation coefficient
R^2	Coefficient of determination
Q^2	Stone-Geisser's predictive relevance
$\rightarrow$	Direct relationship/path
***	$p < 0.001$
**	$p < 0.01$
*	$p < 0.05$
t	t-statistic

LIST OF ABBREVIATIONS

Abbreviations

BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
CAT	Catalase
DPPH	1,1-diphenyl-2-picrylhydrazyl
GSH	Glutathione dismutase
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
TFC	Total flavonoid content
TPC	Total phenolic content

CHAPTER 1

INTRODUCTION

1.1 Research Background

Meat and meat products are a crucial protein source in foods and consumption varies depending on socio-economic factors, ethics or religious beliefs and tradition (Font & Guerrero, 2016). Meat is considered an important source of complete protein because it is rich in all essential amino acids, supplies highly bioavailable iron, zinc, selenium, and vitamin B12 (Klurfeld, 2018).

Meat can be divided into two categories, which are red meat, consisting of lamb, veal, meat and pork, and white meat, consisting of poultry, chickens and turkeys (Bergeron et al., 2019). In Malaysia, Malaysians protein intake consists of 50% of plant proteins, 19% of meat, 12% of fish, 12% of eggs and 7% of dairy. Within the meat portion, 16% out of the 19% is chicken, with meat being 3%. (Siddharta, 2023). In 2022, Malaysians consumed an average of 50kg of poultry individually largely due to poultry meat produces less greenhouse gas compared to beef. On that note however, beef intake has still increased in recent years due to the rise attributed to growing consumer demand for red meat because of its unique flavour, texture and nutritional qualities such as being higher in iron and zinc. Higher income and changing dietary preferences also lead to greater beef consumption despite its higher environmental impact. Meat remains a staple component of the Malaysian diet, contributing significantly to daily protein intake (Siddharta, 2023).

Toughness is one of the most undesirable characteristics that leads to the unacceptability of meat. This is due to the connective tissue structures surrounding each muscle fibre in striated or voluntary muscle tissues (Purslow, 2015). Tenderness, flavours and high quality of the meat are among the most crucial muscle characteristics (Hocquette et al., 2015). Quality of tenderness, juiciness, breed's information and fat content are among the highest priority attributes considered by customers when purchasing meats (Mwashiuya et al., 2018). Meat with high toughness tends to harden after cooking causing them to have poor palatability, chewability become less juicy and overall less desirable (Mohd Azmi et al., 2023). Thus, meat tenderization is an important

method in order for consumers to achieve satisfaction when consuming meat, additional to use of lower grade meat cuts that are known to be tougher but costs significantly lower than higher grade meat cuts (Ismail et al., 2018).

There are various methods in meat tenderization such as the proteolytic enzyme, chemical and mechanical methods (Kim et al., 2019). Utilization of enzymatic treatments, synthetic chemical products and hand tools are available to reduce the toughness of meats but the use of synthetic products may lead to health problems with long term side effects (Omar et al., 2018). Papain, bromelain and ficin are proteolytic enzymes derived from plants that are used as natural source of meat tenderizers (Mohd Azmi et al., 2023).

Jackfruit, also known as *Artocarpus heterophyllus*, is a commonly consumed fruit originated from rainforests in Asian countries. It contains various phytonutrients known to have anticancer, antiulcer, antihypertensive and antiaging properties (Khan et al., 2021). Additionally, dried jackfruit leaves in the form of powder is also found to have antimicrobial potential by having bactericidal properties against *E. coli* and *S. enterica* (Sousa et al., 2022). Jackfruit leaves are also known to have proteases with proteolytic and meat tenderizing properties (Mohd Azmi et al., 2023). Jackfruit protease alongside bromelain are studied to be the most effective natural meat tenderizer as well as preserving meat and fight spoilage (Ramli et al., 2021).

Pineapples, also known as *Ananas comosus*, is a tropical fruit with bold tropical flavours and provides various benefits to health due to being an excellent source of calcium, potassium, fibre and vitamin C (Hossain, 2016). This fruit can be eaten fresh, cooked or even preserved. Pineapples also contain proteolytic enzyme bromelain alongside other types of proteolytic enzymes such as ananain and comosain, in small quantities. Bromelain is well known for its health benefits for its anticoagulant, antimicrobial and anticancer properties (Varilla et al., 2021). Moreover, bromelain has been used to breakdown protein structures of meat causing tough meat to be soften at active temperatures of 50-80°C (Omar et al., 2018).

On the other hand, spices and selected herbs generally contribute to flavour and associate with phenolic compound content of a dish. Common household ingredients and spices used in local Malaysian cuisines are reported to have substantial amounts and become important sources of polyphenols as well as antioxidants (Hanis Mastura et al., 2017). Consumption of diet rich in bioactive compounds and minerals can reduce

risk of developing several degenerative diseases such as cancer and cardiovascular diseases (Bhat et al., 2017).

In the perspective of nutrient profile, commercial meat tenderizers derived from papaya and papain are known to have high content of alkaloids, carpinine, vitamins C and E (Roslan et al., 2019). Other meat tenderizer alternatives such as salt brine 7% solution, soy sauce, cider and apple juice have been known to be used with acceptable sensory but the ingredients did not have a significant influence towards meat tenderness and is either high in sodium or high level of acidity (Aung et al., 2022).

Therefore, this research was conducted to develop a meat tenderizer using the combination of jackfruit leaves, pineapples and selected herbs as natural products. Jackfruit leaves were used in the study because it is believed to contain appropriate proteolytic enzymes for meat tenderization (Mohd Azmi et al., 2023). Determination of antioxidant contents, shelf life, nutrient profile and sensory analysis were conducted in this research to develop a meat tenderizer that has the appropriate nutrient and antioxidant contents.

1.2 Problem Statement

Meat quality and preference are judged by consumers through three main factors, which are tenderness, juiciness and flavour (Bhat et al., 2018). However, commercial meat tenders were created and sold in the market contain a large amount of salt, garlic powder, dextrose, monosodium glutamate and only a small amount of the active ingredient (papain) (Islam & Molinar-Toribio, 2015). Conversely, enzymatic treatments, synthetic chemical products and hand tools able to reduce the toughness of meats but these synthetic products may lead to health problems with long term side effects (Omar et al., 2018). The most important fact is, only a few meat tenderizer available in the market and the number of availability is very limited. Industrial proteases mainly known to have meat tenderizing effects are derived from animal (bovine and porcine) and microorganism (*Rhizopus oryzae*) sources, making their application limited due to religious issues (Ahmad et al., 2019). Otherwise, people need to cook the meat in a longer time to get a tender meat.

Other than that, it is reported that certain restaurants are using Acetaminophen or Paracetamol as meat tenderizers due to its benefit of cost reduction for cooking process due to it being relatively cheap and makes it unnecessary to purchase

commercial meat tenderizers (Sairuji et al., 2017; Ezugwu et al., 2023). In regards of this issue, National Drug Authority (NDA) warned restaurants to stop using Paracetamol as a meat tenderizer. NDA also stated that consumers of food tenderized with Paracetamol might have the possibility of liver and kidney damages (Otago, 2015). Paracetamol, which is a non-opioid analgesic or antipyretic agent is used to treat headaches, toothaches, backaches, flu, fever and menstrual pains, but some people have misused it (Ezugwu, 2023). It is proved that this drug could contribute harm to human health if it is used for other than the original purposes. Additionally, synthetic nitrites and nitrates were also commonly used in meat products to enhance and improve the flavour, colour and tenderness of meat products. However, there is increasing awareness regarding the potential health implications of synthetic food additives (Aung et al., 2022).

Furthermore, meat tenderness being one of the most important characteristics of meat in terms of palatability for consumer satisfaction. The production of tendered meat is required to retain consumer confidence in red meat as purchasing trends and market values increase (Ahmad et al., 2019). Additionally, meat tenderization is also a technique used to make lower-grade meat cuts become tender, which is valuable in satisfying consumer demands of consuming satisfaction while maintaining reasonable cost leading to the increase of commercial value of meats and meat products (Ismail et al., 2018).

1.3 Research Questions

- a) How to develop meat tenderizers from the combination of jackfruit leaves, pineapples and selected herbs?
- b) What are the antioxidant activities of the developed meat tenderizers?
- c) What are the nutritional properties and shelf life of the developed meat tenderizers?
- d) What is the sensory acceptance and meat tenderness using these developed meat tenderizers among adults?

1.4 Research Objectives

1.4.1 General Research Objective

To determine antioxidant activity and nutrient analysis of meat tenderizer developed from jackfruit leaves, pineapples and selected herbs.

1.4.2 Specific Research Objectives

- a) To develop meat tenderizers from the combination of jackfruit leaves, pineapples and selected herbs.
- b) To investigate the antioxidant activity of developed meat tenderizers.
- c) To investigate the nutritional properties and shelf life of the developed meat tenderizers.
- d) To evaluate the sensory evaluation and meat tenderness using these new products among adults.

1.5 Hypothesis

Meat tenderizers developed from a combination of jackfruit leaves, pineapples and selected herbs and spices has significant meat tenderizing effects alongside high antioxidant activities and acceptable sensory attributes.

1.6 Research Justification

The meat tenderizer was developed to provide an alternative for the consumers to choose a healthier food product, made from jackfruit leaf and selected herbs. Currently, there is a high demand from consumers to get the healthier meat products that contain low amounts of salt, nitrites, calories, fat, cholesterol and addition of health-promoting bioactive components such as unsaturated fatty acids, carotenoids, sterols and fibre. It is stated by Martínez-Graciá et al., (2015) that herbs and spices are the good alternatives when the demand for natural food preservatives is increasing.

Furthermore, from this development, people can shift from using Paracetamol as a meat tenderizer to this type of meat tenderizer which consists of jackfruit leaf and

selected herbs. Besides, the toxicity level from Paracetamol also can be avoided. Jackfruit leaf is chosen in this development because there is an increasing demand for discovering natural product from new plant sources of more active, more specific and potent proteolytic enzymes. In addition to the potential functional properties, selected spices were incorporated to enhance flavour, colour and texture to improve consumer acceptability of the final product.

1.7 Significance of study

- a) This study provides a natural alternative to synthetic meat tenderizers by formulating products from jackfruit leaves, pineapples and selected herbs with potential functional benefits.
- b) It enhances understanding of the nutritional value, antioxidant capacity, and shelf life of plant-based tenderizers, supporting the development of health-promoting food products.
- c) The findings contribute to consumer-focused innovations in meat processing by evaluating sensory acceptance and effectiveness in improving meat tenderness.

1.8 Scope of Study

This study will focus on the meat tenderizing effects of jackfruit leaves and pineapples in conjunction to the antioxidant content of the meat tenderizer product. The antioxidants level tested in this study includes the content of DPPH adopted from Ebby-Anuar et al. (2014), Total Phenolic Content (TPC) using Folin-Ciocalteu method, Total Flavonoid Content (TFC) using aluminium chloride method (adapted from Nur Atikah (2021)), SOD using Superoxide Dismutase WST-1 Assay Kit (WST-1 Method), CAT using Catalase (CAT) Assay Kit Ultraviolet Spectrophotometric Method, and GPx using Glutathione Peroxidase (GSH-PX) Assay Kit Colorimetric Method. Additionally, this study also includes the tenderness of meat treated with the meat tenderizer was evaluated by texture analyser model TA.XT plus Stable Micro Systems and also sensory evaluation with eligible panels. Furthermore, this study includes the nutritional analysis of meat tenderizer determined by the nutritional composition of each ingredient referred from The Nutrient Composition of Malaysia 4th Edition 1997.

1.9 Limitations of Study

There are several limitations in this study. The concentration of proteolytic enzymes may vary depending on the origin of the jackfruit leaves and pineapples obtained. For this study, the jackfruit leaves were obtained from jackfruit leaves in Sungai Apong, Sungai Air Tawar, and Sabak Bernam, Selangor. Collection of three different locations were conducted to ensure sufficient sample quantity for analysis. The pineapples were obtained from pineapple farms located in Kampung Seri Merlong, Rengit, Johor. Moreover, the methods of determining antioxidant content are limited to as mentioned.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter presents a comprehensive review of existing literature relevant to the present study. The purpose of this review is to establish a foundation of current knowledge, identify key concepts, theoretical frameworks, and research findings, and to highlight gaps that this study seeks to address. By critically examining scholarly sources, the chapter aims to contextualize the research within the broader academic discourse and justify the need for further investigation. The chapter begins with an overview of meat consumption and tenderization processes, followed by an in-depth discussion of specific themes related to the research objectives. These include meat tenderization methods, antioxidant contents, nutrient contents and shelf life. Each section synthesizes relevant studies, compares differing perspectives, and outlines the evolution of thought in the field, thereby providing a solid grounding for the subsequent methodology and analysis.

2.2 Meat Consumption in Malaysia

Meat and meat products are a crucial protein source in foods and their consumption varies depending on socio-economic factors, ethics or religious beliefs and tradition (Ezugwu et al., 2023). The meat consumption patterns have evolved considerably over the last few decades, closely tied to the country's socioeconomic development and changing dietary behaviours. Historically, Malaysia had lower consumption of meat compared to poultry and seafood, both of which are deeply embedded in traditional Malaysia cuisine. Over time, meat had gained a stronger foothold in the national diet. According to Shaharudin and Tumin (2019), meat consumption in Malaysia increased from 7.7% in 1963 to 9.0% in 2013. This increment represents a significant volume increase when considering the overall growth in meat consumption.

Malaysia's average per capita meat consumption rose from 91 kcal per person per day in 1963 to 266 per kcal per person per day in 2013, indicating a broader trend of increasing reliance on animal protein (Siddharta, 2023). Comparative statistics highlight that, while Malaysia's meat intake per capita (approximately 6.5kg per year) remains below the global average (around 12.2kg per year), it is among the highest in Southeast Asia (Shaharudin & Tumin, 2019). Moreover, the demand for meat has shown steady resilience, even during periods of economic slowdown, suggesting that meat is becoming an essential part of urban Malaysian diets. In 2015, the world meat consumption was 41.3kg per capita and it is expected to increase to 45.3kg per capita in 2030 (Ahmad et al., 2019). The annual wild meat harvest in Sarawak is estimated at 23,500 tonnes with increased commercialisation of wild meat to urban customers (Cantlay et al., 2017). The increase of meat intake is due to the increase of income and diets that were originally composed of mostly crops are now replaced by more animal products, eggs and dairy (Drewnowski et al., 2020)

Additionally, strong economic growth had changed the lifestyle, including food intake, in rural and urban areas with currently Malaysians consuming foods high in calories and proteins, including meats, being more common (Zainal Badari et al., 2015). Several national consumption surveys point towards a diversification of beef products consumed, from traditional dishes like *rendang* and meat soup to modern dishes like beef steaks and burgers (Kamu et al., 2015). Furthermore, due to increase consumption and high demand of meat. Approximately 80% of meat need to be imported from other countries as the self-sufficiency of meat in Malaysia was only 28% (Chamhuri & Batt, 2015). In summary, historical data demonstrate a consistent upward trajectory in meat consumption in Malaysia driven by a combination of economic, social and cultural factors.

One of the primary drivers behind the rising trend in meat consumption in Malaysia is socioeconomic change, notably the increase in disposable income and living standards. As reported by Kumar (2023), Malaysia's gross domestic product (GDP) per capita rose from USD 10,000 in 2010 to over USD 13,000 by 2022, providing more Malaysians with the financial capacity to afford more expensive protein sources like meat. This also means that economic prosperity directly impacts dietary choices. However, as people ascend economically, their diets typically shift from carbohydrate-based staples to protein-rich foods, including red meat. This phenomenon, commonly referred as the "nutrition transition", and it has been documented across many emerging

economies including Malaysia. Urbanization also plays a vital role. Malaysia's urban population grew from 65% in 2000 to over 77% in 2020 (Drewnowski, 2020). Urban dwellers have better access to supermarkets, hypermarkets, and specialty meat stores offering a wider range of meat products, both imported and local.

Urban lifestyles often promote, the consumption of convenience foods, such as ready-to-cook meat meals, beef sausages and processed beef snacks. Education level further influences meat consumption trends. Higher educational attainment correlates with greater awareness of diverse culinary options and a willingness to experiment with meat-based dishes. It also shapes perceptions about the healthiness and prestige associated with meat factors that significantly affect purchasing behaviour (Ahmad Hanis et al., 2015). Gender differences in meat consumption are notable as well. Studies have shown that men tend to consume more meat than women, possibly due to cultural associations of meat with strength and masculinity (Drewnowski, 2020). However, with the growing popularity of female-focused fitness and high-protein diets, meat consumption among women has also seen a modest rise. Collectively, these socioeconomic elements have created an environment conducive to the steady increase in meat consumption across Malaysia.

Malaysia's multi-ethnic and multi-religious composition shapes the nation's dietary trends, including meat intake. The majority Muslim population, constituting approximately 61.3% (Department of Statistics Malaysia (2022)), are abstained from pork consumption due to religious restrictions, therefore leading to a preference for beef, chicken and lamb as primary protein sources. Conversely, the Hindu community, primarily Indians, around 6.3% of the population, generally abstains from beef for religious reasons, considering the cow a sacred animal. The divergence leads to varying patterns of meat consumption across ethnic groups. The Malay population, which forms the majority, exhibits a strong culinary tradition centred on meat, as evidenced in festive dishes like *rendang daging*, a staple during Hari Raya celebrations. Additionally, meat is prominently featured in Malay weddings and religious gatherings, further solidifying its cultural importance (Kamu et al., 2015).

Among the Chinese community, meat consumption is somewhat less prevalent compared to pork and poultry, although beef dishes such as "beef noodles" and "stir-fried beef" are popular in urban centres like Kuala Lumpur and Penang. Exposure to international cuisines through travel and media has further normalized beef consumption among urban Chinese Malaysians (Drewnowski, 2020). Among

indigenous groups, especially in Sabah and Sarawak, meat consumption patterns historically leaned more towards poultry, pork and wild game (Cantlay, 2017). However in recent years, beef has gained popularity due to improved supply chains and changing culinary tastes (Kamu et al., 2015). Religious certification, particularly Halal status, plays an integral role in meat consumption. Consumers, particularly Muslims, demand assurance of Halal compliance, influencing both domestic meat production standards and important practices. This has led to the establishment of rigorous Halal certification systems, enhancing consumer confidence and encouraging greater meat consumption. Thus, cultural and religious factors, while diverse, collectively foster a landscape where meat consumption is not only normalized but celebrated among significant segments of the Malaysian population.

Meat consumption patterns vary significantly between Peninsular Malaysia and East Malaysia (Sabah and Sarawak), largely due to cultural, economic, and logistic factors (Cantlay, 2017). Peninsular Malaysia, particularly in urbanized states like Selangor, Kuala Lumpur, and Johor, exhibits higher meat consumption rates. The presence of a larger Muslim Malay population, high income levels, and easy access to imported meat products contribute to this trend (Kamal, 2023). Supermarkets and specialty stores in these regions regularly stock both fresh and frozen meat, catering to diverse culinary preferences. In contrast, meat consumption in Sabah and Sarawak, while growing, is comparatively lower. Indigenous communities in these states historically relied more on pork, poultry and wild meats.

However, modernization and urbanization have facilitated increased access to meat, especially in major towns like Kota Kinabalu, Kuching and Miri (Kamu et al., 2015). Logistics and supply chain challenges in East Malaysia, including the need for inter-island transportation and cold chain maintenance, historically restricted the availability and affordability of meat. Nevertheless, investments in cold storage infrastructure and improved logistics have gradually enhanced beef accessibility in these regions. Another notable regional trend is the rise of beef consumption during festive seasons and celebrations across Malaysia. In rural areas, beef consumption often peaks during religious festivals like Eid al-Adha, when communal slaughtering and beef distribution are common cultural practices (Shaharudin & Tumin, 2019). Therefore, while beef consumption continues to rise nationwide, regional disparities still persist due to cultural legacies, income differences, and logistical challenges (Drewnowski, 2020).

Additionally, the demand for good quality meat, especially red meat increases over the years with eating satisfaction being the most important factor for choice of meat among consumers (Bhat et al., 2018). Malaysian consumers' for meat are multifaceted, encompassing quality, price, origin, and certification considerations. The three main characteristics when it comes to the sensory enjoyment of meat are flavour, juiciness and tenderness (Bhat et al., 2018). The production of tender meat is considered required to retain consumer confidence in purchasing red meat as the purchasing trends and market values for meat and meat products increase in the years (Ahmad et al., 2019). Price sensitivity remains a crucial factor. Malaysian consumers are highly responsive to meat prices, with quantity often prioritized over premium quality. However, a growing middle class has led to the emergence of a market segment willing to pay more for high-quality meat cuts, such as wagyu and Angus beef, often sourced from Australia or Japan (Kumar, 2023). Freshness is highly valued among Malaysian consumers. Fresh, chilled beef is often preferred over frozen beef, particularly in wet markets and traditional grocery outlets (Bergeron, 2019).

However, due to cold chain limitations, imported frozen beef remains a significant portion of the available supply. The origin of meat is another determinant of purchasing behaviour (Bergeron, 2019). Australian meat enjoys a strong reputation for quality and Halal certification, making it highly preferred among middle- to upper-income consumers. Indian buffalo meat, although cheaper, is more commonly purchased by price-conscious consumers and for mass-catering purposes (Cheah, 2022). Consumer trust in Halal certification significantly influences purchasing decisions. Meat products bearing the official Halal logo from Malaysia's Department of Islamic Development (JAKIM) are more likely to be selected by Muslim consumers. The assurance of Halal slaughtering practices and contamination-free processing are essential considerations (Ahmad Hanis et al., 2015). With the rise of online grocery shopping, particularly accelerated by the COVID-19 pandemic, consumer purchasing behaviours has shifted further. Meat and Livestock Australia (2023) report that approximately 31% of Malaysian consumers purchased beef online at least once a month in 2023, compared to just 13% in 2018. E-commerce platforms provide a wide variety of beef cuts, promotional deals, and the convenience of home delivery, factors increasingly appreciated by tech-savvy younger consumers.

Malaysia's meat supply heavily relies on imports, given its limited domestic meat production capabilities. According to Hamir et al. (2022), approximately 70% of

Malaysia's meat requirements are met through imports, primarily from Australia, India, and New Zealand. Domestic production challenges include limited pasture land, high feed costs, and low productivity rates among local cattle breeds. Most local meats come from small-scale farmers who raise cattle as a secondary source of income rather than through intensive commercial farming. Australian meat enjoys high demand due to its perceived superior quality, stringent Halal certification processes, and reliable supply chains. Indian buffalo meat, labelled as beef in Malaysia, serves the mass market due to its affordability, despite occasional controversies regarding meat quality and religious sensitivities (Kumar, 2023). The Malaysian government has periodically introduced initiatives aimed at improving local meat production, such as through feedlot farming projects. However, these efforts have been marred by logistical challenges, land acquisition issues, and limited technological adoption (Hamir et al., 2022). The supply chain is further supported by advancements in cold chain logistics, which enable the efficient distribution of chilled and frozen beef products across the peninsula and East Malaysia. These improvements are critical to ensuring that imported beef reaches consumers in optimal condition.

The consumption of meat in Malaysia has been a subject of considerable research due to its implications on public health, the environment, and socio-economic trends. Various studies have explored factors such as socio-economic status, cultural influences, and health awareness, all of which contribute to the patterns of meat consumption in the country. Goh et al. (2020) highlights a significant shift in dietary patterns in Malaysia from 1980 to 2014, with a notable increase in meat consumption, particularly red meat. This transition is linked to economic development, increased urbanization, and globalization, which have led to changes in food habits among Malaysians. These socio-economic shifts have made meat more accessible and desirable as an essential part of the diet. According to this study, meat consumption has become more prevalent in urban households, where incomes and access to a variety of food products are higher (Goh et al., 2020). Drewnowski et al. (2020) conducted a study focusing on socio-economic factors affecting food choices in Malaysia. Their research found that education, income, and occupation significantly influence the consumption of animal protein, with higher-income and better-educated individuals opting for higher-quality meats. The study emphasizes how socio-economic status plays a pivotal role in shaping food preferences and choices, particularly in urban Malaysia where meat consumption has become more diversified (Drewnowski et al., 2020).

Ooi et al. (2017) utilized the Linear Approximate Almost Ideal Demand System (LA/AIDS) model to estimate the price and income elasticity for meat products in Malaysia. The findings revealed that meat is considered a normal good, with a positive income elasticity indicating that as incomes rise, meat consumption increases. However, the study also highlighted that meat is price-inelastic, meaning that demand does not respond significantly to price changes. This suggests that while consumers may be willing to purchase more meat as their incomes grow, they are less sensitive to price fluctuations (Ooi et al., 2017). Tan et al. (2015) further explored consumer behaviour related to meat purchasing in Malaysia. Their study, which used a sample selection model, found that factors such as household size, location, ethnicity, and income played a significant role in the likelihood of purchasing and the amount spent on meat. The study concluded that urban households and those with higher incomes were more likely to spend more on both fresh and processed meat products (Tan et al., 2015). The COVID-19 pandemic has significantly altered dietary habits across the world, and Malaysia is no exception. Cheah et al. (2022) examined the dietary patterns of university students in Sarawak during Malaysia's Movement Control Order (MCO). The study found a reduction in the variety of foods consumed, with a particular decline in the consumption of meats due to logistical issues and economic constraints during the lockdown period. While meat is a primary source of protein in the Malaysian diet, access to fresh meat was limited during the pandemic, leading to changes in food consumption patterns among students (Cheah et al., 2022).

A study by Cheah et al. (2022) used a lognormal hurdle model to analyze the socio-demographic factors affecting processed meat consumption in Malaysia. The results indicated that younger households, particularly those headed by younger individuals in the Bumiputera community, were more likely to consume and spend more on processed meats. Additionally, households in urban areas and those with higher incomes were found to have a higher likelihood of purchasing processed meat products. This study sheds light on how socio-demographic factors, including age and location, influence processed meat consumption patterns (Cheah et al., 2022). Kamal and Leby (2023) applied the Theory of Planned Behavior (TPB) to study the factors influencing the willingness of university students in Malaysia to reduce their meat consumption. The study found that attitudes towards health, environmental concerns, and subjective norms were significantly associated with the willingness to reduce meat intake. Health concerns emerged as the most dominant factor, followed by environmental attitudes and

social norms. This suggests that interventions targeting health and environmental benefits could be effective in encouraging Malaysians to reduce their meat consumption (Kamal & Leby, 2023).

According to a report by Kumar (2023), the red meat market in Malaysia is expected to grow at a compound annual growth rate (CAGR) of 3.5%, from USD 2.659 billion in 2024 to USD 3.501 billion by 2032. This growth is driven by an increase in per capita income, heightened awareness of the importance of protein intake, and a rising demand for convenience products such as ready-to-eat and packaged meats. The report suggests that as disposable incomes increase, so does the demand for meat, particularly in urban centres where convenience and quality are highly valued (Kumar, 2023). In Malaysia, halal certification plays a critical role in meat consumption, particularly for the Muslim population, which constitutes the majority of the country. A scandal in 2020 regarding non-halal meat imports raised concerns among Malaysian consumers, leading to greater emphasis on the integrity of halal certification. Trust in halal certification and the supply chain is a significant factor influencing the purchasing decisions of Malaysian consumers, especially in the Muslim community, where adherence to Islamic dietary laws is of utmost importance (Islam et al., 2023). As Malaysia faces increasing demand for meat, the country must consider sustainable approaches to meat production and consumption. Challenges such as the environmental impact of livestock farming, public health concerns, and the ethical treatment of animals are growing areas of focus. The promotion of plant-based diets and the reduction of red meat intake through policies such as the health-promoting planetary diet could help mitigate the environmental and health impacts of excessive meat consumption. Additionally, educational campaigns to raise awareness about the benefits of reducing meat consumption could play a key role in driving changes in dietary patterns in Malaysia (Kamal & Leby, 2023). Furthermore, the increasing focus on food innovation has encouraged the exploration of functional meat products that not only provide basic nutrition but also offer added health benefits. Natural ingredients with antioxidant and bioactive properties are gaining attention as potential alternatives to synthetic additives in meat processing. This aligns with the growing consumer demand for clean-label products that are perceived as safer and more sustainable. In this context, the incorporation of plant-based components such as herbs, fruits, and leaves into meat products has shown promising potential in enhancing both quality and nutritional value. These ingredients may contribute to improved oxidative stability, extended shelf life,

and enhanced sensory characteristics of meat. Therefore, continued research into natural meat enhancement strategies is essential to support the development of healthier and more acceptable meat products in Malaysia's evolving food landscape.

2.3 Meat Tenderization

The importance of meat tenderization cannot be overstated, particularly in the context of Malaysia's diverse culinary landscape. Tenderness significantly influences consumer satisfaction, affecting the palatability, juiciness, and overall acceptability of meat products (Mohd Azmi et al., 2023). Meat tenderness is a critical quality attribute influencing consumer acceptance and marketability. In Malaysia, the meat industry has explored various methods to enhance tenderness, focusing on both traditional and innovative approaches ensuring optimal tenderness is crucial for domestic consumption and the hospitality industries. The country's multicultural population, comprising of Malays, Chinese, Indians, and indigenous groups, has led to a rich tapestry of meat-based cuisines, each with specific textural preferences. Consequently, understanding and implementing effective meat tenderization methods are vital for meeting consumer expectations and enhancing the marketability of meat products. Factors influencing meat tenderization have been studied with variables including management factors like aging, diet, finishing regime, stress and pre-slaughter handling and post-slaughter handling changes like pH decline rate and temperature (Bhat et al., 2018). However, even after all of these attributes are controlled, level of tenderness remains vary proving this characteristic to be a very elusive trait and difficult to manage and manage (Bhat et al., 2018). Meat is often modified externally in order to tenderize its tough muscles. This is done in order to elevate the value of meats within the meat industry by giving the consumers satisfaction of consuming meat. The most common tenderization methods adapted worldwide are mechanical, electrical stimulation, application of exogenous proteolytic enzyme and thermal treatment methods (Woinue et al., 2019).

Mechanical tenderization involves physical processes that disrupt muscle fibres and connective tissues, thereby enhancing meat tenderness. Common techniques include blade tenderization, pounding, grinding, and tumbling. Blade tenderization, for instance, uses sharp blades to cut through muscle fibres, reducing toughness and improving texture (Woinue et al., 2019). This method includes techniques such as needle or blade tenderization, which create small incisions in the meat to break down

connective tissues. Pounding and grinding break down muscle structures, facilitating easier chewing and digestion. Tumbling, often used in industrial settings, involves rotating meat in a drum to loosen muscle fibres and promote the absorption of marinades. In Malaysia, mechanical tenderization is prevalent in both household and commercial settings. Traditional tools like meat mallets are commonly used in home kitchens, while commercial establishments employ advanced equipment for large-scale processing. These methods are favoured for their simplicity and effectiveness, especially when dealing with tougher cuts of meat. A review by Woinue et al. (2019) discussed various mechanical tenderization methods and their effectiveness in improving meat quality. They noted that while mechanical methods can reduce toughness, they may also introduce challenges such as increased microbial contamination and altered sensory attributes. Therefore, mechanical tenderization is often used in conjunction with other methods to achieve optimal results.

Chemical tenderization utilizes substances that break down proteins and connective tissues in meat. Marinades containing acids (e.g., vinegar, citrus juices) or salts are commonly used to alter the meat's structure, enhancing tenderness. Acidic marinades denature proteins, while salts can solubilize muscle proteins and increase water retention, leading to juicier and tenderer meat (Madhusankha & Thilakarathna, 2021). In the Malaysian context, traditional marinades often incorporate ingredients like tamarind, lime juice, and soy sauce, which not only tenderize but also impart distinctive flavours. These methods are deeply rooted in cultural practices and are integral to various regional cuisines. However, the effectiveness of chemical tenderization depends on factors such as marinade composition, duration, and meat type (Amin, 2020).

Advancements in food processing have introduced novel tenderization technologies, including high-pressure processing (HPP) and ultrasound treatment. HPP involves subjecting meat to high pressures, which disrupts muscle structures and inactivates spoilage microorganisms, thereby enhancing tenderness and shelf life (Bernardo et al., 2023). HPP is one such technique that applies high pressure to meat products to improve tenderness without the use of heat. A study by Md Zuki et al. (2025) investigated the combined effect of bromelain and HPP on buffalo meat. The results indicated that while both treatments individually improved tenderness, their combination did not yield significant additional benefits. The study suggested that optimizing the parameters of HPP, such as pressure and treatment time, could enhance its effectiveness in meat tenderization. Ultrasound technology has also been explored

for its potential in meat tenderization. Dong et al. (2022) reviewed the application of ultrasound in meat processing, highlighting its ability to induce cavitation and shear forces that can disrupt muscle fibres and connective tissues. The study noted that ultrasound treatment could improve meat tenderness and quality, but its effectiveness depends on factors such as frequency, intensity, and treatment duration. Ultrasound treatment uses high-frequency sound waves to create cavitation bubbles that break down muscle fibres and connective tissues. In Malaysia, the adoption of these technologies is still in its nascent stages, primarily limited to research institutions and high-end processing facilities. The high costs associated with equipment and operation pose challenges for widespread implementation. Nevertheless, these methods hold promise for the future of meat processing in the country, offering efficient and consistent tenderization outcomes.

In Malaysia, the most common tenderization technique used to modify meat texture is through hydrolysis of muscle tissues by external addition of protease (Ahmad et al., 2019). Degradation of structural or cytoskeletal proteins content in the muscle was occurred through the tenderization process by the endogenous enzymes which are called as calpains (Devine, 2016). Calpains (CAPNs) including calpastatin (CAST) and calpain I (CAPN1) are a large family of 14 cysteine proteases. Both calpastatin (CAST) and calpain I (CAPN1) play a significant role in meat tenderization. However, the function of calpain III (CAPN3) and other family members of CAPN in meat tenderization are still controversial (Sairuji et al., 2017). Besides, several recent studies have provided evidence that caspases and proteasomes could be responsible in the process of meat tenderization as well (Sairuji et al., 2017).

