PRESERVATIVE EFFECTS OF MEDIUM-CHAIN DICARBOXYLIC ACIDS ON MICROBIAL CONTROL AND QUALITY OF BEEF AND SALMON DURING REFRIGERATED STORAGE

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LIST OF SYMBOLS

a_w	Water activity
pKa	Unique dissociation constant

LIST OF ABBREVIATIONS

ADA	Adipic Acid
AZA	Azelaic Acid
DAs	Dicarboxylic Acids
FDA	Food and Drug Administration
FIC	Fractional Inhibitory Concentration
FRAP	Ferric Reducing Antioxidant Power
GLA	Glutaric Acid
GMP	Institut Pengajian Siswazah
GRAS	Generally Recognised as Safe
LAB	Lactic Acid Bacteria
MBC	Minimum Bactericidal Concentration
MCDAs	Medium-Chain Dicarboxylic Acids
MIC	Minimal Inhibitory Concentration
MFC	Minimal Fungicidal Concentration
OAs	Organic Acids
PA	Pimelic Acid
SUA	Succinic Acid
SUBA	Suberic Acid
TVB-N	Total Volatile Base Nitrogen

LIST OF APPENDICES

KESAN PENGAWETAN ASID DIKARBOKSILIK RANTAI SEDERHANA TERHADAP KAWALAN MIKROB DAN KUALITI DAGING LEMBU SERTA IKAN SALMON SEMASA PENYIMPANAN SEJUK

ABSTRAK

Kajian ini meneroka potensi asid glutarik (GLA), asid suksinik (SUA), dan asid azelaik (AZA) sebagai pengawet semula jadi untuk produk daging, dengan menekankan kestabilan fizikokimia dan sifat antimikrobnya. Fasa 1 menilai aktiviti antimikrob (kaedah mikropencairan kaldu) dan *in vitro* aktiviti antioksidan (ujian perencatan radikal bebas DPPH, daya antioksidan penurunan ferik, dan ujian perencatan nitrit) bagi setiap asid dikarboksilik rantai sederhana (MCDA), serta memilih kepekatan paling berkesan untuk pengawetan daging lembu. Walaupun sifat antioksidannya kurang daripada 50% berbanding asid askorbik (AsA), MCDAs menunjukkan kesan bakteriostatik yang kuat, dengan kepekatan perencatan minimum (MIC) dan kepekatan bakterisid minimum (MBC) antara 500–2,000 μg/mL terhadap *Listeria monocytogenes*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Salmonella enterica*, dan *Escherichia coli*. Dalam model daging lembu, kepekatan MCDA yang lebih tinggi (2,000 μg/mL dan 1,456 μg/g) membantu mengekalkan warna, pH, dan kelembapan serta mengurangkan penanda kerosakan seperti jumlah nitrogen bes meruap (TVB-N) (penurunan 8–20 mg/100 g) dan kiraan mikrob. AZA adalah yang paling berkesan, mengatasi prestasi natrium bisulfit (SoB). Fasa 2 menilai kesan antimikrob gabungan GLA, AZA, dan SUA menggunakan ujian papan dam, yang membawa kepada pemilihan nisbah optimum (GLA-AZA 2:1, GLA-SUA 1:1, dan AZA-SUA 2:1, dengan nilai kepekatan perencatan pecahan (∑FIC) antara 0.039–0.5) untuk pengawetan daging lembu.

Gabungan ini menunjukkan aktiviti bakteriostatik yang dipertingkatkan, dengan pengurangan TVB-N sebanyak 2–7 mg/100 g. AZA-SUA (2:1) adalah yang paling berkesan, memberikan panduan lanjut untuk pengawetan salmon. Fasa 3 mengkaji kesan antimikrob MCDAs terhadap *Bacillus cereus*, *Streptococcus pyogenes*, dan *Candida albicans*. Berdasarkan keputusan ini dan dapatan Fasa 1, kepekatan terpilih digunakan dalam pengawetan salmon. MCDAs pada 2,000 μg/mL dan 1,456 μg/g berjaya mengekalkan kualiti salmon dengan pengurangan TVB-N sebanyak 15–24 mg/100 g. AZA sekali lagi lebih berkesan berbanding SoB. Fasa 4 menilai gabungan MCDA untuk pengawetan salmon, dengan memilih GLA-AZA 2:1, GLA-SUA 1:1, dan AZA-SUA 2:1 (nilai ∑FIC antara 0.0625–0.375). Gabungan ini mengurangkan penanda kerosakan dengan ketara (penurunan TVB-N sebanyak 11–20 mg/100 g) serta menurunkan kiraan mikrob. AZA-SUA (2:1) sekali lagi terbukti paling berkesan. Dengan asal semula jadi, sifat antimikrob yang kuat, dan kelebihan keselamatan, MCDAs ini menunjukkan potensi sebagai bahan pengawet daging. Walaupun terdapat cabaran dari segi kos, manfaat yang ditawarkan menunjukkan prospek yang menjanjikan dalam pemeliharaan makanan pada masa hadapan.

