Deanship of Graduate Studies
Al-Quds University

The Impact of Freeze-Dried Pomegranate and Raspberry Powder on the Antioxidant Properties and Quality of Roast Beef

Amir Kamal Alskafi

M.Sc. Thesis

Jerusalem – Palestine

2026 / 1447

The Impact of Freeze-Dried Pomegranate and Raspberry Powder on the Antioxidant Properties and Quality of Roast Beef

Prepared by

Amir Kamal Alskafi

B.Sc. Nutrition and Food Processing

Hebron University. Hebron-Palestine

Supervisor: Prof. Fuad Al-Rimawi

This thesis is submitted in partial fulfillment of the requirements for the degree of Master of Applied and Industrial Technology, Al-Quds University

2026 / 1447

Al-Quds University

Deanship of Graduate Studies

Applied and Industrial Technology

Thesis Approval

The Impact of Freeze-Dried Pomegranate and Raspberry Powder on the Antioxidant Properties and Quality of Roast Beef

Prepared by: Amir Kamal Alskafi

Registration Number: 22220043

Supervisor: Prof. Fuad Rimawi

Master's thesis submitted and accepted, Date: 10-1-2026

The names and signatures of the examining committee are as follows:

Head of Committee: Prof. Fuad Rimawi	**Signature:**
Internal Examiner: Dr. Ziad Ayyad	**Signature:**
External Examiner: Dr. Mohannad Alayoubi	**Signature:** مهند الايوبي

Jerusalem/ Palestine

2026 / 1447

Dedication

" بسم الله الرحمن الرحيم: ﴿يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ﴾ [المجادلة:11]"

Praise be to God, praise befitting His glorious countenance and magnificent dominion, and thanks be to Him for His perfect blessings and complete favor. He has bestowed upon me success, smoothed my path, and inspired me with patience, determination, and perseverance, enabling me to reach this stage of my academic journey and fulfill a dream that has resided in my heart since completing my bachelor's degree.

I dedicate the fruits of this humble effort to:

My first blessings come from God, and I am grateful to my dear parents, who have always been my source of support and strength. You are my foundation, and you embody all that is good.

To my life partner, my beloved wife Roaa Al-Skafi, and to my children, for whom giving is a joy and hope blossoms.

To my beloved brothers, and especially to my brother, Dr. Amin Al-Skafi, who has been my support throughout all stages of my life, my companion on this journey, and my friend in both good times and bad, in its joys and challenges.

And to our righteous martyrs, who watered the soil of Jerusalem and our land with their pure blood, for it is thanks to their sacrifices that we live with hope and build with knowledge and dignity.

With all my love and gratitude,

Amir Alskafi

Declaration

I hereby declare that this thesis, submitted in partial fulfillment of the requirements for the Master's degree, is the product of my independent research, investigation, and analysis, except where explicit references and acknowledgments have been made. To the best of my knowledge and belief, this work has not been submitted previously, either in whole or in part, for any academic degree or qualification at this or any other university or institution.

Amir Kamal Alskafi

Signed:

Date: 10-1-2026

Acknowledgements

First and foremost, I thank God for His countless blessings, who granted me confidence, courage, determination, and guidance throughout my life, especially during my challenging academic journey, and for sending His Prophet Muhammad (peace be upon him), the teacher of teachers and a mercy to all mankind.

I would also like to express my sincere gratitude to my university, Al-Quds University, for opening its doors and enabling me to complete this work and obtain my Master's degree.

I am also grateful to thank the members of my committee, who generously and faithfully shared their expertise and valuable time. A special thank you to Dr. Fouad Al-Rimawi for his countless hours of reflection, reading, encouragement, and the confidence he instilled in me, and most importantly, for his patience and support throughout the entire process. Thank you.

Special thanks are also due to extend my sincere thanks to Dr. Muhammad Abu Al-Hajj, Dean of the Faculty of Science and Technology; And Dr. Mahmoud Al-Khatib, I also extend my sincere thanks and gratitude to the Siniora Food Industries family, represented by my brother and colleague Ahmad Shakarneh and the wonderful staff in the Laboratories, Research and Development Department, for opening the doors of cooperation to complete this study and for not hesitating for a moment to provide assistance and support. Finally, my sincere thanks to my friend and colleague, brother Salah Zamel, for his advice and boundless generosity.

Amir Al-Skafi

Abstract

Lipid oxidation and microbial deterioration are key factors that influence the quality of processed meats, particularly those stored at cold temperatures. This requires discovering efficient and organic techniques and approaches for maintaining these meat products. This study aimed to evaluate the effectiveness of freeze-dried pomegranate powder (FDPP) and freeze-dried red raspberry powder (FDRP) as natural antioxidant alternatives to synthetic additives in enhancing the quality and shelf life of roast beef during refrigerated storage. Three incorporation levels (0.3%, 0.7%, and 1.5%) of each powder were applied and compared with a negative control (NC) sample, without any natural or synthetic antioxidants added, and a positive control (PC) containing sodium erythorbate (100 ppm). The analytical assessments comprised lipid oxidation evaluation using the thiobarbituric acid reactive substances (TBARS) assay, microbiological analyses including total plate count (TPC), total coliforms, and *Escherichia coli*, in addition to monitoring of pH changes. Furthermore, instrumental color parameters (L*, a*, and b*) as well as sensory characteristics were assessed periodically over a 60-day refrigerated storage period, which represents the shelf life of roast beef. The results indicated a progressive increase in lipid oxidation and microbial growth in all treatments throughout storage; however, samples treated with FDPP and FDRP demonstrated lower oxidative deterioration and improved microbiological quality compared to the negative control. Specifically, the study showed that there was significant antioxidant activity at a concentration of 1.5% of FDPP, where it reduced malondialdehyde (MDA) levels by up to 75% compared to NC and achieved TBARS values similar to the PC group. It has also found that FDPP decreased the growth of total microbial levels significantly by over 70% in the treated samples, without affecting sensory characteristics during the storage period. Among the natural treatments, FDPP, particularly at higher concentrations, showed greater effectiveness in maintaining oxidative stability, preserving acceptable pH values, and retaining desirable color and sensory attributes during storage, with performance comparable to that of the positive control treated with sodium erythorbate. Overall, the findings of this study suggest that freeze-dried pomegranate powder is a promising clean-label natural antioxidant capable of improving product quality and extending the refrigerated shelf life of roast beef, thereby supporting its potential application in the meat processing industry. In comparison, FDRP exhibited moderate effectiveness, achieving the best outcomes at 0.3%, whereas elevated concentrations resulted in lower sensory assessments due to minor alterations in color and taste. In general, FDPP, especially at a level of 1.5%, showed notable effectiveness as a natural antioxidant and preservative, providing a pure substitute for synthetic additives such as BHT and BHA in the processing of roast beef.