Enzymatic tenderization involves the application of proteolytic enzymes to break down muscle proteins, thereby improving meat tenderness. Plant-derived enzymes such as bromelain, papain, and ficin have been extensively studied for this purpose. A study by Madhusankha and Thilakarathna (2021) reviewed the mechanisms of meat tenderization and the impact of plant exogenous proteases. They discussed various chemical, physical, and conventional protocols to evaluate meat tenderness, emphasizing the role of proteases in enhancing meat palatability. Despite the commercial production of proteolytic enzymes, many meat products reach the market without any treatment due to a lack of technical know-how and inadequate literature on some proteases. This gap presents opportunities for further research and development in the application of plant proteases for meat tenderization. The enzyme method of meat

tenderization has garnered significant attention in Malaysia due to its effectiveness and alignment with halal dietary requirements. Enzymes such as papain (from papaya), bromelain (from pineapple), and ficin (from figs) are proteolytic, meaning they break down proteins into smaller peptides and amino acids, thereby softening the meat (Mohd Azmi et al., 2023). These plant-derived enzymes are particularly appealing in Malaysia, where halal compliance is essential for the Muslim majority. Research conducted at Universiti Putra Malaysia has explored the use of local plant enzymes for meat tenderization, highlighting their potential in improving meat quality while adhering to religious dietary laws (Mohd Azmi et al., 2023). Additionally, studies have investigated the extraction and application of proteases from indigenous plants, aiming to develop cost-effective and culturally acceptable tenderization solutions (Arshad et al., 2016). The enzymatic method offers several advantages, including specificity, efficiency, and minimal impact on the meat's flavour profile. However, controlling enzyme activity is crucial to prevent over-tenderization, which can result in a mushy texture. Ongoing research focuses on optimizing enzyme concentrations, application methods, and treatment durations to achieve desired tenderness levels without compromising meat quality.

Meat tenderization is a complex process with multiple mechanism. Degradation of collagen protein, decreasing the thickness of muscle fibre bundles, making changes to the sarcomere length during rigor mortis and changes to the chemical and structural properties during aging are the different types of mechanisms directing to meat tenderization. The main mechanisms are protein degradation and oxidation. Consequently, protein constituents in muscle fibre are target components of the enzyme protease. The tenderness of meats depends on its structure, connectivity of muscle tissues and proteolytic enzyme activity. Additionally, the degradation of meat cuts high in collagen solely depends on the cooking method and the end-point temperature. Collagen fibres shrinkage starts at 60-70°C and converts to gelatine at 80°C. The accumulative effects of collagen shrinkage and gelatine conversion all results in meat tenderization but excessive increase in temperature will result in loss of moisture and hardening of myofibrillar proteins which altogether cause meat hardness instead (Madhusankha & Thilakarathna, 2021).

In Malaysia, the use of bromelain, an enzyme extracted from pineapples, has been investigated for its tenderizing effects on buffalo meat. A study by Md Zuki et al. (2025) evaluated the effect of bromelain and high-pressure processing (HPP) on the

physicochemical properties and tenderness of buffalo meat. The results showed that bromelain treatment improved the pH and moisture content of the meat, while HPP further enhanced tenderness by reducing hardness and shear force. However, the sensory panel preferred non-bromelain-treated meat due to the unpleasant taste imparted by the enzyme. This finding suggests that while enzymatic treatments can improve tenderness, their impact on flavour must also be considered. Another study by Ahmad et al. (2019) explored the extraction and optimization of a crude protease from *Manihot esculenta* (cassava) for meat tenderization. The researchers utilized response surface methodology to determine optimal conditions for protease extraction, including pH, calcium chloride concentration, and the presence of additives. The protease exhibited significant proteolytic activity, leading to the degradation of muscle proteins and improvement in meat tenderness. Furthermore, the continued exploration of plant-based proteolytic enzymes presents a promising direction for improving meat tenderization while maintaining consumer acceptability. The use of natural sources such as pineapple, papaya, and other indigenous plants aligns with current trends toward clean-label and functional food products. These natural enzymes not only enhance tenderness but may also contribute additional bioactive compounds, including antioxidants, which can improve the overall quality and shelf life of meat. However, careful optimization of enzyme concentration and application time is essential to prevent undesirable sensory changes such as bitterness or overly soft textures. Therefore, integrating enzymatic tenderization with controlled processing conditions and sensory evaluation is crucial to ensure both technological effectiveness and consumer satisfaction, particularly in a diverse market such as Malaysia where taste preferences vary widely.

2.4 Properties of Commercial Meat Tenderizers

Commercial meat tenderizers are widely utilized to enhance meat texture, palatability, and cooking efficiency. Beyond their proteolytic activity, certain commercial formulations also exhibit antioxidant properties, which contribute to preserving meat quality during processing and storage. Antioxidants are crucial in mitigating oxidative reactions, particularly lipid and protein oxidation, which can otherwise lead to rancidity, colour deterioration, and off-flavours in meat products (Estévez, 2015). Some commercial meat tenderizers incorporate synthetic antioxidants

such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), or natural antioxidants like rosemary extract and tocopherols, to extend shelf life and maintain sensory attributes (Zahoor & Allai, 2020). These ingredients help scavenge free radicals and inhibit oxidative chain reactions in meat tissues. For instance, studies have shown that commercial marinades containing natural plant-derived extracts not only improved meat tenderness but also significantly reduced thiobarbituric acid reactive substances (TBARS), a marker for lipid oxidation (Latoch, Czarniecka-Skubina, et al., 2023).

Protease-based tenderizers remain the most studied approach, particularly plant-derived enzymes such as papain, bromelain, ficin and actinidin. These proteases hydrolyze key myofibrillar and connective tissue proteins (e.g., desmin, titin, tropomyosin-binding subunits), accelerating postmortem proteolysis and reducing shear force across beef, pork and poultry. Recent synthesis highlights expanding interest in underutilized plant proteases and in delivery formats that improve penetration and control over-tenderization to mitigate mushiness and surface “burning” associated with excessive proteolysis (Mohd Azmi et al., 2023). Ultrasound- or pressure-assisted enzyme delivery is increasingly reported as a way to enhance diffusion and uniformity while lowering dosage (Mohd Azmi et al., 2023). Moreover, the incorporation of antioxidant-rich ingredients such as spices and plant-based enzymes into tenderizer formulations have been linked to dual functionalities which are tenderization and oxidative stability. Such multifunctional formulations are particularly valuable in modern clean-label trends where consumers seek products with minimal synthetic additives and enhanced nutritional benefits (Kumar et al., 2015). Despite their potential, the antioxidant activity of commercial meat tenderizers can vary significantly depending on composition, storage conditions, and the meat matrix. Therefore, evaluating antioxidant effectiveness using standardized assays is essential for accurate assessment and product development. In conclusion, commercial meat tenderizers not only modify meat structure but also play an important role in protecting meat from oxidative degradation, thereby improving both shelf life and consumer appeal.

Controlled-dosage trials with papain powders illustrate the narrow operational window for texture benefits. In a study by Ribeiro et al. (2023) on comminute beef loaves, papain powder added at 0.00125–0.005% (w/w) in combination with 1% transglutaminase modulated gelation and tenderness: higher papain levels reduced hardness and cohesiveness while transglutaminase partially rebuilt the protein network,

highlighting how processors can pair proteolysis with crosslinking to steer texture toward target specifications. For intact muscles, processors typically apply papain powders via surface dusting or brine rehydration, using shorter contact times at chill temperatures to limit surface over-softening and purge (Ribeiro et al., 2023). Bromelain powders remain among the most effective dry tenderizers because they act on both myofibrillar proteins and collagen. In whole-muscle applications, bromelain treatments consistently decrease instrumental toughness and can enhance water-holding capacity. A study by Orynbekov et al. (2024) using bromelain delivered from a powdered preparation on horse meat reported lower shear force, improved water-holding capacity, and favourable sensory scores; when paired with selected lactic acid bacteria, pH control and oxidative stability also improved, suggesting combined powder systems that integrate protease and starter cultures for quality and shelf-life co-benefits. These findings support dosage optimization for species with distinct connective tissue architectures (Orynbekov et al., 2024).

Ginger-derived tenderizer powders which is rich in the cysteine protease zingibain have gained traction for tough, high-collagen muscles. In a controlled study by Naqvi et al. (2021) on biceps femoris from older cows, injecting a solution prepared from ginger powder (2 g/L) before sous-vide cooking (65 °C, 8–12 h) reduced shear force and improved trained-panel tenderness without compromising color, indicating that powdered ginger can be formulated for uniform delivery while leveraging long-time, low-temperature cooking to control over-tenderization. Mechanistically, zingibain shows strong collagenolytic preference relative to actomyosin, explaining its suitability for collagen-rich cuts and its compatibility with mild-heat regimes that soften connective tissue while maintaining myofibrillar integrity (Naqvi et al., 2021). Actinidin-containing kiwifruit powders represent another route for clean-label tenderization. Characterization of actinidin from Chinese kiwifruit cultivars demonstrated robust proteolytic activity with potential for generating bioactive peptides; when applied to meat systems, actinidin powders can rapidly reduce toughness, though the literature cautions about over-tenderization if enzyme activity is not thermally inactivated after reaching the desired endpoint (Zhang et al., 2018; Mohd Azmi et al., 2023). Formulators therefore often recommend brief marination times or low inclusion rates for actinidin powders, followed by cooking profiles that denature residual enzyme (Zhang et al., 2018; Mohd Azmi et al., 2023). Comparative work on “synthetic vs. natural” tenderizer powders in restructured poultry further contextualizes

performance. Using spent-hen slices as a tough model matrix, natural enzyme powders outperformed non-enzymatic chemical tenderizers on instrumental tenderness while maintaining acceptable sensory traits, provided that salt levels and holding times were adjusted to limit excessive proteolysis (Kantale et al., 2019). Such studies underscore that carrier salts in commercial powders, often included for followability and even coverage, also influence ionic strength and protein extraction, interacting with enzyme action to shape water-holding capacity, purge, and bite (Kantale et al., 2019).

The primary nutritional content of commercial meat tenderizers consists of minimal amounts of enzymes, salt, and preservatives. In terms of actual nutrients, these products typically contain little to no vitamins, minerals, or proteins, and their contribution to the overall nutritional value of a meal is negligible. Since the purpose of meat tenderizers is to aid in the texture of the meat rather than provide nutritional value, they do not offer any substantial health benefits in terms of macronutrients or micronutrients. In producing commercial meat tenderizers, only extracts of proteolytic agents, whether chemical or natural, are used and formed into powders (Anaduaka et al., 2023). This lack of nutritional content makes meat tenderizers an insignificant contributor to the overall dietary intake.

One concerning aspect of commercial meat tenderizers is their potential for high sodium content. Many tenderizer formulations include salt as a key ingredient to help preserve the product and enhance its flavour. The sodium content can be particularly problematic for individuals monitoring their salt intake, such as those with high blood pressure or kidney disease. Excessive sodium consumption has been linked to an increased risk of hypertension and cardiovascular disease (O'Donnell et al., 2015). Therefore, individuals using these tenderizers should be cautious of their overall sodium consumption, particularly if the tenderizer is used in conjunction with other salty seasonings or processed meats. While commercial meat tenderizers offer culinary advantages, their nutritional contributions are minimal, and their potential high salt content may pose health risks for some individuals. The lack of significant nutrients combined with high sodium levels underscores the need for consumers to use these products in moderation, especially if they are already consuming a diet high in salt.

One significant advantage of these products is their long shelf life, which is made possible by the specific manufacturing processes they undergo. This extended shelf life is crucial for maintaining the product's effectiveness and convenience in both domestic and industrial kitchens. The long shelf life of commercial meat tenderizers is

primarily due to the drying and preservation techniques used during production. Many meat tenderizers are composed of enzymes, such as papain and bromelain, which break down the protein structure of meat fibres. These enzymes are usually stabilized and dried into powder form during production, a process that significantly prolongs their shelf life (Zeeb et al., 2017).

By removing moisture, the risk of microbial growth is minimized, preventing spoilage and allowing the product to remain effective over extended periods. Additionally, many commercial meat tenderizers contain preservatives, such as sodium salts, to enhance their stability and prevent degradation over time. Sodium chloride or salt not only acts as a flavour enhancer but also helps inhibit the growth of bacteria and moulds that might otherwise degrade the product (Teshome et al., 2022). These preservatives, combined with controlled packaging that prevents exposure to moisture and air, ensure that the product can last for months or even years without losing its potency or safety for consumption. The shelf life of these products is further extended through the use of packaging technologies, such as vacuum sealing and the use of inert gases, which minimize exposure to oxygen that could cause oxidative degradation of the active enzymes (Majid et al., 2018). This protective packaging helps maintain the product's enzymatic activity, ensuring that it remains effective when it reaches the consumer. The long shelf life of commercial meat tenderizers is a result of the drying process, the inclusion of preservatives, and advanced packaging techniques. These methods not only ensure the product's safety and stability over time but also enhance its convenience and usability in food preparation.

The application of commercial meat tenderizers is known to influence the sensory attributes of meat, particularly its texture, flavour, and overall mouthfeel. These tenderizers, which typically contain enzymes such as papain and bromelain, work by breaking down the muscle fibres and connective tissues within the meat. As a result, meat treated with these products tends to exhibit distinct sensory characteristics that set it apart from untreated meat. One of the most significant changes in sensory attributes is the texture of the meat. The primary function of commercial meat tenderizers is to soften the muscle fibres, making them easier to chew. Studies have shown that enzyme-treated meat generally exhibits a more desirable tenderness, especially in tougher cuts, such as those from older animals or less marbled meats (Gómez et al., 2020). This improved tenderness enhances the overall eating experience, making the meat more palatable and enjoyable. However, excessive use of tenderizers can result in an overly

soft texture that some consumers may find unappealing, as it can lead to a mushy or mealy mouthfeel (Zhang et al., 2023). In terms of flavour, the impact of meat tenderizers is somewhat more nuanced.

While enzymes like bromelain and papain break down proteins, they can also affect the meat's natural flavour profile by releasing amino acids and peptides that can alter taste. Some studies have noted that enzyme-treated meat may have a slightly altered, more pronounced flavour, which can be perceived as either a positive or negative change depending on the consumer's preferences (Peña-Gonzalez et al., 2019). Additionally, the salt content in some commercial meat tenderizers may influence the perceived flavour, contributing a slightly saltier taste, which could be beneficial in certain culinary contexts but might not be desirable for consumers limiting their sodium intake. Finally, the visual appearance of the meat can also be affected by the application of commercial meat tenderizers. The breakdown of muscle fibres and collagen often results in a slightly more moisture-retentive meat, which can lead to a juicier product. However, overuse of these tenderizers can result in an unattractive, mushy texture that diminishes the visual appeal of the meat (Gómez et al., 2020). Commercial meat tenderizers significantly improve the texture and mouthfeel of meat, their impact on flavour and appearance depends on the extent of application and the specific product used. Their effects are generally positive in terms of tenderness but require careful use to avoid undesirable sensory changes.

Proteolytic enzymes, such as bromelain and papain, function by breaking down muscle proteins, thereby reducing meat toughness. However, excessive application can lead to over-tenderization, resulting in a mushy or undesirable texture. This over-tenderization occurs when the enzymes continue to act on the meat proteins beyond the optimal point, leading to a breakdown of the meat's structural integrity. The challenge lies in achieving a balance where the meat is tenderized sufficiently without compromising its texture (Mohd Azmi et al., 2023). The application of meat tenderizers can influence the sensory attributes of meat, including flavor, aroma, and juiciness. Improper use may lead to off-flavors or a loss of natural meat taste, affecting consumer acceptance. Additionally, the enzymatic activity can alter the meat's water-holding capacity, potentially leading to changes in juiciness and mouthfeel (Bolumar et al., 2021; Madhusankha & Thilakarathna, 2021). Enzymatic tenderization can also affect the nutritional profile of meat. The breakdown of proteins may lead to the loss of essential amino acids, impacting the meat's overall nutritional value. Furthermore, the

process may result in the formation of bioactive peptides that, while beneficial in some contexts, could have unintended physiological effects (Ismail et al., 2018; Gómez et al., 2020; Dong et al., 2022).

Consumer perception plays a crucial role in the acceptance of tenderized meat products. Some consumers may have reservations about the use of chemical additives or enzymes in food processing, preferring natural or minimally processed products. This preference can influence market demand and acceptance of tenderized meat products. The use of commercial meat tenderizers is subject to regulatory oversight to ensure food safety. Improper application or use of non-approved substances can lead to food safety issues, including the potential for allergic reactions or other health concerns. Ensuring compliance with food safety standards is essential to mitigate these risks. The cost of commercial meat tenderizers can be a significant factor for meat processors. While they can improve product quality, the expense of these additives must be weighed against the benefits. Additionally, the potential for over-tenderization leading to product loss or consumer dissatisfaction can impact the economic viability of using such products (Mohd Azmi et al., 2023). Furthermore, the limitations associated with commercial meat tenderizers highlight the need for alternative approaches that balance functionality, safety, and consumer acceptance. The reliance on synthetic additives and high sodium content has raised increasing concerns regarding long-term health implications, particularly among health-conscious consumers. As a result, there is growing interest in developing natural tenderizing agents derived from plant-based sources that can provide similar proteolytic effects while offering additional nutritional or functional benefits. Natural tenderizers may also contribute antioxidant properties, potentially improving oxidative stability and extending shelf life without compromising sensory quality. Therefore, future research should focus on optimizing natural formulations that ensure controlled enzymatic activity, minimize undesirable sensory changes, and meet regulatory standards, thereby supporting the development of safer and more sustainable meat tenderization strategies in the modern food industry.

2.5 Study on Meat Tenderization

Mechanical methods of tenderization rely on physically disrupting muscle structure, reducing sarcomere length, or redistributing connective tissue so that shearing force and perceived toughness decline. Two of the most widely used mechanical

interventions in industry and research are blade tenderization and maceration. Blade tenderization pierces the muscle with closely spaced blades or needles, physically cutting muscle fibres and connective tissue and thereby shortening cooking time and decreasing shear force (Arnold et al., 2018). Cubing or maceration uses blunt blades to shear and fragment surface tissue, it is common in processing to produce more uniform bite-size pieces with improved palatability (Arnold et al., 2018). Multiple surveys of processing operations also note that mechanical methods including tumbling, injection-enhancement, and cubing are often used in combination to produce intended sensory effects while adding moisture and flavour components (Arnold et al., 2018). These processes are effective at reducing measured shear force and improving consumer sensory scores for tough cuts. However, mechanical disruption can also increase food safety risk because surface pathogens may be translocated into the interior of the muscle when needles or blades penetrate intact surfaces, a practical consequence requiring modified risk management and cooking recommendations (Arnold et al., 2018).

Beyond needle and cubing, mechanical strategies include tumbling, injection, and novel physical treatments such as ultrasonic or impact tenderization. Tumbling mechanically agitates muscle in a brine or marinade, promoting salt-mediated solubilization of myofibrillar proteins and mechanical disruption of fibers, combined with brine chemistry, tumbling can improve water holding and tenderness while retaining juiciness (Ismail et al., 2018). Injection distributes brines and functional ingredients (salt, phosphates) deep into muscle, which can increase perceived tenderness but is technically a combined chemical–mechanical intervention because the mechanical act of injecting facilitates the distribution of tenderizing agents. Studies show that tumbling and injection often produce tenderness improvements secondary to moisture and protein solubilization rather than gross fibre severing alone (Ismail et al., 2018). Mechanical approaches are attractive for processors because they are relatively simple, rapidly produce measurable improvements, and are widely adopted. Yet the literature emphasizes trade-offs: integrity of whole-muscle appearance, product yield, and microbiological safety must be weighed against sensory improvements. Regulatory guidance and industry best practices consequently recommend labelling of mechanically tenderized products and altered cooking recommendations to compensate for potential interior contamination from surface microbes (Arnold et al., 2018).

Electrical methods span classical electrical stimulation (ES) applied to carcasses post-slaughter and emerging pulsed electric field (PEF) technologies used as pre-

treatments for tenderization or process intensification. Electrical stimulation (low-voltage or high-voltage) accelerates post-mortem glycolysis and rigor onset, preventing cold shortening and promoting early proteolytic activity that results in reduced toughness, improved colour, and shortened aging requirements (Zhang et al., 2018). High-voltage electrical stimulation (HVES) typically uses brief, high amplitude pulses applied within the first hour after slaughter; it produces rapid muscle contractions that stretch fibres and accelerate pH decline, but timing and intensity must be controlled because excessively rapid pH fall can reduce calpain activity and paradoxically impair tenderness (Zhang et al., 2018). The industry literature and experimental studies make clear that ES is effective for many red meat species and carcass classes but requires parameter optimization to balance tenderization benefits against negative biochemical side effects.

PEF treatment is a non-thermal processing technology that uses short, high-voltage pulses to soften cell membranes (electroporation). In meat science, PEF has been explored as a tenderization pre-treatment because membrane disruption can alter muscle ultrastructure, accelerate ionic fluxes, and enhance subsequent proteolysis or thermal denaturation patterns. Recent reviews and experimental studies find that PEF effects on tenderness are condition-dependent: parameters such as field strength, pulse duration, pulse number, and sample temperature determine whether PEF produces significant shear-force reductions. Some studies report improved texture and reduced cutting force after PEF treatment, while others show minimal change likely reflecting heterogeneity in muscle type, PEF uniformity, and subsequent processing steps (Guo et al., 2024). Importantly, PEF can be synergistic with other treatments, and because it is non-thermal it can enable tenderness modification with less protein denaturation if optimised correctly. Still, scale-up and industrial uniformity remain active research topics. Electro-based methods also have food safety and quality interactions: ES reduces aging time and can improve colour stability, while PEF may also provide some microbial inactivation depending on energy delivered. However, both methods require careful integration into the kill-floor/carcass chain because timing and species differences materially affect outcomes (Zhang et al., 2018).

Thermal tenderization encompasses a range of heat-based treatments that transform collagen and myofibrillar proteins to reduce toughness. At the molecular level, thermal tenderization is primarily driven by collagen solubilization which occurs typically in the range 60–70 °C depending on collagen crosslinking and

denaturation/aggregation of myofibrillar proteins, myosin denatures 40–60 °C, both of which influence texture and juiciness (Latoch, Głuchowski, et al., 2023). Conventional braising and slow cooking use sustained moist heat to solubilize collagen in tough cuts, producing measurable reductions in shear force and improved palatability. However, these methods can incur large cooking losses and flavour changes. In contrast, controlled low-temperature long-time (LTLT) thermal methods especially sous-vide precisely control temperature to exploit collagen and protein denaturation windows, balancing tenderness with retained moisture and improved colour and flavour retention (Noh et al., 2025). Recent experimental work on stepwise sous-vide strategies reports meaningful shear-force reductions while limiting moisture losses compared with some conventional protocols. Thermal methods are also increasingly studied in combined or sequential processes. For example, high-pressure processing (HPP) combined with sous-vide or other thermal steps has been shown to affect collagen solubilization and myofibrillar structure in ways that can enhance tenderness without extensive heating, and combinations of PEF and mild heat are being explored as well (Chen et al., 2024). These hybrid approaches aim to preserve sensory and nutritional qualities while achieving safety and tenderness goals, but the complexity of interactions, pressure, temperature, time, and muscle biochemistry, makes empirical optimization necessary for each product class.

Across mechanical, electrical, and thermal strategies, a recurring theme in the literature is trade-offs between tenderness, yield, safety, and sensory identity. Mechanical methods deliver clear textural gains but raise interior contamination risk and may alter appearance; electrical methods can reduce aging time and prevent cold shortening but require precise timing and voltages; thermal methods provide robust collagen solubilization but vary in yield and flavour outcomes depending on temperature and time. Moreover, research increasingly emphasizes combined processes to exploit synergies and minimize downsides. Implementation decisions in commercial settings therefore rest on product targets, regulatory constraints, and cost considerations and the literature suggests that an evidence-based integration of process parameters yields the best balance of quality and safety (Zhang et al., 2018).

The application of proteases in meat had been known to cause protein degradation of muscles and connective tissues which causes high-molecular weight of polypeptides to reduce into smaller molecular weight of protein ultimately making the meat tenderer. However, industrial proteases are derived from animals and

microorganism sources, making their application limited due to religious issues related to food authenticity and adulteration (Ahmad et al., 2019). Use of exogenous enzymes from plants or microbial sources may also degrade both myofibrillar and connective tissue proteins, which if unregulated, will result in over-tenderized meat producing unfavourable tastes (Bhat et al., 2018).

Nonetheless, its use in the meat industry to improve meat tenderness has been an increasing focus of interest (Bhat et al., 2018). Papain, bromelain and ficin are cysteine proteases usually referred as thiol or sulfhydryl proteases are most studied for meat tenderization. These proteases are endopeptidases with low substrate specificity and are able to catalyse the hydrolysis of a wide range of bonds. Papain and papain-like proteases are synthesised in plants as inactive precursors (Bekhit et al., 2015; Bhat et al., 2018). Plant proteases break down the muscle proteins in the connective tissues of meat by hydrolysing the peptidyl bonds in the proteins into peptides and into amino acid components resulting in meat becoming tender (Mohd Azmi et al., 2023). Bromelain, ficin and papain derived from plants are currently approved as generally recognized as safe for improving tenderness by USDA's Food Safety Inspection Service (Bhat et al., 2018)

A study by Mohd Azmi et al. (2023) found papain, a cysteine protease extracted from papaya (*Carica papaya*) latex, is widely used as a meat tenderizing component due to its ability to break down myofibrillar proteins and connective tissue. It was also concluded with increasing concentration of papain is used, there was a relative decrease in the content of alpha-helix and increase in irregular coil structures of meat structures, causing meat to be tenderer. Papain can also withstand in a wide range of temperature and pH making it the perfect choice for industrial use (Mohd Azmi et al., 2023). A review by Arshad et al. (2016) revealed that papain also comes with a recommended dose of 0.6% for better texture and quality, exceeding the limit will alter them. In a different study, papain was reported to cause meat structures to be mushy and produce abnormal flavours (Bhat et al., 2018).

On the other hand, Bromelain which is a mixture of cellulose, peroxidase, phosphates, protease inhibitors, and glycoproteins extracted from pineapples, is also used for meat tenderization (Bekhit, 2015). Freeze-dried bromelain powder is used to improve the tenderness and nutritional value of tough meat by decreasing sheer force and increasing amino acid content in the treated meat (Mohd Azmi, 2023). However, the enzymatic activity and performance of bromelain is affected by pH and temperature.

The optimum temperature range for enzymatic activity is 10-20°C, 30-40°C and 40-60°C for acidic, alkaline and neutral conditions, respectively (Mohd Azmi et al., 2023). Purified bromelain also completely ruptures the myofibril tissues in meat showing higher tenderizing effects (Arshad et al., 2016). Additionally, Bromelain also assists in digestion of protein-rich foods, helping elderly to digest consumed meats and lowers occurrence of abdominal upset (Aung et al., 2022).

Ficin, a plant protease obtained by from figs latex (*Ficus carica*), is also used in meat tenderization by hydrolysing the peptide bonds at aromatic residues. The use of ficin also extends from meat tenderization to use of dairy industry due to its ability to coagulate milk. It is reported ficin works optimally at pH 8.0 and temperature 60°C. However research on ficin extraction is limited (Mohd Azmi et al., 2023). Consequently, ficin can be inhibited by oxidizing agents and divalent metals but this inhibition can be changed (Arshad et al., 2016).

Moreover, a more controlled tenderizing action from actinidin, a protease derived from kiwifruit has also been reported. Fresh kiwifruit juice is effective in solubilizing selected connective tissues but its actions on collagen is very limited (Erdem, 2025), signifying the potential of said protease can only improve tenderness of specific cuts of meat. It is also reported pure actinidin with a dosage of 0.5mg/100g of pork or rabbit meat reduces shear force by more than half. Kiwifruit juice infused to lamb also improved its tenderness partly to the activation of calpain 2 (Bhat et al., 2018)

Microbial collagenases derived from microbial sources are also capable of degrading collagen which makes up 80% of connective tissues which results in tender meat and also proposed as a non-plant source meat tenderizer (Bhat et al., 2018). A study also revealed that a bacterial protease derived from *Bacillus subtilis* displays significant enzymatic activity (80-190 U/g) as compared to commercial meat tenderizers that had no enzymatic activity (0 U/g) (Bureros et al., 2020). However, due to their lack of safety and risks of undesirable effects, these proteases were not successfully utilized for meat tenderization (Bhat et al., 2018).

All of these plant and microbial proteases have been studied and reported for meat tenderization are mesophilic and thermophilic, with 50 to 80°C being optimal temperature and retain only 10% of their highest activity at 20°C. These proteases' ability to tenderize meat structures at room temperature but remain stable during cooking which may cause over-tenderization. Ideal proteases meant for meat tenderization are preferably to retain their meat tenderizing activity at cold or room

temperature and inactivated at cooking temperature to avoid over-tenderization (Bhat et al., 2018). Furthermore, the selection of appropriate proteases for meat tenderization requires careful consideration of their enzymatic stability, specificity, and inactivation behaviour during cooking. Ideal tenderizing enzymes should exhibit sufficient activity at low or ambient temperatures to allow controlled tenderization during marination, while becoming inactivated at higher cooking temperatures to prevent excessive degradation of muscle structure. This balance is crucial in maintaining desirable texture and preventing the development of mushy or unappealing meat. Additionally, the source of proteases plays an important role in consumer acceptance, particularly in regions where religious and cultural considerations influence food choices. Therefore, plant-derived enzymes are increasingly preferred due to their safety, availability, and compatibility with dietary requirements. Continued research into enzyme optimization and application techniques is essential to enhance efficiency and ensure consistent meat quality in both industrial and domestic settings.

2.6 *Artocarpus Heterophyllus*

Jackfruit, also known as *Artocarpus heterophyllus*, is a commonly consumed non-seasonal fruit originated from rainforests in Asian countries. It is a tropical fruit native to South and Southeast Asia and has received growing attention not only for its large, fibrous, and edible fruit but also for its various parts, including the seeds, bark, and notably, its leaves. While much of the research and culinary interest has traditionally focused on the fruit, emerging studies highlight the potential of jackfruit leaves in various applications, particularly in meat processing. These leaves, often discarded as agricultural waste, are now being re-evaluated for their biochemical properties that can serve functional purposes, such as meat tenderization. The jackfruit tree grows in warm and moist regions. The jackfruit tree's productivity is relatively high, yielding fruits from a range of 10 to 200 fruits in its lifespan.

Jackfruits are borne in the main and side branches of the tree (Ranasinghe et al., 2019). The use of plant-based tenderizers is not novel. Traditional food systems have long used natural enzymes and phytochemicals from fruits such as papaya and pineapple to break down tough meat fibers. However, with increasing interest in sustainable and cost-effective alternatives, jackfruit leaves have come under scrutiny for their proteolytic and antioxidative capabilities. Jackfruit leaves are simple, alternate,

and elliptical with a leathery texture, making them resilient and capable of withstanding harsh climatic conditions. Their structural features contribute to their unique phytochemical profile. Microscopically, the leaves exhibit a dorsiventral structure with well-developed palisade and spongy parenchyma layers. They also contain trichomes and oil glands, which are responsible for various biochemical secretions.

Phytonutrients contained in the fruit such as lignans, flavones and saponins have anticancer, antiulcer, antihypertensive and antiaging properties (Khan et al., 2021). A study conducted by Sousa et al. (2022) was also done to evaluate the antimicrobial potential of jackfruit leaves. Using the leaves in form of dried powder, it was found there is present bactericidal effect of jackfruit leaves on *E. coli* and *S. enterica*. Phytochemical analyses have demonstrated that jackfruit leaves are rich in bioactive compounds such as flavonoids, tannins, saponins, alkaloids, and phenolic compounds (Buddhisuharto et al., 2021). These compounds contribute not only to the plant's natural defence system but also exhibit significant antioxidative and proteolytic activities. Proteolytic enzymes such as cysteine proteases and other plant-derived endopeptidases have been identified in preliminary screenings (Puente et al., 2019). These enzymes are central to the tenderization process when the leaves are used in meat processing. Another structural aspect is the high concentration of cellulose and lignin in the leaves, which encapsulates the bioactive components. For practical extraction, various methods such as aqueous, ethanolic, or enzymatic extractions are employed to obtain the phytochemicals in a usable form for meat tenderization.

Jackfruit has emerged as a versatile food resource in which nearly every fraction which are the pulp, unripe “vegetable” flesh, seeds, rind, and leaves has documented techno-functional or nutritional applications. Recent studies have focused on upgrading by-products into value-added ingredients and exploring leaf-derived proteins and phenolics for direct food uses or food-contact applications. Collectively, this literature shows how both jackfruit and jackfruit leaves can enrich staple foods, support plant-based “meat” formats, and contribute natural antioxidants or structure-building proteins to diverse matrices. Unripe jackfruit as a meat analogue ingredient has gained momentum due to its fibrous texture and neutral flavour once cooked and seasoned. A study by Samutsri and Thimthud (2025) on young jackfruit-based meatballs reported that thermal pre-treatments meaningfully tune texture; boiling for about 45 minutes optimized juiciness, springiness, and overall acceptability on sensory panels, supporting its use as a plant-based “meat” component. Complementary process work shows that

drying method affects physicochemical attributes of young jackfruit chicken-style analogues; vacuum freeze-drying preserved superior sensory qualities relative to hot-air drying (Leelawat & Taikerd, 2024). These process-focused papers align with formulation studies where jackfruit was combined with wheat gluten and/or soy protein isolate to balance chewiness and cohesiveness: increasing gluten elevated hardness, whereas higher jackfruit fractions reduced chewiness and improved acceptability within limits (Leelawat & Taikerd, 2024). Together, this evidence base indicates that unripe jackfruit can serve as a structural bulk phase in meat analogues, with process parameters and protein complements as key levers for texture design (Leelawat & Taikerd, 2024; Samutsri & Thimthud, 2025).

Beyond texture, substitution of unripe jackfruit in hybrid animal–plant foods has been tested in comminute formats. For example, incorporating unripe jackfruit into chicken balls altered colour, darkness increased with higher jackfruit, and modestly adjusted moisture and fat distribution without compromising consumer liking when optimized (Samutsri et al., 2023). Such hybrid approaches may ease sensory transitions for consumers while capturing fibre and phytochemical benefits intrinsic to jackfruit (Samutsri et al., 2023). Seeds, which are often discarded, are now well-characterized as flours and starches with promising techno-functional traits (water absorption, swelling power) and protein contributions. A systematic review by Mohammed et al. (2024) concluded that jackfruit seed flour fortifies extruded snacks and bakery products nutritionally but can depress expansion ratio and lighten texture and volume at high substitution due to gluten dilution, underscoring the need for optimization (Mohammed et al., 2024). In extruded products, jackfruit seed flour generally increases water solubility and absorption indices owing to starch gelatinization, while reducing oil holding, which can assist fat reduction strategies; in pasta, 18% JSF yielded acceptable cooking quality and sensory profile, with 24% penalizing texture (Mohammed et al., 2024). Formulation-specific studies echo these patterns. When jackfruit seed flour replaces part of wheat flour in cookies or cakes, moderate inclusion (10–12.5%) often achieves favourable sensory scores while enhancing protein and fibre; higher inclusion can increase hardness or grittiness (Mohammed et al., 2024). This performance aligns with compositional data showing seed starches with useful swelling/bulk density and adequate oil and water absorption, attributes that can stabilize batters and doughs if levels are tuned carefully. As a by-product valorization route, jackfruit seed flour therefore offers a credible path to nutritional enrichment in staples, provided the

rheology and gluten network are managed via blends or processing (Mohammed et al., 2024).