PRESERVATIVE EFFECTS OF MEDIUM-CHAIN DICARBOXYLIC ACIDS ON MICROBIAL CONTROL AND QUALITY OF BEEF AND SALMON DURING REFRIGERATED STORAGE

ABSTRACT

This study explores the potential of glutaric acid (GLA), succinic acid (SUA), and azelaic acid (AZA) as natural preservatives for meat products, highlighting their physicochemical stability and antimicrobial properties. Phase 1 evaluated the antimicrobial (microdilution broth method) and *in vitro* antioxidant activities (DPPH free radical scavenging, ferric reducing antioxidant power, and nitrite scavenging assays) of individual medium-chain dicarboxylic acids (MCDAs), selecting the most effective concentrations for beef preservation. Although their antioxidant properties were less than 50% of ascorbic acid (AsA), they demonstrated strong bacteriostatic effects, with minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values ranging from 500–2,000 µg/mL against *Listeria monocytogenes*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Escherichia coli*. In beef models, higher MCDA concentrations (2,000 µg/mL and 1,456 µg/g) helped maintain colour, pH, and moisture while reducing spoilage indicators such as total volatile base nitrogen (TVB-N) (8–20 mg/100 g decrease) and microbial counts. AZA was the most effective, outperforming sodium bisulphite (SoB). Phase 2 assessed the combined antimicrobial effects of GLA, AZA, and SUA using checkerboard tests, leading to the selection of optimal ratios (GLA-AZA 2:1, GLA-SUA 1:1, and AZA-SUA 2:1, with fractional inhibitory concentration (∑FIC) values of 0.039–0.5) for beef preservation. These combinations demonstrated enhanced bacteriostatic activity,

reducing TVB-N by 2–7 mg/100 g. AZA-SUA (2:1) was the most effective, offering insights for salmon preservation. Phase 3 examined the antimicrobial effects of MCDAs on *Bacillus cereus*, *Streptococcus pyogenes*, and *Candida albicans*. Based on these and Phase 1 results, selected concentrations were applied to salmon preservation. MCDAs at 2,000 μg/mL and 1,456 μg/g effectively maintained salmon quality, reducing TVB-N by 15–24 mg/100g. AZA again outperformed SoB. Phase 4 evaluated MCDA combinations for salmon preservation, selecting GLA-AZA 2:1, GLA-SUA 1:1, and AZA-SUA 2:1 (∑FIC values 0.0625–0.375). These combinations significantly reduced spoilage markers (TVB-N decrease of 11–20 mg/100 g) and microbial counts. AZA-SUA (2:1) was the most effective. With natural origins, strong antimicrobial properties, and safety advantages, these MCDAs show promise as meat preservatives. Despite cost challenges, their potential benefits indicate a promising future for food preservation.

CHAPTER 1

INTRODUCTION

1.1 Research background

Meat is a good source of micronutrients, such as zinc, vitamin B_{12}, iron, phosphorus and proteins, which are very sensitive to oxidative degradation and microbial contamination (Ndlovu, 2010). Oxidation, especially in lipids and microbial growth, leads to deterioration in meat's nutritional quality, flavour, texture, colour and storage time (Dave & Ghaly, 2011). Antioxidants and antimicrobials are commonly used to preserve meat and meat products, and their functions often overlap. Some antioxidants can have antimicrobial properties, while some antimicrobials can also act as antioxidants, making them versatile in extending the shelf life and maintaining the quality of meat products (Martelli & Giacomini, 2018).

Sorbic acid, benzoic acid, sodium bisulphite (SoB), and nitrites are widely used synthetic preservatives in the meat industry due to their effectiveness, low cost, and minimal impact on taste, flavour, colour, and texture. Despite these benefits, there are growing concerns among consumers about the potential health risks associated with synthetic preservatives (Yu et al., 2021). Some studies have linked these substances to various health issues, such as allergic reactions, asthma, and even a potential increase in cancer risk with prolonged exposure to nitrites and nitrosamines (Valencia, 2023; Yu et al., 2021). To reassure consumers that food additives were safe for consumption, the E number system was introduced in the 1960s. However, misleading claims in some publications raised concerns about the safety of these compounds, leading to skepticism among consumers. Besides, as life expectancy increased, so did awareness of overall well-being, driving a greater demand for healthier food choices. The well-established link between nutrition and

health further reinforced this shift in consumer behaviour. One notable trend associated with this perspective is the rise of "clean labels," which emphasize natural attributes such as "free-range," "less processed," "organic," or "biological" foods (Faustino et al., 2019). This reflects a growing preference for products perceived as free from synthetic chemicals, making "natural" ingredients a key selling point in the food industry. In response, the industry is increasingly focusing on innovative alternatives that provide the same technological benefits as conventional additives but are perceived more favourably by consumers. One promising approach involves utilising bioactive compounds derived from natural sources. In particular, agro-food byproducts, which are often considered low-value waste, have gained attention as a potential source of bioactive and functional ingredients that could serve as natural additives (Valencia, 2023).

Natural preservatives derived from animals (e.g., lysozyme, lactoferrin, lactoperoxidase, and ovotransferrin), plants (e.g., extracts from rosemary, sage, chestnut, grapefruit seeds, and turmeric), and microorganisms (e.g., organic acids (OAs), bacteriocins, and bacteriocin-like inhibitory substances) have been studied as alternatives to synthetic chemical preservatives (Kanatt et al., 2008). However, natural preservatives face challenges such as higher production costs, reduced effectiveness in certain foods, lack of standardisation, insufficient safety research, and potential negative impacts on taste and texture. These factors make them less versatile and sometimes less effective than synthetic preservatives (Yu et al., 2021).

The routine use of OAs in meat processing offers a practical, strategically cost-effective, and safe method for extending the shelf life and ensuring the safety of meat products (Lues, 2005). The U.S. Food and Drug Administration (FDA) generally recognises them as safe, and no specific daily intake limits have been

established, supporting their widespread use (Anyasi et al., 2017). Many dicarboxylic acids (DAs) and their salts have been reported to be employed as food additives, either purposefully or inadvertently, due to their safety and other properties (Spaggiari et al., 2023).