Keywords: Roast beef, Freeze-Dried pomegranate powder, Freeze-Dried raspberry powder, natural antioxidants, lipid oxidation, TBARS, MDA, sensory attributes.

Contents

List of tables

List of Figures:

List of Abbreviations, Symbols, and Terminology:

$^{1}O_2$	Singlet oxygen
a*	Redness
ABTS	2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
b*	Yellowness
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
CFU	Colony-Forming Units
D	Dilution
DPPH	2,2-Diphenyl-1-picrylhydrazyl
FD	Freeze- drying
FDA	Food and Drug Administration
FDPP	Freeze-Dried Pomegranate Powder
FDRP	Freeze-Dried Raspberry Powder
GAE	Gallic Acid Equivalents
GRAS	Generally Recognized as Safe
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric Acid
L*	Lightness
LDL	Low-Density Lipoprotein
LSD	Least Significant Difference
MDA	Malondialdehyde
MFC	Minimal fungicidal
Mg/kg	Milligram per kilogram
N	Number
n=3	Number of replicates
n=50	Number of panelists
NC	Negative Control
Nmol	Nano Molar
O_2•	Superoxide anion radicals
•OH	Hydroxyl radicals
•OOH	Hydroperoxyl radical
PCA	Plate Count Agar
PC	Positive Control
PPE	Pomegranate peel extract
Ppm	Parts Per Million
PV	Peroxide value
RH	Unsaturated fatty acid
RNS	Reactive Nitrogen Species
ROO•	Peroxyl radical
ROOH	Hydroperoxides
ROOR	Organic peroxide
ROS	Reactive Oxygen Species

SD	Standard Deviation
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric Acid Reactive Substances
TBHQ	Tert-Butylhydroquinone
TCA	Trichloroacetic Acid
TPC	Total Plate Count
µg/ml	Microgram per milliliter
US	United States
V	Volume
VRBA	Violet Red Bile Agar

Chapter One

Introduction

1.1 Background

Since the dawn of prehistory and the evolution of hunting methods, meat has been an essential component of the human diet. The early development of preservation techniques, such as drying and curing methods, was spurred by the naturally perishable nature of meat and its clearly seasonal presence in ancient times. Due to the relatively high cost of meat and the demands of an expanding population, many products were created, such as meat pies and sausages, which effectively utilized almost every anatomical part of the animal. These two events ultimately resulted in the development of a significant meat products industry, which continues to hold significant economic significance in modern society (Varnam et al. 1995).

Through the development of novel products aimed at consumers who prioritize quality and health, this industry has adeptly addressed both general concerns regarding meat consumption and specific concerns regarding sodium content and preservatives, particularly nitrates in meat products. However, this advancement has occasionally sparked questions regarding the microbiological safety of specific contemporary meat products (Varnam et al. 1995).

Meat is a highly perishable commodity, providing an ideal environment for the proliferation of various microorganisms. Specifically, spoilage bacteria like *Pseudomonas* and *Lactic Acid Bacteria* are responsible for the degradation of fats and proteins, resulting in off-odors and slime formation. Moreover, the growth of pathogens such as *Escherichia coli* not only compromises quality but also poses health risks. Consequently, implementing effective spoilage control measures is crucial to extending shelf life while preserving the nutritional integrity and sensory attributes of the meat. This has led to an increasing interest in natural alternatives, such as pomegranate and raspberry powders, to inhibit these microbial activities (Dave et al. 2011).

Reducing bacterial activity in processed meat involves a combination of physical and chemical strategies designed to inhibit microbial proliferation and ensure food safety. Conventional methods include temperature control (refrigeration and freezing), vacuum packaging, and the use of synthetic additives such as nitrites, benzoates, and sodium erythorbate, which act as potent antimicrobials and preservatives. However, due to growing consumer concerns regarding the potential health risks of synthetic chemicals, there is a significant shift toward 'clean-label' strategies. These involve the application of natural antimicrobial agents derived from plant extracts, such as essential oils and polyphenolic-rich powders. These natural compounds exert antimicrobial effects by disrupting bacterial cell membranes, inhibiting enzyme activity, and interfering with metabolic pathways. Recently, the incorporation of fruit-based powders like pomegranate and raspberry has emerged as an effective approach to suppress the growth of spoilage and pathogenic bacteria, thereby extending the shelf life of meat products without compromising their nutritional quality (Burt, S. 2004; Dahham et al. 2010).

1.2 Oxidation

The production of superior products with a long shelf life is the main goal of the meat processing industry. But there are problems with lipid oxidation and microbial contamination, which can affect the safety and quality of food. The result is a reduction in undesirable changes and deteriorations in nutritional quality, such as variations in texture, color, and shelf life. Lipid peroxidation poses serious health risks, such as cellular damage and genetic changes, producing serious health issues (Rodionova, K. 2022).

The main factor causing meat quality degradation and shorter meat product shelf life is oxidation, which also negatively affects the nutritional content, sensory, and physicochemical characteristics of meat. Synthetic antioxidants have been used extensively up to this point to prevent oxidation, which may be harmful to human health and increase the toxicity of products. However, since plants and plant materials are rich in bioactive compounds that act as natural antioxidants and have potential health benefits, natural antioxidants could be a good substitute for this problem (Horbańczuk et al. 2019).