Rind and rag are similarly being repurposed. Jackfruit rind powder, rich in dietary fibre and phenolics, has been incorporated into cookies to produce fibre and antioxidant-enriched formulations with acceptable sensory quality at optimized inclusion. One recent development study reported improved antioxidant status in finished cookies and feasible consumer acceptance after recipe adjustment (Ngo et al., 2024). Another trial added jackfruit rind powder to wheat cookies and documented elevated crude fibre with acceptable colour and texture under optimized substitution (Samutsri & Thimthud, 2025). These studies reinforce rind's role as a clean-label fibre and antioxidant booster for baked goods, though colour darkening and texture toughening remain constraints at higher loads (Ngo et al., 2024; Samutsri & Thimthud, 2025). Peel-derived pectin further expands the ingredient toolbox. Ultrasound-assisted extraction has yielded high-quality jackfruit peel pectin suitable for jams and confectionery, with greener process metrics and competitive gelling performance (Chan et al., 2023). Such pectin enables fruit-by-product circularity and offers functional fibre benefits alongside textural control (Chan et al., 2023). On the phytochemical front, cultivar and ripening state strongly influence pulp phenolic and antioxidant capacity; a study by Cheng et al. (2025) across 21 cultivars identified 60+ phenolic and found positive correlations between antioxidant activity and phenolic content, with certain cultivars exhibiting particularly strong antioxidant potential (Cheng et al., 2025). Simulated digestion work indicates that gastrointestinal conditions can increase measurable free phenolic and antioxidant capacity from jackfruit matrices, which is relevant to in vivo efficacy (Cheng et al., 2025). These findings support using jackfruit fractions not only for structure or macronutrients but also as sources of bioactive antioxidants in foods (Cheng et al., 2025).

Jackfruit leaves, long used traditionally, now feature in modern food science as sources of proteins with foaming/emulsifying capacity and as phenolic-rich extracts for beverages or biopreservatives. Protein concentrates from leaves can be fractionated (albumin, globulin, prolamin, glutelin) and show functional properties relevant to beverages, bakery, and emulsions. A study by Calderón-Chiu et al. (2024) characterized protein concentrate fractions, reporting glutelin-rich fractions with strong emulsifying activity and prolamin-rich fractions with high foaming capacity; functional and thermal properties correlated with amino-acid composition and protein content. Follow-up

structural modification via enzymatic hydrolysis improved emulsifier performance, demonstrating how targeted proteolysis tunes interfacial behaviour (Calderón-Chiu et al., 2024). Most recently, jackfruit leaf protein hydrolysates produced with pepsin and pancreatin showed antioxidant activity and in-vitro antiproliferative effects alongside low cytotoxicity—evidence that leaf peptides may contribute both function and bioactivity in food systems (Calderón-Chiu et al., 2024). Leaf phenolics are being positioned in beverages and as natural preservatives. A Malaysian study optimizing aqueous extraction from leaves (via response surface methodology) sought maximal phenolics and antioxidant capacity, reflecting interest in developing safe, water-based extracts for consumption (Sayuti et al., 2020). Building on this, fermentation platforms have integrated leaf and pulp substrates: lactic-acid fermentation of jackfruit material increased total phenolics and antioxidant activity, suggesting fermented beverages or fermented leaf infusions could deliver enhanced bioactives (Hoang et al., 2024). A safety assessment of fermented jackfruit pulp and leaf extracts in rats found no toxicity across a 28-day oral study at tested doses, supporting their potential as ingestible ingredients (Sabidi et al., 2020). Meanwhile, ethanolic jackfruit leaf extracts have demonstrated antifungal activity against phytopathogens using isothermal microcalorimetry, indicating promise as a natural antimicrobial that could be translated into food preservation contexts pending further work on foodborne targets and sensory impact (Sabidi et al., 2020).

Beyond direct ingestion, jackfruit leaf-derived peptides have been incorporated into edible and biodegradable films for food packaging, conferring antioxidant and antimicrobial properties that could extend shelf life via active packaging concepts (Vazquez-Gonzalez et al., 2020). While these films are food-contact rather than food ingredients, migration of benign antioxidant peptides into the headspace or surface could complement formulation strategies for lipid-rich or oxidation-prone foods (Vazquez-Gonzalez et al., 2020). Leaf applications in beverage formats are also evolving beyond traditional teas. Early explorations have tested kombucha frameworks in which jackfruit leaves contribute phenolics and potential antioxidant activity to the fermented drink; these studies point to viable flavour and functional profiles, though they require optimization of fermentation parameters, leaf load, and sensory acceptance (Bueno-Rojas et al., 2025). Traditional herbal infusions using mature leaves have also been investigated for phenolic content, antioxidant capacity, and preliminary glycemic effects in acute postprandial tests, suggesting potential for functional drinks. However,

these trials are small and require rigorous randomized designs before making health claims (Biworo et al., 2015).

Jackfruit leaves are also found to have proteases with proteolytic properties, extracted by turning the leaves into powder and marinated into meat to have meat tenderizing properties (Mohd Azmi et al., 2023). Another study by Ramli et al. (2021) found that jackfruit protease alongside bromelain in pineapple are one of the accepted and effective natural meat tenderizer that also help in preserving meat and fight bacterial spoilage. The use of jackfruit leaves in meat processing involves direct application in various forms: powdered, paste, or aqueous extract. Among these, aqueous extraction is the most common method, wherein fresh or dried leaves are soaked or boiled in water to extract soluble enzymes and phytochemicals. This extract is then either applied to the surface of the meat or used as a marinade.

Studies have shown that marinating meat with jackfruit leaf extract for a specified duration (commonly 4–12 hours) significantly enhances tenderness, as measured by shear force and sensory evaluation (Ketnawa & Rawdkuen, 2015). The extract helps break down the tough collagen and muscle fibres, rendering the meat softer and easier to cook. Jackfruit leaves, made in a form of paste alongside other marinating ingredients such as onions and green papayas to help tenderize meat and make it juicier (Zainol, 2015). A study proven that the content of bioactive compounds, quality and antifungal activity differ among different extraction methods with extract of jackfruit leaves obtained by HHP showed the best antifungal activity against *C. gloeosporioides* and *P. italicum* (Vazquez-Gonzalez et al., 2020). Additionally, jackfruit leaf extract may be incorporated into brine solutions or used in sous-vide cooking processes to enhance its effects. The extract's high antioxidant content also contributes to the stabilization of meat color and reduction of lipid oxidation during cooking and storage, adding further value beyond mere tenderization (Reddy et al., 2018).

The leaves of jackfruit are abundant in secondary metabolites. A study by Amadi et al. (2018) reported high concentrations of alkaloids (12.43 mg/100g), tannins (0.07 mg/100g), and flavonoids (3.91 mg/100g) in the leaves, surpassing those found in the pulp and seeds. These compounds are associated with antioxidant, anti-inflammatory, and antimicrobial activities. Additionally, research by Bungi and Dunggio (2022) identified lignin and flavonoids in jackfruit leaf extracts, further confirming the presence of bioactive compounds that may contribute to the plant's medicinal properties. Jackfruit leaves exhibit notable antioxidant properties, attributed to their high phenolic

and flavonoid content. A study by Yuniarti et al. (2025) demonstrated that methanolic extracts of jackfruit leaves possess strong antioxidant activity, which is essential for combating oxidative stress-related diseases. Furthermore, Ragazzo-Calderón et al. (2024) reported that high-intensity ultrasound treatment enhances the antioxidant capacity of jackfruit leaf proteins, suggesting potential applications in functional food development.

The antimicrobial potential of jackfruit leaves has been extensively studied. Sousa et al. (2022) found that methanolic and ethanolic extracts of dried jackfruit leaves exhibit significant antibacterial activity against *Escherichia coli* and *Salmonella enterica*, with minimum inhibitory concentrations as low as 7.2 mg/mL. Similarly, Soni et al. (2024) reported that methanolic extracts of jackfruit leaves inhibit the quorum sensing and biofilm formation of *Pseudomonas aeruginosa*, a pathogen known for its resistance to antibiotics. Nutritionally, jackfruit leaves are a valuable source of essential minerals (Adan et al., 2020). Amadi et al. (2018) noted that the leaves contain 0.52 g/100g of calcium, 0.21 g/100g of potassium, and 59.5 mg/100g of iron, making them a potential supplement for addressing mineral deficiencies. Traditional uses of jackfruit leaves include their application in managing diabetes. Tyagi (2015) also mentions that compounds in jackfruit leaves may help control blood sugar levels postprandially. Moreover, the antidiabetic properties of jackfruit components were also highlighted, noting their potential in the prevention and control of diabetes (Tyagi, 2015). The leaves of jackfruit are also utilized in traditional medicine for their wound healing and anti-inflammatory effects. Tyagi (2015) documented the use of heated jackfruit leaves applied to wounds to promote healing and alleviate pain. These therapeutic applications are supported by the presence of bioactive compounds that modulate inflammatory pathways and enhance tissue repair mechanisms (Indrianingsih et al., 2024). Furthermore, the utilization of jackfruit leaves as a natural meat tenderizer presents a sustainable approach by valorising agricultural by-products that are often underutilized or discarded. This not only contributes to waste reduction but also supports the development of eco-friendly food processing techniques. The presence of diverse bioactive compounds in jackfruit leaves, including phenolic and flavonoids, enhances their functionality beyond tenderization by providing antioxidant and antimicrobial benefits. These properties may help in extending the shelf life of meat products while maintaining their sensory and nutritional quality. Additionally, the adaptability of jackfruit leaf extracts in various forms such as powders, pastes, or aqueous solutions

offers flexibility in application across different processing methods. Therefore, jackfruit leaves hold significant potential as a multifunctional ingredient in modern meat processing and functional food development.

2.7 *Ananas Comosus*

Pineapple, also known as *Ananas comosus*, is a tropical fruit with excellent quality of juiciness, bold tropical flavours and various health benefits. Pineapple plants are herbaceous plants, with main parts of the plant includes the stem, leaves, peduncle, crown, shoots and adventitious roots. Pineapple plants grow in warm and humid climates, most well in tropical and subtropical climates ranging from mild coastal climates up to an altitude of 1000 meters provided the area is free from hoarfrost. Plants grown on higher altitudes than 1000 meters result in smaller fruits, increased sour taste and less attractive in colour and flavour. Most optimal growth of pineapples are in between 23-24°C but can be stretched up to 20-30°C (Hossain, 2016). Pineapples have garnered substantial attention not only for its sweet flavour and nutritional value but also for its significant enzymatic properties.

Native to South America and now cultivated in tropical and subtropical regions worldwide, pineapple is widely consumed fresh, juiced, or canned. While its dietary and culinary roles are well established, pineapple is also noted for its industrial applications, particularly in the food processing sector. One of the most significant uses of pineapple, especially in culinary and food science domains, is as a natural meat tenderizer. The proteolytic enzyme bromelain, found abundantly in the fruit and stem of pineapple, is the critical component responsible for this tenderizing action. The practice of using fruit-based enzymes for tenderizing meat dates back centuries and is still prevalent in many cultures. Among various enzymatic sources, pineapple stands out due to the potency and activity of bromelain, which has been studied extensively for its biochemical properties and impact on meat proteins. As the demand for clean-label and natural food products increases, the interest in plant-based meat tenderizers like pineapple continues to rise. Beyond its culinary uses, extensive research has highlighted its diverse health benefits, largely attributed to its rich phytochemical composition, including vitamins, minerals, and bioactive compounds.

Pineapple fruits contain sugar, digestive enzymes known as bromelain, citric acid, malic acid and is a good source of vitamins, minerals, calcium, potassium, and

fibre. Ripened fruits are best eaten fresh with its crown, rind, eyes and core removed. Pineapples can also be consumed cooked, preserved or jammed in order to prolong its shelf life. Outer peel and core of pineapples unused by the industry can be utilized as animal feed. Cattles preferred pineapples wastes fermented as compared to fresh pineapple wastes due to its higher acidity (Hossain, 2016; Mohd Ali et al., 2020). Pineapple is recognized for its rich nutrient profile, comprising high levels of vitamin C, manganese, dietary fibre, and antioxidants. According to The Nutrient Composition of Malaysia 4th Edition 1997, 100 grams of pineapple provide about 47.8 mg of vitamin C, which is approximately 80% of the recommended daily intake (Health, 2025). This high ascorbic acid content contributes to immune health and collagen synthesis. Pineapples also contain a significant amount of dietary fibre, which promotes gastrointestinal health, and manganese, which plays a role in bone formation and metabolic functions. Pineapple is a low-calorie fruit that provides a significant amount of essential nutrients. A typical serving contains approximately 82 calories, 2 grams of dietary fibre, 169 milligrams of potassium, 74 milligrams of vitamin C, and 50% of the recommended daily value of manganese. These nutrients contribute to various health benefits, including improved digestion, enhanced immune function, and reduced risk of chronic diseases .

In addition to its nutritional profile, pineapple contains various bioactive compounds with anti-inflammatory, antioxidant, and antimicrobial properties. Several studies have shown that bromelain, the enzyme complex in pineapple, exhibits multiple therapeutic effects, including modulation of immune responses and facilitation of wound healing (Maurer, 2021). These benefits have expanded pineapple's applications beyond culinary uses to include roles in nutraceuticals and traditional medicine. Culinary applications of pineapple are diverse. The fruit is used in salads, desserts, juices, and as an ingredient in marinades. Canned and dried pineapple products are widely available and serve as convenient sources of flavour and nutrition. Among these uses, the enzymatic tenderization of meat remains one of the most functionally significant contributions of pineapple to food science. In medical purposes, pineapples being rich in ester compounds including methyl-2-methylbutanoate, methyl hexanoate, methyl-3-(methylthiol)-propanoate, methyl octanoate, and 2-methoxy-4-vinyl phenol have served in folk medicine, being used to arouse appetite, an effective diuretic, contraceptives, the discharge of intestinal helminths prevent ulcers, enhance fat burning (Hossain, 2016; Mohd Ali et al., 2020). The health benefits of pineapples are further

improved with the presence of different phytochemicals bioactive compounds, phenolic compounds and flavonoids (Mohd Ali et al., 2020).

Bromelain is a mixture of proteolytic enzymes extracted primarily from the stem and fruit of the pineapple plant. It has been studied for its potential therapeutic applications, including anti-inflammatory, antioxidant, and anticancer properties. It belongs to a class of enzymes known as cysteine proteases, which break down protein molecules by hydrolyzing peptide bonds. The enzyme's efficacy in tenderizing meat arises from its ability to degrade collagen and muscle fibers, thereby improving meat texture and palatability. According to Ketnawa et al. (2018), bromelain's enzymatic activity depends on factors such as pH, temperature, and duration of exposure. The optimal pH for bromelain activity is between 6.0 and 8.0, and it functions effectively at temperatures ranging from 50°C to 60°C. When applied to meat, bromelain hydrolyzes collagen and myofibrillar proteins, leading to a softer and more palatable product. This makes bromelain particularly useful in the preparation of tougher cuts of meat that are otherwise difficult to cook and consume. Comparative studies have demonstrated that bromelain is more effective in tenderizing meat than other plant-derived proteases such as papain from papaya and ficin from figs. A study by Ha et al. (2016) compared the tenderizing effects of bromelain, papain, and ginger extract on beef and found that bromelain-treated samples showed significantly greater reduction in shear force values, indicating enhanced tenderness. Bromelain is commonly used in commercial meat tenderizer powders and marinades. It can be applied in fresh or processed pineapple juice form or as a lyophilized powder. When used in marinades, pineapple juice not only tenderizes the meat but also imparts a characteristic flavour that is appreciated in many cuisines. Varilla et al. (2021) indicates that bromelain can modulate immune responses, reduce inflammation, and inhibit tumour growth in animal models. Additionally, it may enhance the bioavailability of certain drugs and reduce the adverse effects of multiple antibiotics (Varilla et al., 2021).

In culinary practices, pineapple juice or pulp is often used to marinate meat for a few hours before cooking. This is especially common in Asian and Caribbean cuisines, where pineapple marinades are part of traditional recipes. In industrial settings, bromelain is incorporated into meat tenderizer powders or enzymatic hydrolysis processes to improve the quality of meat products. Research by Arshad et al. (2016) investigated the use of bromelain as a natural meat tenderizer in chicken and beef and found that it significantly improved tenderness without adversely affecting flavour or

nutritional quality. The enzyme's action led to a noticeable breakdown of muscle fibres, which was evident under microscopic examination. In meat processing, controlled enzymatic treatment is crucial to ensure consistent product quality. Excessive exposure to bromelain can result in overly mushy textures, which are generally undesirable. Therefore, the enzyme concentration, duration of treatment, and storage conditions must be optimized. Recent innovations have explored the encapsulation of bromelain to regulate its release and activity during meat processing. Microencapsulation techniques using alginate or chitosan matrices have been proposed to provide a controlled enzymatic action while preserving the enzyme's stability (Lourenço et al., 2020).

While the use of pineapple and bromelain as meat tenderizers offers numerous benefits, certain precautions must be considered. First, excessive use of bromelain can lead to over-tenderization of meat, resulting in a mushy and unpalatable texture. This is especially true in home cooking scenarios where enzyme concentrations and exposure times are not accurately measured. Secondly, bromelain can cause allergic reactions in sensitive individuals. Symptoms may include skin rashes, gastrointestinal discomfort, and, in rare cases, anaphylaxis. According to Pavan et al. (2017), individuals with known allergies to pineapple or latex may exhibit cross-reactivity with bromelain, and its use should be avoided or monitored closely in such populations.

Additionally, bromelain may interact with certain medications, including anticoagulants and antibiotics. Its anticoagulant properties, while beneficial in some therapeutic contexts, can pose risks of bleeding when combined with blood-thinning medications. Therefore, individuals on such medications should consult healthcare professionals before consuming bromelain-containing meat tenderizers or supplements. Microbial contamination is another concern in the use of fresh pineapple juice or pulp in meat marinades. Pineapple, being a perishable fruit, can harbour microbial flora that may contaminate meat during marination. Ensuring hygiene and proper storage conditions is essential to prevent foodborne illnesses. The use of pineapple by-products for bromelain extraction has positive environmental implications. Traditionally, the stem and peels of pineapple are considered agricultural waste. However, these parts contain high concentrations of bromelain and can be valorized through enzyme extraction. Utilizing pineapple waste for bromelain production not only adds value to the fruit but also reduces agricultural waste, promoting a circular bioeconomy model (Ketnawa et al., 2018). From an economic standpoint, the adoption of natural meat

tenderizers like bromelain can reduce reliance on synthetic additives, thereby aligning with consumer preferences for clean-label and natural food products. This shift can enhance market competitiveness for meat processors and contribute to sustainable food processing practices.

Pineapple contains various antioxidants, including flavonoids and phenolic acids, which help combat oxidative stress and reduce the risk of chronic diseases such as cardiovascular disease and cancer. A study by Seenak et al. (2021) demonstrated that daily intake of pineapple reduced cardiac oxidative stress and improved lipid profiles in rats with hypercholesterol. Pineapple exhibits antimicrobial properties due to the presence of compounds like bromelain, saponins, and phenolic acids. Studies have shown that pineapple extracts can inhibit the growth of various pathogenic bacteria, including *Escherichia coli* and *Pseudomonas aeruginosa*. These properties suggest potential applications in food preservation and therapeutic treatments (Castro et al., 2025). The pineapple industry generates significant amounts of waste, including peels, cores, and stems. Recent studies have focused on valorising these by-products to produce value-added products such as biofuels, bioplastics, and functional foods. For instance, pineapple peel waste has been utilised in the production of vinegar and prebiotic flour. Additionally, innovative bio refinery techniques have been employed to extract bioactive compounds like pectin, citric acid, and bromelain from pineapple waste (Ayu Tania Putrisma, 2025). Furthermore, the utilization of pineapple as a natural meat tenderizer highlights its multifunctional role in both food processing and nutritional enhancement. Beyond its proteolytic activity, the presence of antioxidants and bioactive compounds in pineapple contributes to improved meat quality by reducing oxidative deterioration during storage and cooking. This dual functionality supports the development of value-added meat products that align with current consumer demand for natural and health-promoting ingredients. Additionally, the potential to utilize pineapple by-products, such as peels and stems, for bromelain extraction presents an environmentally sustainable approach by reducing agricultural waste. However, careful control of enzymatic activity remains essential to ensure optimal tenderness without compromising texture or safety. Therefore, pineapple-based tenderization represents a promising strategy in advancing sustainable, clean-label, and functional meat processing technologies.

2.8 Herbs and Spices

Herbs and spices have been fundamental to human societies for thousands of years, serving multiple purposes in culinary, medicinal, and ritualistic contexts. Derived from plants, herbs typically refer to the green, leafy parts, while spices originate from other parts such as seeds, bark, roots, and fruits. Their use is deeply embedded in various cultures and continues to play a crucial role in food preparation and preservation, contributing to both flavour and health. In Southeast Asia, and particularly in Malaysia, herbs and spices are not only culinary enhancers but also cultural icons that reflect the rich tapestry of ethnic diversity. With its strategic location in the spice route and a confluence of Malay, Chinese, and Indian communities, Malaysia boasts a vibrant culinary heritage where herbs and spices are central to flavour development and identity. Use of spices and herbs have been reported to have climbed exponentially over the course of the last 500 years according to the U.S. Department of Agriculture, especially spices such as chilli pepper, cinnamon, ginger, black pepper, turmeric, fenugreek, rosemary, and garlic (Jiang, 2019). In Malaysia, the most commonly used spices in local dishes are cardamom, pepper, clove, star anise, cumin, fennel, nutmeg and exotic vegetables made finely ground or in form of paste. Local Malaysian dishes are characterised with being rich in herbs and spices, coconut milk being used for most dishes and usually spicy (Raji et al., 2017).

Spices have been essential in the world of cooking for the longest time, mainly for the use of flavouring, colouring, preserving food as well as medicinal purposes (Jiang, 2019). It is also further explained to have the main advantage of being residue free and generally food grade safe thus being potential alternatives to chemical additives (Martínez-Graciá et al., 2015). Historically, the value of herbs and spices transcended mere culinary uses. They were among the first commodities traded across continents, often valued as highly as gold and precious stones. In ancient civilizations such as Egypt, China, and India, spices played critical roles in medicine, preservation, and religious practices. The ancient Egyptians used herbs such as cumin and coriander in embalming, while Indian Ayurvedic texts documented the therapeutic properties of turmeric, ginger, and black pepper (Srinivasan, 2014). In the context of Malaysia, the historical significance of spices is underscored by the region's prominence in the ancient maritime spice trade. The Malacca Sultanate, which flourished in the 15th century, served as a pivotal trading port that attracted Arab, Chinese, Indian, and European

merchants seeking spices such as cloves, nutmeg, cinnamon, and pepper. This exchange not only facilitated economic growth but also introduced a range of spices and culinary traditions that were assimilated into local practices (Jayaraj, 2020). Indigenous herbs, such as *serai* (lemongrass), *kunyit* (turmeric), and *daun kesum* (Vietnamese coriander), have long been cultivated and utilized by native communities, contributing to a deeply rooted tradition of spice usage.

Malaysian culinary tradition is richly infused with herbs and spices, which serve as essential agents of flavor and aroma. *Persicaria minor*, also known locally as *kesum* or *laksa* leaf, plays a central role in traditional dishes, delivered both as a fresh and dried ingredient. It is commonly incorporated into *laksa*, *nasi kerabu*, *asam pedas*, and *tom yam*, and is also consumed as tea or *ulam* (edible raw salad herbs) and used for topical medicinal applications in Malay culture. Pharmacological investigations have provided evidence of its antioxidant, anti-inflammatory, antimicrobial, digestive-enhancing, anti-ulcer, cognitive-enhancing, immunomodulatory, and acetylcholinesterase-inhibiting activities. Moreover, *kesum* has the highest phenolic and antioxidant concentrations compared to other Malaysian herbs and has been explored in bioactive edible packaging films to extend food shelf life (Khairul Ikram et al., 2024).

Malaysia's culinary landscape is a fusion of Malay, Chinese, Indian, and indigenous influences, each contributing unique spice blends and cooking techniques. Herbs and spices serve as the backbone of Malaysian cuisine, not merely as seasoning but as essential elements that define a dish's identity. Their usage reflects cultural practices and is instrumental in creating complex flavour profiles characterized by spiciness, earthiness, and aromatic intensity. In Malay cuisine, the foundational flavouring base often begins with a *rempah*; a spice paste comprising blended shallots, garlic, ginger, galangal, chilies, turmeric, and candlenuts. This paste is sautéed until aromatic and forms the basis for dishes such as *rendang*, *asam pedas*, and *laksa*. Additionally, the broader culinary landscape in Malaysia features pervasive use of lemongrass, galangal, *belacan* (shrimp paste), coconut milk, and an array of spices in both powdered and whole form. These ingredients are foundational to flavouring Malay dishes and reflect deep cultural roots in regional cuisine (Zainal Abidin et al., 2020).

Lemongrass and kaffir lime leaves are commonly added for their citrusy notes, while *daun kunyit* (turmeric leaves) imparts a subtle earthiness to dishes like *nasi ulam*. Indian Malaysians incorporate spices such as cumin, fennel, mustard seeds, curry leaves, and various masala blends into their cuisine. Dishes like chicken curry, dal, and

biryani are seasoned with intricate spice combinations that are dry-roasted or tempered in oil to release essential oils that deepen the flavours. Chinese Malaysians tend to use ginger, garlic, star anise, and five-spice powder to infuse broths, stir-fries, and braised meats with warmth and complexity. A notable feature of Malaysian spice usage is the layering of flavours through sequential addition and heat control. This method allows volatile aromatic compounds to be preserved or enhanced, creating the distinctive depth seen in Malaysian cooking. Herbs and spices are not only added for flavour but also for texture and visual appeal. For instance, fresh curry leaves are often added at the end of cooking to provide a burst of aroma and colour.

The flavour-enhancing properties of herbs and spices are due to their phytochemical constituents, including essential oils, terpenoids, flavonoids, and alkaloids. These compounds interact with taste receptors and olfactory neurons to produce a wide spectrum of sensory experiences. Capsaicin in chili peppers, for example, elicits a spicy sensation, while eugenol in cloves imparts a sweet, warming flavour (Cedillo-Olivos et al., 2024). Beyond flavour, many herbs and spices offer preservative properties. Turmeric and ginger contain antimicrobial compounds that inhibit the growth of bacteria, contributing to food safety and shelf-life extension. Clove oil, rich in eugenol, has been used as a natural preservative in traditional foods. Spices also stimulate appetite and digestion, making them indispensable in tropical climates where maintaining appetite and digestion amid heat and humidity is essential. In traditional Malay households, dishes such as *ulam*, a raw herb salad made from *pegaga*, *daun selom*, and *daun kesum*, are consumed not only for their taste but also to aid digestion and detoxification.

A cross-sectional study conducted by Jiang (2019) involving 703 adults examined consumers' perception towards spices and their uses. The results of the study showed 48% of the consumers were interested in learning the health benefits of spices, 51% were willing to use spices as alternative and complementary. This is further supported by a review written by Martínez-Graciá et al. (2015) reporting these herbs and spices possessing antimicrobial activity and a high correlation between antimicrobial efficacy and level of phenolic components present in the herbs and spices.

Studies have highlighted the potential of herbs and spices as functional foods and nutraceuticals. Curcumin, the active compound in turmeric, has been shown to possess anti-inflammatory, antioxidant, and anticancer properties. It modulates signalling pathways involved in chronic diseases, including metabolic syndrome and

neurodegenerative disorders (Sharma et al., 2015). Similarly, gingerol from ginger and allicin from garlic are associated with cardiovascular health benefits, including blood pressure regulation and cholesterol reduction. These spices are frequently used in Malaysian dishes not only for their flavour but also for their perceived health benefits. The antioxidant capacity of herbs like curry leaves, lemongrass, and *pandan* has also been documented. These herbs contain polyphenolic compounds that scavenge free radicals and may reduce oxidative stress, which is implicated in aging and various chronic illnesses (Zainal Abidin et al., 2020). While herbs and spices are generally recognized as safe when used in culinary amounts, there are potential risks associated with their overconsumption or inappropriate use. Some spices, such as nutmeg, can cause hallucinogenic effects and toxicity in high doses due to compounds like myristicin. Overuse of chili peppers can lead to gastrointestinal irritation and exacerbate conditions such as gastritis. Furthermore, a 2019 Malaysian-based systematic review highlighted rising consumer interest in herbal-based food products, driven by perceptions of safety and naturalness. The review noted significant challenges in ensuring product quality and regulatory oversight in Malaysia's growing herbal food sector (Zainal Abidin et al., 2020).

Another area of concern is allergic reactions. Spices such as mustard, sesame, and celery are known allergens and can trigger hypersensitivity reactions in susceptible individuals. Even commonly used herbs like coriander and cumin have been reported to cause allergic responses in rare cases (Sokovic et al., 2020). Furthermore, certain spices may interact with medications. Turmeric, for example, can potentiate the effects of anticoagulants, increasing the risk of bleeding. Garlic supplements can interfere with blood pressure medications, while high doses of cinnamon have been linked to liver toxicity due to coumarin content (Cedillo-Olivos et al., 2024). The issue of adulteration also poses a significant risk. Unscrupulous practices may involve the addition of non-food-grade dyes, fillers, or contaminants such as heavy metals to bulk up spices. In Malaysia, the Food Safety and Quality Division under the Ministry of Health conducts regular surveillance, but consumer awareness remains vital. Pregnant women, children, and individuals with chronic illnesses are advised to exercise caution when consuming herbal supplements. While culinary usage is typically safe, concentrated extracts can pose health risks if taken without medical supervision.

In food preservation, several Malaysian herbs and spices have demonstrated remarkable antimicrobial and antioxidant properties, suggesting their role as natural

biopreservatives. *Polygonum minus* leaf extracts or *kesum* have exhibited strong antibacterial, antifungal, and anti-inflammatory capabilities, notably inhibiting pathogens such as *Salmonella enteritidis*, *S. typhi*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*. These effects are attributed to a high content of phenolic and flavonoid compounds (Saripah et al., 2015). Chitosan-based edible films infused with extracts of *kesum* and curry leaf were developed for meat packaging. When applied to chicken breast stored at 4 °C, films containing 2.0% (w/v) of both extracts significantly reduced microbial counts and pH decline over 14 days, extending shelf life by at least two days compared to controls (Saripah et al., 2015). Beyond *kesum*, research on other spices common in Malaysian contexts such as cinnamon, clove, and black pepper reveal their broad-spectrum antimicrobial and antioxidant capacities, aligning with global findings on their utility in preserving food quality (Aziz et al., 2018). Furthermore, the incorporation of herbs and spices into meat processing systems extends beyond flavour enhancement, offering functional benefits that align with modern food innovation trends. Their natural antioxidant and antimicrobial properties contribute to improved shelf life, reduced microbial spoilage, and enhanced safety of meat products. This is particularly relevant in tropical climates such as Malaysia, where food preservation remains a significant concern. Additionally, the use of herbs and spices supports the development of clean-label products by reducing reliance on synthetic additives while maintaining desirable sensory attributes. However, the effectiveness of these natural ingredients depends on factors such as concentration, extraction method, and interaction with the meat matrix. Therefore, optimizing their application is essential to achieve consistent quality and consumer acceptance, reinforcing their potential as multifunctional ingredients in both traditional and industrial meat processing.

2.9 Antioxidants

Antioxidants are defined as substances that significantly delay or prevent the oxidation of a substrate when present in low concentrations relative to the substrate (Halliwell & Gutteridge, 2015). These substances can be enzymatic or non-enzymatic. Enzymatic antioxidants include superoxide dismutase, catalase, and glutathione peroxidase, which catalyze the breakdown of ROS into less harmful molecules. Non-enzymatic antioxidants, commonly obtained through diet, include vitamins C and E,

carotenoids, flavonoids, and phenolic acids. The antioxidant capacity of these compounds lies in their ability to donate electrons to free radicals, stabilizing them and preventing them from initiating chain reactions that lead to cellular damage. Antioxidants are molecules that inhibit the oxidation of other molecules, thereby protecting the body from the deleterious effects of oxidative stress.

Oxidative stress results from an imbalance between ROS and the body's ability to detoxify them or repair the resulting damage. ROS can damage cellular components such as DNA, proteins, and lipids, and are implicated in the pathogenesis of various chronic diseases including cardiovascular diseases, cancer, diabetes, and neurodegenerative disorders (Pham-Huy et al., 2018). With increasing awareness of the impact of oxidative stress on human health, dietary antioxidants have garnered significant attention due to their natural occurrence and safety profile. Among the wide array of antioxidant-rich foods, fruits are particularly noteworthy for their high content of vitamins, polyphenols, and other bioactive compounds. Antioxidants can be found in two forms which are natural and synthetic antioxidants. The natural antioxidants that can be obtained from plants possess more significant benefit compared to the synthetic ones. This is because the use of natural antioxidants does not cause side effects, while the use of synthetic antioxidants was found to have genotoxic effects (Donkor, 2015).

Phytochemicals are bioactive compounds in foods with the protective role as antioxidants by assisting the body by reacting to free radicals and oxygen species. This antioxidant activity proves to be beneficial because of the excess production of ROS and reactive nitrogen species which causes imbalance and oxidative damage to the human body (Amadi et al., 2018). The protective effects of antioxidants are primarily due to their ability to neutralize free radicals by donating an electron or hydrogen atom. This stabilizes the free radical and terminates chain reactions that can lead to cellular injury.

Some antioxidants also chelate metal ions such as iron and copper, which catalyze the formation of ROS via Fenton reactions. Others upregulate the expression of endogenous antioxidant enzymes through the activation of transcription factors like nuclear factor erythroid 2-related factor 2 (Nrf2). Polyphenols, one of the most abundant groups of antioxidants in plant-based foods, exert their effects through direct scavenging of ROS, modulation of enzyme activity, and interaction with cell signaling pathways (Panche et al., 2016). Flavonoids, a subclass of polyphenols, possess a structural configuration that allows them to donate hydrogen atoms or electrons and

delocalize the resulting radical within their aromatic ring structure. Carotenoids such as beta-carotene and lycopene quench singlet oxygen and peroxy radicals, contributing to the reduction of oxidative damage.