Medium-chain dicarboxylic acids (MCDAs) are OAs characterized by the presence of two carboxyl groups and a medium-length carbon chain, typically between 4 to 10 carbon atoms. Key MCDAs include sebacic (SA), azelaic (AZA), pimelic (PA), suberic (SUBA), adipic (ADA), glutaric (GLA), and succinic (SUA) acids. Due to their versatile chemical properties, they are widely utilised across various industries, including pharmaceuticals, polymers, lubricants, cosmetics, and food processing (Li et al., 2020). However, despite their broad industrial applications, only SUA and ADA are currently used in the food industry. SUA is primarily employed as an antimicrobial agent and flavour enhancer (Patsalou et al., 2017). Nevertheless, its antimicrobial efficacy remains controversial, as some studies suggest that it may not consistently inhibit microbial growth under all conditions (Kang et al., 2003; Patsalou et al., 2017). In contrast, ADA has been recognised for its ability to inhibit food spoilage microorganisms since as early as 1987 (Yamamoto et al., 1987). Its use in food preservation has gained increasing attention in recent years due to its high safety, stability, and compatibility. Further, ADA is incorporated into food packaging materials to enhance freshness and extend shelf life (Hosseini et al., 2021).

Currently, only ADA, SUA, GLA and AZA of these MCDAs have been studied more clinically or pharmacologically. In contrast, little has been reported on using GLA and AZA in food additives. The “medicine and food homology” aspect is the breakthrough that allows them to cross over to the food sector, as SUA has done

(Hosseini et al., 2021; Alkhaibari & Alanazi, 2022). “Medicine and food homology” refers to the concept that certain natural substances possess both nutritional and medicinal properties, allowing them to be used for health maintenance and disease prevention. These substances are considered to have both nutritional value and medicinal effects, supporting health, preventing illness, and sometimes treating diseases. So far, SUA, GLA, and AZA are among the most desirable and practical substances used in the pharmaceutical industry because of their remarkable biological properties, such as glucose regulation, anti-inflammatory, antioxidant and antimicrobial properties (Kang et al., 2003; Patsalou et al., 2017). Fatty acids demonstrate their antimicrobial mechanisms through the suppression of cellular energy generation, inhibition of DNA/RNA replication, disruption of enzyme function, disturbance of nutrient intake, formation of peroxidation and autooxidation breakdown products, and disruption of the cytoplasmic membrane, ultimately leading to cell damage and microbial inhibition (Alkhaibari & Alanazi, 2022).

According to the FDA, carboxylic acids are classified as Generally Recognized as Safe (GRAS) (Voguri, 2010). More recently, aliphatic diacids have been considered for food applications due to their inclusion in the GRAS list as approved salt formers (Bandaru et al., 2024). Due to their excellent safety profile, GLA, AZA, and SUA have the potential to be developed as alternative preservatives. Currently, they can be produced through industrial synthesis or microbial fermentation (Chae et al., 2020). Besides, they are strategically cost-effective, stable, and easily standardised, making them superior to many natural and conventional preservatives (Lues, 2005). In brief, their safety profile and potential health benefits position them as promising yet underexplored candidates for food preservation.

1.2 Research objectives

This study aims to investigate the preservation effects of AZA, SUA, and GLA, individually and in different combinations in refrigerated raw meats. To achieve this aim, this study embarks on the following objectives:

1. To determine the effects of GLA, AZA and SUA individually on the quality of sliced and minced beef during refrigerated storage.
2. To determine the effects of GLA, AZA and SUA in different combinations on the quality of sliced and minced beef during refrigerated storage.
3. To determine the effects of GLA, AZA and SUA individually on the quality of sliced and minced salmon during refrigerated storage.
4. To determine the effects of GLA, AZA and SUA in different combinations on the quality of sliced and minced salmon during refrigerated storage.

1.3 Overview of the study

This study will cover 4 phases:

1. Individual application of GLA, AZA, and SUA for preserving sliced and minced beef during refrigerated storage.
2. Application of GLA, AZA, and SUA in different combinations for preserving sliced and minced beef during refrigerated storage.
3. Individual application of GLA, AZA, and SUA for preserving sliced and minced salmon during refrigerated storage.

4. Application of GLA, AZA, and SUA in different combinations for preserving sliced and minced salmon during refrigerated storage.

Phase 1: The antimicrobial properties of GLA, AZA, and SUA were assessed individually using a microdilution broth method, while their antioxidant potential was determined through DPPH free radical scavenging, ferric reducing antioxidant power (FRAP), and *in vitro* nitrite scavenging assays. Based on these results, the most effective concentrations were selected for testing in beef preservation models. The impact of these selected MCDAs on microbial counts, colour, pH, weight loss, and total volatile base nitrogen (TVB-N) in sliced and minced beef was analysed over 12 days of refrigerated storage.

Phase 2: Checkerboard tests were used to evaluate the antimicrobial effects of GLA, AZA, and SUA in combination, alongside antioxidant assessments using the same assays as in Phase 1. The most effective MCDA ratios were selected for further testing in beef preservation models, with their impact on key quality parameters monitored during 12 days of refrigerated storage. Findings from Phases 1 and 2 provided a foundation for subsequent studies on salmon preservation.

Phase 3: The antimicrobial properties of GLA, AZA, and SUA were re-evaluated individually using the microdilution broth method. Effective concentrations, determined from Phases 1 and 3 antimicrobial and antioxidant tests, were applied to salmon preservation models. The quality of sliced and minced salmon was assessed over 12 days of refrigerated storage.

Phase 4: Checkerboard tests were conducted to analyse the antimicrobial properties of GLA, AZA, and SUA in combination, with antioxidant properties evaluated as in previous phases. Optimal MCDA ratios, determined from Phases 2

and 4, were tested in salmon preservation models, and their effects on microbial counts, colour, pH, weight loss, and TVB-N were monitored over 12 days.

CHAPTER 2

LITERATURE REVIEW

2.1 Meat spoilage and preservation techniques

Meat is one of the primary sources of protein used in human food, but because of its complex chemical makeup, it can become contaminated by microorganisms. This can lead to the production of volatile compounds, colour changes, the formation of superficial slime, increased risk of foodborne illness, and financial losses (da Silva et al., 2021).