Lipids are one of the most important components of meat, providing its softness, taste, juice, and aroma. At the same time, lipids are susceptible to the oxidation process, which can deteriorate the quality of meat. Symptoms of lipid oxidation can be clear due to color changes, off-odors, off-flavors, and potential poisoning. For this reason, the characteristics of meat become less acceptable for human consumption. The use of antioxidants can help eliminate lipid oxidation. Which can be natural or synthetic (Bariya et al. 2020).

Accordingly, by postponing oxidative reactions, the food industry making the use of antioxidants, whether natural or synthetic, the main target for preserving the quality of perishable products. Propyl gallate, butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), sodium erythorbate, sodium ascorbate, and curing agents' nitrite and nitrate are some of the synthetic antioxidants that are more regularly used. Every one of these substances has toxicological and carcinogenic consequences (Ribeiro et al. 2019).

The increasing demand for plant-passed ingredients has inspired the food venture to find a natural option for synthetic antioxidants. These natural antioxidants, obtained from plants, can help in the control of lipid oxidation, increase the nutritional value of food products, and eliminate the use of potentially dangerous synthetic additives. By including natural antioxidants in products, food ventures can expand shelf life and cater to the preferences of consumers for cleaner and natural food options (Mishra et al. 2023).

Nevertheless, natural antioxidants could be an alternative response to these problems, as plants and plant-based products are rich in bioactive molecules acting similarly to natural antioxidants, which might show beneficial pharmacological activities. Furthermore, the consumers' awareness of using natural products is growing. The high probability is that in the future, one will replace synthetic antioxidants and preservatives with natural substances (Horbańczuk et al. 2019).

The mechanism of action involves the above-mentioned aspects of phenolic components for metal chelating, lipoxygenase inhibition, and free radical scavenging, and thereby the ability to act as antioxidants. In most carriers, phenolic compounds can suspend or eliminate autooxidation by sequestering free radicals that promote oxidation. In other conditions, they act as antioxidants and eventually produce even more stable compounds that are more resistant to autooxidation. And thereby preserve low-density lipoproteins that would be oxidized in the human body. From time immemorial, fruit and vegetable consumption has been related to benefits due to phenolic compounds with antioxidant activity that may lower risks related to cancer and cardiovascular diseases (Pabón-Baquero et al. 2018).

1.2.1 Fat Oxidation

Fats mostly undergo larger changes due to reactions with ROS (reactive oxygen species), forming an array of different aldehydes. Because of their affinity to macromolecules, especially proteins, aldehydes also form end-products of advanced oxidation processes that spoil food. Co-oxidation can also cause extensive damage. Another problem that arises during freezing and thawing, as well as heat treatment of meat, is protein oxidation, which also impairs its quality and nutritional value. The loss of some essential amino acids due to various mechanisms and heat treatment into carbonyl compounds is one of the changes which are both physical and chemical (Al-Shibli et al. 2023).

An increase in the concentration of these carbonyl compounds indicates the progression of protein oxidation. Factors such as heat treatment will cause myoglobin to denature, brown color of cooked meat, which is heat-denatured myoglobin, is affected by numerous variables. Active protein aggregates with maillard products formed from the interaction of the oxidation products of polyunsaturated fatty acids and the glycation/glycoxidation of carbohydrates are also formed. It is important to know the extent of the oxidative changes in the energy-providing polyunsaturated fats as well as the protein, since these are essential and significant components of the food, and especially meat products (Al-Shibli et al. 2023).

1.2.2 Mechanism of Lipid Oxidation

All living tissues experience a variety of chemical reactions to achieve cellular homeostasis, particularly oxidation-reduction reactions, which maintain specific compound concentrations. When homeostatic mechanisms within a tissue cease functioning properly, reactive oxygen species (ROS), free radicals, and reactive nitrogen species (RNS) may become confined within the tissue, causing damaging interactions with proteins and lipids (Moylan et al. 2014). In the meat industry, the oxidation by-products of these interactions become significant problems because they reduce the shelf life of meat, cause undesirable sensory changes, and produce toxic by-products. Consequently, the oxidation of lipids and proteins is considered the second most common cause of spoilage in meat (Manessis et al. 2020).

Aging is associated with many diseases, some of which are the result of amino acid alterations, protein modifications, and oxidative stress. Cancer, disorders of the immune system, and heart diseases are some of these disorders. Eye diseases, diabetes, and obesity are metabolic disorders. Others are inflammatory disorders like arthritis, cardiovascular problems such as atherosclerosis, and diseases of the kidneys and lungs. As for neurodegenerative diseases, they include Alzheimer's and Parkinson's disease. All these disorders are linked to oxidative stress (Falowo et al. 2014; Hadidi et al. 2022).

Singlet oxygen, prooxidant metal ions, and gamma radiation exposure also encourage the production of ROS, therefore boosting free radical formation. ROS, RNS, and metal ions, among other factors, can cause lipid oxidation. In this process, unsaturated fatty acids interact indirectly with oxygen, which needs to be activated first (Domínguez et al. 2019).

Usually, this activation produces hydroxyl radicals (•OH), superoxide anion radicals (O2•), or hydrogen peroxide (H2O2). The created free radicals rapidly transform into nonradical molecules, including conjugated dienes (chains of carbon with two double bonds separated by a single bond), and the major results of lipid oxidation are hydroperoxides (ROOH). (Fig. 1 .1) These compounds degrade into secondary products, including aldehydes, ketones, alcohols, and carbonyl compounds in the last stage of oxidation. Among these are important secondary products since they quickly interact with proteins, resulting in undesirable flavor and nutritive quality changes in meat, among aldehydes like alkenals, alkadienals, and hydroxyalkenals (Guyon et al 2016).

Furthermore, dangerous byproducts of oxidation connected to health problems, including inflammatory disorders, malignancies, and atherosclerosis, are α, βunsaturated aldehydes, alcohols, and oxidized cholesterol. Common techniques employed in fat oxidation include measuring peroxide value, thiobarbituric acid reactive substances (TBARS) tests, and chromatographic investigation. Promising results also come from modern methods, including chemiluminescence, fluorescence emission, Raman spectroscopy, infrared spectroscopy, and magnetic resonance (Manessis et al. 2020).