Several analytical techniques have been developed to evaluate the antioxidant activity of food-derived compounds. These assays vary in terms of mechanism, specificity, sensitivity, and applicability. The most common methods include DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay, Ferric Reducing Antioxidant Power (FRAP) assay, and Oxygen Radical Absorbance Capacity (ORAC) assay. The DPPH assay is a colorimetric method that measures the ability of antioxidants to reduce the DPPH radical, which is purple in colour, to a yellow-coloured diphenylpicrylhydrazine. The degree of discoloration indicates the scavenging potential of the antioxidant (Shahidi & Zhong, 2015). Similarly, the ABTS assay involves the generation of a blue-green ABTS radical cation, which is reduced by antioxidants, resulting in a decrease in absorbance. The FRAP assay assesses the reducing power of antioxidants based on their ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), forming a coloured complex with TPTZ (2,4,6-tripyridyl-s-triazine).

ORAC measures the inhibition of peroxy-radical induced oxidation by antioxidants and is considered more biologically relevant due to the use of a free radical generator that mimics *in vivo* conditions. These assays, when used in combination, provide a comprehensive understanding of the antioxidant potential of food samples. However, due to their different mechanisms, results from different assays may not always correlate, highlighting the need for careful interpretation and standardized protocols. DPPH assay is most generally used to assess antioxidant capacity of food products and plants, delivering consistent results under varying concentrations, organic solvents, antioxidant/free radical volume ratios and reaction times. DPPH assay is normally expressed as EC_{50} antiradical power, stoichiometric value, free radical inhibition, remaining antiradical activity or ascorbic acid equivalent antioxidant capacity. DPPH assay has been used for various organic solvents. When semi-aqueous medium is used, the reaction can be completed in seconds, the predictability of antioxidant properties of analysed compounds is elevated (Musa et al., 2016).

Based on a study conducted by Othman et al. (2015), four Malaysian local herbs were investigated for TPC, TFC and AA using DPPH radical scavenging. It was found all herbs showed positive correlation between TPC and DPPH. However low correlation

between TFC and AA showing that phenolic compounds were responsible for antioxidant activities. This is due to DPPH is a part of phenolic content thus they correlate between each other while flavonoids and antioxidants, though having similar function, are independent of each other. This is further supported by a study by Abdullah and Mazlan (2020), investigating TPC and TFC of herbal tea and revealed that the two were positively to the antioxidant activity suggesting phenolic compounds and flavonoids being responsible for antioxidant activities. Another study by Hanis Mastura et al. (2017) revealed that TPC and antioxidant capacities can have an increment due to cooking process and observed linear relationship between TPC and DPPH antioxidant capacity, FRAP and antioxidant capacity. A positive relationship between TPC and antioxidant capacities shown that polyphenol is one of the sources of antioxidants in mix spice cooking pastes.

Dietary antioxidants are predominantly obtained from plant-based foods, with fruits being among the richest sources. Fruits contain a wide array of antioxidant compounds, including vitamin C (ascorbic acid), vitamin E (tocopherols and tocotrienols), carotenoids, and polyphenols such as flavonoids and phenolic acids. Berries, such as blueberries, strawberries, and raspberries, are exceptionally high in anthocyanins, which contribute to their vibrant colour and potent antioxidant activity. Studies have shown that regular consumption of berries can improve oxidative stress biomarkers and reduce inflammation (Abdullah & Mazlan, 2020). Citrus fruits like oranges, lemons, and grapefruits are excellent sources of vitamin C, a water-soluble antioxidant that plays a vital role in collagen synthesis and immune function. Tropical fruits, especially those grown in Southeast Asia, are also rich in antioxidants. Mangoes contain beta-carotene and mangiferin, a xanthonoid with strong free radical scavenging properties.

Papayas provide vitamin C and carotenoids, while pineapples offer phenolic compounds and flavonoids. Guava is particularly notable for its high vitamin C content, exceeding that of oranges in some varieties. The Malaysian fruit *rambutan* (*Nephelium lappaceum*) has been reported to possess antioxidant-rich peel and seed extracts, which are increasingly explored for functional food development (Thitilertdech et al., 2018). Grapes and pomegranates are other fruits with significant antioxidant potential, containing polyphenols like resveratrol and ellagitannins, respectively. These compounds have been associated with cardiovascular protection, improved lipid profiles, and anti-cancer properties. Apples and pears, commonly consumed in

temperate regions, are rich in quercetin and catechins, which contribute to their antioxidant capacity. From a study by Amadi et al. (2018), Vitamin C, an effective antioxidant, content is low within the jackfruit pulp, seed and leaves. However, the content varied depending on variety and maturation. Meanwhile in the same study, phytochemicals in jackfruit pulp are significantly lower than the seeds and leaves. In a review by Mohd Ali et al. (2020), it has been reported that the phenolic content of pineapple comprised of 51% methanol, 14% ethyl acetate and 3% water extract with determination of antioxidant activity through a β -carotene-linoleate acid antioxidant assay at 100 ppm of concentration yielding similar results showing that pineapples are a good source of antioxidants, signifying a high content of phenolic in the fruit.

Regular consumption of antioxidant-rich fruits has been linked to numerous health benefits. Epidemiological studies consistently demonstrate an inverse relationship between fruit intake and the risk of chronic diseases, largely attributed to the antioxidant content of fruits. One of the most well-established benefits is cardiovascular protection. Antioxidants such as flavonoids and vitamin C improve endothelial function, reduce blood pressure, and inhibit LDL oxidation, a key step in atherogenesis (Morelli et al., 2020). The anti-inflammatory properties of fruit antioxidants further contribute to the prevention of atherosclerosis. Antioxidants also play a role in cancer prevention by protecting DNA from oxidative damage and modulating signalling pathways involved in cell proliferation and apoptosis. Polyphenols such as quercetin and resveratrol have been shown to inhibit the growth of various cancer cell lines in vitro and reduce tumour formation in animal models. In the context of neurodegenerative diseases, antioxidants from fruits may help delay cognitive decline by reducing oxidative stress and inflammation in the brain. For example, studies suggest that the polyphenols in berries can improve memory and cognitive performance in aging populations.

Antioxidants also support immune function, skin health, and metabolic regulation. Vitamin C enhances immune responses, carotenoids support skin integrity, and polyphenols influence glucose metabolism and insulin sensitivity. While dietary antioxidants from fruits are generally considered safe and beneficial, there are certain precautions and implications associated with their consumption, particularly in supplemental form or in excess. One concern is the potential for antioxidant supplements to interfere with physiological ROS signalling. Although ROS are often viewed as harmful, they also serve as signalling molecules in various biological

processes, including cell proliferation and immune responses. Excessive antioxidant supplementation may blunt these processes and have unintended consequences (Poljsak et al., 2018). Furthermore, some studies have reported that high doses of certain antioxidant supplements, such as beta-carotene and vitamin E, may increase the risk of adverse health outcomes, including lung cancer in smokers and haemorrhagic stroke, respectively. This highlights the importance of obtaining antioxidants primarily from whole foods rather than isolated supplements, unless medically indicated. Another consideration is the bioavailability of antioxidants.

The health benefits of antioxidants depend not only on their intake but also on their absorption, metabolism, and distribution within the body. Factors such as food matrix, digestion, gut microbiota, and individual variability influence the bioavailability of dietary antioxidants. For instance, the polyphenols in fruits often exist in glycosylated forms that require hydrolysis by intestinal enzymes or microbiota before absorption. Additionally, the stability of antioxidants during food processing and storage is a concern. Heat, light, and oxygen can degrade sensitive compounds like vitamin C and polyphenols, reducing their effectiveness. Therefore, consuming fresh fruits or minimally processed products is recommended to maximize antioxidant intake. In populations with allergies or sensitivities, certain fruits may pose risks. For example, some individuals may experience allergic reactions to citrus fruits or stone fruits. Those with fructose malabsorption or irritable bowel syndrome (IBS) may also need to moderate fruit intake to avoid gastrointestinal symptoms. Finally, while antioxidant-rich fruits are an important component of a healthy diet, they should be consumed in the context of a balanced diet that includes other food groups. Relying solely on fruits without considering overall dietary patterns may lead to nutritional imbalances. Furthermore, the integration of antioxidant-rich ingredients into food systems, particularly in meat products, has gained increasing importance due to their ability to enhance both nutritional value and product stability. Antioxidants play a crucial role in inhibiting lipid and protein oxidation, which are primary factors contributing to quality deterioration during storage and processing. By incorporating natural sources of antioxidants, such as fruits, herbs, and plant extracts, it is possible to improve the shelf life, colour stability, and sensory attributes of meat products without relying on synthetic additives. Additionally, the synergistic effects between different antioxidant compounds may further enhance their protective capabilities. However, variations in antioxidant activity depending on extraction methods, processing conditions, and food

matrices highlight the need for standardized evaluation techniques to ensure consistency and effectiveness in practical applications within the food industry.

2.10 Nutrient Content

Understanding nutrient content is a foundational aspect of nutritional science, food technology, and public health. Nutrient content refers to the quantitative and qualitative presence of essential substances in food that contribute to physiological health, metabolic functioning, and overall well-being. These substances include macronutrients such as carbohydrates, proteins, and fats as well as micronutrients like vitamins and minerals. The nutritional profile of food directly influences diet quality, nutritional status, and long-term health outcomes (Paraginski et al., 2016).

Particularly in the context of meat and meat-based products, understanding nutrient content becomes crucial due to the dual role of meat as a rich source of essential nutrients and a dietary component associated with potential health risks when consumed inappropriately. Nutrient content encapsulates the presence of biologically essential components required to sustain life and promote health. Macronutrients provide the energy necessary for basic physiological activities and include proteins, fats, and carbohydrates. Proteins, for instance, are crucial for tissue building and repair and are composed of amino acids, some of which are essential and must be obtained from the diet. Meat, especially red meat and poultry, is considered a complete protein source as it contains all essential amino acids in adequate proportions (Kumar et al., 2015).

Fats serve as energy stores and are carriers of fat-soluble vitamins such as A, D, E, and K. Carbohydrates, although not significantly present in most unprocessed meats, can be introduced through marinades or preparation techniques. Micronutrients, though required in smaller quantities, are no less important. Iron, zinc, selenium, and B vitamins (notably B12) are abundantly found in meat products. Iron from meat (heme iron) has higher bioavailability compared to non-heme iron from plant sources, making meat an effective dietary source for addressing anaemia (Hurrell & Egli, 2015). Likewise, vitamin B12, essential for neurological function and red blood cell formation, is almost exclusively available in animal-derived products, emphasizing the nutritional significance of meat in omnivorous diets.

Accurate understanding of nutrient content has several vital uses. In clinical settings, it aids healthcare providers in crafting individualized nutrition plans,

particularly for patients with specific dietary needs such as those with metabolic disorders, cardiovascular diseases, or nutritional deficiencies. For instance, patients with chronic kidney disease require controlled protein intake, necessitating precise knowledge of protein levels in consumed foods, especially meats (Ikizler et al., 2020). From a public health perspective, nutrient profiling of commonly consumed foods informs dietary guidelines and fortification strategies. Governments and health organizations rely on nutrient composition data to develop food labelling systems, dietary reference intakes, and food assistance programs (Drewnowski et al., 2020).

In food technology and product development, nutrient analysis supports the creation of healthier food products by modifying recipes to enhance or reduce specific nutrients, such as reducing sodium or increasing omega-3 fatty acid content in processed meats. Furthermore, in consumer education, knowledge about nutrient content empowers individuals to make informed dietary choices, improving nutritional literacy and promoting healthier eating behaviours. In meat consumption, this translates into awareness about saturated fat content, cholesterol levels, and the benefits of lean cuts versus processed meats. Balanced nutrition, defined as the consumption of food containing essential nutrients in appropriate proportions, is critical for sustaining physiological function, maintaining health, and preventing disease. Imbalanced diets, whether due to undernutrition or over nutrition, can lead to a host of health issues, including stunted growth, weakened immunity, obesity, diabetes, and cardiovascular diseases (Micha et al., 2017). Meat, as a dense source of high-quality protein and micronutrients, plays a pivotal role in achieving balanced nutrition, particularly in diets where plant-based sources may not suffice for certain nutrients.

However, the consumption of meat must be moderated and contextualized within the overall dietary pattern. The Dietary Guidelines Malaysia emphasize lean meat choices and recommend limiting intake of processed meats, which are often high in sodium, saturated fats, and preservatives like nitrites. Including meat as part of a balanced diet involves not just focusing on its nutrient content but also considering preparation methods, portion sizes, and complementary food combinations. In developing countries, meat serves a critical role in combating undernutrition and micronutrient deficiencies, particularly among vulnerable populations such as children and pregnant women. In contrast, in high-income settings, excessive meat consumption, especially of red and processed varieties, has been linked to chronic health issues, highlighting the need for dietary balance and diversity (Richi et al., 2015).

The application of marinades to meat prior to cooking is a common culinary practice intended to enhance flavour, tenderness, and shelf life. However, marinades can also significantly influence the nutrient content of meat, both positively and negatively, depending on their composition and the marination process. Marinades are typically composed of acidic components, oils, salts, herbs, spices, and sometimes enzymes or dairy products. These ingredients can interact chemically and physically with meat tissues, resulting in alterations in moisture content, pH levels, and protein structures. Acidic marinades, for example, can denature surface proteins, improving texture but also potentially leaching out water-soluble vitamins and minerals, especially during prolonged marination or high-temperature cooking following marination (Domínguez et al., 2019).

Conversely, marinades rich in antioxidants such as those containing rosemary, thyme, garlic, or ginger have been shown to protect meat lipids from oxidative degradation during cooking. Lipid oxidation is a primary cause of nutrient loss in meat, particularly affecting unsaturated fatty acids and fat-soluble vitamins. Antioxidant-rich marinades thus help preserve the nutritional quality of meat by inhibiting the formation of harmful oxidative compounds (Mancini & Hunt, 2015). The addition of herbs and spices in marinades can also contribute to the overall nutrient profile by adding phytochemicals, although their quantitative contribution to total nutrient intake is often minimal. However, the health-promoting effects of certain compounds such as curcumin from turmeric or allicin from garlic should not be disregarded, particularly when consumed regularly. Nonetheless, the use of commercial marinades requires caution. Many pre-packaged marinades contain high levels of sodium, sugars, and artificial preservatives, which can negatively impact the healthfulness of the final dish. For individuals managing hypertension, diabetes, or other diet-related conditions, such additives can undermine the benefits of lean meat consumption. Therefore, the choice and preparation of marinades must be aligned with health-conscious cooking practices to avoid compromising nutrient quality.

A study by Aung et al. (2022) revealed that adding meat tenderizers to meat or processed meat products do change the nutrient content of the product. In the study, it was shown that adding plant based meat tenderizers (pineapple and tomato powder) makes higher processing yield, higher rehydration capacity, lower fat content, higher moisture and lower pH and thiobarbituric acid reactive substances (TBARS). Contrarily, another study using salt brine 7% solution, soy sauce, cider and apple juice as meat

tenderizers on boneless shoulder meat, using these ingredients have made the meat more tender and acceptable in sensory, but the ingredients used did not have significant influence over meat tenderness and they were either high in sodium, unfavourable for health, or high level of acidity, which affects taste (Stiintifice, 2018).

Natural plant-based tenderizers, by contrast, often show more favourable outcomes, combining tenderizing effects with preservation of quality and sometimes enhanced nutritional value. A study on beef treated with proteolytic enzymes extracted from pineapple and jackfruit by-products demonstrated effective tenderization without compromising quality parameters; even cooking yield and moisture retention remained acceptable, indicating that agricultural waste-derived natural tenderizers may enhance both sustainability and meat quality (Ramli et al., 2021). Ginger-derived zingibain, a cysteine protease, offers particular advantages. Unlike papain, zingibain preferentially targets collagen and actomyosin, minimizing over-hydrolysis of other proteins and avoiding the mushy texture associated with less specific proteases (Ramli et al., 2021). Its high collagenolytic activity suggests it can tenderize efficiently while maintaining structural meat integrity—a desirable trait aligning both sensory and nutritional preservation.

The nutrient content of meat on the other hand, based on a study by Bujang et al. (2016) incorporating Cassava leaves in meat floss, adding ingredients to meat changes the nutrient content, lowering total protein percent and increasing fiber content due to the addition of the leaves. This is due to adding more ingredient in meat increases the total weight of the meat without adding any more protein which causes the protein percent to be lower, the total protein content is actually unchanged. However, in another research done by Susilowati et al. (2022) of soaking meat in HSFI-8 crude protease, an enzyme that can be obtained by sea cucumber, it is found that the meat that had been soaked in the enzyme had higher concentration of protein as compared to control meat after being analysed by using spectrophotometry due to the protein content of the crude protease which seeps in the meat after three hours of soaking. Certain meat tenderizer treatments also proves to improve the nutrient content of meat, a study by Bureros et al. (2020) shows meat treated with bacterial protease derived from *Bacillus subtilis* had significantly higher crude protein (37%) as compared to negative control and commercial meat tenderizer (34% and 31% respectively). Nutrient content of pineapples primarily contains carbohydrates, water, dietary fiber, sugars, organic acids, vitamins and minerals (Mohd Ali et al., 2020).

Natural extracts may offer antioxidant and antimicrobial benefits that bolster nutritional quality. In semi-dried goat jerky intended for elderly consumers, a group often affected by compromised digestion, addition of a plant-based tenderizer resulted in enhanced moisture, reduced shear force, inhibition of *E. coli*, lower lipid oxidation, and improved fatty acid profile (lower SFA and increased PUFA with better health indices) (Aung et al., 2022). This demonstrates that ingredient-based tenderizers can improve both digestibility and nutritional composition, not merely tenderness. Likewise, natural extracts rich in antioxidants can enhance storage stability and quality of meat products. In meatball formulations, plant extract significantly suppressed lipid oxidation (up to 30 % reduction in TBARS) compared to control, while maintaining texture and sensory quality (Ha et al., 2016). Such protective effects preserve meat's nutritional value over storage, enhancing shelf life without resorting to synthetic additives.

Despite the apparent benefits of consuming nutrient-rich foods such as meat, improper management of nutrient content can lead to adverse health outcomes. Overconsumption of certain nutrients, particularly saturated fats and cholesterol prevalent in some meat cuts, has been associated with increased risks of atherosclerosis, obesity, and metabolic syndrome (Pan et al., 2016). Furthermore, excessive intake of iron, especially from heme sources, may contribute to oxidative stress and has been tentatively linked to an elevated risk of colorectal cancer, although findings remain inconclusive and context-dependent (Hurrell & Egli, 2015). Marinades, when misused, can become vehicles for nutrient loss or unwanted nutrient excess. For example, prolonged marination in salt-heavy solutions can significantly increase sodium levels in meat, posing risks for individuals with hypertension. Similarly, using acidic or enzymatic marinades improperly can result in texture breakdown and nutrient leaching. It is also important to consider the impact of cooking methods such as grilling, frying, or boiling post-marination can differentially affect nutrient retention, with high-heat methods generally leading to higher nutrient degradation.

Another concern is the formation of potentially harmful compounds such as heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs) during high-temperature cooking of marinated meats. Certain marinade ingredients may exacerbate or mitigate the formation of these compounds. For instance, antioxidant-rich marinades can inhibit HCA formation, whereas sugary marinades may promote caramelization and increase PAH levels when grilled over open flames (Sinha et al.,

2018). From a food safety perspective, marinating meat also necessitates attention to hygiene and temperature control. Cross-contamination from raw meat marinades can pose microbiological hazards if not properly managed. Thus, consumers and food handlers must be educated about safe marination practices, including discarding used marinades or boiling them before reuse. Moreover, individuals with allergies or intolerances must scrutinize marinade ingredients, as common allergens like soy, dairy, or certain spices can be hidden components in commercial preparations. Accurate labelling and consumer awareness are essential to prevent adverse reactions.

The use of synthetic meat tenderizers, including calcium compounds and broadly acting commercial enzymatic powders, has raised concerns regarding both nutritional quality and sensory outcomes. For instance, inclusion of calcium chloride in restructured spent hen meat slices (RSHS) led to altered physico-chemical properties: pH shifts and uncertain sensory impacts, raising doubts about the overall nutritional and textural acceptability of treated products (Kantale et al., 2019). Beyond pH effects, studies indicate that weak specificity of commercial enzymes like papain and bromelain may result in over-tenderization and mushy textures, which compromise meat integrity and potentially affect digestibility and mouthfeel adversely (Madhusankha & Thilakarathna, 2021). Furthermore, the relationship between meat tenderization and nutrient retention highlights the importance of selecting appropriate processing methods and ingredients to preserve both quality and health value. While tenderization techniques aim to improve texture and palatability, their influence on nutrient composition must be carefully evaluated to avoid unintended losses or imbalances. Natural tenderizers, particularly those derived from plant sources, offer a more favourable approach by enhancing tenderness while maintaining or even improving nutritional properties such as moisture retention and oxidative stability. Additionally, the incorporation of antioxidant-rich ingredients can mitigate nutrient degradation during cooking and storage. However, improper application of tenderizers or marinades may lead to excessive sodium intake, nutrient leaching, or formation of undesirable compounds. Therefore, optimizing tenderization processes is essential to ensure that both sensory quality and nutritional integrity of meat products are effectively maintained.

2.11 Shelf Life

Shelf life is a fundamental concept in food science and technology, directly impacting food quality, safety, consumer acceptability, and economic value. Particularly in food paste products such as curry pastes, meat marinades, tomato paste, and herbal blends understanding and managing shelf life is crucial due to their semi-solid nature, nutrient content, and potential for microbial spoilage. These products are widely used in both domestic and commercial food preparation, and their stability over time is pivotal to ensure safety and retain desired sensory and nutritional attributes (Corradini, 2018). Shelf life is defined as the time span under defined storage conditions in which a food is still acceptable for consumption in terms of safety, nutritional attributes and maintain their chemical, physical, microbiological and sensory characteristics after packing and manufacture (Haouet et al., 2018). Shelf life represents the period during which a product is expected to remain suitable for sale and consumption. In practical terms, it is the interval between the date of production and the point at which the product becomes unacceptable for consumption due to spoilage or significant quality deterioration. In the context of food paste products, shelf life encompasses both microbiological and physicochemical stability (Corradini, 2018).

Due to their semi-solid consistency, high moisture content, and often nutrient-rich formulations (such as spices, oils, and protein derivatives), food pastes are particularly vulnerable to microbial growth and oxidative changes. Shelf life in this category of foods may range from a few days (for fresh, unpreserved pastes) to several months (for heat-treated or chemically preserved versions), depending on ingredients, packaging, and storage conditions (Corradini, 2018; Haouet et al., 2018). All food products are composed by organic substances and undergo degradation or spoilage after a certain amount of time (Haouet et al., 2018). The accurate determination and utilization of shelf life in food paste products serve several important purposes. First and foremost, it ensures food safety, preventing the consumption of products that may harbour pathogenic microorganisms such as *Salmonella* spp., *Listeria monocytogenes*, or *Clostridium botulinum*. Foodborne illnesses pose significant public health risks and can have severe consequences, especially when products are widely distributed or used in institutional settings such as restaurants and catering services (Corbo et al., 2019).

Second, shelf life labelling supports regulatory compliance and consumer protection. Food laws in many countries require clear indication of expiry or best-before

dates to help consumers make informed purchasing and consumption decisions. Mislabelling or mismanagement of shelf life data can lead to legal penalties, recalls, and brand damage. In the highly competitive food market, credibility regarding product stability is essential for building consumer trust and loyalty (Kuswandi et al., 2015). Third, optimizing shelf life contributes to economic sustainability. Food waste is a major global issue, and improper estimation or management of shelf life can result in unnecessary product disposal or consumer reluctance to purchase items perceived as perishable. For producers, extending shelf life while maintaining product quality means reduced losses, increased distribution range, and better inventory control. For consumers, it equates to longer usability and convenience, especially in the case of multi-use pastes. Lastly, nutritional preservation is a key benefit of effective shelf life management. Food paste products often contain sensitive nutrients such as vitamin C, polyunsaturated fatty acids, and bioactive compounds from herbs and spices. Degradation of these compounds during storage reduces the health value of the product. Thus, managing shelf life also serves nutritional objectives (Ekanem & Ekanem, 2018).

Numerous interrelated factors determine the shelf life of food paste products, broadly categorized into intrinsic and extrinsic factors. Intrinsic factors are properties inherent to the food itself. Key intrinsic factors include water activity, pH level, nutrient composition, and the presence of natural antimicrobial compounds. Food pastes typically have high water activity, creating a conducive environment for microbial growth. Lowering a_w through salt, sugar, or dehydration can inhibit spoilage (Rahman & Labuza, 2017). Acidic pastes like tamarind or tomato paste generally have longer shelf lives due to low pH levels that inhibit bacterial growth. Neutral or slightly alkaline pastes are more susceptible to spoilage. The presence of proteins, carbohydrates, and lipids provides a substrate for microbial growth and chemical degradation. Lipids in particular are prone to oxidation, leading to rancidity and flavour loss. Ingredients such as garlic, turmeric, and chili contain phytochemicals with antimicrobial properties that can enhance stability. However, their effect is concentration-dependent and may vary with formulation (Ali et al., 2015). Extrinsic factors pertain to the environmental conditions surrounding the food product.

These include temperature, packaging, storage duration and handling, and preservation method. Storage temperature has a profound effect on microbial growth and chemical reactions. Refrigeration slows spoilage, whereas higher temperatures accelerate microbial activity and enzymatic degradation (Corradini, 2018). Packaging

affects oxygen permeability, light exposure, and moisture retention. Vacuum sealing or modified atmosphere packaging can significantly extend the shelf life of paste products. Repeated opening and exposure to air or contaminants shorten shelf life. Inconsistent cold chain practices during distribution also contribute to early spoilage, especially for perishable pastes. Whether the paste has been heat-treated, frozen, acidified, or chemically preserved makes a difference in its stability. The combination of hurdles is often more effective than a single preservation method. Based on Kusumaningrum et al. (2015) in her study, factors that affect the shelf life of tropical fruits are microbial contamination, physiological disorders, mechanical damage and level of postharvest treatment. Another study stated that temperature is the most common factor for degradation acceleration in chemicals, pharmaceuticals, biological products and foods characterized by the Arrhenius equation, a formula for the temperature dependence of reaction rates (Haouet et al., 2018).

Several techniques are employed to extend the shelf life of food paste products, ranging from traditional methods to advanced technological interventions. Heat treatment through pasteurization or sterilization is one of the most common methods used to prolong shelf life. Thermal processing kills pathogenic and spoilage microorganisms and inactivates enzymes that contribute to deterioration. Retort processing (used for canned or pouch-packed pastes) can significantly extend shelf life up to one year or more under ambient conditions. However, thermal degradation of sensitive nutrients and flavour compounds is a potential drawback (Ali et al., 2015).

Cold storage is effective for short- to medium-term preservation, especially for fresh, minimally processed pastes. Refrigeration slows down microbial growth and oxidation, while freezing halts enzymatic activity altogether. The challenge with freezing lies in maintaining texture and preventing phase separation upon thawing, which is particularly relevant for emulsified or oil-based pastes. Modified atmosphere packaging (MAP) and vacuum sealing involve replacing the air in the packaging with gases like nitrogen or carbon dioxide, or removing air altogether. Oxygen reduction slows oxidation and microbial spoilage. MAP has proven especially effective for extending the shelf life of fat-containing and herb-based pastes (Sivertsvik et al., 2022). However, MAP requires specialized equipment and careful selection of compatible packaging materials.

Natural compounds such as essential oils, plant extracts, organic acids, and fermented products are increasingly used to enhance shelf life. These substances can be

incorporated into the paste or applied as coatings or additives. Their benefits include consumer appeal, lower toxicity, and synergistic antimicrobial effects. Nonetheless, standardization and regulatory approval are challenges for widespread adoption (Gyawali & Ibrahim, 2015). HPP is a non-thermal method that inactivates microorganisms by applying very high pressures (up to 600 MPa). It has gained popularity in preserving heat-sensitive products while retaining nutritional and sensory attributes. In food pastes, HPP can extend shelf life significantly without the "cooked" flavour associated with thermal treatment (Balasubramaniam et al., 2015). Lowering pH through the addition of vinegar, citric acid, or fermentation is a classical preservation method. Acidified pastes are less prone to bacterial spoilage and often show improved colour stability.

However, not all pastes are compatible with acidification due to sensory constraints. The mismanagement of shelf life, whether through incorrect estimation, labelling, or handling, can have serious consequences for public health, food safety, business operations, and consumer trust. The most critical implication is the potential for foodborne illness due to consumption of expired or improperly stored paste products. Pathogens such as *Clostridium botulinum* can thrive in anaerobic, low-acid pastes if improperly processed or stored, leading to life-threatening illness. Undetected spoilage can also result in outbreaks, especially in commercial foodservice operations. Incorrect shelf life estimation or poor packaging can lead to premature spoilage, resulting in financial losses from unsold stock, recalls, or liability claims. Small-scale producers of artisanal pastes are particularly vulnerable due to limited quality control infrastructure. Off-flavours, discoloration, and texture changes in pastes due to oxidation or microbial growth can deter repeat purchases.

Inaccurate or unreadable expiration dates diminish consumer confidence and may lead to reputational damage, especially in competitive food categories. Authorities require adherence to shelf life regulations, including scientific validation of expiry claims. Non-compliance can lead to sanctions, market withdrawal, or business closure, especially for exported goods subject to international food safety standards (Hazards, 2015). Premature disposal of food due to conservative shelf life estimation or spoilage contributes to environmental degradation through increased waste and carbon footprint. Sustainable shelf life extension strategies are thus essential for environmentally conscious food production. One of the methods used to extend shelf life studied by Immaculate et al. (2015) is sun drying, a simple and cheap method needing the least

methodological expertise. From that study, it was concluded that dried fish has lower spoilage indicator TVB-N and microbial load during the summer as compared to monsoon season as dried fish tend to absorb moisture from the atmosphere which promotes microbial growth. Another method is by using high-power pulsed light (HPPL) which prohibited microbial contamination and increased the shelf life of strawberries by two days (Luksiene et al., 2016).

A study by Sharif et al. (2018) involving random sampling of spicy shrimp paste revealed preservatives, for example; benzoic or ascorbic acid, were not compulsory in order to have an extended shelf life. The pH reading, total plate counts and microbiological analysis were acceptable and the product's shelf life could be extended up to a year. However this is contradicted in a different study, where adding sodium benzoate, a kind of preservative, was necessary to extend a food product and can extend its shelf life to up to two years without making any changes in taste and quality alongside good results of physicochemical and nutritional analysis but the product tested was spicy soy sauce which is a different food product and in form of liquid which is known to have a shorter shelf life as compared to paste due to higher moisture content (Mat Sharif et al., 2017). Furthermore, the development of effective shelf life strategies for food paste products requires a holistic approach that integrates formulation, processing, packaging, and storage conditions. The use of multiple preservation techniques, often referred to as hurdle technology, has proven to be more effective than relying on a single method. By combining factors such as reduced water activity, controlled pH, refrigeration, and natural antimicrobial agents, it is possible to significantly inhibit microbial growth and delay quality deterioration. Additionally, advances in packaging technologies, including vacuum sealing and modified atmosphere systems, further enhance product stability by limiting exposure to oxygen and contaminants. However, maintaining a balance between extended shelf life and preservation of sensory and nutritional qualities remains a key challenge. Therefore, continuous optimization and validation of preservation methods are essential to ensure both product safety and consumer acceptance.

2.12 Sensory Evaluation

Sensory evaluation of food is a scientific discipline used to measure, analyze, and interpret reactions to food characteristics as perceived by the human senses: sight,

smell, taste, touch, and hearing. It plays an integral role in food science and product development by providing data on consumer preferences, product quality, and market viability. Sensory attributes such as appearance, aroma, flavour, texture, and overall acceptability are essential in determining a product's success, especially in complex formulations such as meat marinated in food pastes. These marinated products are growing in popularity due to their convenience, enhanced flavour, and cultural relevance, particularly in Asian cuisines where spice pastes are commonly used for meat preparation. Sensory evaluation is a test using all five human senses which are smell, sight, touch or feel and taste to evaluate colour, texture, appearance and flavour of a food or product.

Positive sensory acceptance is important within a product as the intensity of flavour present in food can serve as an early signal of the food's content, good flavour signalling nutrients while foul flavours signalling toxicity. Sensory function also affects the process of satiation and subsequent food consumption (Teo et al., 2018). The importance of sensory evaluation in the food industry, especially for marinated meat products, cannot be overstated. Sensory testing allows producers to fine-tune recipes and processing methods to meet consumer expectations and enhance product acceptability. For meat products marinated in spice pastes, where flavour and tenderness are key drivers of consumer satisfaction, sensory evaluation helps assess how different marinade formulations affect sensory perception. According to Stone et al. (2019), sensory analysis contributes not only to quality assurance but also to innovation in food design, helping manufacturers align product characteristics with targeted consumer segments.

This is particularly critical in marinated meats, where balance in flavour, juiciness, and texture is vital. It is also used to compare new formulations with existing products or competitors, making it a strategic tool for product positioning in the marketplace. Sensory evaluation further supports food safety and consistency by detecting off-flavours or changes that may result from spoilage or ingredient variability. Consequently, it forms a bridge between consumer science and food technology, ensuring that both product quality and consumer satisfaction are simultaneously addressed. From a processing standpoint, natural proteases often align better with sensory preservation. Enzyme application increases tenderness without significant changes to organoleptic traits in certain products such as mortadella or turkey thighs,

indicating that proper dosage and selection of enzyme can yield tender meat while maintaining flavour and texture (Bujang et al., 2016).

Several factors influence the outcome and reliability of sensory evaluation, especially in complex food matrices like marinated meat. These factors can be categorized into product-related, panel-related, and methodological influences. Product-related factors include the nature of the marinade (its pH, salt concentration, enzymatic activity, and spice composition), the type of meat (species, muscle type, fat content), marination duration, and cooking method. For instance, enzymatic marinades containing papain or bromelain can significantly alter the texture of meat by breaking down connective tissue, leading to a softer mouthfeel (Ashie et al., 2015). The presence of spices such as garlic, ginger, turmeric, and chili in pastes not only affects the flavour profile but also interacts chemically with meat proteins, influencing colour and aroma. Moreover, cooking methods such as grilling, roasting, or steaming contribute to the formation of Maillard reaction products, further enhancing the sensory appeal through the development of complex flavours and appealing browning. Panel-related factors involve the composition and training of the sensory panel.