2.1.1 Factors causing meat spoilage

Meat spoilage can be viewed as an ecological process that includes modifications to the available substrates (such as low molecular components) brought about by the microbial growth that makes up the meat's microbial community (Doulgeraki et al., 2012). The prevalence of a specific microbial community in meat is determined by variables that endure throughout processing, distribution, and retail storage (Nychas et al., 2008). It is commonly known that there are five types of ecological factors in any food ecosystem (e.g., intrinsic, processing, extrinsic, implicit, and emergent impacts). These affect the rate at which a stable microbial community is established and influence the formation of specific microbial relationships. Among these, both intrinsic and extrinsic factors (Figure 2.1) play a crucial role in influencing the shelf life of meat during distribution and retail storage (Nychas et al., 2008).

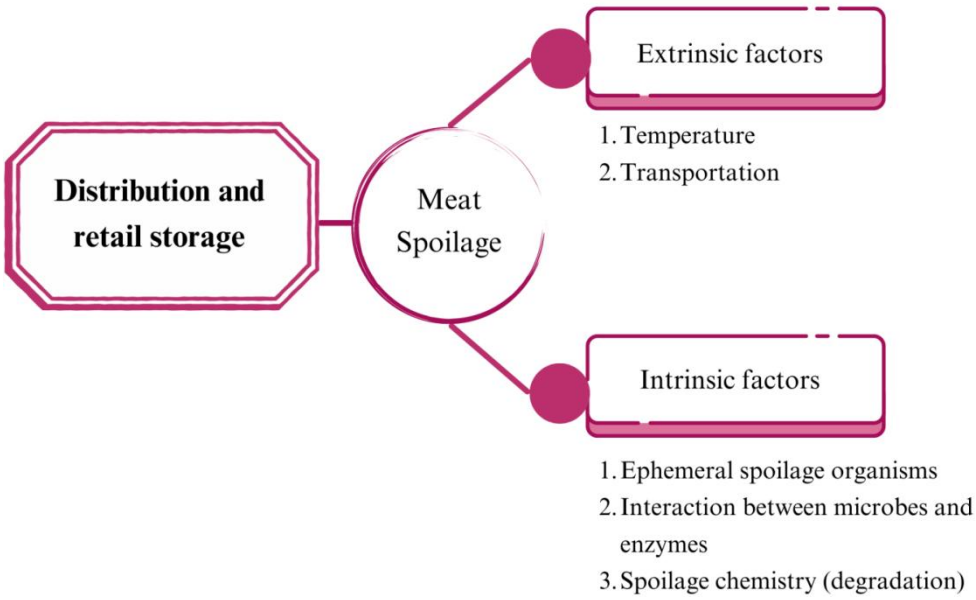

Figure 2.1 Major factors affecting the shelf-life of raw meat products during distribution and retail storage.

2.1.1(a) Intrinsic factors

2.1.1(a)(i) Ephemeral spoilage organisms

Meat deterioration during distribution can be viewed as an ecological phenomenon that includes the alterations of the available substrates (such as low molecular components). At the same time, a specific microbial association, known as specific spoilage organisms (SSO), predominates. A far smaller portion of specific spoilage organisms, known as ephemeral spoilage organisms (ESO), is responsible for meat deterioration (Nychas et al., 2008). The animal's physiological state at slaughter, the rate at which infection spreads throughout the slaughter and processing, temperature, and other storage and distribution circumstances all affect the microbiological quality of meat (Taormina, 2021). Some microorganisms originate from the animal's digestive system and its environment, either before or during the slaughter process (Coombs et al., 2017).

Meat deterioration is typically caused by the metabolic activity of the transient microbial association, which develops in the meat's ecosystem under specific aerobic conditions or results from contamination during processing. The microbial association's type, composition, and population, along with the kind and availability of energy substrates such as glycogen, proteins, lipids, and nitrogenous compounds in the meat, are linked to these alterations or spoilage (Nychas et al., 2008). The availability and metabolism of low-molecular-weight compounds, such as lactate and glucose, influence microbial growth dynamics and, consequently, the type and extent of meat spoilage (Nychas et al., 2008).

Meat held aerobically at varying temperatures (from 1 to 25 °C) is typically spoilt by a group of microorganisms, most commonly led by *Pseudomonas* spp. It is currently widely known that *Pseudomonas fragi*, *Pseudomonas fluorescens*, and *Pseudomonas lundensis* are the three most significant species when stored aerobically. Slime and off-odour generation have been linked to the arbitrary level of 10^{7-8} CFU/g of the pseudomonad population (Pateiro et al., 2018). In actuality, though, both of these traits show up when the pseudomonads start to metabolise nitrogenous substances like amino acids after they have used up all of the meat's glucose and lactate. Although they are present on refrigerated meat kept aerobically, cold-tolerant Enterobacteriaceae (such as *Hafnia alvei*, *Serratia liquefaciens*, and *Enterobacter agglomerans*) do not contribute significantly to the microbial associations (Nychas et al., 2008). Enterobacteriaceae have been identified as indicators of food safety, despite being rarely detected in meat and meat products and contributing minimally to spoilage microbiota. When spoilage progresses to an advanced stage, secondary metabolic processes, such as the breakdown of

nitrogenous compounds and amino acids, become the primary contributors (Nychas et al., 2008).

2.1.1(a)(ii) Microbial and enzymatic interactions

Following slaughter, the animal's natural enzymes drive post-mortem glycolysis until the final pH falls between 5.4 and 5.5. Beyond this point, microbial activity surpasses the role of native enzymes in meat deterioration. It is essential to notice meat's natural lipolytic and proteolytic enzymes may not even be sufficient to influence meat conditioning (ageing) (Nychas & Tassou, 1997). Regarding the function of proteolysis, Nychas and Tassou (1997) unequivocally demonstrated that autolysis—that is, the use of native proteolytic enzymes—did not play a part in spoiling.