The previously mentioned oxygen activation is affected by various factors, including transition metals, prooxidant agents, existing ROS, and energy inputs (light and temperature). Lipid oxidation occurs through enzymatic catalysis, autoxidation, and photooxidation, but the distinctions among them lie in the activation process. Autoxidation is crucial for the meat industry as it represents the main process through which oxygen interacts with unsaturated fatty acids, leading to the deterioration of meat and meat products. (Hadidi et al. 2022, Falowo et al. 2014).

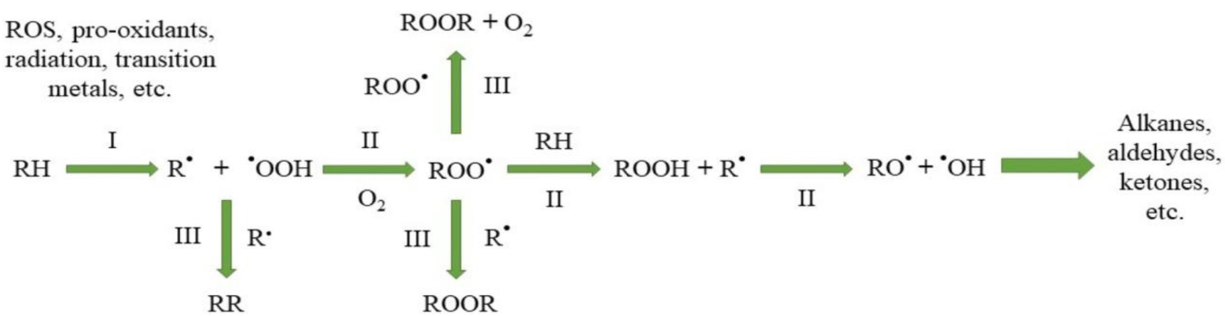

Fig 1.1 General lipid oxidation mechanism: I) initiation, II) propagation, and III) completion (Hadidi et al 2022).

- RH: unsaturated fatty acid
- ROS: reactive oxygen species
- R•: alkyl radical, RR: non-radical product
- ROO•: peroxyl radical
- ROOR: organic peroxide
- ROOH: hydroperoxides
- •OOH: hydroperoxyl radical
- RO•: alkoxy radical
- •OH: hydroxyl radical
- O2: oxygen

Stages of lipid oxidation

1- Initiation: an unsaturated fatty acid, a hydrogen atom is removed to produce an alkyl radical, which may interact with yet another alkyl radical to create a nonradical product or continue onto the propagation stage.

2- propagation: alkali reacts with radical molecular oxygen peroxide radicals, which are highly reactive and can interact to create hydroperoxide and more alkali radicals (primary product) with unsaturated fatty acids (removal of a hydrogen atom). In addition, peroxide radicals can react with other peroxide radicals or alkyl radicals to create an organic peroxide. The formation of hydroperoxides also decomposes, resulting in alkoxy radical and hydroxyl radical formation. Sometimes, alkoxy radicals are decomposed to create volatile aromatic compounds such as aldehydes, ketones, and alkanes (secondary products). All these free radical chain reactions can increase lipid oxidation.

3- Compilation: reactions between free radicals can give rise to non-cutting compounds, and thus, prevent their own spread. There are also reactions between free radicals and antioxidants in this stage (Hadidi et al. 2022).

1.3 The concept of antioxidants and their role in preventing oxidation

By stabilizing the generated radical, a compound or antioxidant system's primary function is to prevent or detect a chain of oxidative propagation, thereby reducing oxidative damage in humans (Vinson, J. A. 1998).

Listed the characteristics of antioxidants and provided a classification. Antioxidants can be divided into two categories

1- Primary (which breaks the chain reaction and scavenges free radicals) and secondary (also known as preventive).
2- The secondary antioxidant mechanisms include metal deactivation, lipid hydroperoxide inhibition by preventing the production of unwanted volatiles, primary antioxidant regeneration, and singlet oxygen removal are examples of secondary antioxidant mechanisms. Thus, "those substances that, in low quantities, act by preventing or greatly retarding the oxidation of easily oxidizable materials such as fats" is how antioxidants are defined (Santos-Sánchez et al. 2019).

Antioxidants, functioning as hydrogen atom donors, are utilized in meat products to reduce or halt oxidative reactions. The majority of antioxidants incorporated into animal products, such as meat, are synthetic; however, because of the increasing movement to minimize synthetic food additives and the rising demand for natural ingredients, further research is essential to identify new natural extracts with potential uses for meat and meat products (Lorenzo et al. 2019).

To combat the oxidative instability of proteins and lipids in meat, it is crucial to investigate natural alternatives, such as plant-based antioxidants, due to their affordability and health advantages (Munekata et al. 2020).

The agro-food sector generates a significant volume of plant by-products each year during the growing and processing of food products. These by-products include a variety of natural antioxidants. Numerous plant components, including seeds, skins, foliage, husks, stems, and roots, act as unexplored sources of natural antioxidants. Extracting antioxidant-rich compounds from these by-products is especially attractive, as the non-edible portions of fruits and vegetables frequently possess greater bioactive content than the edible sections (Lourenço et al. 2019).

Tocopherols, carotenoids, and phenolic compounds are bioactive constituents found in plant sources, categorized as “Generally Recognized as Safe" (GRAS) food additives for human use, providing pharmaceutical, antimicrobial, and antioxidant benefits**,** as per the US Food and Drug Administration (FDA) (Brandolini et al., 2022).

Consequently, identifying natural sources, such as antioxidants from plants, is essential for addressing the issues caused by the oxidative instability of lipids and proteins in meat and meat products (Pateiro et al, 2018). Encapsulation technology, which involves enclosing bioactive materials within tiny capsules formed by a material wall, serves as an effective approach to enhance the delivery of bioactive components into meat products. It enables the regulated discharge of their contents over prolonged durations under certain circumstances. This review sought to investigate the use of bioactive natural compounds derived from plant by-products as natural antioxidants to slow down oxidative processes in these products (Hadidi et al, 2022).