Trained panels are capable of discerning subtle differences in sensory attributes and providing consistent and reproducible results. However, the use of untrained consumer panels may better reflect real-world preferences, although such panels are more susceptible to bias and variability (Meilgaard et al., 2016). Cultural background, dietary habits, and individual sensitivity to flavours or aromas can affect panellist responses. For example, consumers from spice-rich culinary traditions may rate pungency and aroma more favourably compared to those less accustomed to strong seasonings. Methodological factors include the testing environment, sample presentation, and data collection techniques. Sensory evaluations must be conducted under controlled conditions to minimize extraneous influences such as lighting, temperature, and ambient odours. Randomized and blind sample presentation is essential to reduce bias. The use of appropriate evaluation scales—such as hedonic scales, ranking tests, or descriptive analysis—determines the granularity and usefulness of the collected data. Statistical tools like analysis of variance (ANOVA) and principal component analysis (PCA) are often used to interpret sensory data and identify patterns or significant differences among samples (Stone et al., 2019).

A growing body of literature has examined the sensory attributes of meat marinated with various food pastes, aiming to optimize formulation and enhance

consumer acceptability. A study by Jeong et al. (2023) investigated the sensory quality of pork loins marinated with a traditional Korean fermented paste (gochujang) and found significant improvements in flavour and tenderness compared to unmarinated controls. The fermentation-derived umami compounds in the paste contributed positively to taste perception, while the acidity and salt content improved water retention, enhancing juiciness. Similarly, Oktafa et al. (2023) explored the effects of herbal meat tenderizer on broiler chicken, revealing that spices such as rosemary and thyme increased overall acceptability and imparted a desirable aroma. The study highlighted that marination not only altered the sensory profile but also increased consumer willingness to purchase. Another relevant study by Munekata et al. (2021) assessed the sensory impact of turmeric-based paste marinades on beef.

Their findings showed that the colour and aroma of beef improved with paste application, particularly due to the visual appeal of turmeric and the masking of undesirable meat odours. However, overuse of turmeric led to bitterness, underscoring the need for balanced formulation. In addition, sensory acceptability was higher when marination was combined with vacuum tumbling, suggesting that uniform marinade penetration improves palatability. Research by Kim et al. (2019) focused on soy-based marinades and found that the inclusion of sugar, garlic, and sesame oil enhanced sweetness and umami, resulting in increased consumer preference scores. These studies underline the complex interplay between marinade composition, meat type, and processing method in determining sensory outcomes. They also emphasize the importance of optimizing not just the flavour profile but also texture and juiciness, which are often improved through the action of acids or enzymes in the paste.

Moreover, the effect of marination time is critical. Short marination may lead to insufficient flavour absorption, while excessively long marination may result in textural degradation or flavour imbalances. Thus, the sensory characteristics of marinated meat are influenced by a matrix of formulation variables and process controls. In a study by Al-Nabulsi et al. (2015), it was found mango pulp can improve the colour, appearance, flavour, taste, mouth feel and overall acceptability of soy milk-based yogurt which in conclusion, improved sensory evaluation. Meanwhile in a study by Bujang et al. (2016), incorporating Cassava leaves in meat floss to alter its nutrient content, in the perspective of sensory attributes, the Cassava leaves substitution did not alter the texture, taste and overall acceptability of the meat floss. Differently from a study by Aung et al. (2022), adding plant based meat tenderizers, pineapple and tomato powder, improves sensory

acceptance of semi-dried goat meat jerky, which is a meat product. The addition of these tenderizers makes the meat more chewable, digests easier for elderly, reduces its smell and improves overall acceptance.

Similarly, a study of using papaya peel as meat tenderizer showed meat marinated with 30% of green papaya peel for two hours at room temperature had high levels of acceptance in terms of texture and flavour making papaya peel a great potential to be used as a low-cost but effective meat tenderizer (Islam & Molinar-Toribio, 2015). In a study using bacterial protease derived from *Bacillus subtilis*, it was revealed meat treated with 0.35% *B. subtilis* is tenderer as compared to negative control and commercial meat tenderizer derived from plant protease extract and did not over-tenderize proving its potential as a meat tenderizer to replace plant-derived commercial meat tenderizers (Bureros et al., 2020). However, as stated before, microbial proteases raises the issue of side effects and safety thus were not further developed (Bhat et al., 2018). Papain and Bromelain are also widely used natural tenderizers, with demonstrated effects on connective tissue breakdown. Bromelain, especially, reduces meat firmness and increases water-holding capacity and tenderness in various meats, although its broad specificity can lead to over-tenderization if not dosed carefully (Madhusankha & Thilakarathna, 2021). When optimally applied, for instance, 10 mg per 100 g beef with controlled marination and temperature, bromelain improved beef tenderness without detrimental structural breakdown (Font & Guerrero, 2016).

On the other hand, while enzymes such as papain, bromelain, and ficin improve tenderness generally, they may undermine juiciness and other organoleptic attributes. Font and Guerrero (2016) evaluated seven enzyme treatments including papain and bromelain and found that although tenderness improved, juiciness and taste scores sometimes dropped compared to untreated controls. Such degraded sensory quality can indirectly affect nutritional intake: if meat becomes less palatable, individuals may consume less or substitute with less nutritious options.

Precautions in sensory evaluation must be considered to ensure the validity and reliability of findings, particularly in meat products where food safety is a concern. First, hygiene and safety protocols must be strictly followed during sample preparation and presentation to avoid contamination or spoilage, which could compromise both panellist health and data integrity. Second, the freshness of ingredients, particularly meat and paste components, must be verified to avoid off-flavours or misleading results. Third, sensory fatigue can affect panellist performance, especially during extended

testing sessions or when evaluating strongly flavoured products. Limiting the number of samples per session and providing palate cleansers like water or crackers can mitigate this issue (Stone et al., 2019). Another implication is the subjectivity inherent in sensory data. Even with trained panels, personal preferences and psychological factors may influence responses. Hence, combining sensory data with instrumental analysis such as texture profile analysis, colorimetric, or gas chromatography for aroma profiling can provide a more comprehensive understanding of product quality.

Sensory evaluation, while invaluable, should be viewed as one part of a holistic quality assessment strategy. Additionally, cross-cultural sensory evaluations may reveal differing preferences that affect market targeting. For example, a paste-marinated meat product optimized for Southeast Asian consumers may not be equally accepted in Western markets due to differing taste preferences. Therefore, regional sensory panels and market segmentation are important when developing products for international markets. Finally, the use of novel ingredients in pastes such as plant-based antimicrobials or unconventional spices requires cautious sensory evaluation to ensure acceptability. Ingredients with strong functional properties may impart unfamiliar or undesirable tastes, thus necessitating pilot testing and consumer validation. Ethical considerations must also be addressed, ensuring that panellists are fully informed of ingredients and any potential allergens. This is particularly crucial in products that include nuts, dairy derivatives, or soy-based ingredients commonly found in food pastes. Furthermore, the integration of sensory evaluation with instrumental and analytical techniques enhances the reliability and applicability of research findings in meat product development. While sensory analysis provides valuable insights into consumer perception, it is inherently subjective and can be influenced by individual preferences and environmental factors. Therefore, combining sensory data with objective measurements such as texture profile analysis, colorimetric, and chemical analysis allows for a more comprehensive evaluation of product quality. This integrated approach enables researchers to better understand the relationship between formulation variables and sensory outcomes, facilitating the optimization of meat products. Additionally, incorporating consumer feedback into product development ensures that the final product aligns with market expectations. Ultimately, a balanced approach that integrates both sensory and instrumental evaluation is essential for producing high-quality, acceptable, and competitive meat products in the food industry.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Introduction

The development of a high-quality meat tenderizer paste presents a unique opportunity to enhance culinary experience. This study explores the formulation of a novel meat tenderizer paste that not only enhances textures of meat but also incorporates significant antioxidant properties while maintaining a palatable experience among users. By evaluating the developed meat tenderizer's effectiveness, antioxidant activities, shelf life, nutrient content and acceptability, this study aims to create a product that functional, beneficial and acceptable.

3.2 Research Design

An experimental study was intended to develop meat tenderizers by using jackfruit leaves, pineapples and selected herbs. Figure 3.1 shows the research design of the study

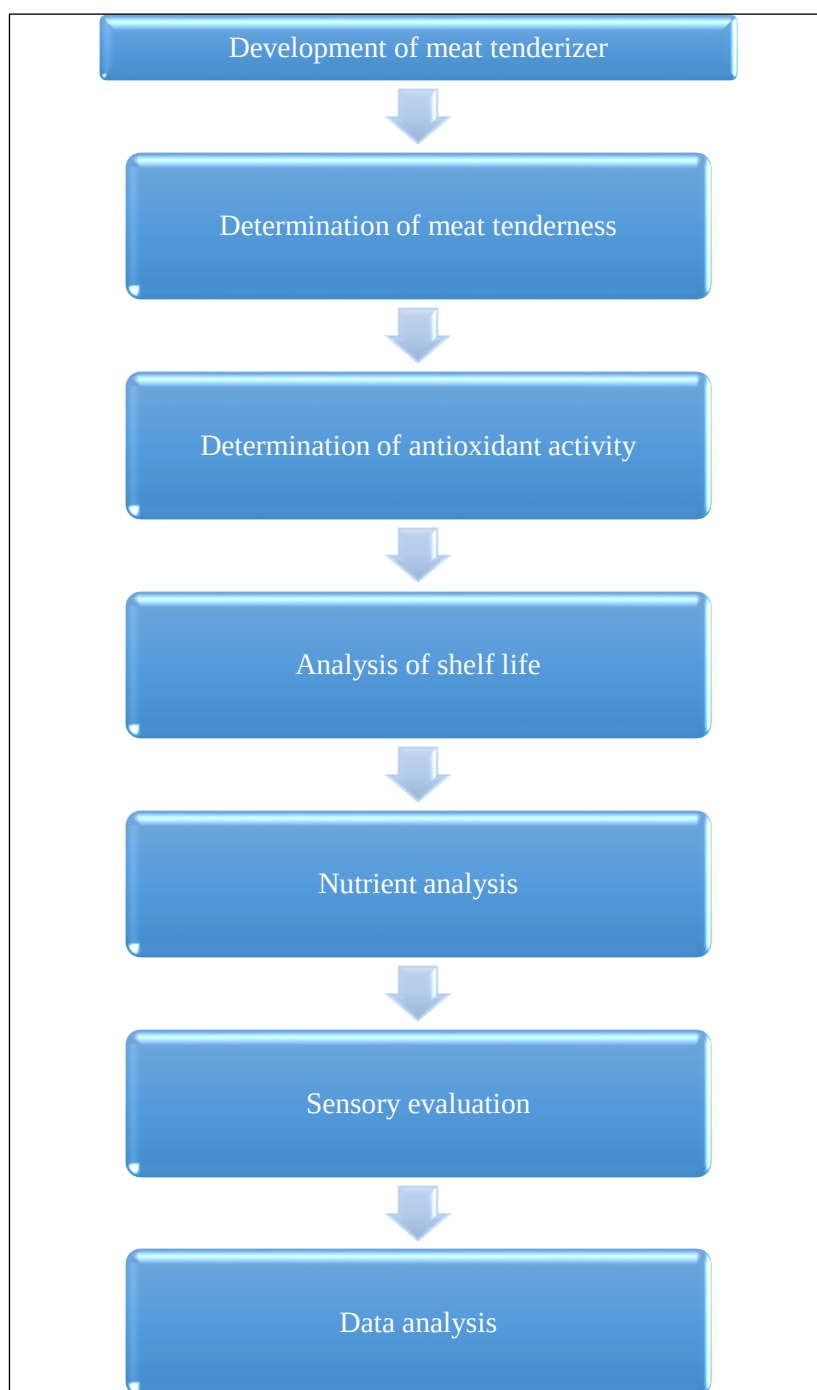


Figure 3.1 Steps in Study of Meat Tenderizer

3.3 Study Location

This study was conducted at Universiti Teknologi Mara (UiTM) Selangor, Puncak Alam Campus. Specifically, development of the meat tenderizers was conducted at Food Preparation Laboratory, Centre of Dietetics Studies. The tenderness of cooked meats was conducted at Faculty of Pharmacy. Sensory evaluation, pH

analysis and determination of moisture was conducted at Food Sensory Laboratory, Food Microbiological Laboratory and Food Analysis Laboratory, Centre of Dietetics Studies. Antioxidant activity analysis was conducted at Postgraduate Laboratory, Faculty of Health Sciences.

3.4 Sampling

3.4.1 Meat Sample

Meat sample (Biceps femoris/Bottom round) was purchased from a local meat supplier and instantly moved to the Food Preparation Laboratory for visible connective tissue and external fat trimming. The samples were kept frozen in the commercial refrigerator (-20°C) until further use. Upon use, the meat was thawed until manageable temperature (10-20°C) and was cut into chunks of 2cm x 5cm x 2cm. The bottom round part of meat comes from the rear leg of the animal. This is a tough part of the meat because it is composed of well-exercised muscles with a high content of connective tissue and collagen. These muscles support the animal's weight and are used extensively in movement, which results in denser muscle fibres and reduced intramuscular fat (Purslow, 2015). Tough portions of meats are chosen because their dense muscle fibres and high collagen content present a clear challenge to tenderness, making them ideal for evaluating the effectiveness of tenderizing agents.

3.4.2 Leaf Sample

Jackfruit leaves were collected in farms located in Sungai Apong, Sungai Air Tawar, and Sabak Bernam, Selangor. In order to have a standard content of phytonutrients and proteases of leaves, the leaves were taken from the same tree. The selected leaves must be green or dark green, elliptic in shape without any physical distortion and not sun scalded or damaged due to extensive exposure to the sun. The leaves were washed and cut into halves. Then they were dried in an oven at 50°C to prevent the deterioration of present compounds from too high temperature. Then, the sample was ground in the food processor (model Kenwood Food Processor FP120) into powder and kept in zip lock plastic bags.

3.4.3 Pineapple Sample

Pineapple samples were collected at pineapple farms located in Kampung Seri Merlong, Rengit, Johor. The fruits were peeled, cut up into smaller pieces (to blend later), kept in air-tight plastic containers and frozen at -20°C until further use.

The samples were collected at specific location with purpose of choosing fruits with optimal growth conditions and are carefully nurtured which matches the quality of fruits being sold in markets. Optimal growth conditions include adequate sunlight exposure, optimal soil pH, consistent water availability, proper fertilization schedules and maturity stage at harvest.

3.4.4 Spice Samples

Spices were purchased from the hypermarket Econsave Cash and Carry Sdn. Bhd. or Lotus (Malaysia) Puncak Alam Branch, Selangor. Spices such as cumin, fennel, black pepper, coriander and cinnamon were readily available in powder form. On the other hand, spices such as clove, star anise and cardamom are not available in powder form thus these spices were purchased and were finely ground in a spice grinder to become powder and were kept in sealed plastic bags.

3.4.5 Commercial Meat Tenderizer Sample

A commercial meat tenderizer was chosen from the local market to serve as control for this experiment. For this study, Knorr Meat Tenderizer Powder (Vo Fon Trading Sdn. Bhd.) was used.

3.4.6 Preparation of Other Ingredients

Other ingredients that is necessary for the development of the product such as vegetable oil, lemongrass, ginger, turmeric, sugar, salt, garlic and onions were purchased from the hypermarket Econsave and Lotus, Puncak Alam, Selangor and stored in Food Preparation Laboratory.

3.5 Development of Meat Tenderizers

Meat tenderizer of meat tenderizer was developed from jackfruit leaves, pineapples, lemongrass, sugar, garlic, onion, black pepper, chili powder, cayenne, paprika, cumin, fennel, ginger, turmeric, coriander seed, vegetable oil and salt. All of these ingredients were made into paste and kept in sealed zip lock plastic bags. Four recipes (traditional, fusion, Josapine and MD2) were developed using the listed ingredients in different proportions. Pineapples were only included in Josapine and MD2 recipes. This is due to evaluate the effectiveness of the developed meat tenderizer pastes with only jackfruit leaves as the proteolytic substance.

Jackfruit leaves were oven dried at 50°C overnight. Once dried, they are grinded using a coffee grinder into a fine powder. Then, alongside other herbs and spices, they were processed together using a food processor into a paste. Two recipes were made with only jackfruit leaves act as proteolytic agents which are the traditional recipe and the fusion recipe. The fusion recipe contained additional ingredients such as rosemary and thyme. Differently, two other recipes were developed by adding pineapples respective of their variants. Following the traditional recipe, Josapine variant pineapples were added to develop the Josapine recipe and MD2 variant pineapples were added to develop the MD2 recipe.

The development of the meat tenderizer pastes started off by drying the jackfruit leaves in an oven at 50°C overnight to completely dry. This was due to enzymes are mostly stable at 50-60°C (Ahmad et al., 2019). Then the dried jackfruit leaves were pulverized into a powder and were processed with the herbs and spices listed in methodology. For the recipes including pineapples, two different variants of pineapples were added to their corresponding recipes. The development of the meat tenderizers were meticulous as all of the raw ingredients needed to be washed, peeled if necessary, cut up into usable pieces before being processed in the food processor. Hence, all four recipes of the meat tenderizer meat tenderizers were successfully developed.

3.6 Enzyme Treatment and Marinade

The meat was cut into 5 chunks of 2cm x 5cm x 2 cm. The meat sample groups will consisted of

- a) B0 – Control meat sample without treatment of meat tenderizer
- b) B1 – Meat sample treated with 5g of commercial meat tenderizer per 500g of meat (based on instruction)
- c) B2 – Meat sample treated with 7g meat tenderizer of meat tenderizer (traditional flavour)
- d) B3 – Meat sample treated with 7g meat tenderizer of meat tenderizer (fusion flavour)
- e) B4 – Meat sample treated with 7g meat tenderizer of meat tenderizer (tropical flavour)
- f) B5 – Traditional recipe tenderizer
- g) B6 – Fusion recipe tenderizer
- h) B7 – Josapine recipe tenderizer
- i) B8 – MD2 recipe tenderizer

After 60 minutes of marination in fridge temperature (20°C), the meat chunks (in room temperature) were baked in a pre-heated oven for 8-10 minutes at 190°C or until the internal temperature of the meat chunks reaches 57°C measured with a thermometer.

3.7 Measurements

3.7.1 Determination of Hardness

The hardness of meat was measured using texture analyser (model TA.XT plus Stable Micro Systems, England) using a blade probe with a load cell of 5 kg applied at a crosshead speed of 60 mm/min. The immersion depth of meat chunk was 5 mm. The hardness of meat was expressed in gram (g) with higher reading indicating greater force needed hence tougher meat. The amount of meat, cooking method and type of meat were kept constant for every sample.

3.7.2 Antioxidant Activity

3.7.2.1 1,1-diphenyl-picrylhydrazyl radicals (DPPH)

The AA of samples were evaluated for the radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH). The method was adopted from Ebby-Anuar Bahari et al. (2014). 5 mg of meat tenderizer was mixed with 1 ml of absolute ethanol for sample preparation. 0.5 ml of sample was added to 1 ml of 0.45 mM of DPPH ethanolic solution. The mixture was mixed thoroughly and incubated in the dark at room temperature for 30 minutes. The absorbance was then read at 517 nm by using a spectrophotometer. Antioxidants in the sample (deep violet colour) converts 1,1-diphenyl-2-picrylhydrazyl hydrazine with decolourization into yellow colour. The scavenging effect was directly proportional to scavenging inhibition. AA was calculated using the following formula:

$$(\%) \text{ of AA} = [(A_c - A_s) / A_c] * 100 \quad (3.1)$$

Where,

A_c = Absorbance of the control

A_s = Absorbance of the sample after 30 minutes

3.7.2.2 Total Phenolic Content

The total phenolic content (TPC) of the meat tenderizer were determined using the Folin-Ciocalteu method as described by Hanis Mastura (2017). 100µl of the extract samples were added to 2.5ml of Folin-Ciocalteu reagent (x10 dilution) and the mixture was left to react for 5 minutes before adding 2.5ml of 7% sodium carbonate solution. The mixture was left to stand at room temperature for 1 hour. The absorbance was measure at 765nm with UV-visible spectrophotometer using distilled water as a blank. The results were expressed as mg gallic acid equivalents per 100 gram DW and each analysis was conducted in duplicates.

3.7.2.3 Total Flavonoid Content

As for total flavonoid content (TFC), the aluminium chloride colorimetric method was adapted from Nur Atikah Anwar (2021). 1mg of crude extracts were diluted with 1ml MeOH to produce a stock solution of 1mg/ml. 100µl of this stock solution was mixed thoroughly with 500µl of distilled water and 100µl of 5% sodium hydroxide and be left to stand for 6 minutes. Then 150µl of 10% aluminium chloride solution and 200µl of 1M sodium hydroxide was added to the mixture and was left to react for 5 minutes. The absorbance was measured in duplicates at 510nm using a spectrophotometer. The activity potential of sample extracts was expressed as the quercetin equivalence (mg QE/100g), and the TFC of each sample was calculated using this formula:

$$TFC = \frac{cV}{M} \times 100 \quad (3.2)$$

3.7.2.4 Superoxide Dismutase (SOD)

Methods followed the Superoxide Dismutase WST-1 Assay Kit (WST-1 Method) instructions as comes with the product. The kit came with four reagents; Reagent 1: buffer, Reagent 2: substrate stock solution, Reagent 3: enzyme stock solution, Reagent 4: enzyme diluent. In order to prepare the substrate working solution, 0.15ml of Reagent 2 was diluted with 30ml of Reagent 1 at ratio 1:200 and was mixed sufficiently. Then, to prepare the enzyme working solution, 0.3ml of Reagent 3 was diluted with 3ml of Reagent 4 at a ratio of 1:10 and mixed sufficiently.

Sample preparation

9 samples were tested with the following kit; 1 cooked sample of B1, 4 cooked and uncooked samples of B1, B2, B3 and B4, respectively. 10g of meat were used for each sample. Four of the samples were marinated in 5g of each recipe of meat tenderizers for one day. Before testing were carried out, all meat samples were cooked thoroughly by frying on a non-stick cooking pan without the addition of any extra fats.

Each sample were then weighed and only 200mg of meat were taken to prepare tissue homogenate for the assay. For the meat tenderizer samples, 200mg of each recipes were taken to prepare tissue homogenates. 1.8ml of normal saline was added to each

samples to create a mass(g)-volume(ml) ratio of 1:9 in 2ml Eppendorf tubes. Then all samples were centrifuged at 2500rpm for 10 minutes. Only supernatant was used for the assay as 10% homogenate.

As for the four meat tenderizer samples, each of the pastes were weighed accurately at 200mg and diluted with 1.8ml of 0.1ml/L pH 7 phosphate buffer at ratio 1:9 and centrifuged at 3500rpm for 10 minutes. The supernatant of the samples were taken as 10% homogenate.

A 96-well microplate was used for the assay. A contrast well was prepared by adding 20 μ l of double distilled water, 20 μ l of enzyme working solution and 200 μ l of substrate working solution. A contrast blank well was prepared by adding 20 μ l of double distilled water, 20 μ l of enzyme diluent and 200 μ l of substrate working solution. Sample wells were prepared by adding 20 μ l of sample, 20 μ l of enzyme working solution and 200 μ l of substrate working solution. Sample blank wells were prepared by adding 20 μ l of sample, 20 μ l of enzyme diluent and 200 μ l of substrate working solution.

Each of the nine samples had different individual sample wells and sample blank wells. Next the microplate was read at 450nm using a microplate reader (POLARstar omega). Each reading was taken at triplicates. SOD inhibition percentage was calculated using the following formula:

$$\begin{aligned} \text{SOD inhibition percentage} & \quad (3.3) \\ = & \frac{(\text{Contrast} - \text{Contrast Blank}) - (\text{Sample} - \text{Sample Blank})}{(\text{Contrast} - \text{Contrast Blank})} \times 100\% \end{aligned}$$

SOD activity of each of the nine samples were calculated using the following formula:

$$\begin{aligned} \text{SOD activity} \left(\frac{\text{U}}{\text{mgprot}} \right) & \quad (3.4) \\ = & \text{SOD inhibition percentage} \div 50\% \\ & \times \text{Reaction system dilution times} \left(\frac{0.24\text{ml}}{0.02\text{ml}} \right) \\ & \times \text{Protein concentration in sample to assay} \left(\frac{\text{mgprot}}{\text{ml}} \right) \end{aligned}$$

3.7.2.5 Catalase

Methods followed the Catalase (CAT) Assay Kit (Visible Light Method) instructions as comes with the product. The kit came with 4 reagents, Reagent 1 100ml, Reagent 2 10ml and Reagent 4 10ml in liquid form in its own bottles, Reagent 3, the chromogenic agent, in powder form. Double distilled water was added to reconstitute Reagent 3 until volume reached 100ml and then dissolved sufficiently.

a) Sample Preparation

9 samples were tested with the following kit; a cooked control meat that is not treated with anything, four cooked meat samples that are treated with the four recipe of meat tenderizers and four recipes of the meat tenderizers. 10g of meat were used for each sample, four of the treated samples were marinated in 5g of each recipes of meat tenderizers for one day. Before testing were carried out, all meat samples were cooked thoroughly by frying on a non-stick cooking pan without the addition of any extra fats.

Each five meat samples were then weighed and only 200mg of meat were taken to prepare tissue homogenate for the assay. For the meat tenderizer samples, 200mg of each recipe were taken to prepare tissue homogenates. 1.8ml of Normal saline was added to each sample to create a mass(g)-volume(ml) ratio of 1:9 in 2ml Eppendorf tubes. Then all of the samples were centrifuged at 2500rpm for 10 minutes. Only supernatant was used for the assay.

Before starting the test, all four reagents were warmed in a water bath at 37°C as per instruction. Two tubes will be needed for each of the nine sample, one for contrast, another for sample. Contrast tubes were made to differentiate sample antioxidant activity from the reagents. In Contrast tubes, 1ml of reagent 1 and 0.1ml of reagent 2 were added. They were then mixed sufficiently and were left to react at 37°C for 1 minute. Then 1ml of reagent 3, 0.1ml of reagent 4 and 0.05ml of homogenates were added to the tubes and mixed sufficiently. For sample tubes, 0.05ml of homogenates, 1ml of Reagent 1 and 0.1ml of Reagent 2 were mixed sufficiently and were left to react at 37°C for 1 minute. Then 1ml of Reagent 3 and 0.1 of Reagent 4 were added and mixed sufficiently.

For each nine sample homogenates, concentrations used were 0.125%, 0.25%, 0.5%, 1%, 2% and 5% for the assay testing for Contrast Tubes and Sample Tubes. Double distilled water was used as blank to adjust to zero. All samples were transferred to 0.5cm light path cuvettes and all OD values were read at 405nm using a spectrophotometer. Each reading was taken at triplicates. Optimal concentration of each samples were used for CAT activity calculation. CAT activity was calculated using the following formula:

$$\begin{aligned} \text{CAT activity} = & (\text{OD}_{\text{Contrast}} - \text{OD}_{\text{Sample}}) \times 271 \\ & \times \frac{1}{60 \times \text{Sample Volume}} \\ & \times \text{Protein Concentration in Tissue Homogenate } \left(\frac{\text{mg}}{\text{ml}}\right) \end{aligned} \quad (3.5)$$

3.7.2.6 Glutathione Peroxidase

Methods followed the Glutathione Peroxidase (GSH-PX) Assay Kit (Colorimetric Method) instructions as comes with the product. The kit came with seven reagents that were needed to be prepared in order to obtain their working solutions.

Nine samples were used for this assay; control meat that is untreated with anything, four meat samples treated with the four meat tenderizers of each recipe, and four meat tenderizers of each recipe. The four meat samples were marinated with the four recipes of meat tenderizer overnight and all of the meat samples were cooked thoroughly in a cooking pan with no additional fats.

Each five meat samples were then weighed and only 200mg of meat were taken to prepare tissue homogenate for the assay. For the meat tenderizer samples, 200mg of each recipes were taken to prepare tissue homogenates. 1.8ml of Normal saline was added to each samples to create a mass(g)-volume(ml) ratio of 1:9 in 2ml Eppendorf tubes. Then all of the samples were centrifuged at 3000rpm for 10 minutes. Only supernatant was used for the assay.

Using a 2ml Eppendorf tube, 0.2ml of 1mmol/L GSH was warmed using a water bath at 37°C for 5 minutes. Then, 0.1ml of Reagent 1 working solution was added and left to react in a water bath at 37°C for 5 minutes. Next, 2ml of Reagent 2 and 0.2ml of homogenate supernatant were added and mixed sufficiently and centrifuged at 3500rpm

for 1 minute. 1ml of this supernatant was used for chromogenic reaction. These steps were repeated in order to obtain the supernatant for all nine samples.

Subsequently, blank tube was prepared by adding 1ml of GSH standard solvent working solution, 1ml of Reagent 3 working solution, 0.25ml of Reagent 4 working solution and 0.05ml of Reagent 5 working solution. A standard tube was prepared by adding 1ml of 20µmol/L GSH standard solution, 1ml of Reagent 3 working solution, 0.25ml of Reagent 4 working solution and 0.05ml of Reagent 5 working solution to compare with the samples. The sample tubes were prepared by adding 1ml of the previously prepared supernatant, 1ml of Reagent 3 working solution, 0.25ml of Reagent 4 working solution and 0.05ml of Reagent 5 working solution, all repeated for all nine samples.

All of the tubes were mixed sufficiently, placed at room temperature for 15 minutes, transferred into 1cm light path cuvettes and then measured OD values at 412nm using a spectrophotometer adjusted zero by double distilled water. Calculation of GSH-PX was as follows:

$$\begin{aligned} \text{Tissue GSH – PX activity} & \quad (3.6) \\ &= \frac{\text{ODSample}}{\text{ODStandard} - \text{ODBlank}} \\ &\times \text{Standard Solution concentration} \times \text{Dilution times} \\ &\div \text{Reaction time length} \div (\text{Sample volume} \\ &\times \text{Protein content in sample}) \end{aligned}$$

3.7.3 Shelf-Life Analysis

The developed meat tenderizers were stored in a sealed plastic bag at two different conditions, room temperature and fridge temperature (Model LR-1050). They were analysed five times for the pH activity and moisture content in order to investigate the shelf life. The analysis was done on the 1st day, 20th day, 40th day and 80th day of production. The sampling intervals (Day 1, 20, 40 and 80) were selected to represent baseline, early, intermediate and extended storage phases. This progressive monitoring approach allows observation of physiochemical changes over time and facilitates evaluation of the product's stability and potential shelf life under different storage conditions.

3.7.3.1 pH Activity

10 g of the samples were homogenized with 50 ml of distilled water. The pH values were measured with a digital pH meter (Model pH-Meter 765 Calimatic, Knick Instrument). The readings were taken in triplicates.

3.7.3.2 Determination of Moisture

The method that was executed for the determination of moisture is an air-oven method. Aluminium dishes with covers were dried in an oven at 105°C for 3 hours. Then, the dishes were cooled in a desiccator and weighed at room temperature. 5g of the homogenized samples were weighed and put into dishes with covers that were dried previously for 3 hours. The dishes with samples were placed uncovered in an oven at 60°C overnight. The dish covers were placed over the dishes while still in the oven. After that, the samples were cooled in the desiccator and weighed at room temperature. The % of moisture (wt/wt) was calculated by using the following formula:

$$\begin{aligned} \text{\% of moisture (wt/wt)} = & \hspace{15em} (3.7) \\ & \frac{(\text{Weight of wet sample + dish}) - (\text{Weight of dried sample + dish})}{(\text{Weight of wet sample + dish}) - (\text{Weight of dish})} \\ & \times 100 \end{aligned}$$

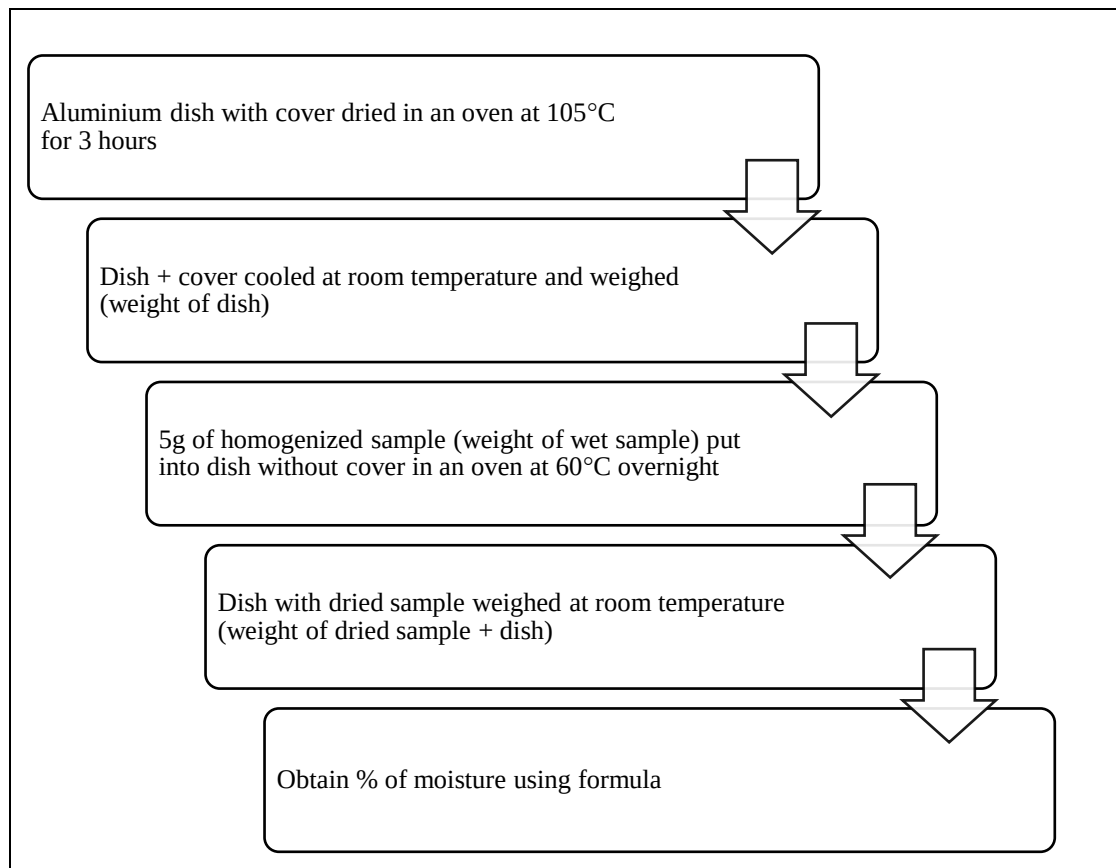


Figure 3.2 Flow Chart of the Steps of Determination of Moisture of Sample

3.7.4 Nutrient Analysis

Nutritional values of the meat tenderizer were determined by sum of the nutritional values of each components or ingredients proportionate to the quantity of the ingredient used. The nutritional composition of each ingredient was referred to The Nutrient Composition of Malaysia 4th Edition 1997.