The term "autolysis" refers to a series of post-mortem chemical changes that occur in animal tissues due to the activity of lipolytic, amylolytic, and proteolytic enzymes. These enzymes, which are involved in animal metabolism, break down proteins, fats, and carbohydrates after slaughter. Amylolytic enzymes convert glycogen into lactic acid, while lipolytic enzymes facilitate the breakdown or oxidation of fat. These biochemical modifications occur in the early stages of meat storage (Singh & Anderson, 2004). Proteolytic enzymes play a key role in protein degradation by breaking down proteins into amino acids, which are further converted into amino nitrogen or non-protein nitrogen, increasing the amount of soluble nitrogen compounds in meat. One of the most important proteolytic systems in post-mortem muscle is the calpain system, a family of calcium-dependent proteases responsible for muscle protein degradation and tenderization. This system consists of at least three enzymes: ε-calpain, m-calpain, and calpain p94, the latter being

exclusive to skeletal muscle. Among these, ε-calpain is primarily responsible for post-mortem tenderization (Singh & Anderson, 2004). In addition to calpains, aminopeptidases also contribute to meat quality by influencing its flavour. These enzymes hydrolyze amino acids from the N-terminus of peptides and proteins, generating free amino acids during meat processing, which contribute to taste development. The activity of enzymes such as aminopeptidases, cathepsins, and calpains is significantly influenced by temperature and pH. Following slaughter, as pH declines and enzymes are released, lysosomal membranes become more permeable, leading to further enzymatic activity. The rate of post-mortem pH decline impacts meat tenderness. On this occasion, rapid pH decline (from 6.9 to 5.8 within 3 h) and slow pH decline (from 6.9 to 6.6 within 3 h) both result in less tender meat. Moderate pH decline (from 6.9 to 6.2 within 3 h) allows for greater post-mortem protein degradation and improved tenderization (Singh & Anderson, 2004). To prevent or delay spoilage and deterioration, acids and curing salts are commonly used to inhibit the activity of autolytic enzymes (Singh & Anderson, 2004).

The investigation revealed that the ephemeral spoilage groups of the final microbial interaction govern the deterioration pattern. These patterns, which were visible regardless of the microbial populations even in the early stages of preservation, could only be ascribed to the microbial proteolytic activity rather than the natural autolysis of meat enzymes (Nychas et al., 2008). Without a doubt, the most significant factor determining the alterations that lead to meat deterioration is microbial activity. It is important to note that food deterioration is primarily caused by the accumulation of metabolic byproducts. Rather than the direct action of microbial enzymes, it is the overall microbial activity and growth that drive this

process (Pateiro et al., 2018). Therefore, it is crucial to consider the relationships between microbial proliferation and enzyme activity when discussing meat rotting.

While microbial growth plays a dominant role, endogenous enzymes also contribute to the degradation process by facilitating biochemical reactions that break down proteins, fats, and carbohydrates. These enzymatic activities, although not the primary drivers of spoilage, can accelerate quality deterioration under certain conditions. The tissues' complex molecules (protein, lipids, and carbohydrates) are broken down into simpler ones during the autolysis process, causing the flesh to soften and turn greenish. Proteolysis and lipid hydrolysis are two autolytic processes essential for microbial breakdown. Excessive autolysis is referred to as "souring" (Singh & Anderson, 2004). Tissue proteases cause the postmortem breakdown of polypeptides, which gives meat its flavour and texture variations. Tenderization is the outcome of post-mortem ageing of red meat. All animal tissues undergo post-mortem autolysis. However, the pace varies depending on the organ—it happens more quickly in glandular tissue, like the liver, and more slowly in striated muscle. By breaking down the z-line proteins of the myofibril, the enzymes calpains, cathepsins, and aminopeptidases are found to be in charge of the post-mortem autolysis of meat (Nychas et al., 2008). Calpains are one of these enzymes that have been identified as a starting point for the proteolytic tenderization of meat. It was also discovered that tenderization at low pH is facilitated by cathepsins. Because proteolytic enzymes are active at low temperatures (5 °C), the growth of microorganisms and the formation of biogenic amines cause the quality of meat to deteriorate (Singh & Anderson, 2004).

2.1.1(a)(iii) Chemical spoilage processes

Lipid autoxidation and free radical formation are naturally occurring processes that affect fatty acids, leading to oxidative degradation of meat and the development of off-flavours (Kanatt et al., 2008). After slaughter, when blood flow ceases, metabolic processes gradually decline, allowing fatty acids in the tissues to undergo oxidation. Oxygen's reaction with fatty acid double bonds is known as lipid oxidation. It involves the initiation, propagation, and termination of three stages of free radical processes. Lipid hydrolysis in meat can occur either non-enzymatically or enzymatically (Singh & Anderson, 2004). Lipolysis, sometimes known as fat deterioration, is the word for the enzymatic hydrolysis of fats that is controlled by particular enzymes, including phospholipase, lipases, and esterases. Lipolytic enzymes can come from psychrotrophic bacteria or be indigenous in the food product (like meat) (Kanatt et al., 2008). Animal tissue, blood, and skin all contain lipase enzymes. Lipases break down glycerides during lipolysis to produce free fatty acids, which are commonly known as rancidity and cause an off-flavour (Coombs et al., 2017). The two primary enzymes involved in the breakdown of animal fats are phospholipase A1 and A2. Three biosynthetic processes are involved in the regiospecific process of lipid hydrolysis: acyl migration, triacylglycerol cleavage, and 1-monoacyl-sn-glycerol cleavage (Nychas et al., 2008).

It is commonly known that the three types of materials that the microbial association uses are (1) compounds involved in the glycolytic pathway (such as glucose, pyruvate, lactate, and glycogen), (2) metabolic products (such as gluconate, gluconate-6-phosphate, pyruvate, and lactate), and (3) nitrogen energy sources (such as proteins and amino acids) (Singh & Anderson, 2004). In that order, all the microorganisms in the meat microflora catabolize glucose, lactic acid, and certain

amino acids, followed by nucleotides, urea, and water-soluble proteins. Despite being far less common than proteins, the former substances are the primary energy sources for the massive proliferation of microcosms in meat (Singh & Anderson, 2004). Research indicates that the concentration of these chemicals can impact the type (such as saccharolytic and proteolytic) and rate of spoiling (Coombs et al., 2017). Moreover, these compounds appear to be the primary precursor(s) of the microbial metabolite(s) that cause spoiling (Nychas et al., 2008).