1.3.1 Types of antioxidants

Compounds with antioxidant activity are used in meat systems to lessen the impact of free radicals. These substances are classified as "antioxidants," a term that is currently used to refer to any substances, even when added in small amounts, that slow down or stop the oxidation of biomolecules in food. The meat industry uses either synthetic or natural antioxidants. The origin of natural antioxidants (plants, animals, or bacteria) and their chemical makeup (phenols, tocopherols, or vitamin C) can be used to further categorize them (Halliwell, B. 1996).

Natural antioxidants are derived from plant parts, such as vegetables, fruits, seeds, cereals, herbs, spices, teas, and trees. Depending on the species of plant and the particular antioxidant compound, different plant organs, including leaves, flowers, fruits, stems, and roots, accumulate high concentrations of antioxidants. The plant parts mentioned above are either used directly in food products (like fruit juice or puree) or after the antioxidant compounds they contain have been extracted and purified, like rosemary extract (Shah et al. 2014)

Antioxidants' primary mode of action and other characteristics are linked to their chemical structure. The main categories of antioxidant substances according to their molecular structures are phenols, flavonoids, tannins, anthocyanins, isoflavonoids, vitamin C, tocopherols, lignans, stilbenes, and carotenoids (Manessis et al. 2020).

Antioxidant-rich fruit and pomace extracts can be used to boost the antioxidant content of meat products naturally. These antioxidants include water-soluble vitamin C, flavonoids, and fat-soluble vitamins and precursors like tocopherols and carotenoids. These products can be used as natural food additives (antioxidants, antimicrobials, colors, flavors, and thickeners) due to the high levels of bioactive compounds found in fruits (vitamin C, carotenoids, tocopherols, phenolic compounds, and fiber (Abd El-Khalek et al. 2013)

As natural antioxidants, numerous researchers have attempted to incorporate different fruits and their byproducts into meat products. By lowering the dosage of currently used synthetic antioxidants and adding natural antioxidants to meat products, their nutritional value could be improved, benefiting consumers' health. Plant-based food processing by-products pose a significant disposal challenge for impacted industries, but because of their advantageous nutritional or technological qualities, they also present a promising source of compounds (Peiretti et al. 2015).

Numerous bioactive compounds exist in whole grains, fruits, and vegetables. Among their highly diverse class of compounds are polyphenolic compounds, carotenoids, tocopherols, phytosterols, and organosulfur compounds, each having distinct chemical structures, natural distributions, concentrations in food and the human body, potential action sites, effectiveness against oxidative species, specificity, and biological activities (Bariya et al. 2020).

The significant attention directed toward (poly)phenolic compounds is attributed to their exceptional ability to neutralize harmful free radicals. Among these substances, flavonoids are a diverse group that includes subgroups like anthocyanins, flavanones, and flavonols. A common 15-carbon structure with two aromatic rings connected by a 3-carbon connector unites them all. Phenolic acids, which are separated into hydroxycinnamic acids (like ρ-coumaric and ferulic acids) and hydroxybenzoic acids (like gallic and ellagic acids), constitute an important class of phenolic compounds (Baenas et al. 2018).

1.3.1.1 Phenols

Phenols are byproducts of plant metabolism originating from the aromatic amino acid phenylalanine, via the shikimic acid (phenylpropanoids) and acetic acid (simple phenols) routes. They play a role in the color, flavor, and astringency of plants. Phenolic compounds can be categorized according to their:

- Origin of plant species
- Chemical composition (Table 1.1), the quantity and organization of hydroxyl groups, unsaturation in the carbon rings, and the kind and extent of alkylation and/or glycosylation
- water solubility, which influences their nutritional, metabolic, and physiological effects; a shared trait is the presence of a hydroxy-substituted benzene ring in their structure (Rice-Evans et al. 1996).

Phenolic compounds serve as hydrogen donors, effectively neutralizing free radicals, or they can transfer single electrons to reduce chemically reactive substances with oxidative properties. In addition to their antioxidant properties, phenolic compounds have an essential functional and biological role in plant physiology, serving as structural polymers, UV light protectors, pollination attractants, non-specific defense mechanisms, phytoalexins, and possibly as signaling molecules for systemic acquired resistance. Flavan monomers, dimers, and polymers, along with derivatives of cinnamic acid, constitute the primary portion of natural phenolic compounds, which are typically classified (Manessis et al. 2020).

Table 1.1 Classification of phenolic compounds based on their carbon chains (Manessis et al. 2020).

Class	Basic Skeleton	Plant Sources
Phenols and benzoquinones	C6	Sorghum, berries, fruit wines, olive oil.
Phenolic acids	C6-C1	Blueberries, blackberries, apple juice, cider, cherry, canola meal, oranges, and rye.
Acetophenones and Phenylacetic acids	C6-C2	Cocoa powder, pine, Nicotiana, and Lycopersicon
Hydroxycinnamic acids, phenylpropenes, coumarins, isocoumarins, and chromones	C6-C3	Berries, pomes, herbs, seeds, cereal grains, leafy greens, asparagus, cinnamon, cloves, and potatoes
Naphthoquinones	C6-C4	Oranges, Plumbaginaceae, Droseraceae, Ebenaceae
Xanthones	C6-C1-C6	Guttiferae, Moraceae, Clusiaceae, and Polygalaceae
Stilbenes and Anthraquinones	C6–C2–C6	Grapes, pine, peanuts, sorghum, rheum, Rubiaceae, and black pepper
Flavonoids	C6–C3–C6	Celery, parsley, red prickly pears, olives, acerola, litchis, avocadoes, green and black tea, cherries, raspberries, strawberries, grapes, and red wine
Lignans and neolignans	C6–C3	Wheat, oats, rye, barley berries
Lignins	(C6–C3) n	Spruce, wattle, birch, rice, eucalyptus, pine

- **Tannins**

Tannins are polyphenolic substances that are water-soluble and astringent, formed through the polymerization of phenylpropanoid compounds. Historically, tannins were utilized for protein precipitation because they can engage with a minimum of two protein molecules and create insoluble, cross-linked complexes of tannin and protein.