Proximate analysis was also be carried out using the methods adapted from Hassan Pyar, 2018. The parameters that were determined were moisture, crude protein, crude fat, ash, carbohydrate and energy. Water content was measured using the oven drying method. Crude protein was measured using the Kjeldahl method. Crude fat was measured using the Soxhlet extraction method. Total ash was measured using the dry ashing method. Carbohydrate of samples were calculated by differences of total weight and all of the sum of other macronutrients. Energy was calculated using the Atwater factors formula.

3.7.5 Sensory Evaluation

The sampling method chosen was convenience sampling. The sensory evaluation test was conducted to evaluate the people's acceptance of these new products and to compare it with the commercial meat tenderizer. 25 participants were involved in this experiment. Criteria for selecting participants are as followed below:

Table 3.1
Criteria for Selecting Participants

Inclusion Criteria	Exclusion Criteria
• Aged between 21-45 • Healthy people	• Smokers • People with colour-blindness

25 panels were chosen to evaluate 6 meat samples that have been cooked, 1 without any treatment, 1 treated with commercial meat tenderizer and the other 4 with the developed meat tenderizers of each recipes. Six important criteria were evaluated by the participants such as colour, flavour, appearance, texture, aroma and overall acceptance of the samples by using 9-point hedonic scale where 1 indicated extreme dislike and 9 indicated extreme like. Participants cleansed their palates between samples with water. For hedonic tests involving untrained panellists, a sample size between 20 to 30 participants is commonly used in food science research. It provides enough observation to perform basic statistical analysis such as ANOVA and mean comparison tests. 25 panels generate 150 total evaluations (25 x 6) which improves data reliability and reduces random variability (Gómez et al., 2020).

3.8 Flow Chart of Research Activities

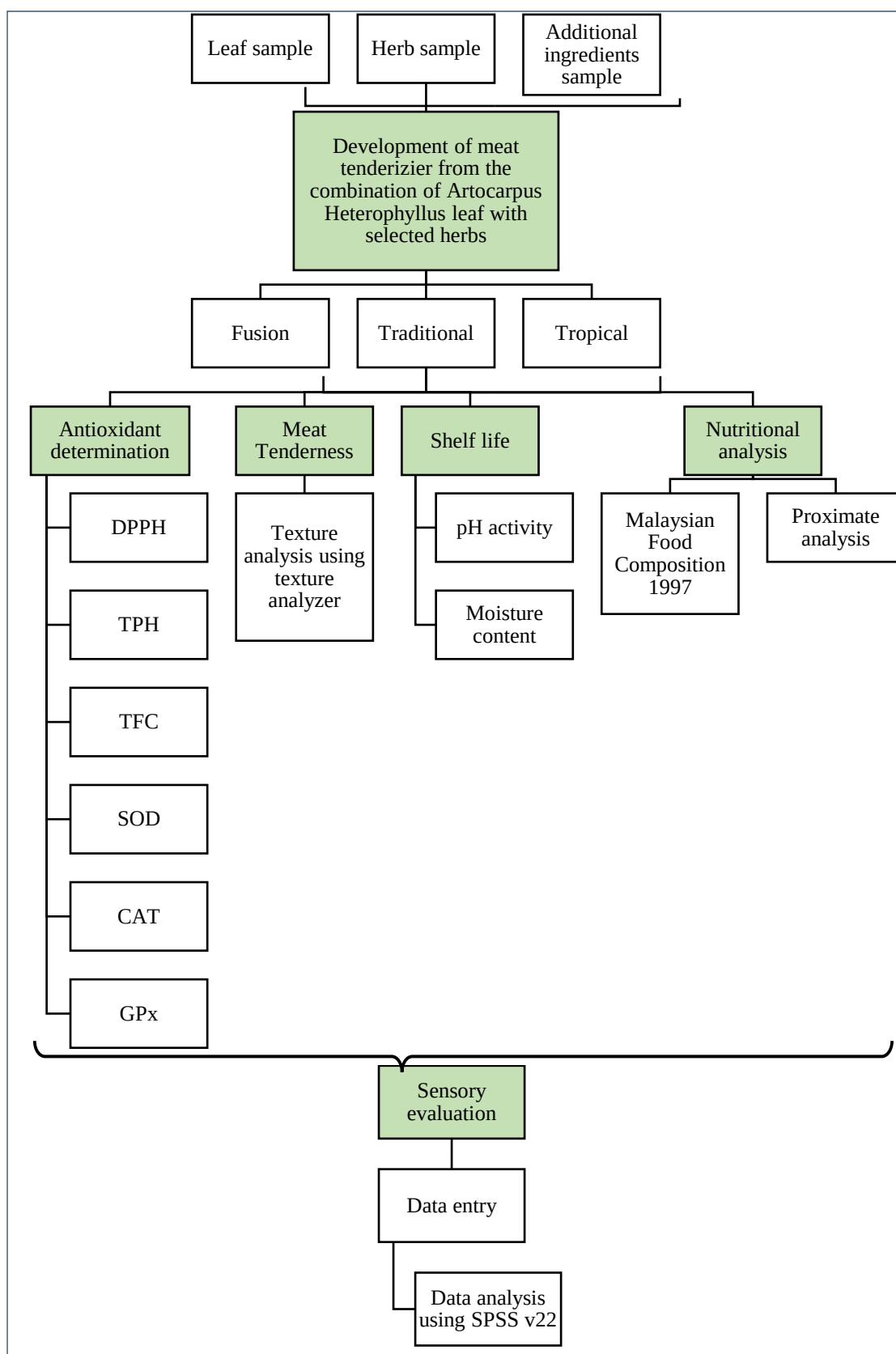


Figure 3.3 Flow Chart of the Research

3.9 Data Analysis

All of the data were analysed by using Statistical Package for Social Science 22 (SPSS 22). Analysis of Variance (ANOVA) was used to establish the significant differences between means followed by post hoc test. Data was presented as mean value $\pm$ standard deviation. Values of $p < 0.05$ were regarded as statistically significant.

3.10 Ethical Consideration

The data collection commenced after obtaining ethical permission from the Research Committee of the Faculty of Health Sciences and Universiti Teknologi MARA (UiTM) and the Department of Social Welfare with reference number 600-IRMI(5/1/6). Only participants who agreed and gave permission to participate were considered for study. The participants were provided with informed consent forms as written proof of their agreement to participate.

CHAPTER 4

RESULTS

4.1 Introduction

The assessment of hardness, antioxidant activity, shelf life and nutrient analysis is crucial in understanding the overall quality, stability and health benefits of the meat tenderizers. This section presents the findings of all these parameters and provide important information of the function, usability and health benefits of the meat tenderizers and also be a guide for improvements.

4.2 Development of Meat Tenderizer

The development of meat tenderizer paste involved optimizing the four recipes to achieve desired appearance, taste and function. These formulations of meat tenderizer are used in determination of antioxidants, hardness, shelf life, nutrient analysis and sensory evaluation. 292g of each recipe was made as this amount reflects a manageable batch size typical for small-scale experiments. It is sufficient to stimulate real-use scenarios without exceeding the capacity of a standard household kitchen. The final product of meat tenderizer in form of meat tenderizer is shown in Plate 1.1.



Plate 4.1 The Final Product of Meat Tenderizer in Form of Meat Tenderizer

The traditional flavour meat tenderizer was made with the most common of ingredients found and used in Malaysia (Raji et al., 2017). This recipe was aimed to have the familiarity towards people in order for easier acceptance with the additional ingredient of jackfruit leaves. The other ingredients are used as to create familiarity in flavour with what Malaysians are used to. Table 4.1 refers to the recipe of the traditional flavour:-

Table 4.1
Recipe for Traditional Flavour of Meat Tenderizer

Ingredients	Amount (g) / 292 g	Percentage (%)
Jackfruit leaf	21	7.19
Garlic	16	5.48
Lemongrass	47	16.10
Turmeric	3	1.03
Ginger	6	2.05
Coriander	6.5	2.23
Cumin	4.7	1.61
Fennel	6.7	2.29
Brown sugar	19	6.51
Black pepper	2.5	0.86

Ingredients	Amount (g) / 292 g	Percentage (%)
Onion	76	26.03
Chili powder	12	4.11
Cayenne pepper	1.7	0.58
Paprika powder	1.7	0.58
Sunflower oil	32	10.96
Sugar	30	10.27
Salt	6.2	2.12

As for the fusion recipe of meat tenderizer, additional ingredients are added which are thyme and rosemary. These herbs are less commonly used in Malaysia but are well-known herbs to be used in Western countries. Rosemary has a woody aroma, with slightly bitter flavour with hints of pine and adds depth and complexity to a dish while thyme has slightly minty, earthy flavours with an aromatic sense (Ozogul, 2016). These herbs, though widely accepted worldwide in terms of flavours and aroma, may cause a sense of unfamiliarity among some Malaysians. This recipe was made in order to cater the preference of those who favour international flavours or to those using the meat tenderizer to make international meat dishes. Table 4.2 refers to the recipe of the fusion flavour:-

Table 4.2
Recipe for Fusion Flavour of Meat Tenderizer

Ingredients	Amount (g) / 292 g	Percentage (%)
Jackfruit leaf	21	7.13
Garlic	16	5.43
Lemongrass	47	15.96
Turmeric	3	1.02
Ginger	6	2.04
Coriander	6.5	2.21
Cumin	4.7	1.59
Fennel	6.7	2.28
Brown sugar	19	6.45
Black pepper	2.5	0.85

Ingredients	Amount (g) / 292 g	Percentage (%)
Onion	76	25.81
Chili powder	12	4.07
Cayenne pepper	1.7	0.58
Paprika powder	1.7	0.58
Thyme	1.1	0.37
Rosemary	1.4	0.47
Sunflower oil	32	10.87
Sugar	30	10.19
Salt	6.2	2.10

Next, for Josapine recipe for the meat tenderizer, the same recipe as the traditional flavour was applied with the exception of addition of pineapple of the Josapine variant, processed together in the paste. This is a development of a new recipe as pineapples contain bromelain which is known to have its own meat tenderizing effect (Bekhit et al., 2015; Ramli et al., 2021; Mohd Azmi et al., 2023). Moreover, pineapples also contain sugars that caramelizes during the cooking process due to Maillard reaction, adding sweetness and hints of tanginess to the paste. Table 4.3 refers to the recipe of the Josapine flavour:-

Table 4.3
Recipe for Josapine Flavour of Meat Tenderizer

Ingredients	Amount (g) / 292 g	Percentage (%)
Jackfruit leaf	21	7.13
Garlic	16	5.43
Lemongrass	47	15.96
Turmeric	3	1.02
Ginger	6	2.04
Coriander	6.5	2.21
Cumin	4.7	1.59
Fennel	6.7	2.28
Brown sugar	19	6.45
Black pepper	2.5	0.85

Ingredients	Amount (g) / 292 g	Percentage (%)
Onion	76	25.81
Chili powder	12	4.07
Cayenne pepper	1.7	0.58
Paprika powder	1.7	0.58
Pineapple (Josapine variant)	2.5	0.84
Sunflower oil	32	10.87
Sugar	30	10.19
Salt	6.2	2.10

Lastly, MD2 recipe followed the same recipe as Josapine recipe but replaced the Josapine variant with MD2 variant. MD2 was used as an alternative to Josapine in this study because preferable by consumers as compared to other to other variants in terms of aroma, sweetness and colour (Syahrin, 2021). Table 4.4 refers to the recipe of the MD2 flavour:-

Table 4.4
Recipe for MD2 Flavour of Meat Tenderizer

Ingredients	Amount (g) / 292 g	Percentage (%)
Jackfruit leaf	21	7.13
Garlic	16	5.43
Lemongrass	47	15.96
Turmeric	3	1.02
Ginger	6	2.04
Coriander	6.5	2.21
Cumin	4.7	1.59
Fennel	6.7	2.28
Brown sugar	19	6.45
Black pepper	2.5	0.85
Onion	76	25.81
Chili powder	12	4.07
Cayenne pepper	1.7	0.58

Ingredients	Amount (g) / 292 g	Percentage (%)
Paprika powder	1.7	0.58
Pineapple (MD2 variant)	2.5	0.84
Sunflower oil	32	10.87
Sugar	30	10.19
Salt	6.2	2.10

4.3 Determination of Hardness

The result showed that B0 gave the highest reading of hardness of meat (414.31 ± 29.66) followed by B1 (350.92 ± 43.30), B2 (297.79 ± 27.67), B4 (280.00 ± 7.82), B3 (279.94 ± 31.18) and B5 (246.37 ± 19.11). There was a significant difference ($p < 0.05$) between untreated control meat sample with the other five treated meat samples. Moreover there were also significant differences between B0 with other samples and B1 with B2, B3 and B4. This means that all of the developed meat tenderizers were significantly more effective in reducing the hardness of meat than the control and commercial meat tenderizer. The results of the experiment is shown below in Table 4.5 and Figure 4.1.

Table 4.5
The Hardness of Meat Control and Treated Meat Samples

	Hardness of meat (g)	p-value
B0	414.31 ± 29.66^a	
B1	350.92 ± 43.30^b	0.034
B2	297.79 ± 27.67^c	0.001
B3	279.94 ± 31.18^c	0.001
B4	280.00 ± 7.82^c	0.001
B5	246.37 ± 19.11^c	0.001

Note: Values are expressed as mean $\pm$ standard deviation. Data were analysed using one-way ANOVA, followed by Tukey's HSD post hoc test at $p < 0.05$. Different superscript letters within the same column indicate significant differences.

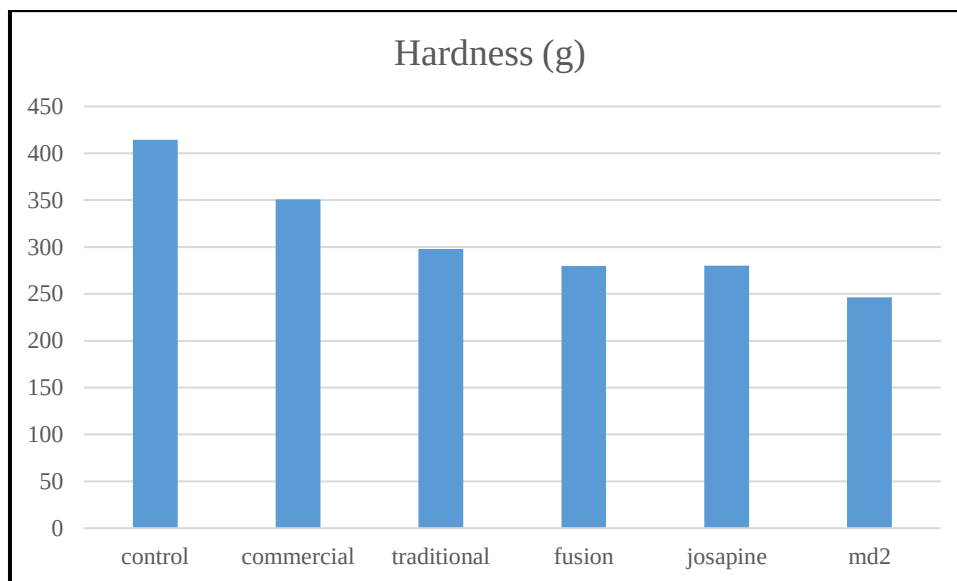


Figure 4.1 Hardness of Meat Treated with Meat Tenderizers

4.4 Antioxidant Activity

4.4.1 DPPH

All meat tenderizers have significantly lower ($p < 0.05$) antioxidant activity as compared to ascorbic acid. Ascorbic acid on the other hand, showed substantially higher DPPH scavenging ability, confirming its role as a strong positive control in antioxidant studies. Table 4.6, Figure 4.2 and Figure 4.3 shows the antioxidant activities of standard ascorbic acid and the meat tenderizers via DPPH.

Table 4.6
Antioxidant Activities of Meat Tenderizers via DPPH

Sample	DPPH (%)	p-value
Ascorbic Acid	98.654 ± 0.91 ^a	
Traditional Recipe	31.837 ± 0.088 ^b	0.0001
Fusion Recipe	27.119 ± 0.095 ^c	<0.0001
Josapine Recipe	27.616 ± 0.129 ^c	<0.0001
MD2 Recipe	33.786 ± 0.054 ^d	0.0001

Note: Values are expressed as mean ± standard deviation. Data were analysed using one-way ANOVA, followed by Tukey's HSD post hoc test at $p < 0.05$. Different superscript letters within the same column indicate significant differences

4.4.2 Total Phenolic Content

The highest value of TPC of meat tenderizer is the MD2 recipe (0.446 ± 0.03) followed by josapine recipe (0.436 ± 0.043), fusion recipe (0.347 ± 0.021), and traditional recipe (0.273 ± 0.066). Meat that is treated with the meat tenderizer have significantly lower levels of TPC as compared with the meat tenderizers themselves, with the highest value of TPC is meat treated in MD2 recipe (0.189 ± 0.053) followed by meat treated in josapine recipe (0.163 ± 0.008), fusion recipe (0.079 ± 0.0352) and traditional recipe (0.063 ± 0.071) with the lowest TPC level is the control meat untreated with anything. One way ANOVA analysis revealed a significant difference in polyphenolic content ($p < 0.05$) between control meat with meat treated with meat tenderizers. This shows an increase in TPC in meat when marinated with the developed meat tenderizers. Table 4.7 shows the polyphenolic content of the four meat tenderizers via TPC analysis

Table 4.7
Polyphenolic Content of the Four Meat Tenderizers via TPC Analysis

Sample	TPC (mg GAE/g)	p-value
Control Meat	0.001 ± 0.032^a	
Meat treated with Traditional Recipe	0.063 ± 0.071^b	0.001
Meat treated with Fusion Recipe	0.079 ± 0.0352^b	<0.001
Meat treated with Josapine Recipe	0.163 ± 0.008^c	<0.001
Meat treated with MD2 Recipe	0.189 ± 0.053^c	<0.001
Traditional Recipe Paste	0.273 ± 0.066^d	<0.001
Fusion Recipe Paste	0.347 ± 0.021^d	<0.001
Josapine Recipe Paste	0.436 ± 0.043^e	<0.001
MD2 Recipe Paste	0.446 ± 0.03^e	<0.001

Note: Values are expressed as mean $\pm$ standard deviation. Data were analysed using one-way ANOVA, followed by Tukey's HSD post hoc test at $p < 0.05$. Different superscript letters within the same column indicate significant differences.

4.4.3 Total Flavonoid Content (TFC)

The highest level of TFC among the meat tenderizers is the traditional recipe (30.664 ± 1.845), followed by fusion recipe (25.265 ± 1.541), josapine recipe

(18.785±1.268) and MD2 recipe (16.694±0.521). The level of TFC in meat treated with the meat tenderizers are significantly lower than the meat tenderizers themselves with meat treated with traditional recipe (15.439±0.523) have the highest TFC level, followed by fusion recipe (13.231±1.203), MD2 recipe (12.849±0.452), josapine recipe (12.323±2.556) with untreated control meat (9.311±0.715) having the lowest TFC level. Significant differences between control meat and meat treated with meat tenderizers ($p<0.05$). This shows an increase in TFC level when marinating meat in the developed meat tenderizers particularly when using traditional recipe. Table 4.8 shows the polyphenolic content of meat samples treated in the meat tenderizers and the polyphenolic content of the four meat tenderizers.

Table 4.8
Polyphenolic Content of the Four Meat Tenderizers via TFC Analysis

Sample	TFC (mg QE/g)	p-values
Control Meat	9.311 ± 0.715 ^a	
Meat treated with Traditional Recipe	15.439±0.523 ^b	<0.001
Meat treated with Fusion Recipe	13.231 ± 1.203 ^c	<0.001
Meat treated with Josapine Recipe	12.323 ± 2.556 ^c	<0.001
Meat treated with MD2 Recipe	12.849 ± 0.452 ^c	<0.001
Traditional Recipe Paste	30.664 ± 1.845 ^d	<0.001
Fusion Recipe Paste	25.265 ± 1.541 ^d	<0.001
Josapine Recipe Paste	18.785 ± 1.268 ^e	<0.001
MD2 Recipe Paste	16.694 ± 0.521 ^b	<0.001

Note: Values are expressed as mean ± standard deviation. Data were analysed using one-way ANOVA, followed by Tukey's HSD post hoc test at $p<0.05$. Different superscript letters within the same column indicate significant differences.

4.4.4 Superoxide Dismutase (SOD)

Table 4.9 shows SOD inhibition of meat samples and meat tenderizers. The SOD inhibition for the four meat tenderizers had significant differences ($p<0.05$) with highest value of SOD inhibition when comparing control meat to meat treated with the developed meat tenderizers. Meat treated with MD2 flavour meat tenderizer was not significantly different from control meat indicating insignificant changes in SOD

inhibition. Post hoc Tukey HSD test revealed marinating meat with Fusion recipe meat tenderizer significantly inhibits SOD among other recipes.

Table 4.9

SOD Inhibition of Control Meat, Four Meat Samples Marinated with the Meat Tenderizers and the Four Meat Tenderizers

Sample	SOD Inhibition	p-values
Control meat	23.50±6.66 ^a	
Meat marinated with Traditional flavour	37.58±6.80 ^b	<0.001
Meat marinated with Fusion flavour	50.10±2.48 ^c	<0.001
Meat marinated with Josapine flavour	38.62±4.53 ^b	<0.001
Meat marinated with MD2 flavour	22.94±3.96 ^a	0.748
Traditional	40.36±9.13 ^b	<0.001
Fusion	27.85±3.25 ^b	0.012
Josapine	36.85±8.78 ^b	<0.001
MD2	29.46±12.09 ^a	0.061

Note: Values are expressed as mean ± standard deviation. Data were analysed using one-way ANOVA, followed by Tukey's HSD post hoc test at p<0.05. Different superscript letters within the same column indicate significant differences.

The SOD activity for the four meat tenderizers have significantly higher values with highest value of SOD belongs to josapine recipe followed by traditional recipe, MD2 recipe and fusion recipe as compared to control meat and meat marinated in the four meat tenderizers with meat treated with fusion recipe having highest SOD level among the meat followed by josapine recipe, traditional recipe, control untreated meat and lastly MD2 recipe. Significant differences (p<0.05) were observed between traditional recipe, fusion recipe and Josapine recipes. However, there is no significant differences observed in MD2 recipe. Post hoc Tukey HSD test revealed fusion recipe has significantly higher SOD activity compared to traditional and Josapine. The meat tenderizers themselves has significantly high SOD activity which suggest that it does not translate when marinating meat and is loss after cooking. Table 4.10 shows SOD activity of meat samples and the four meat tenderizers.

Table 4.10

SOD Activity of Control Meat, Four Meat Samples Marinated with the Meat Tenderizers and the Four Meat Tenderizers

Sample	SOD Activity	p-values
Control meat	225.64±63.98 ^a	
Traditional + meat	360.81±131.25 ^b	0.04
Fusion + meat	480.98±23.77 ^c	<0.001
Josapine + meat	370.71±139.45 ^b	0.03
MD2 + meat	220.22±134.04 ^a	0.88
Traditional	2421.33±548.08 ^c	<0.001
Fusion	1821.15±212.64 ^c	<0.001
Josapine	2843.49±677.73 ^c	<0.001
MD2	2055.28±843.26 ^c	<0.001

Note: Values are expressed as mean ± standard deviation. Data were analysed using one-way ANOVA, followed by Tukey's HSD post hoc test at p<0.05. Different superscript letters within the same column indicate significant differences.

4.4.5 Catalase (CAT)

Table 4.11, shows spectrophotometer reading of meat samples and the four meat tenderizers in different concentrations.

Table 4.11

Spectrophotometer Reading of Control Meat, The Four Meat Samples Marinated with the Meat Tenderizers and the Meat Tenderizers in Different Concentrations

Concentration (%)	0.125	0.250	0.500	1.000	2.000	5.000
Control meat	0.014	0.024	0.056	0.128	0.204	0.213
Meat marinated with Traditional flavour	0.011	0.0326	0.061	0.12	0.241	0.261
Meat marinated with Fusion flavour	0.011	0.031	0.051	0.106	0.212	0.242
Meat marinated with Josapine flavour	0.016	0.032	0.07	0.107	0.254	0.257
Meat marinated with MD2 flavour	0.009	0.032	0.061	0.112	0.229	0.239
Traditional	0.018	0.034	0.071	0.154	0.301	0.314

Concentration (%)	0.125	0.250	0.500	1.000	2.000	5.000
Fusion	0.002	0.029	0.082	0.144	0.296	0.316
Josapine	0.009	0.031	0.088	0.162	0.302	0.326
MD2	0.017	0.032	0.077	0.161	0.31	0.324

The highest CAT activity was found in the josapine recipe, followed by MD2 recipe, fusion recipe and traditional recipe. Meat treated with the meat tenderizers and untreated control meat have significantly lower CAT activity ($p < 0.001$). In comparison with control, all other meat samples and meat tenderizers were significantly different ($p < 0.05$) Table 4.12 and Figure 4.15 shows CAT activity of meat samples and the four meat tenderizer.

Table 4.12

CAT Activity of Control Meat, Four Meat Samples Marinated with the Meat Tenderizers and All Four Meat Tenderizers

Sample	CAT Activity	p-values
Control meat	0.001843 ^a	
Meat marinated with Traditional flavour	0.002177 ^b	<0.001
Meat marinated with Fusion flavour	0.001915 ^b	<0.001
Meat marinated with Josapine flavour	0.002294 ^b	<0.001
Meat marinated with MD2 flavour	0.002069 ^b	<0.001
Traditional	0.016994 ^b	<0.001
Fusion	0.018214 ^b	<0.001
Josapine	0.02193 ^b	<0.001
MD2	0.020351 ^b	<0.001

Note: Values are expressed as mean $\pm$ standard deviation. Data were analysed using one-way ANOVA, followed by Tukey's HSD post hoc test at $p < 0.05$. Different superscript letters within the same column indicate significant differences.

4.4.6 Glutathione Peroxidase (GSH)

Meat treated with the meat tenderizers and untreated control meat have significantly higher GSH activity as compared to the meat tenderizers themselves with

the highest GSH activity being meat treated with josapine recipe followed by traditional recipe, fusion recipe, MD2 recipe and lastly control meat. The meat tenderizers however have significantly lower GSH activity with traditional recipe having the highest GSH activity. T-test analysis revealed significant differences ($p<0.05$) between control meat and meat treated with meat tenderizers. Table 4.13 shows the GSH activity of meat samples and the four meat tenderizers

Table 4.13
GSH Activity of Meat Samples and Meat Tenderizers

Sample	GSH Activity	p-values
Control meat	533.58±2.66 ^a	
Meat marinated with Traditional flavour	647.88±11.88 ^b	<0.001
Meat marinated with Fusion flavour	608.97±4.12 ^b	<0.001
Meat marinated with Josapine flavour	735.00±3.66 ^b	<0.001
Meat marinated with MD2 flavour	584.24±4.01 ^b	<0.001
Traditional	179.03±0.70 ^c	<0.001
Fusion	51.70±0.63 ^c	<0.001
Josapine	33.85±0.23 ^c	<0.001
MD2	42.95±0.78 ^c	<0.001

Note: Values are expressed as mean ± standard deviation. Data were analysed using one-way ANOVA, followed by Tukey's HSD post hoc test at $p<0.05$. Different superscript letters within the same column indicate significant differences.

4.5 Shelf Life Analysis

4.5.1 pH Activity

On day 1, the pH of all samples started around 4 to 5. Overtime, the pH value drops sharply for samples in room temperature as compared to chiller temperature. Overall the pH of samples at room temperature changed significantly ($p<0.05$) after 20 days which means these meat tenderizers are unsafe for consumption. In contrast, samples stored in the chiller maintained relatively stable without significant changes even up to 80 days. Clusters in the Table 4.14 labelled with the letter “a” signifies it is

still safe to consume. Table 4.14 shows pH of all four meat tenderizers left in room temperature and chiller at day 1, day 20, day 40 and day 80

Table 4.14
pH of Meat Tenderizers Stored in Room Temperature and Chiller at day 1, 20, 40, and 80

Sample	Temperature	Day 1	Day 20	Day 40	Day 80
Traditional	Room (23°C)	5.23±0.50 ^a	3.50±0.64 ^c	2.12±0.23 ^d	1.91±1.25 ^c
	Chiller (4°C)	4.80±0.93 ^a	4.36±1.04 ^a	4.75±1.42 ^a	4.64±0.71 ^a
Fusion	Room (23°C)	4.82±1.25 ^a	1.99±1.04 ^c	1.98±0.64 ^b	1.96±0.26 ^c
	Chiller (4°C)	4.67±0.26 ^a	4.33±0.93 ^a	4.71±1.04 ^a	4.57±0.71 ^a
Josapine	Room (23°C)	4.74±0.42 ^a	1.96±0.26 ^b	1.99±0.26 ^b	2.01±0.56 ^c
	Chiller (4°C)	4.68±1.43 ^a	4.12±0.23 ^a	4.51±0.86 ^a	4.44±0.87 ^a
MD2	Room (23°C)	4.73±0.45 ^a	3.62±0.81 ^c	2.13±0.48 ^d	2.21±0.42 ^b
	Chiller (4°C)	4.74±1.34 ^a	4.06±1.33 ^a	4.35±0.43 ^a	4.38±0.32 ^a

Note: Values are expressed as mean ± standard deviation. Data were analysed using two-way ANOVA, followed by Tukey's HSD post hoc test at $p < 0.05$. Different superscript letters within the same column indicate significant differences.

4.5.2 Determination of Moisture

Each meat tenderizer starts off at Day 1 with different moisture content of each recipe due to the difference in recipes. However, for each recipe, their moisture content remains relatively similar even when kept in room temperature and chilled temperatures. On Day 20, the moisture content of all meat tenderizers increased values with room temperature having a more significant increase as compared to chiller temperature. On Day 40, the moisture content of all meat tenderizers regardless of temperature decreases and on Day 80, the moisture content mostly increased for the meat tenderizers of both temperatures. Also on Day 20, the meat tenderizers in room temperature were observed damaged while the meat tenderizers in room temperature still remain edible after Day 80. Table 4.15 and shows moisture content of all four meat tenderizers left in room temperature and chiller at day 1, day 20, day 40 and day 80.

Table 4.15

Moisture Content of All Four Meat Tenderizers Left in Room Temperature and Chiller at Day 1, Day 20, Day 40, Day 80

Sample	Temperature	Day 1	Day 20	Day 40	Day 80
Traditional	Room (23°C)	39.20±0.89 ^c	44.36±0.71 ^c	44.36±1.04 ^c	46.95±0.98 ^c
	Chiller (4°C)	38.65±1.20 ^c	40.34±0.60 ^c	31.74±1.07 ^c	38.90±0.47 ^c
Fusion	Room (23°C)	40.72±1.03 ^c	45.49±0.66 ^c	45.49±0.66 ^c	44.25±0.64 ^c
	Chiller (4°C)	40.56±0.90 ^c	41.11±0.55 ^c	37.04±1.01 ^c	39.71±1.17 ^c
Josapine	Room (23°C)	44.36±0.73 ^b	59.45±0.57 ^b	59.45±0.39 ^b	59.68±0.96 ^b
	Chiller (4°C)	44.81±0.86 ^b	49.36±0.97 ^b	47.11±0.56 ^b	51.59±0.91 ^b
MD2	Room (23°C)	53.80±0.62 ^a	69.82±0.3 ^a	69.82±0.69 ^a	67.60±0.59 ^a
	Chiller (4°C)	53.00±0.80 ^a	55.15±0.53 ^{ab}	53.78±0.72 ^{ab}	57.17±0.53 ^a

Note: Values are expressed as mean ± standard deviation. Data were analysed using two-way ANOVA, followed by Tukey's HSD post hoc test at $p < 0.05$. Different superscript letters within the same column indicate significant differences.

4.6 Nutrient Analysis

4.6.1 Reference to The Nutrient Composition of Malaysia 4th Edition 1997

Table 4.16 to 4.19 shows the nutrient content of all ingredients of all four meat tenderizers.