2.1.1(b) Extrinsic factors

2.1.1(b)(i) Temperature

The efficient monitoring of time and temperature variables that impact meat's safety and overall quality is a crucial component of the distribution and consumption of fresh (raw) meat (Singh & Anderson, 2004). The European industry, retailers, food authorities, and consumers alike acknowledge that the weakest links in refrigerated perishable food management are found to be numerous phases of the real refrigerated supply chain, such as transfer stations or storage rooms. Meat products spoil quickly if they are not properly transported, stored, or packaged (Nychas et al., 2008).

The primary and secondary refrigerating are the first two phases in the meat refrigerated supply chain. Both phases are crucial for the sake of eating quality, production yield, and microbiological stability (Doulgeraki et al., 2012). The technique of lowering the body temperature of slaughtered animal carcasses to that of refrigeration is known as primary refrigerating. Microorganisms that cause spoiling as well as pathogenic growth may proliferate quickly during primary refrigerating (Nychas et al., 2008). There is no publicly available data specifying the

maximum allowable time for meat to cool before transportation or processing. Nevertheless, EU legislation (Regulation (EC) No 853/2004) mandates that meat must reach a core temperature of 5 °C or lower before it can be transported or further processed (Singh & Anderson, 2004). The shelf life of a product can be extended, and microbial development can be inhibited by quickly cooling the carcass surface. It is evident that quick refrigerating has several other benefits in terms of production costs and product quality. Any handling after the initial refrigerating, like chopping and dicing, will raise the meat's temperature; therefore, a secondary refrigerating is necessary to get the temperature below 5 °C (Singh & Anderson, 2004). Additionally crucial is secondary refrigerating when it comes to pre-cooked beef items. Following the cooking procedure, the temperature of the meat should be quickly lowered from 60 to 5 °C in order to stop the growth of pathogens that have survived the heat process or from recontaminating the product (Nychas et al., 2008). Furthermore, it is critical for cooked meat items to cool quickly to prevent quality issues brought on by overcooking that happens when cooling slowly (Nychas et al., 2008).

2.1.1(b)(ii) Transportation

Meat and meat products are kept in rails, retail cabinets and residential refrigerators during the marketing (transport) process to the final consumer, where they are prepared and consumed. These factors significantly affect the safety and quality of meat (Singh & Anderson, 2004). Indeed, the features and functions of industrial and track chambers vary. When evaluating cold store requirements, the size of the cabinets, the starting temperature of the arriving meat, the intended temperature of storage, the surrounding temperatures, mechanical characteristics (the location of refrigeration machinery, compressors, ventilation, and insulation), and

energy/cost issues should be given top priority (Nychas et al., 2008). The “First in, first out” (FIFO) principle is a widely used inventory management strategy in the meat industry to ensure proper stock rotation and minimise spoilage. In most (though not all) circumstances, this management strategy is also rigorously followed at every point of the cold chain thanks to well-thought-out handling procedures in the refrigerated storage rooms. For the meat’s overall quality and safety, the various stages of transport—from cold storage to the retail location and finally to the customer refrigerator—are crucial (Taormina, 2021). In general, the vehicle used for transportation must be equipped with an effective refrigeration system. Further, the time required to transfer a product from the point of sale to the customer’s home refrigerator presents another challenge in the distribution process (Nychas et al., 2008).

2.1.2 Quantitative assessment of meat spoilage

The most common methods for determining whether meat and meat products are safe, spoilt, or fresh are sensory and microbiological studies (European Commission, 2005). Although sensory analysis is likely the most appropriate and acceptable method, it has drawbacks. It is expensive and unappealing for routine analysis because it depends on highly trained panellists (Singh & Anderson, 2004). However, despite their retrospective nature and specific limitations, microbiological analysis—either using conventional figures, such as total viable—or molecular methods, like as PCR (polymerase chain reaction), RT-PCR (reverse transcription polymerase chain reaction), and DGGE (denaturing gradient gel electrophoresis)—is frequently employed for the accurate evaluation of meat deterioration (Taormina, 2021).

Predictive microbiology, often known as quantitative microbiology, generally deals with the quantitative expression of microbial growth responses to environmental stimuli through mathematical equations. Databases can be used to store and retrieve models and data that are utilised to interpret how microbial growth is affected by distribution, processing, and storage settings (Coombs et al., 2017). However, temperature changes could often occur when storing and distributing food. Therefore, testing the model at varying temperatures is crucial to assess its performance in forecasting shelf life in actual refrigerated chain scenarios (Singh & Anderson, 2004).

It is often known that opinions on the early indicators of meat spoiling must be in accord. The difficulty of objectively evaluating this issue is mostly caused by advancements in meat preservation technology, such as vacuum and changed atmospheres (Singh & Anderson, 2004). Spoilage occurs when specific substrates are metabolised, leading to the accumulation of metabolites associated with meat deterioration. Microbial metabolites produced during microbial growth have long been recognised as indicators for assessing meat quality (Nychas et al., 2008). Regardless of the method used to quantify spoilage for control purposes, key factors include (1) food structure and physicochemical properties (e.g., type, concentration, diffusivity, and nutrient availability); (2) microbial competition; (3) understanding microbial ecology and determining the mechanisms underlying microbial growth and survival in stressful food environments, including microbial communication and the functional role of genes (genomics); and (4) the effects of dynamic storage conditions, such as temperature fluctuations, vacuum packaging (VP), modified atmosphere packaging (MAP), and film permeability (Nychas et al., 2008). By identifying the origin of specific metabolites (metabolomics), understanding their

regulation at the cellular level (genomics–proteomics), and evaluating the influence of meat characteristics and microbial interactions on the rate and type of metabolite formation, we can develop strategies to improve meat preservation and quality control for the benefit of the industry, regulatory authorities, and consumers (Nychas et al., 2008).