Tannins fall into categories of:

- Condensed tannins (proanthocyanidins), which are polymers composed of catechin, epicatichins, prodelphinidins, profisetinidins, and prorobinetidins.

- Hydrolysable tannins, capable of being broken down by weak acids or bases, consist of combinations of carbohydrates with gallic and ellagic acid (gallotannins and ellagitannins). Proanthocyanidins provide hydrogen and electrons (main antioxidant function), bind to iron, and suppress the activity of cyclooxygenase (additional antioxidant function). The intake of minor amounts of tannins positively influences lipid metabolism and immune response regulation, lowers blood pressure, and exhibits anticarcinogenic and antimutagenic effects. Foods high in tannins possess reduced nutritional value because tannins can flocculate proteins, leading to lower digestibility (Manessis et al. 2020).

- **Flavonoids**

  Flavonoids and isoflavonoids are present in numerous plants and originate from the aromatic amino acids, phenylalanine and tyrosine, as well as from malonate. The fundamental framework of flavonoids is the flavan core, made up of three carbon atom rings (C6-C3-C6). The degree of oxidation and the arrangement of carbon ring substitution are utilized to differentiate the categories of flavonoids (flavones, flavanones, isoflavones, flavonols, flavanonols, flavan-3-ols, anthocyanidins, biflavones, chalcones, aurones, and coumarins) (Panche et al. 2016).

  The action of flavonoids as antioxidants includes:

  1- The prevention of ROS generation by blocking enzymatic processes and binding elements that contribute to free-radical formation.
  2- The removal of ROS. Flavonoids have demonstrated considerable antioxidant potential in laboratory studies and are believed to be linked to a lower risk of developing cardiovascular diseases, hypertension, Alzheimer’s disease, and specific cancers. Nonetheless, the intestinal absorption of flavonoids in humans is restricted; hence, these advantages may remain underutilized (Panche et al. 2016).

- **Lignans**

  Lignans are phytoestrogens, which are diphenol compounds. After being substituted, cinnamic alcohols dimerize they are derived from the amino acid phenylalanine. Lignans have antioxidant properties because they function as complex divalent transition metal cations and hydrogen donors. Linseed, pumpkin and sesame seeds, broccoli, soybeans, and certain berries are the primary sources of lignans. Atherosclerosis and colon, breast, endometrial, and prostate cancer are prevented by the colon microbiota's conversion of secoisolariciresinol diglucoside, the primary lignan of linseeds, into enterodiol and enterolactone. In lipid peroxidation systems, sesame lignans boost vitamin E's antioxidant activity and radical scavenging capacity, sesamin (Gülçin et al. 2006)

- **Stilbenes**

  Stilbenes are characterized by a 1,2-diphenylethylene structure. They are categorized into monomeric and oligomeric stilbenes, with their main dietary sources being grapes and wine, peanuts, and some types of berries. Stilbenes benefit chronic diseases such as cancer, cardiovascular problems, and neurodegenerative disorders by modulating redox balance, cell proliferation, mitochondrial activity, and the production of inflammatory markers. Resveratrol, present in red wine, is the most studied stilbene due to its beneficial effects on cardiovascular health. Even with the reduced bioavailability of resveratrol from metabolic activities and the effects of gut microbiota, its metabolites are still associated with specific health benefits (Shen et al. 2009).

1.3.1.2 Vitamin E

Compounds in the Vitamin E group consist of tocopherols and tocotrienols. They are phenolic compounds produced by plants (such as cabbage, oregano, and paprika) and are important elements in the diets of humans and animals. Compounds in the Vitamin E group feature a chroman head, which comprises a phenolic ring and a heterocyclic ring, linked to a phytyl chain (Kamal-Eldin et al. 1996)

Tocopherols exist in four forms: α, β, γ, and δ, differentiated by the quantity of methyl groups on the saturated phytyl chain and the patterns of substitution on the phenolic ring. Tocotrienols are distinct from tocopherols due to the presence of three trans-double bonds in their hydrocarbon tails (Munné-Bosch et al. 2002).

The antioxidant properties of tocopherols depend on their ability to donate hydrogen to lipid-free radicals, and their antioxidant effectiveness has been demonstrated through both in vitro and in vivo studies. In both study types, the greatest antioxidant activity was observed for α-tocopherol, followed by β, γ, and δ. The greatest antioxidant ability of α-tocopherol, in vivo, is shown because of its favored retention and distribution in animal species (Kamal-Eldin et al. 1996).

Vitamin E derivatives safeguard membrane lipids by neutralizing peroxy-radicals and either extinguishing or engaging with singlet oxygen 1O2 or ROS. At the same time, the oxidative products of vitamin E compounds act as pro-oxidants, enhancing peroxidation unless reduced by vitamin C. Tocopherols positively influence protein kinase C and protein phosphatase 2 activity, as well as gene expression and cell growth, whereas tocotrienols demonstrate neuroprotective, anticancer, and cholesterol-lowering properties. These effects can be partially, if not entirely, ascribed to the antioxidant properties of vitamin E compounds (Traber et al. 2007).

1.3.1.3 Carotenoids

Carotenoids are pigment-rich, lipophilic compounds produced naturally by plants and microorganisms (such as cabbage, paprika, carrots, and acerola) but not by animals. Carotenoids have a polyisoprenoid structure, characterized by a carbon chain with conjugated carbon bonds, and exhibit a nearly bilateral symmetry around the central double bond. Cyclic end-groups are connected to the central chain and can be replaced with functional groups that have oxygen (Rao et al. 2007).