Table 4.16

Nutrient Content of all Ingredients of Traditional Flavour Meat Tenderizer

Ingredient	Weight (g)	Water (g)	Energy (kcal)	Protein (g)	Fat (g)	CHO (g)	Fibre (g)	Ash (g)	Calcium (mg)	Iron (mg)	Phosphorus (mg)	Potassium (mg)	Sodium (mg)	Vit C (mg)
Jackfruit leaves	21	1.14	65.73	1.60	1.76	15.75	5.52	0.76	175.14	9.03	23.73	111.09	4.83	9.77
Garlic	16	9.39	25.44	0.69	0.02	5.63	0.13	0.14	1.60	0.13	17.76	52.64	1.76	2.13
Lemongrass	47	39.01	28.20	0.33	0.33	6.02	0.94	0.38	13.16	0.52	13.63	135.36	0.94	0.00
Turmeric	3	0.43	10.05	0.07	0.15	2.10	0.10	0.16	4.38	0.56	8.52	69.33	0.96	0.00
Ginger	6	5.17	3.12	0.13	0.06	0.52	0.10	0.03	1.02	0.15	3.78	7.50	0.18	0.32
Coriander	6.5	0.62	18.14	0.72	0.02	3.77	0.81	0.55	39.33	2.24	24.12	118.24	6.05	0.00
Cumin	4.7	0.56	12.08	0.55	0.63	1.06	1.59	0.31	51.37	1.29	17.95	61.15	0.71	0.00
Fennel	6.7	0.74	22.38	1.28	1.65	0.60	1.88	0.55	50.65	4.05	25.66	128.64	7.17	0.00
Brown sugar	19	1.94	67.07	0.25	0.04	16.45	0.00	0.32	35.53	0.51	7.60	71.63	2.66	0.00
Black pepper	2.5	0.27	9.05	0.25	0.07	1.87	0.03	0.02	18.30	0.12	1.10	2.55	0.10	0.00
Onion	76	63.23	47.12	0.91	0.00	10.87	0.46	0.53	56.24	0.84	6.08	212.04	9.12	9.42
Chili powder	12	1.70	29.16	1.54	0.54	4.55	3.07	0.60	12.84	0.17	18.36	20.76	2.88	5.16
Cayenne powder	1.7	0.14	5.41	0.20	0.29	0.96	0.46	0.10	2.52	0.13	4.98	34.17	0.51	1.30
Paprika powder	1.7	0.19	4.79	0.24	0.22	0.92	0.59	0.13	3.89	0.36	5.34	38.76	1.16	0.02
Oil	32	0.00	288.00	0.00	32.00	0.00	0.00	0.00	1.28	0.00	0.00	0.00	1.60	0.00
Sugar	30	0.15	119.40	0.00	0.00	29.82	0.00	0.03	0.60	0.03	0.00	1.50	0.30	0.00
Salt	6.2	0.22	0.00	0.00	0.00	0.00	0.00	6.14	1.92	0.05	0.00	6.76	2428.73	0.00
Total	292	124.91	755.13	8.74	37.77	100.88	15.68	10.76	469.77	20.17	178.61	1072.11	2469.64	28.11
per 100g		42.78	258.61	2.99	12.94	34.55	5.37	3.68	160.88	6.91	61.17	367.16	845.77	9.63

Table 4.17

Nutrient Content of all Ingredients of Fusion Flavour Meat Tenderizer

Ingredient	Weight (g)	Water (g)	Energy (kcal)	Protein (g)	Fat (g)	CHO (g)	Fibre (g)	Ash (g)	Calcium (mg)	Iron (mg)	Phosphorus (mg)	Potassium (mg)	Sodium (mg)	Vit C (mg)
Jackfruit leaves	21	1.14	65.73	1.60	1.76	15.75	5.52	0.76	175.14	9.03	23.73	111.09	4.83	9.77
Garlic	16	9.39	25.44	0.69	0.02	5.63	0.13	0.14	1.60	0.13	17.76	52.64	1.76	2.13
Lemongrass	47	39.01	28.20	0.33	0.33	6.02	0.94	0.38	13.16	0.52	13.63	135.36	0.94	0.00
Turmeric	3	0.43	10.05	0.07	0.15	2.10	0.10	0.16	4.38	0.56	8.52	69.33	0.96	0.00
Ginger	6	5.17	3.12	0.13	0.06	0.52	0.10	0.03	1.02	0.15	3.78	7.50	0.18	0.32
Coriander	6.5	0.62	18.14	0.72	0.02	3.77	0.81	0.55	39.33	2.24	24.12	118.24	6.05	0.00
Cumin	4.7	0.56	12.08	0.55	0.63	1.06	1.59	0.31	51.37	1.29	17.95	61.15	0.71	0.00
Fennel	6.7	0.74	22.38	1.28	1.65	0.60	1.88	0.55	50.65	4.05	25.66	128.64	7.17	0.00
Brown sugar	19	1.94	67.07	0.25	0.04	16.45	0.00	0.32	35.53	0.51	7.60	71.63	2.66	0.00
Black pepper	2.5	0.27	9.05	0.25	0.07	1.87	0.03	0.02	18.30	0.12	1.10	2.55	0.10	0.00
Onion	76	63.23	47.12	0.91	0.00	10.87	0.46	0.53	56.24	0.84	6.08	212.04	9.12	9.42
Chili powder	12	1.70	29.16	1.54	0.54	4.55	3.07	0.60	12.84	0.17	18.36	20.76	2.88	5.16
Cayenne powder	1.7	0.14	5.41	0.20	0.29	0.96	0.46	0.10	2.52	0.13	4.98	34.17	0.51	1.30
Paprika powder	1.7	0.19	4.79	0.24	0.22	0.92	0.59	0.13	3.89	0.36	5.34	38.76	1.16	0.02
Oil	32	0.00	288.00	0.00	32.00	0.00	0.00	0.00	1.28	0.00	0.00	0.00	1.60	0.00
Sugar	30	0.15	119.40	0.00	0.00	29.82	0.00	0.03	0.60	0.03	0.00	1.50	0.30	0.00
Salt	6.2	0.22	0.00	0.00	0.00	0.00	0.00	6.14	1.92	0.05	0.00	6.76	2428.73	0.00
Thyme	1.1	0.09	3.04	0.10	0.08	0.70	0.41	0.13	20.79	1.36	2.21	8.95	0.61	0.55
Rosemary	1.4	0.13	4.63	0.07	0.21	0.90	0.60	0.09	17.92	0.41	0.98	13.37	0.70	0.86
Total	294.5	125.12	762.80	8.91	38.06	102.48	16.69	10.98	508.48	21.95	181.80	1094.43	2470.95	29.52
per 100g		42.49	259.02	3.03	12.92	34.80	5.67	3.73	172.66	7.45	61.73	371.62	839.03	10.02

Table 4.18

Nutrient Content of all Ingredients of Josapine Flavour Meat Tenderizer

Ingredient	Weight (g)	Water (g)	Energy (kcal)	Protein (g)	Fat (g)	CHO (g)	Fibre (g)	Ash (g)	Calcium (mg)	Iron (mg)	Phosphorus (mg)	Potassium (mg)	Sodium (mg)	Vit C (mg)
Jackfruit leaves	21	1.14	65.73	1.60	1.76	15.75	5.52	0.76	175.14	9.03	23.73	111.09	4.83	9.77
Garlic	16	9.39	25.44	0.69	0.02	5.63	0.13	0.14	1.60	0.13	17.76	52.64	1.76	2.13
Lemongrass	47	39.01	28.20	0.33	0.33	6.02	0.94	0.38	13.16	0.52	13.63	135.36	0.94	0.00
Turmeric	3	0.43	10.05	0.07	0.15	2.10	0.10	0.16	4.38	0.56	8.52	69.33	0.96	0.00
Ginger	6	5.17	3.12	0.13	0.06	0.52	0.10	0.03	1.02	0.15	3.78	7.50	0.18	0.32
Coriander	6.5	0.62	18.14	0.72	0.02	3.77	0.81	0.55	39.33	2.24	24.12	118.24	6.05	0.00
Cumin	4.7	0.56	12.08	0.55	0.63	1.06	1.59	0.31	51.37	1.29	17.95	61.15	0.71	0.00
Fennel	6.7	0.74	22.38	1.28	1.65	0.60	1.88	0.55	50.65	4.05	25.66	128.64	7.17	0.00
Brown sugar	19	1.94	67.07	0.25	0.04	16.45	0.00	0.32	35.53	0.51	7.60	71.63	2.66	0.00
Black pepper	2.5	0.27	9.05	0.25	0.07	1.87	0.03	0.02	18.30	0.12	1.10	2.55	0.10	0.00
Onion	76	63.23	47.12	0.91	0.00	10.87	0.46	0.53	56.24	0.84	6.08	212.04	9.12	9.42
Chili powder	12	1.70	29.16	1.54	0.54	4.55	3.07	0.60	12.84	0.17	18.36	20.76	2.88	5.16
Cayenne powder	1.7	0.14	5.41	0.20	0.29	0.96	0.46	0.10	2.52	0.13	4.98	34.17	0.51	1.30
Paprika powder	1.7	0.19	4.79	0.24	0.22	0.92	0.59	0.13	3.89	0.36	5.34	38.76	1.16	0.02
Oil	32	0.00	288.00	0.00	32.00	0.00	0.00	0.00	1.28	0.00	0.00	0.00	1.60	0.00
Sugar	30	0.15	119.40	0.00	0.00	29.82	0.00	0.03	0.60	0.03	0.00	1.50	0.30	0.00
Salt	6.2	0.22	0.00	0.00	0.00	0.00	0.00	6.14	1.92	0.05	0.00	6.76	2428.73	0.00
Pineapple (Josapine)	52	45.66	23.40	0.26	0.05	5.51	0.31	0.21	12.48	0.73	3.12	50.44	16.12	7.90
Total	344	170.56	778.53	9.00	37.82	106.40	15.99	10.96	482.25	20.90	181.73	1122.55	2485.76	36.01
per 100g		49.22	224.68	2.60	10.92	30.71	4.62	3.16	139.18	6.03	52.45	323.97	717.39	10.39

Table 4.19

Nutrient Content of All Ingredients of MD2 Flavour Meat Tenderizer

Ingredient	Weight (g)	Water (g)	Energy (kcal)	Protein (g)	Fat (g)	CHO (g)	Fibre (g)	Ash (g)	Calcium (mg)	Iron (mg)	Phosphorus (mg)	Potassium (mg)	Sodium (mg)	Vit C (mg)
Jackfruit leaves	21	1.14	65.73	1.60	1.76	15.75	5.52	0.76	175.14	9.03	23.73	111.09	4.83	9.77
Garlic	16	9.39	25.44	0.69	0.02	5.63	0.13	0.14	1.60	0.13	17.76	52.64	1.76	2.13
Lemongrass	47	39.01	28.20	0.33	0.33	6.02	0.94	0.38	13.16	0.52	13.63	135.36	0.94	0.00
Turmeric	3	0.43	10.05	0.07	0.15	2.10	0.10	0.16	4.38	0.56	8.52	69.33	0.96	0.00
Ginger	6	5.17	3.12	0.13	0.06	0.52	0.10	0.03	1.02	0.15	3.78	7.50	0.18	0.32
Coriander	6.5	0.62	18.14	0.72	0.02	3.77	0.81	0.55	39.33	2.24	24.12	118.24	6.05	0.00
Cumin	4.7	0.56	12.08	0.55	0.63	1.06	1.59	0.31	51.37	1.29	17.95	61.15	0.71	0.00
Fennel	6.7	0.74	22.38	1.28	1.65	0.60	1.88	0.55	50.65	4.05	25.66	128.64	7.17	0.00
Brown sugar	19	1.94	67.07	0.25	0.04	16.45	0.00	0.32	35.53	0.51	7.60	71.63	2.66	0.00
Black pepper	2.5	0.27	9.05	0.25	0.07	1.87	0.03	0.02	18.30	0.12	1.10	2.55	0.10	0.00
Onion	76	63.23	47.12	0.91	0.00	10.87	0.46	0.53	56.24	0.84	6.08	212.04	9.12	9.42
Chili powder	12	1.70	29.16	1.54	0.54	4.55	3.07	0.60	12.84	0.17	18.36	20.76	2.88	5.16
Cayenne powder	1.7	0.14	5.41	0.20	0.29	0.96	0.46	0.10	2.52	0.13	4.98	34.17	0.51	1.30
Paprika powder	1.7	0.19	4.79	0.24	0.22	0.92	0.59	0.13	3.89	0.36	5.34	38.76	1.16	0.02
Oil	32	0.00	288.00	0.00	32.00	0.00	0.00	0.00	1.28	0.00	0.00	0.00	1.60	0.00
Sugar	30	0.15	119.40	0.00	0.00	29.82	0.00	0.03	0.60	0.03	0.00	1.50	0.30	0.00
Salt	6.2	0.22	0.00	0.00	0.00	0.00	0.00	6.14	1.92	0.05	0.00	6.76	2428.73	0.00
Pineapple (MD2)	126.5	111.07	56.93	0.63	0.13	13.41	0.76	0.51	30.36	1.77	7.59	122.71	39.22	19.23
Total	418.5	235.98	812.06	9.38	37.90	114.29	16.44	11.26	500.13	21.95	186.20	1194.82	2508.86	47.34
per 100g		56.05	192.89	2.23	9.00	27.15	3.91	2.67	118.80	5.21	44.23	283.80	595.93	11.24

4.6.2 Proximate Analysis

Table 4.20 shows the proximate test results of all four meat tenderizers.

Table 4.20
Proximate Test Results of all Four Meat Tenderizers

Nutrient per 100g	Traditional	Fusion	Josapine	MD2
Water (g)	43.4±1.0 ^c	44.8±0.9 ^c	49.5±0.6 ^b	57.4±0.7 ^a
Energy (kcal)	265.0±0.9 ^b	274.0±0.7 ^a	237.0±1.1 ^c	211.0±0.7 ^d
Protein (g)	3.6±1.1 ^a	3.3±0.9 ^a	2.8±1.0 ^a	3.1±0.8 ^a
Fat (g)	10.9±1.1 ^b	13.8±0.6 ^a	9.8±0.7 ^b	10.3±0.8 ^b
Carbohydrates (g)	38.0±1.1 ^a	34.1±1.0 ^b	34.4±0.9 ^b	26.4±1.0 ^c
Ash (g)	4.1±0.9 ^a	4.0±0.6 ^a	3.5±0.8 ^{ab}	2.8±0.6 ^b

Note: Values are expressed as mean ± standard deviation. Data were analysed using one-way ANOVA, followed by Tukey's HSD post hoc test at $p < 0.05$. Different superscript letters within the same row indicate significant differences

The nutrient composition of the four formulations show notable variations. Water content was the highest in the MD2 recipe (57.4g), suggesting that it has the highest moisture content. Inversely, fusion recipe has the highest caloric value (274.0 kcal) which is due to it having the highest fat content (13.8g) which contributes to its high energy level. Protein levels were relatively consistent ranging from 2.8g (josapine recipe) to 3.6g (traditional recipe) which indicates minor sources of protein. Carbohydrates and ash were the highest in traditional recipe (38.0g and 4.1g relatively) as compared to other recipes.

4.7 Sensory Evaluation

The highest mean score for appearance was the MD2 recipe followed by josapine, fusion, traditional, control meat and lastly commercial meat tenderizer. For taste, texture and acceptability, the highest mean score was the josapine recipe followed by MD2, fusion, traditional, commercial meat tenderizer and lastly the control meat. For aroma, the highest mean score was the josapine followed by MD2, fusion, traditional, control meat and lastly the commercial meat tenderizer. There were

significant differences ($p<0.05$) for the mean score of every sensory attribute between the meat tenderizers and the control meat. However, for all parameters, there were no significant differences ($p>0.05$) for the commercial meat tenderizers. Table 4.21 and Figure 4.2 shows the average scores of sensory parameters for all meat samples. Overall, the post hoc analysis demonstrated that all natural meat tenderizers treatments outperformed the commercial tenderizer and untreated control across all sensory attributes. Josapine consistently scored the highest for taste, texture and overall acceptability, while MD2 was particularly effective for appearance and aroma. These results suggest that fruit-based marinades offer superior sensory compared to conventional tenderizing methods.

Table 4.21
Average Scores of Sensory Parameters for Meat with Meat Tenderizers

Parameter	Control	Commercial	Traditional	Fusion	Josapine	MD2
Appearance	3.36±2.08 ^c	3.12±1.77 ^c	6.20±1.30 ^b	6.28±1.48 ^b	6.80±1.14 ^{ab}	7.12±1.07 ^a
Taste	2.84±1.71 ^c	3.04±1.97 ^c	5.88±1.48 ^b	5.96±1.87 ^b	7.48±1.24 ^a	6.68±1.64 ^{ab}
Texture	2.40±1.72 ^c	2.84±1.71 ^c	4.40±2.00 ^b	5.44±2.08 ^b	7.20±1.67 ^a	6.52±2.16 ^{ab}
Aroma	3.08±1.74 ^c	2.96±1.71 ^c	6.88±1.68 ^{ab}	5.88±1.86 ^b	7.00±1.47 ^a	6.92±1.29 ^a
Acceptability	2.80±1.70 ^c	2.88±1.70 ^c	5.72±1.46 ^b	5.80±1.79 ^b	7.24±1.30 ^a	6.88±1.58 ^a

Note: Values are expressed as mean ± standard deviation (n=25). Data were analysed using two-way ANOVA, and mean comparisons with the control were performed using Tukey's HSD post hoc test at $p<0.05$. Different superscript letters within the same row indicates significant differences.

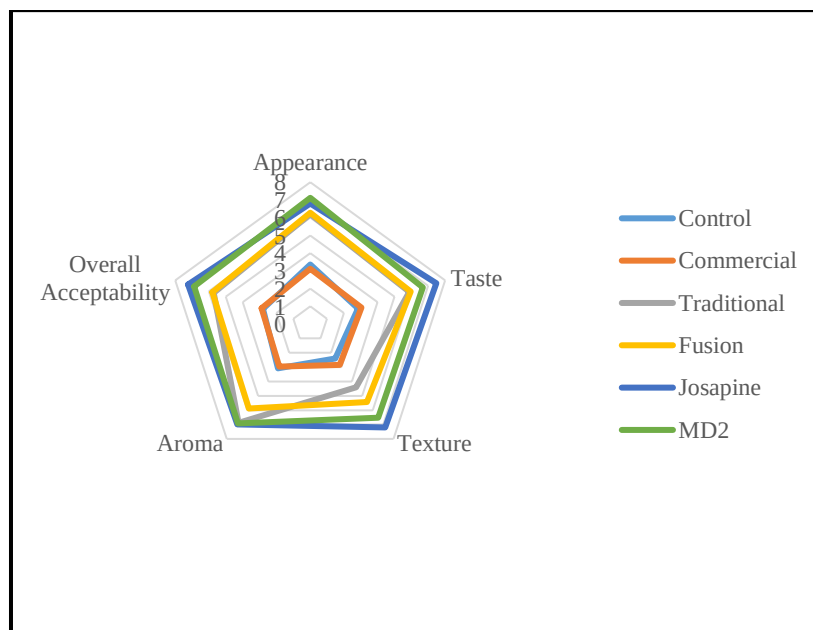


Figure 4.2 Average Scores of Sensory Parameters for Meat Treated with Meat Tenderizers

The sensory evaluation was conducted using a hedonic rating scale ranging from 0 to 8, where the numerical values represent the degree of preference for each sensory attribute assessed. A score closer to 0 indicates very low acceptability, whereas a score closer to 8 indicates very high acceptability. Panelists evaluated the meat samples based on appearance, taste, texture, aroma, and overall acceptability, and the average scores obtained were plotted in a radar chart to visually compare the performance of the control sample, commercial tenderizer, and the developed meat tenderizer formulations. In this scale, 0 represents extremely dislike, 2 represents dislike, 4 represents moderate acceptance, 6 represents like, and 8 represents extremely like.

CHAPTER 5

DISCUSSION

5.1 Introduction

This section analyses the study's findings in relation to the research objectives, relevant literature and applications. Development, effectiveness, antioxidant activity, nutrient analysis and sensory evaluation of the meat tenderizer pastes would be further explored and discussed.

5.2 Development of Meat Tenderizer

Four recipes of meat tenderizer pastes made from jackfruit leaves, pineapples and selected herbs have been established in this study. After several trials and error, the amount of ingredients for each recipe is optimized and developed as mentioned in Table 4.1, 4.2, 4.3 and 4.4. Four recipes of the meat tenderizer were made which were traditional flavour, fusion, josapine and MD2. Multiple flavours were developed to create a variety of food choices for consumers. Jackfruit leaves and pineapples were selected for their content of plant enzymes which were extensively researched to improve meat tenderness (Bekhit et al., 2015). It is also important that the meat tenderizers do not contain extensive amount of these ingredients as uncontrolled application can lead to over-tenderizing of meat causing them to have mushy texture (Bureros et al., 2020).

The selection of herbs and spices used in the meat tenderizer pastes was made due to a few factors including availability, acceptability, flavour and aroma. The selected of herbs and spices are widely available and used in Malaysia thus giving consumers a sense of familiarity when using the meat tenderizer paste. This factor plays an essential role in the selection of ingredients for development and since all the ingredients are readily available in the market, the product development process can proceed smoothly without interruption. Moreover, it is also crucial that the selected herbs and spices are widely used by consumers.

Four distinct recipes were formulated to explore both traditional and innovative approaches to enhancing meat texture and flavour, with consideration for cultural and biochemical perspectives. The first recipe, a traditional Malaysian formulation, was designed to align with local culinary preferences and utilized a blend of local herbs and spices commonly used in Malaysian cuisine. This paste reflects the ethnic richness of Malaysian cooking, where natural enzymes from ingredients such as the jackfruit leaves play a key role in meat tenderization while simultaneously imparting strong aromatic qualities (Shahrai et al., 2023). The second recipe adopted a fusion approach aimed at appealing to an international palate. It incorporated rosemary and thyme offering a herbal complexity. This approach not only expanded the flavour profile but also integrated enzymes and acids that work synergistically with jackfruit leaves to break down muscle fibres and connective tissues (Khan et al., 2021).

The remaining two recipes were formulated to assess the tenderizing effects of different pineapple variants, specifically Josapine and MD2. Pineapples are rich in bromelain, a proteolytic enzyme that has been widely studied for its potent ability to degrade myofibrillar proteins and collagen in meat tissues (Ha et al., 2016). The Josapine variant, native to Malaysia, is characterized by a slightly acidic and fibrous composition, which contributed not only to meat softening but also to a distinctively tangy flavour in the resulting product. On the other hand, the MD2 pineapple, a globally popular cultivar known for its high sweetness and juiciness, introduced a milder acidity and a more rounded flavour profile while maintaining significant tenderizing capacity due to its higher bromelain content. These comparative formulations allowed for an exploration of how enzymatic activity from natural fruit sources could be optimized according to varietal differences, thereby enhancing both texture and flavour dimensions in meat dishes. The integration of traditional, fusion, and fruit-enzyme-based approaches provides a multifaceted framework for meat tenderizer development, addressing both sensory and functional quality. Future studies could further investigate the enzymatic kinetics and shelf stability of these pastes under various storage conditions to support commercial scalability and consumer acceptability.

5.3 Determination of Hardness

Untreated meat sample showed significantly ($p < 0.05$) high measurements indicating harder texture as compared to other meat samples. The application of

exogenous proteolytic enzymes from jackfruit leaves and pineapples to the meat tenderizers. It was proven that proteolytic enzyme-treated meat samples produced significantly more tender meat due to the protein tissues of meat being lysed by the enzymes. Meat samples treated with jackfruit leaves and pineapple-based pastes exhibited significantly lower hardness values compared to untreated controls, affirming the effectiveness of these natural agents in proteolysis. The tenderizing effect is attributed to the enzymatic activity inherent in both ingredients. Pineapple, particularly due to its bromelain content was capable of hydrolyzing muscle proteins and weakening connective tissue structures, leading to softer textures (Mohd Azmi et al., 2023). Similarly, jackfruit leaves have been reported to contain protease-like compounds that contribute to muscle fiber degradation, although their tenderizing efficacy remains underexplored relative to commercial enzymes.

This is in line with a study using protease reduces the protein bands of muscle proteins creating softer and more tender meat due to the breaking of tissue fibres and loosening of myofibrils (Ahmad et al., 2019). This finding also complements a study by Manohar et al. (2016), Aung et al. (2022), Razali et al. (2023) and Ahmad Danial et al. (2024) proving proteolytic enzymes can degrade collagen fibres of meat and can be used as an effective meat tenderizer. Use of proteases such as bromelain, papain, ficin, actinidin and calpain in the food industry have been an efficient method for meat tenderization (Bekhit et al., 2015; Arshad et al., 2016; Manohar et al., 2016; Ismail et al., 2018; Ahmad et al., 2019; Bureros et al., 2020; Gagaoua et al., 2021; Mohd Azmi et al., 2023; Ahmad Danial et al., 2024).

A study by Ranasinghe et al. (2019) demonstrated a marked reduction in shear force values in meat marinated with jackfruit leaf extract, comparable to the effect of synthetic tenderizers, thus supporting its application in natural meat processing. To assess hardness quantitatively, texture profile analysis (TPA) was employed, a method widely recognized for its precision in evaluating meat textural attributes such as hardness, cohesiveness, and chewiness. TPA provides mechanical measurements that simulate the biting process, and its reliability in determining meat hardness has been validated in numerous studies (Kantale et al., 2019). In the current investigation, TPA revealed a consistent trend of decreased hardness in samples treated with combined jackfruit leaf and pineapple pastes, underscoring the synergistic enzymatic effect. Additionally, microscopic evaluations of muscle fibres indicated structural disintegration, correlating well with instrumental measurements. These findings align

with previous research by Mohd Azmi et al. (2023), which highlighted the effectiveness of natural fruit-derived enzymes in disrupting the integrity of myofibrillar and collagen networks in meat.

On the other hand, even with different methods of evaluating meat hardness, the results align with the results of this study. Roslan et al. (2019) evaluated meat hardness by measuring shear force with higher shear force indicating harder texture of meat and from their study, the use of plant proteases has reduced the shear force values of cooked meat after cooked as compared to meat without the use of plant proteases. A similar method implemented by Sairuji et al. (2017) that determined the harness of meat by using a texture analyser had found results that align with this study. They found that use of meat tenderizer that contained proteolytic enzymes from jackfruit leaves had significantly reduced ($p < 0.05$) the toughness of cooked meat as compared to untreated meat.

Another factor that contributes to the effectiveness of the developed meat tenderizer is the drying temperature of jackfruit leaves. The jackfruit leaves were dried at 50°C. Based on a study done by Ahmad et al. (2019) and Vidinamo et al. (2022), the most optimal temperatures for enzymatic activity is between 50-60°C, this means the proteolytic enzymes of the jackfruit leaves are at most active during the development of the meat tenderizer. Proteolytic enzymes deactivation starts at above 70°C which means the enzymatic properties are lost during cooking since the cooking temperature is 190°C (Zeeb, 2017). Proteolytic enzymes are heat-sensitive proteins that begin to denature at temperatures above approximately 60–70 °C. Thermal denaturation disrupts the tertiary structure of the enzyme, leading to distortion of the active site and loss of catalytic activity. Consequently, at typical cooking temperatures, enzymatic activity is completely inactivated. Therefore, meat tenderization by proteolytic enzymes occurs primarily during the marination stage prior to cooking, as further proteolysis cannot proceed once the enzyme is thermally denatured. Regardless, the proteolytic activity already took place during the margination period of the meat.

5.4 Antioxidant Activity

DPPH (1,1-diphenyl-2-picrylhydrazyl) is a deep violet and it is considered as a very stable organic free radical. Presence of ascorbic acid and other antioxidants will react with DPPH which makes the deep violet colour become clearer. In all of the

experiments, extraction was done with methanol is was proven to be the best solvent for antioxidant extraction (Bhat et al., 2017). For DPPH, the antioxidant content of the four developed meat tenderizers were significantly low as it does not reach EC₅₀ ascorbic acid equivalent antioxidant activity even at max concentration of 5%. This shows poor capability of the meat tenderizers to inhibit DPPH.

Next, to detect Total Phenolic Content (TPC), the principle of the assay relies on the electron transfer between phenolic compounds of the food sample and the Folin-Ciocalteu reagent. The phenolic hydroxyl groups (-OH) in the food sample undergoes oxidation by the Folin-Ciocalteu reagent which is the reactive free radical containing a mixture of molybdate and tungstate ions. This oxidation leads to a reduction of the ions forming a blue complex. The intensity of the blue complex was measured using a spectrophotometer and the total phenolic content was expressed as Gallic Acid Equivalent (mg GAE/g) using a standard calibration curve of Gallic Acid for quantification. Using the developed meat tenderizers significantly increased ($p < 0.05$) the level of TPC especially when using the Josapine or MD2 recipe.

Subsequently, the aluminium chloride colorimetric method was used to detect Total Flavonoid Content (TFC) for the same reason of being widely used and a reliable method for food samples. The assay principle was based on the formation of flavonoid-aluminium chloride complex where the hydroxyl and keto groups in flavonoids react with aluminium chloride forming a yellow complex. The intensity of yellow colour is directly proportional to the flavonoid concentration and can be measured spectrophotometrically. A standard curve of quercetin was used to quantify TFC expressed as mg Quercetin Equivalent per gram of sample (mg QE/g). Using the developed meat tenderizers significantly increase the level of TFC especially higher when using the traditional recipe

Based on the results, the four recipes of meat tenderizers were found to contain both TPC and TFC, indicating presence of bioactive compounds. These compounds are known for their potential antioxidant properties, which contribute to the functional and preservative qualities of the meat tenderizer. The quantification of TPC and TFC provided insight into the phytochemical composition of the pastes, which influences their overall effectiveness and stability.

In addition, it was displayed that all four recipes of the meat tenderizers on their own contain high levels of SOD and CAT. The traditional, fusion and josapine recipe showed significant increased in SOD activity while MD2 did not have any significant

difference in SOD activity. On the other hand, all four meat tenderizers show significant increase in CAT and GSH levels. However upon marinating meat samples and testing SOD and CAT content of the meat samples, it was found the content were significantly lower than the meat tenderizers themselves. This is due to several limiting factors. Firstly, antioxidants within the meat tenderizer primarily only remain on the surface of the meat rather than penetrating inside due to their solubility and molecular size (Abderrahim, 2015). Secondly, high temperatures during the cooking process may also cause degradation of heat sensitive antioxidants such as vitamin C and certain polyphenols. Additionally, the marinated meat samples release juices during the cooking process. These lost juices contain water soluble compounds which include antioxidants and results in a significant reduction of antioxidant content. Conversely, the antioxidant contents of the meat tenderizers are also bound to the reactive oxidative agents of the meat. These bound antioxidants have limited bioavailability after the cooking process that made antioxidant detection by assay resulted in less antioxidant content in the end.

While the herbs and spices like turmeric or garlic are known to have high levels of antioxidants (Latif et al., 2021). They are normally used in small quantities in food and mixed with other ingredients as well, which may lead to small amounts of functional antioxidants. Cooking processes also causes increment in TPC by and antioxidant capacities of food pastes (Hanis Mastura et al., 2017). This is in line with a study by Vidinamo et al. (2022) who proved cooking process prevents loss of phenolic acids resulting to an increase of total phenolic content and stronger antioxidant activity. The leaves of the jackfruit plant also contains the highest phenol content present as proven by Ilmi et al. (2020) using ethanol leaf extracts. Addition of herbs and spices also contribute to antioxidant activity as they contain sulphur containing compounds, phenolic, flavonoids and polyphenols especially clove and rosemary that have high phenolic compound content (Hanis Mastura et al., 2017; Jiang, 2019). There are significant correlations between antioxidant activities, total phenolic and flavonoids contents as evident by Donkor (2015) that contribute important health benefits to consumers.

5.5 Shelf Life Analysis

All of the meat tenderizer samples were deteriorated by day 20 of room temperature storage with the presence of microorganisms growing on the meat tenderizers indicating it is no longer safe for consumption. These plant-based components, while effective for meat tenderization due to their rich proteolytic enzyme profiles, are prone to microbial degradation, enzymatic browning, and chemical instability over time. Studies have shown that natural extracts with enzymatic activity tend to lose efficacy and safety due to microbial spoilage, pH shifts, and oxidation reactions unless properly preserved (Lisboa et al., 2024). For instance, bromelain is known to degrade rapidly at room temperature, especially in aqueous formulations, which significantly reduces the functionality and shelf stability of pineapple-based pastes (Das & Bhattacharyya, 2017). Similarly, jackfruit leaves, which contain chlorophyll, polyphenols, and other phytochemicals, are susceptible to oxidation and microbial proliferation, particularly in non-acidified environments.

This deterioration is due to several factors. First of all, the moisture content of each product is relatively high, ranging from 39.2% to 53.8% on the first day of the meat tenderizer development. The moisture and room temperature conditions were optimal for microbial growth which lead to perceivable microbial growth on the 20th day. Nevertheless, no additional water was added during development of meat tenderizer pastes to ensure less moisture content of the final product with vegetable oil being used as a substitute for moisture to allow the combination of ingredients to be processed together. Regardless, all the detected moisture came from the tenderizer ingredients itself such as onions, garlic and pineapple which normally contains high moisture content on their own. Ingredients such as pineapples contain up to 85-92% moisture content which leads to high moisture content of the end product (Vidinamo et al., 2022). Inadequate conditions such as optimal microbial temperatures 25-30°C or relatively high humidity of 40-60% are key factors that accelerates food deterioration (Immaculate et al., 2015; Kusumaningrum et al., 2015; Mat Sharif et al., 2017; Corradini, 2018; Sharif et al., 2018).

The pH of each recipe ranged from 4.5 to 5. Most bacteria thrive in neutral to slightly acidic pH ranging from 6 to 7.5 which made the pH of the meat tenderizers ideal for inhibiting microbial growth without producing strong acidic flavours. However, based on observation of the meat tenderizers, all the pastes that are kept in

room temperature were spoiled and unsafe to consume after 20 days with the presence of fungal growth and significant drop in pH. This is due to the pH value being low enough to inhibit bacterial growth but still within optimal temperature for fungal growth which is 4 to 7. The significant drop of pH was due to the breakdown of organic compounds from the fungus creating acidic by-products.

Validated methods for determining shelf life of such natural pastes typically involve monitoring microbial growth, pH changes, and enzyme activity over time under controlled storage conditions. Accelerated shelf-life testing (ASLT), combined with periodic analysis of microbial load (total plate count, yeast and mould counts), and biochemical assessments such as enzyme residual activity and lipid oxidation are widely accepted for predicting degradation kinetics (Chiarelli et al., 2024). In comparative studies, enzyme-rich plant pastes without preservatives showed spoilage within 5 to 7 days at refrigeration temperatures (4°C), primarily due to bacterial growth and the loss of protease activity. In the current context, jackfruit leaf–pineapple pastes stored under refrigeration showed visible microbial growth and a substantial decline in pH after one week, accompanied by a notable drop in tenderizing efficacy, indicating enzymatic denaturation and microbial instability. These observations align with prior findings that natural bio compounds often require encapsulation, pH adjustment, or natural antimicrobial additives to extend their usability (Ravindran & Jaiswal, 2016).

Moreover, a study by Mat Sharif et al. (2017) proved the shelf life can be improved by adding chemical preservative such as sodium benzoate without jeopardizing the taste of a product. However, this contradicts with the purpose of developing a chemical-free meat tenderizer. Use of herbs and spices were supposed to help extend the shelf life of the developed meat tenderizer meat tenderizers but only high concentrations able to inhibit microbial growth (Martínez-Graciá et al., 2015). This is evident in a study by Singh et al. (2015) that powdered clove can be used as an effective antimicrobial to prolong storage period in chilled temperature. A study by Mills et al. (2015) also demonstrated vacuum packing chilled food items can extend its shelf life beyond the recognised time of 70 days due to bacterial species having poor adaptability to grow under anaerobic conditions. Thus, while natural meat tenderizers offer desirable functional and clean-label benefits, their limited shelf life remains a critical constraint. Future work should focus on enhancing stability through mild preservation technologies such as vacuum packaging, incorporation of edible coatings,

or natural antimicrobial compounds like essential oils to prolong their effectiveness and ensure safety during storage.

5.6 Nutrient Analysis

The proximate analysis results revealed significant variations of proximate composition of the four meat tenderizers. When compared between the two methods. The differences in the nutrient content of each parameter between the samples were observed minimal. The difference of water content using the two methods was at most 2.35g, for energy was 18.11kcal, for protein the difference at most was 0.87g, for carbohydrates was 3.69 and for ash the difference at most was 0.42g. These minimal differences indicate both methods provide reliable results.

The water content was the highest in the MD2 recipe indicating the highest moisture followed by josapine recipe. The traditional and fusion recipes had low water content. This is due to the two recipes containing pineapples which had high water content up to 85-92% (Vidinamo et al., 2022). This also resulted in the two recipes; josapine and MD2 to be a bit more watery as compared to traditional and fusion recipe which were grainy and drier. Nonetheless, the difference on moisture did not affect the effectiveness in meat tenderizing and the difficulty to smear the product on raw meat.