Indeed, to determine the most suitable processing techniques and preservation strategies for raw materials, the meat industry must act swiftly to quantify and assess these factors in order to predict the shelf life of meat products. These practical techniques are essential for retailers and wholesalers to guarantee the safety and freshness of their goods as well as to utilise them in the event of a disagreement between customers and sellers (Singh & Anderson, 2004). Before retail and consumption, it is desirable to indicate the safety and quality status of meat reliably. Therefore, it is crucial to use preservation technology to efficiently monitor freshness and safety to maintain quality from the perspectives of customers, industry, and scientists alike (Singh & Anderson, 2004).

2.1.3 Meat preservation techniques

The two main objectives of preservation techniques are to: (1) prevent microbial deterioration and (2) reduce oxidation and enzymatic deterioration. Modern meat preservation techniques include chemical, biopreservative, and nonthermal procedures have supplanted traditional methods like drying, smoking, brining, fermenting, refrigerating, and canning. The three main categories of meat preservation techniques used today are (1) temperature control, (2) water activity management, and (3) chemical or preservative use. It is possible to slow down the spoiling process by combining several preservation methods (Nychas et al., 2008).

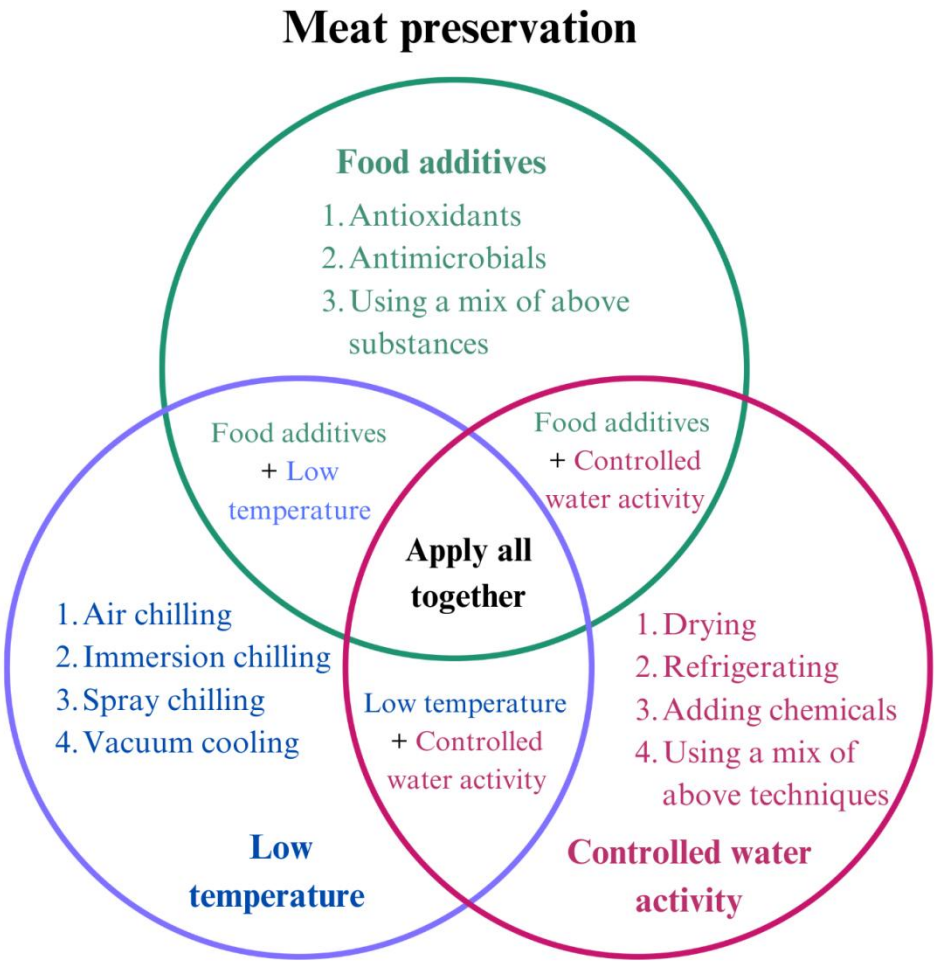

Figure 2.2 Main categories of raw meat preservation techniques used today during distribution and retail storage.

2.1.3(a)(i) Low temperature

Since temperatures below the optimum range can inhibit microbial development, the primary goal of cooling strategies is to halt or limit the rate of spoiling. Two stages of low-temperature storage are often employed: refrigerating and freezing. Each of these levels contributes to inhibiting or ceasing microbial growth (Doulgeraki et al., 2012). However, low temperatures do not completely stop the growth of psychrophilic bacteria, yeasts, and moulds. Further, both enzymatic and non-enzymatic changes continue, though at a significantly slower rate (Nychas et al., 2008).

Before being transported, meat and meat products are chilled using a variety of techniques, including (1) air chilling, (2) immersion chilling, (3) spray chilling, and (4) vacuum cooling (Singh & Anderson, 2004). The weight, external fat layer, air temperature and velocity, relative humidity, and product loading all influence the effectiveness of air chilling applications. In contrast, immersion chilling is likely the least expensive method and offers high-speed cooling without the risk of freezing (Taormina, 2021). Spray chilling is an alternate method to immersion chilling that has gained popularity, particularly in the USA. It involves using a combination of sprays and air for the first part of the chilling cycle and air alone for the remaining chilling time (Nychas et al., 2008). Lastly, Hoover Chilling is a quick batch method that uses moisture evaporation under Hoover to cool moist items containing free water. The primary benefit of this technology is that, after being stored for a few days, quick cooling under a vacuum can dramatically lower the microbial populations of mesophiles and psychrophiles. The biggest drawback of vacuum cooling is the significant weight loss of the meats (Singh & Anderson, 2004).