Carotenoids are further categorized into carotenes (like β-carotene and lycopene, consisting solely of carbon and hydrogen) and oxycarotenoids (xanthophylls), which include at least one oxygen atom. The carotenoids that have been most researched are lycopene (featuring eleven conjugated double bonds and two acyclic end-groups) and β-carotene (which has eleven conjugated double bonds and two cyclohexene-type groups) (Stahl et al. 2003).

The antioxidant capacity of carotenoids relies on the count of conjugated double bonds and is expressed through their ability to neutralize singlet oxygen $^1O_2$ and peroxyl radicals via physical quenching. In addition, carotenoids exhibit provitamin A activity and are linked to the control of lipoxygenases and connexin 43 gene expression. (Stahl et al. 2003).

Carotenoids show positive effects against cataracts and macular degeneration and are known to lower the risk of certain cancers (such as lung cancer) in the general population. Providing higher doses of carotenoids to high-risk groups, including smokers and asbestos workers, raises the likelihood of developing lung cancer (Stahl et al. 2005).

1.3.1.4 Vitamin C

Vitamin C, or L-ascorbic acid, is a lactone made up of six carbon atoms. It comes from glucose and is considered a vitamin for only a few vertebrate species (humans, primates, and guinea pigs) due to the absence of l-gulonolactone oxidase caused by mutations in the gene encoding the enzyme (Padayatty et al. 2003)

Vitamin C functions as an electron donor, thus counteracting free radicals and preventing the oxidation of additional compounds; it can donate two electrons from a double bond located between C2 and C3 of the six-carbon chain. Vitamin C is well-known for its role in both preventing and addressing scurvy. Furthermore, it benefits the innate and adaptive immune systems by boosting the cellular functions of neutrophils and epithelial barrier cells and encouraging the differentiation and generation of B- and T-cells. Vitamin C is essential for iron absorption, regulating hypertension, and preventing vascular disorders and stomach cancer (Linster et al.2007).

Despite its remarkable antioxidant capacity being shown in vitro, epidemiological and in vivo research suggest that healthy people are unlikely to gain further health benefits if daily intake exceeds the recommended amount. In this situation, excess vitamin C is excreted through urine due to its soluble nature in water and the restricted capacity of cells and tissues to store vitamins (Manessis et al. 2020).

1.4 Freeze-Dried Pomegranate and Raspberry

Pomegranates, *Punica granatum L*, are celebrated for their high levels of bioactive substances, especially phenolic compounds including punicalagins, ellagic acid, gallic acid, and different flavonoids such as catechin, quercetin, and rutin. Among these, punicalagins, hydrolysable tannins (Table 1.2, Fig. 1.2), are particularly prevalent and have shown notable health advantages, such as anti-inflammatory, antitumor, and antioxidant effects. The fruit also contains a significant amount of anthocyanins, which contribute to its bright red hue and strong antioxidant properties (Venkitasamy et al. 2019).

Pomegranate seed oil is a good source of punicic acid, which is a special type of fatty acid that has anti-inflammatory and anti-cancer qualities. It also contains other fatty acids, sterols, vitamins, and minerals that make it nutritious and useful for health. The many different plant chemicals in pomegranates offer health benefits and make them useful for making special foods, dietary supplements, and natural preservatives (Malik et.al. 2005).

Recently, there has been an increasing demand for pomegranates around the world. This is because pomegranates have strong antioxidant, anti-mutagenic, and blood pressure-lowering properties, and they can also help protect the liver. In traditional Asian medicine, like Ayurveda and Unani, pomegranates are seen as a valuable food for treatment (Sumner et al.2005).

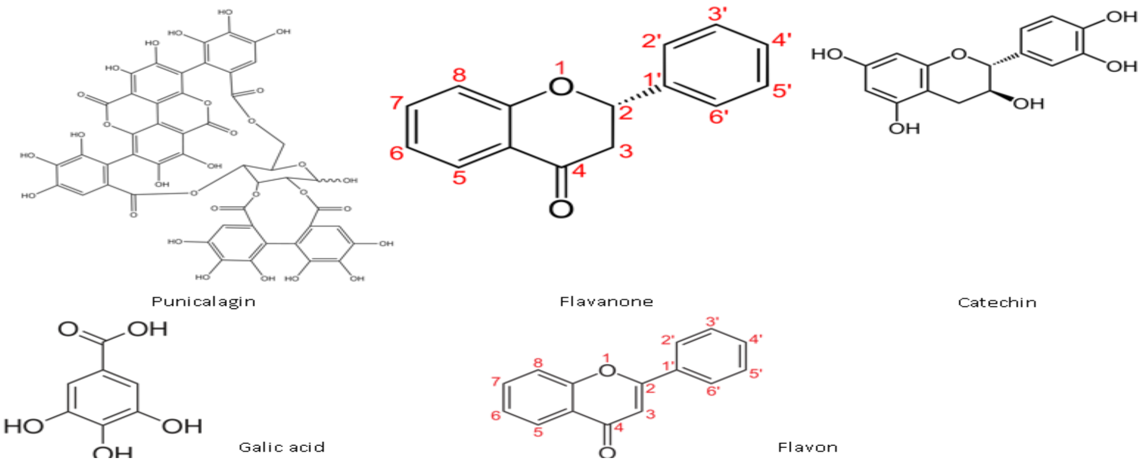

Fig. 1 .2 Representative polyphenolic compounds contributing to antioxidant function in pomegranate powders. (Celiksoy et al.2021, Manessis et al. 2020)

They are used to help with problems like stomach pain, colic, colitis with diarrhea, dysentery, vaginal discharge, paralysis, and headaches. They are also believed to help with long-term stomach issues and have anti-inflammatory and anti-atherosclerotic effects, which may help with osteoarthritis, prostate cancer, heart disease, and HIV-1 (Venkitasamy et al. 2019).