Moreover, the energy and fat values of each meat tenderizer also varied with the fusion recipe containing the highest calories and fat values. The high energy value is primarily due to the elevated content of fats which contributed to its total energy value. The relatively higher fat content observed in the formulation may be attributed to the incorporation of rosemary and thyme, which are known to contain lipid-soluble essential oils. Mechanical processing such as grinding can facilitate the release of these oil fractions, which may subsequently be co-extracted during crude fat analysis, thereby contributing to the overall fat content of the product (Khademi Doozakhdarreh et al., 2022). In contrast, the lowest calorific value among the four recipes belong to the MD2 recipe. This is due to the addition of pineapples which contributed a lot of water and moisture. The addition of water contributed to the total volume and weight of the paste which leads to a decrease of energy value and fat when compared per 100g of weight with other recipes.

The protein content across all four meat tenderizers remains relatively consistent, ranging from 2.8g to 3.6g per 100g with traditional recipe containing highest

amount of protein. However, the amount of protein in all of the meat tenderizers were low to remain relevant for consumption. Nevertheless, the amount of carbohydrates varied widely, with traditional recipe having the highest amount per 100g. This is because all four recipes contain the same amount of sugar despite all of them having different volumes at the end of their formulations. Since traditional recipe has the least total volume, it has the most sugar to weight ratio leading to the highest amount of carbohydrates per 100g. On the other hand, MD2 had the least amount of carbohydrates even when the recipe contain pineapples. This is because the addition of pineapples increases the amount of water and weight of the end product significantly. This results in the end product the highest weight and having the least sugar to weight ratio since the sugar amount remains the same regardless of total weight of all the recipes.

A study by Amadi et al. (2018) revealed jackfruit leaves contain significantly high amounts of fibre and ash, which can also be seen in this study. Pineapples also contain considerable amount of nutrients such as essential vitamins and dietary fibre (Mohd Ali et al., 2020). The four developed meat tenderizer recipes had moderate amounts of fibre and can reach up to 5.67g of fibre per 100g weight. However only small amount of these pastes were used for meat marination before cooking. Thus the pastes themselves will not be directly consumed and the benefits of fibre will be lost.

5.7 Sensory Evaluation

Sensory evaluation is an evaluation including various parameters in determining public acceptance towards a development of a new product. A higher score on a given parameter given by consumers during the sensory evaluation test indicates that a product is acceptable in terms of that parameter. These parameters include colour, appearance, taste, texture and overall acceptance. If a product has relatively low scores given by consumers, the product simply needs improvements before it is ready to be commercialized.

Each of the meat samples marinated have significantly ($p < 0.05$) higher mean scores of colour, appearance, taste and overall acceptance compared to control meat. The tenderizing action, primarily driven by bromelain from pineapples and protease-like compounds potentially present in jackfruit leaves, contributed to the breakdown of muscle fibres and connective tissue, resulting in a notably softer texture as perceived by panellists. This is due to the additions of a mixture of ingredients, including proteolytic

enzymes from jackfruit leaves and/or pineapples that have the ability to separate proteins and amino acids by breaking peptide bonds which tenderizes the texture of meat making them softer and multiple herbs and spices giving an attractive colour and improved flavour (Hanis Mastura et al., 2017; Jiang, 2019; Rani et al., 2022; Razali et al., 2023; Mohd Azie & Nurufairah, 2024).

The presence of pineapples also improves colour on cooked meat samples. The presence of sugars from the pineapples promote the Maillard reaction and gave the meat more browning color during cooking process as compared to untreated meat samples which turned mostly grey. This evidence align with a study by Omar et al. (2018) that proved the addition of pineapples shows significant difference with ($p < 0.05$) for colour value which showed improvement of colour of meat as compared to untreated samples. This finding complements with a study by Ismail et al. (2018) and Ramli et al. (2021) who also did a sensory evaluation and found positive results on meat marinated in plant proteases at a given time and at room temperature to have the highest sensory acceptance due to the enzymes having maximal activity in optimal temperature. This is also in line with a study by Omar et al. (2018), Manohar et al. (2016), (Roslan et al., 2019) and Susilowati et al. (2022) showing the use of bromelain to degrade collagen fibres of meat, decreasing the pH slightly improving the taste by having extra acidity and improve water holding capacity which means the meat retains moisture better during the cooking process. Higher water holding capacity of meat results in juicier meat after cooking process and higher cooking yield while retaining its softer texture (Omar et al., 2018).

These findings are also consistent with previous studies where natural enzymes like bromelain, papain, and ficin were used to improve meat palatability, often yielding favourable consumer responses (Mohd Azmi et al., 2023). According to validated sensory evaluation methods outlined by the International Organization for Standardization (ISO 13299:2016), sensory testing requires consistent panellist training, controlled conditions, and appropriate scaling methods to ensure reliability and repeatability. In the present study, these standards were adhered to, supporting the credibility of the outcomes. Supporting evidence from a study by Mohd Azmi et al. (2023) demonstrated that enzymatically treated meat exhibited improved textural and sensory qualities, reinforcing the current findings. Additionally, tenderized meat samples retained better moisture, which enhances perceived juiciness, a trait closely linked to consumer preference. In another investigation by Shin et al. (2019), bromelain-

treated meat samples showed higher sensory ratings for tenderness and flavour due to efficient enzymatic hydrolysis of tough meat proteins, which mirrors the results obtained in the present study. Notably, the jackfruit leaf component not only enhanced tenderness but also contributed a mild, herbal note to the meat, which was well-received by the panellists and did not mask the natural meat flavour.

However, these results contradict with a study by Rashid et al. (2024) that claimed addition of proteases significantly lowers water holding capacity and cooking yield. Nevertheless, consumers still scored protease treated meat higher in sensory evaluation due to the preference of tenderness outweigh loss of moisture. The result showing the recipe containing josapine has an average softer texture score as compared to MD2 variant is also in line with a study by Lim (2018) proving that bromelain from josapine have the stronger proteolytic ability than MD2.

A study revealed the use of additional flavourings and colourings within a product can further improve acceptability of a product (Al-Nabulsi et al., 2015). Furthermore, texture is also an important parameter to take into account among consumers especially elderly consumers who dislike meat textures that are hard and overly chewy (Aung et al., 2022). Achieving a good score in texture of the meat tenderizer paste can ensure acceptability and can also promote elderly adults to consume more proteins. Overall, the combination of jackfruit leaves and pineapples resulted in meat products with better consumer acceptability than untreated controls, supporting the potential for natural tenderizers to enhance both functional and sensory meat quality. In summary, the best formulation is the MD2 recipe for having the best average scores in the sensory evaluation with high scores in all parameters.

CHAPTER 6

CONCLUSION

6.1 Conclusion

As a conclusion, the present study successfully developed natural meat tenderizer pastes formulated from jackfruit leaves in combination with pineapples and selected spices. The findings demonstrate that the formulated tenderizers possess effective proteolytic activity, contributing to a significant reduction in meat hardness and improvement in tenderness compared to untreated samples. This confirms the potential of plant-based enzymatic sources as viable alternatives to commercial or synthetic tenderizers.

The developed formulations also exhibited measurable antioxidant activity, indicating the presence of bioactive compounds that may contribute to product stability and additional health-related benefits. Shelf life evaluation through periodic pH and moisture content analysis revealed that storage temperature plays a crucial role in maintaining product quality. Samples stored under refrigerated conditions showed greater physicochemical stability compared to those kept at room temperature, suggesting that chilled storage effectively extends the product's shelf life.

Furthermore, nutrient analysis confirmed that the developed tenderizers contain identifiable nutritional components suitable for nutritional labelling, enhancing their potential for commercialization. Sensory evaluation results indicated general consumer acceptability in terms of tenderness, flavour, aroma, juiciness, and overall preference, supporting the practical application of the developed pastes in meat preparation.

Overall, this study demonstrates that jackfruit leaf–pineapple-based meat tenderizer pastes offer a natural, functional, and consumer-acceptable alternative to excessive salt usage, synthetic chemicals, and animal-derived tenderizing agents, while also reducing the need for prolonged cooking times to achieve desirable meat tenderness.

6.2 Recommendation

For further research, different packaging methods should be tested in order to improve their shelf life. As known, these products can be stored in the chiller but for commercial availability, it is much more convenient if these products can be sold in shelves of market places rather than being in the chiller sections. Existing commercial meat tenderizers are already being sold in room temperature on the shelves of market places and are also sold online making them more convenient since they can last long in room temperature. As a recommendation, retort packaging may be introduced to these new meat tenderizer pastes to ensure the products are commercially sterile significantly reducing microbial activity and tested for shelf life.

Future studies should also focus on a detailed quantitative analysis of bromelain from pineapple and other proteolytic enzymes present in jackfruit leaves that contribute to meat tenderization. Enzyme extraction, purification, and activity assays are recommended to determine precise protease concentration, enzymatic activity, and their effects on muscle proteins such as collagen and myofibrillar structures. Further research may also include enzyme kinetics, stability under different pH and temperature conditions, extended microbial shelf life evaluation, and larger-scale sensory assessments to enhance product optimization and support potential commercialization of the developed natural meat tenderizers.

6.3 Limitation

One of the limitations of this study was the limited accessibility to a texture analyser within the Faculty of Health Sciences. The instrument required for objective measurement of meat hardness was only available at the Faculty of Pharmacy, Puncak Alam. Consequently, arrangements had to be made to utilize their facility, which involved scheduling constraints and payment per sample analysis which has slowed the study progress and increased the study budget. This external dependency affected the workflow and timeline of the research, as sample testing had to be conducted according to equipment availability rather than immediate experimental needs.

In addition, the cost incurred per sample limited the number of meat samples that could be analysed, although all measurements to ensure reliability. The financial and logistical considerations therefore restricted the overall sample size for texture

analysis, which may affect the breadth of data obtained. If the equipment were readily accessible within the faculty, a larger number of samples could have been tested with greater flexibility, potentially enhancing data precision, reducing cost burden, and improving the efficiency of the research process.

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APPENDICES

APPENDIX 1

Letter Ethical Clearance of Study

www.irmi.uim.edu.my

Institut Pengurusan Penyelidikan & Inovasi
Institute of Research Management & Innovation

Universiti Teknologi MARA
Jalan 1, Puncak Alam, Selangor
42300 Puncak Alam, Selangor, MALAYSIA
Tel: (603) 7511 2011 Fax: (603) 7511 2019
E-mail: irm@uitm.edu.my

Reference : 600-IRM (5/1/6)
Our reference : REC/236/17
Date : 5 July 2017

Mr. Razif Dasimon
Faculty of Health Sciences
Universiti Teknologi MARA
Puncak Alam Campus
42300 Puncak Alam
Selangor

Dear Mr. Razif,

ETHICS APPROVAL BY UITM RESEARCH ETHICS COMMITTEE

Title: Development of Meat Tenderizer by Using Combination of Artocarpus Heterophyllus Leaf with Selected Herbs.

Trial Site: UITM Shah Alam Campus & Puncak Alam Campus

Thank you for your research ethics application and presentation on 20 June 2017. We would like to inform that the UITM Research Ethics Committee had deliberated your proposal.

It is our pleasure to inform you that the Research Ethics Committee has agreed to grant an ethics approval for the said study.

Please submit progress report of the study to the REC Secretariat 12 months after the date of approval letter and annually until the study has completed. Please kindly notify the REC for any amendments to the relevant documents for this study. Notification should be made to the REC for any protocol deviation and in case of serious adverse event, please notify the REC within 48 hours from the time known. A summary of the final report should be submitted at the end of your study.

The UITM Research Ethics Committee operates in accordance to the ICH Good Clinical Practice Guidelines, Malaysia Good Clinical Practice Guidelines and the Declaration of Helsinki.

Attached is the list of members of the Committee.

Thank you.

Yours truly,

PROFESSOR DR HADARIAH BAHRON
Assistant Vice Chancellor (Research & Innovation)
Chairman of UITM Research Ethics Committee

Research with Integrity & Accountability

RMC
Research Management Committee
Chairman: Professor Dr. Hadariah Bahron

RIBU
Research Innovation & Business Unit
Chairman: Professor Dr. Hadariah Bahron

APPENDIX 2

Consent Form for Sensory Evaluation

Consent Form

To become a subject in the research, you or your legal guardian is advised to sign this Consent Form.

I herewith confirm that I have met the requirement of age and am capable of acting on behalf of myself/* as a legal guardian as follows:

1. I understand the nature and scope of the research being undertaken.
2. I have read and understood all the terms and conditions of my participation in the research.
3. All my questions relating to this research and my participation therein have been answered to my satisfaction.
4. I voluntarily agree to take part in this research, to follow the study procedures and to provide all necessary information to the investigators as required.
5. I may at any time choose to withdraw from this research without giving reasons.
6. I have received a copy of the Subjects Information Sheet and Consent Form.
7. Except for damages resulting from negligent or malicious conduct of the researcher(s), I hereby release and discharge UiTM and all participating researchers from all liability associated with, arising out of, or related to my participation and agree to hold them harmless from any harm or loss that may be incurred by me due to my participation in the research.

Name of Subject/Legal Guardian

Signature

I.C. No

Date

Name of Witness

Signature

I.C. No

Date

Name of Consent Taker

Signature

I.C. No

Date

APPENDIX 3

Sensory Evaluation Form for Participants

Sensory Evaluation Form

Directions: Check (☑) one rating for each of the following: Appearance, Taste, Texture, Aroma and Overall Acceptability

Rating Scale:-

1. Dislike Extremely
2. Dislike Very Much
3. Dislike Moderately
4. Dislike Slightly
5. Neither Like or Dislike
6. Like Slightly
7. Like Moderately
8. Like Very Much
9. Like Extremely

Recipe A

	1	2	3	4	5	6	7	8	9
Appearance									
Taste									
Texture									
Aroma									
Overall Acceptability									

Recipe B

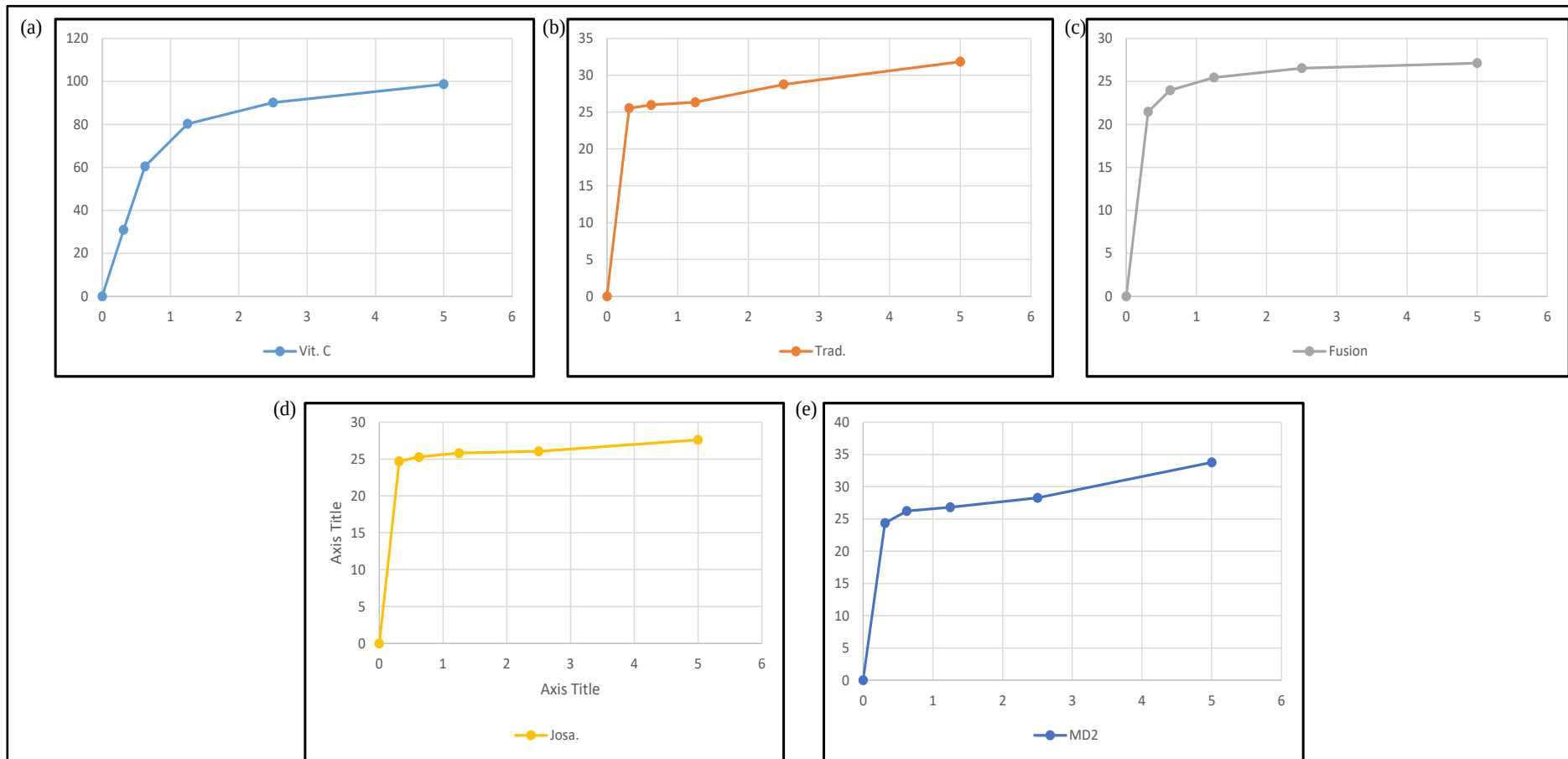
	1	2	3	4	5	6	7	8	9
Appearance									
Taste									
Texture									
Aroma									
Overall Acceptability									

Recipe C

	1	2	3	4	5	6	7	8	9
Appearance									
Taste									
Texture									
Aroma									
Overall Acceptability									

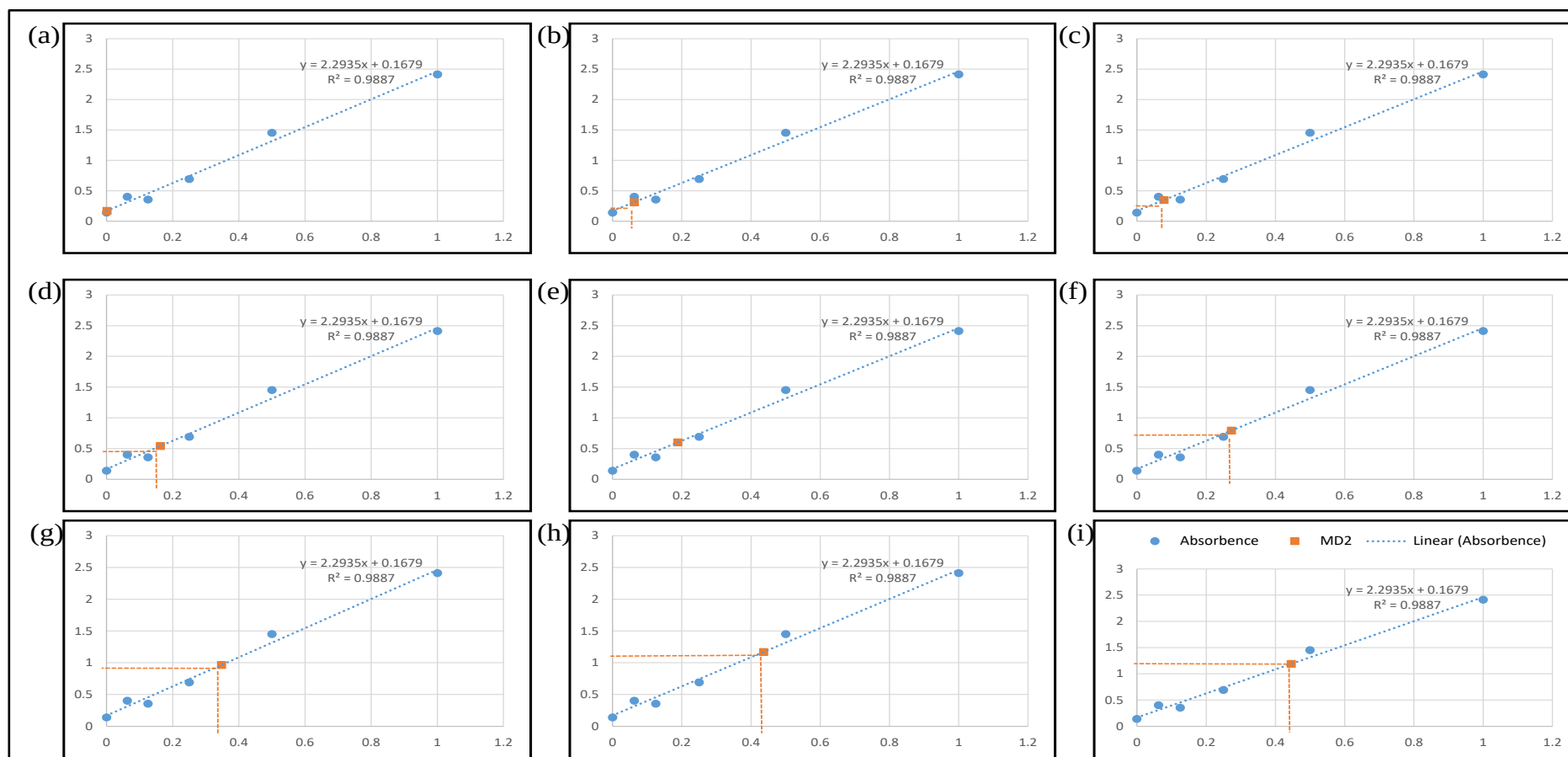
APPENDIX 4

Antioxidant Activity of Vitamin C and the Four Recipes Meat



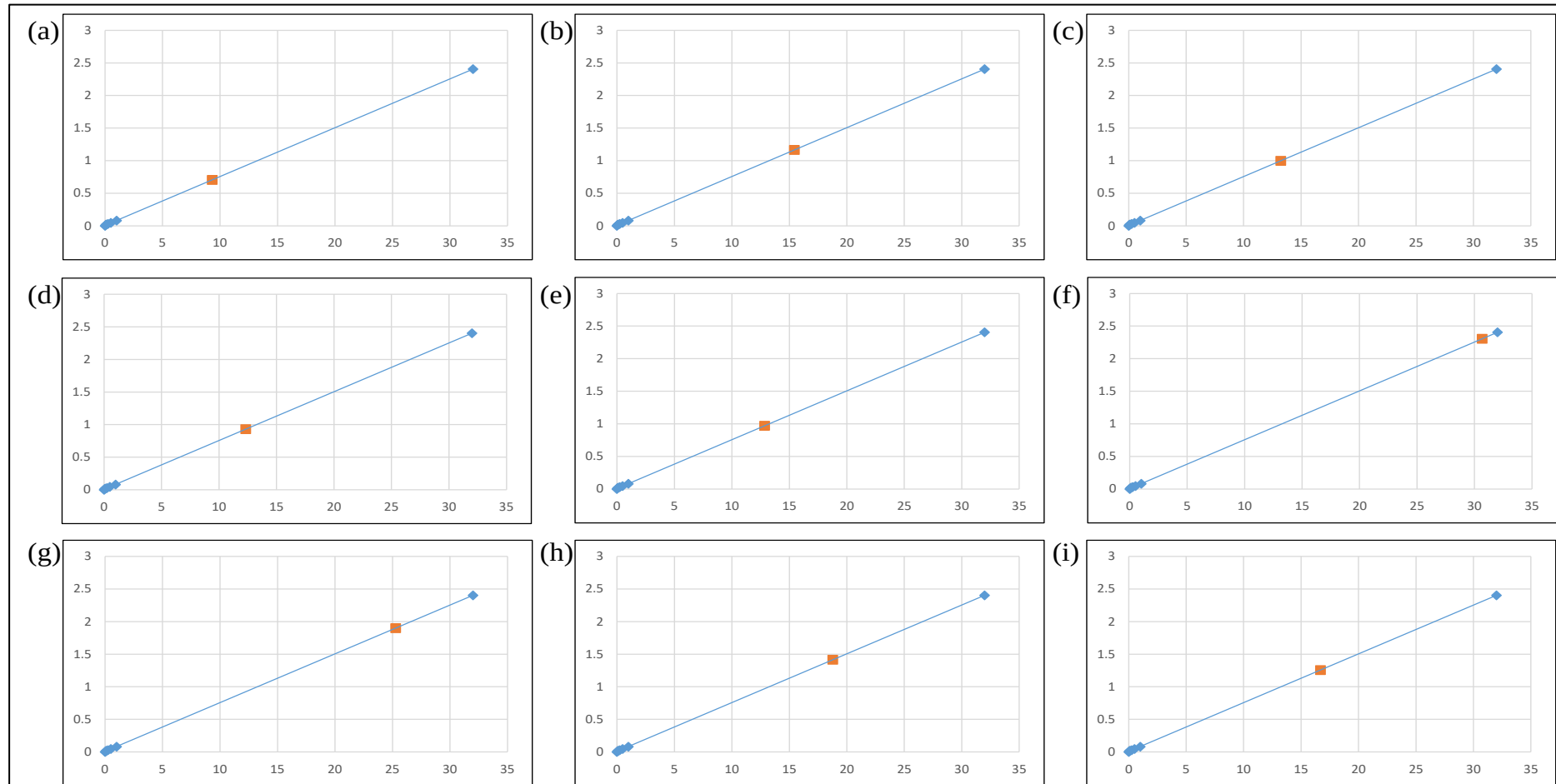
APPENDIX 5

Total Phenolic Content of Meat Treated With Meat Tenderizer and the Meat



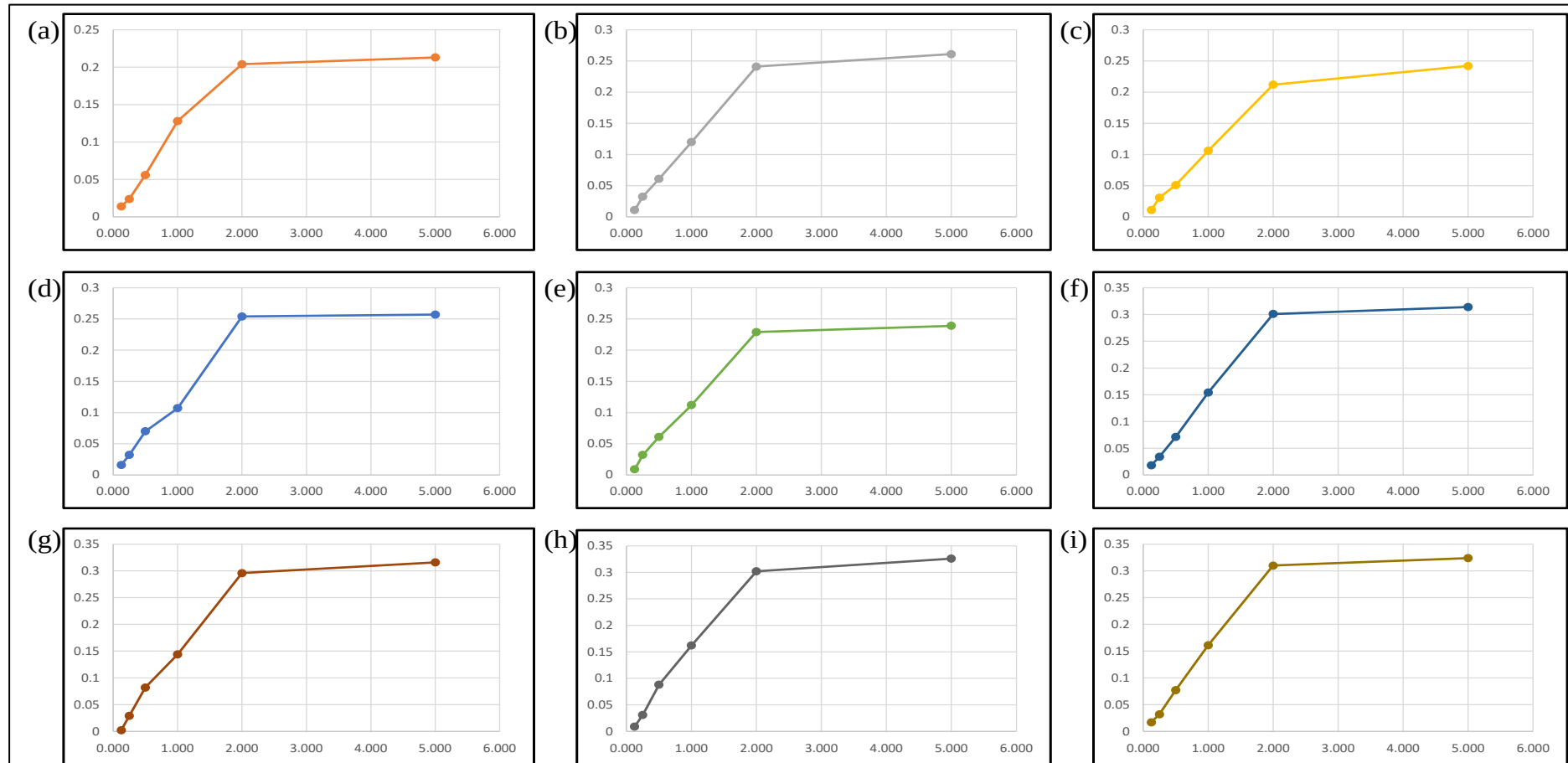
APPENDIX 6

Polyphenolic Content via TFC Analysis Of Meat Treated with of Meat Tenderizers and the Meat Tenderizers



APPENDIX 7

Spectrophotometer Reading of Meat Treated with the Meat Tenderizers and the Meat Tenderizers



APPENDIX 8

Pictures

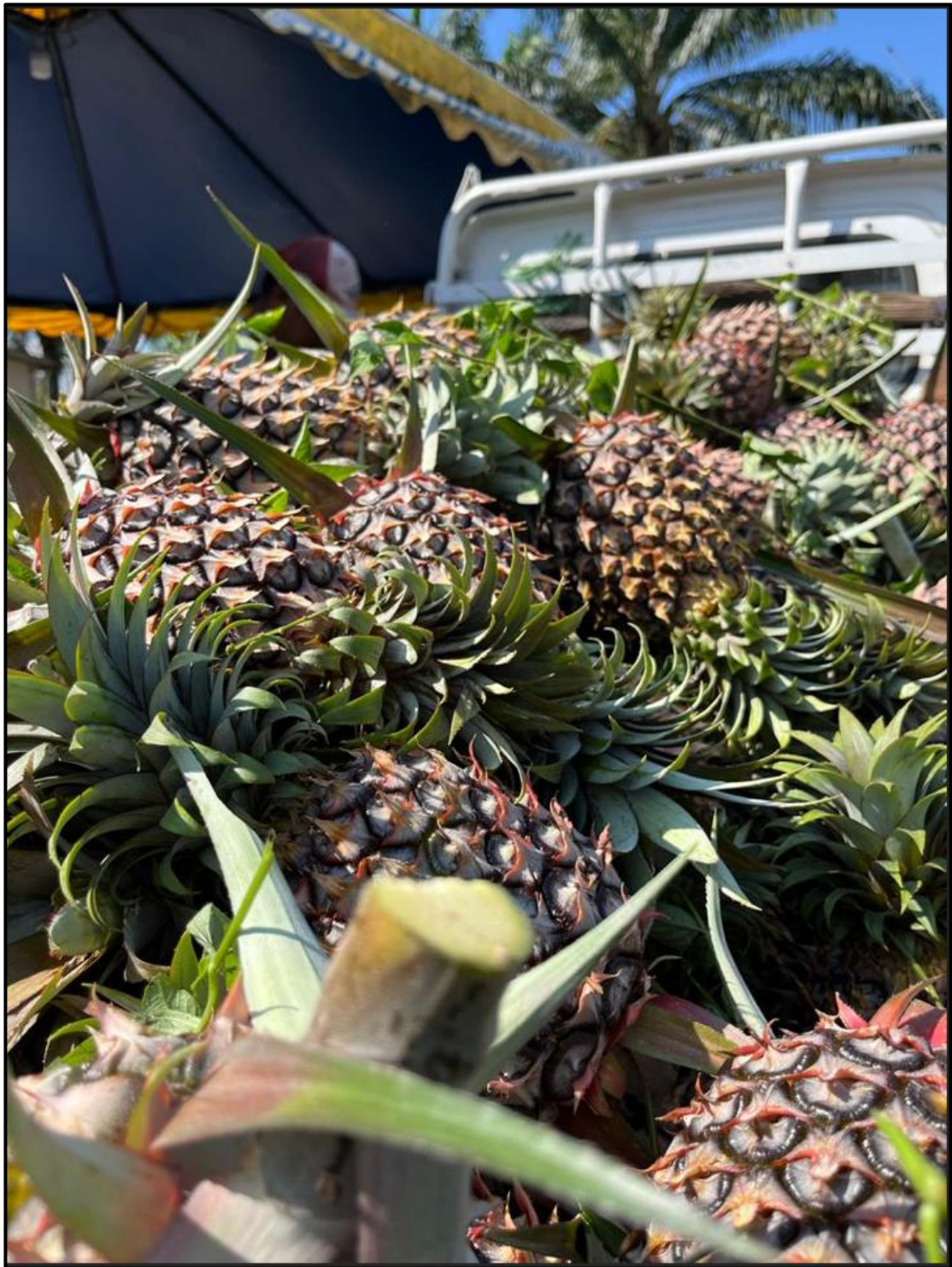
A. Jackfruit leaves



B. Drying process of jackfruit leaves



C. Pineapples samples



D. Sample storage for shelf life evaluation



E. pH testing of samples for shelf life evaluation



AUTHOR'S PROFILE



Muhammad Arif Fadluddin Bin Md Dzahir is a dedicated and motivated graduate with a strong academic background in nutrition and dietetics, particularly in the field of food innovation and product development. He has a degree in Dietetics (2021) and a diploma in Pharmacy (2017). He has demonstrated a keen interest in natural food applications through his research on natural meat tenderizers formulated from plant-based ingredients such as jackfruit leaves, pineapple, herbs, and spices. His work emphasizes the development of healthier and more sustainable food alternatives while focusing on nutritional value, antioxidant properties, and consumer acceptance. Known for his commitment to academic excellence, analytical skills, and passion for research, Muhammad Arif Fadluddin continues to expand his knowledge and contribute to advancements in food science and nutrition. He has also published “Consumer’s Preferences between Natural MD2-Based and Commercial Meat Tenderizers” in Environment-Behaviour Proceeding Journal, E-BPJ 10(33), Sep (2025) and joined conferences including International Medical Laboratory and Health Sciences Symposium (IMLHS 2024), The 2nd SEAMEO International Conference on Food and Nutrition (ICFN) and 13th Asia-Pacific International Conference on Environment-Behaviour Studies.