Chilling is used at slaughterhouses both during storage and transit just after the animal is killed. After the carcass has been eviscerated, it must be cooled to 4 °C within 4 h of the killing (Nychas et al., 2008). For meat to be safe, hygienic, have a long shelf life, and maintain its nutritional value, it must be chilled. There are two techniques used to cool it: (1) immersion chilling, where the product is submerged in water that has been chilled to between 0 and 4 °C, and (2) air chilling, where the carcasses are misted with water in a room that has chilly air circulating (Nychas et al., 2008). Air chilling lowers the temperature of the carcass surface more quickly, enhancing drying and reducing microbial deterioration. In terms of microbial count, the air-chilling method was more secure than the water-chilling method. Regarding

shelf life, spoiling was less likely to occur at 0 °C than at 4 and 5 °C (Nychas et al., 2008). According to Zhou et al. (2019), quick freezing also prevents the denaturation of proteins, which can be dangerous for microorganisms because they are more vulnerable to denatured proteins than native proteins. On the other hand, rapid freezing of pre-rigour beef may cause cold-shortening and toughening.

Freezing is a great way to preserve the original qualities of fresh meat (Coombs et al., 2017). Depending on the species, meat can contain between 50 and 75 per cent water by weight. When meat freezes, the majority of the water turns into ice. Nearly 75% of tissue fluid freezes at -5°C, demonstrating the speed at which meat freezing occurs. The rate of freezing increases with decreasing temperature; at -20°C, about 98% of water begins to freeze, and by -65°C, crystal formation is complete. Nevertheless, over 10% of muscle-bound water—which is chemically bonded to specific locations like protein amino acids and carbonyl bonds—will not freeze. The slow or fast freezing rate significantly impacts the quality of frozen meat (Pateiro et al., 2018). Meat frozen quickly is of higher quality than meat frozen slowly. Large ice crystals that develop during gradual freezing harm cells and denature proteins. The process of protein denaturation is controlled by the concentration of enzymes and the presence of other substances. Because the physical, chemical, or biological changes that occur in animal tissues after slaughtering do not wholly halt after cold treatment, the ability of frozen meat to be preserved is limited (Singh & Anderson, 2004). At -12 °C, microbiological growth halts; below -18°C, mammalian tissues experience complete cellular metabolic inhibition. Meat can be kept from completely changing in quality down to -55 °C (Nychas et al., 2008). Enzymatic processes, oxidative rancidity, and ice crystallisation will still be significant factors in spoiling. About 60% of the viable microbial population perishes

after freezing, but the growing population steadily grows during frozen storage (Nychas et al., 2008).

2.1.3(a)(ii) Controlled water activity

Water activity (a_w) directly affects the microbiological safety of food (Comi, 2017). Water that is not attached to food molecules and can promote the growth of microorganisms is referred to as a_w. It shows the proportion of the food's water vapour pressure to that of pure water under the same circumstances. The relative humidity of the air in equilibrium with the product is equal to the water activity of meat products (Comi, 2017). With a water activity greater than 0.85 and falling into the moist food group, the majority of fresh meats, fruits, and vegetables need to be refrigerated or kept behind another barrier to prevent the formation of microorganisms. There are minimum, optimal, and maximum water activities for every microorganism. In general, most microorganisms grow best at a_w values between 0.980 and 0.995, while bacterial growth is significantly inhibited below a_w 0.900, though some fungi and halophilic bacteria can tolerate lower a_w conditions. Moulds and yeasts can grow at low a_w values of 0.6 (Nychas et al., 2008). However, at an a_w of 0.85, the growth of many foodborne pathogens is significantly reduced, though some may still survive or remain dormant. Meat can reduce its water activity by drying, refrigerating, adding chemicals, or using a mix of these techniques. Water activity has been regulated by sugar and sodium chloride because free water binds to these substances, creating an osmotic imbalance that ultimately inhibits cell growth (Nychas et al., 2008). Notably, at the water activity level from 0.99 to 0.97, microorganisms that are resistant to salt, like yeasts and lactic acid bacteria (LAB), could proliferate (Kanatt et al., 2008). On the other hand, sodium chloride, which

hastens the development of lipid oxidation and, consequently, the degradation of value-added products, is hampered by sodium chloride prooxidant activity (Nychas et al., 2008).

Sugars have the ability to attach to moisture in food, which lowers its water activity. When processing dried meat, sugars or carbohydrates such as maltodextrins, sucrose, brown sugar, corn syrup, lactose, honey, molasses, and starches are typically added to improve flavour, lessen the harshness of the salt, and decrease water activity (Singh & Anderson, 2004).

2.1.3(a)(iii) Food additives

Microbial/enzymatic and oxidative spoiling cannot be stopped by freeze storage. Therefore, chemical preservation techniques are highly advantageous for maximising stability and product quality while preserving freshness and nutritional value when used in conjunction with refrigeration. This method addresses multiple aspects of meat quality, including microbial safety, texture, colour, flavour, and nutritional value, making it a highly effective strategy in modern food preservation (Coombs et al.,2017). Although chemicals have been used as food additives to preserve meat, each nation has set limitations and created restrictions to minimise adverse effects on people. Another solution to the problem of preserving meat and meat products may indeed be the development of effective antioxidants, antimicrobials, or mixtures of both. Antioxidant and bacteriostatic capabilities are vital when assessing the effectiveness of additives in extending the shelf life and safety of meat products. Antioxidants help prevent the oxidation of fats, which can lead to rancidity and off-flavours. At the same time, bacteriostatic agents inhibit the