Table 1.2 Chemical Constituents of Different Parts of the Pomegranate Plant. Dhineshkumar et al. (2016)

Plant part	Constituents
Pomegranate Juice	Anthocyanins, glucose, ascorbic acid, ellagic acid, gallic acid, caffeic acid, catechin, Minerals, amino acids, quercetin, rutin
Pomegranate seed oil	95% punicic acid, ellagic acid, sterols
Pomegranate pericarp (peel, rind)	Phenolic punicalagins, gallic acid, catechin, flavones, flavonones, anthocyanidins
Pomegranate leaves	Tannins, flavone glycosides, luteolin, apigenin, Pomegranate flower, Gallic acid, ursolic acid, triterpenoids including maslinic, and asiatic acid
Pomegranate roots and bark	Ellagitannins, punicalin, and punicalagin, piperidine alkaloids

Raspberries *R. idaeus L.* are also rich in various bioactive compounds, including anthocyanins, ellagic acid, flavonoids, and dietary fiber, which all contribute to their well-known health benefits. A variety of studies revealed that different phytochemical components in berry fruits display a broad spectrum of biological activities, such as antioxidant, anticarcinogenic, vasodilatory, and antimicrobial effects. Berry fruits are regarded as a premier category of nutritious food because of their antiproliferative and anticancer properties (Vulić et al. 2014).

These substances aid in neutralizing free radicals, safeguard cells from oxidative damage, and are associated with a lower risk of developing chronic illnesses such as cancer and heart disease (Deighton et al. 2000)

Anthocyanins give raspberries their distinctive hue and serve as powerful antioxidants, aiding in the neutralization of free radicals and the reduction of oxidative stress. Ellagic acid, a polyphenol found in raspberries, shows anti-cancer and anti-inflammatory effects, akin to those in pomegranates. The intake of these berries is projected to rise significantly soon as their importance in everyday nutrition is highlighted. Flavonoids like quercetin and kaempferol also promote anti-inflammatory and heart health. Moreover, the dietary fiber found in raspberries is important for digestive health, and vitamins such as vitamin C boost immune function. Both fruits display a combination of bioactive compounds that support their healing properties, rendering them important elements of health-promoting diets and functional foods (Graham et al. 2007).

The abundant phytochemical profiles of both fruits render them beneficial functional foods and dietary sources of antioxidants, aiding health and disease prevention through their inherent bioactive compounds:

- Polyphenols: These diverse antioxidants, including punicalagins and gallic acid found in pomegranates, as well as different flavonoids present in raspberries, contribute to decreasing oxidative stress, hindering tumor development, and minimizing inflammation. They have been demonstrated to enhance heart health and safeguard against specific chronic diseases.

- Anthocyanins: These compounds give fruits their bright red to purple hues and possess powerful antioxidant properties, aiding in the neutralization of free radicals, diminishing inflammation, and possibly aiding in the prevention of neurodegenerative disorders and some cancers.

- Ellagitannins (e.g. punicalagins): commonly found in pomegranate, these substances display anti-mutagenic, anti-inflammatory, and heart-protective characteristics, and are associated with a lower risk of cardiovascular diseases and some cancers (Venkitasamy et al. 2019, Graham et al. 2007).

Global food security is a significant issue, particularly due to the swift increase in the global population and the unpredictability of future food availability. Alongside population growth, factors like heightened consumer awareness about nutritional value and food safety, climate change, and the exhaustion of natural resources complicate the task of securing food availability (Santos et al. 2025).

Many fresh plants and numerous food products are extremely perishable because of their high moisture content. Numerous fruits and vegetables contain over 80% water, making them prone to spoilage, including microbial growth and enzyme-driven chemical reactions, resulting in rapid modifications in their makeup. Therefore, eliminating and/or decreasing the water content in plants or foods has consistently been a crucial strategy for stabilizing these biomaterials, both in food processing and for future advancement as functional or nutraceutical products (Belwal et al. 2022).

Furthermore, hot-air or convective drying is commonly employed because of its affordability and ease of use. Although widely utilized, this method is linked to the deterioration of the physical, structural, and chemical properties of food items, such as their appearance, porosity, texture, microstructure, color, and the existence of bioactive compounds. It also poses difficulties concerning energy use because of prolonged processing durations (Santos et al. 2025).

Dehydrating is among the most efficient techniques for preserving fragile fruits that have elevated moisture levels and a limited harvest period. It is well recognized that freeze-drying preserves valuable food compounds more effectively than conventional drying methods (Vulić et al. 2014).

The key concept in freeze-drying is sublimation, the transition from a solid straight into a gas. Similar to evaporation, sublimation takes place when a molecule acquires sufficient energy to escape from the surrounding molecules. Water will transition from a solid (ice) to a gas (vapor) when the molecules possess sufficient energy to escape, yet the circumstances are not suitable for liquid formation (Shukla, S. 2011).

The lack of liquid water and the low temperatures needed for the process halt most decay and microbial reactions, resulting in an excellent quality final product (Vulić et al. 2014).

Freeze-drying procedure

The entire freeze-drying procedure consists of three phases:

1- Freezing: The process of freezing involves solidifying the substance. In a laboratory, this process is typically achieved by putting the substance in a freeze-drying flask and spinning the flask in a bath known as a shell freezer, which is cooled using mechanical refrigeration, dry ice with methanol, or liquid nitrogen.

Typically, the freezing temperatures range from -50°C to -80°C. This freezing stage is the most essential in the entire freeze-drying procedure, as the product may be damaged if not executed properly.

2- Primary drying: In the primary drying stage, the pressure is reduced (to a few millibars), and sufficient heat is provided to the substance to allow the water to sublimate. The quantity of heat required can be determined by using the latent heat of sublimation of the sublimating molecules. During this preliminary drying stage, approximately 95% of the moisture in the substance is sublimated. During this stage, pressure is managed by utilizing a partial vacuum. The vacuum accelerates sublimation, rendering it beneficial as an intentional drying method.

3- Secondary drying: The purpose of the secondary drying stage is to eliminate unfrozen water molecules, as the ice was already removed during the primary drying stage. This segment of the freeze-drying procedure is controlled by the adsorption isotherms of the material.

Once the freeze-drying process is finished, the vacuum is typically released with an inert gas like nitrogen prior to sealing the material. By the conclusion of the process, the remaining water content in the product is approximately 1% to 4%, indicating a very low level (Shukla, S. 2011).

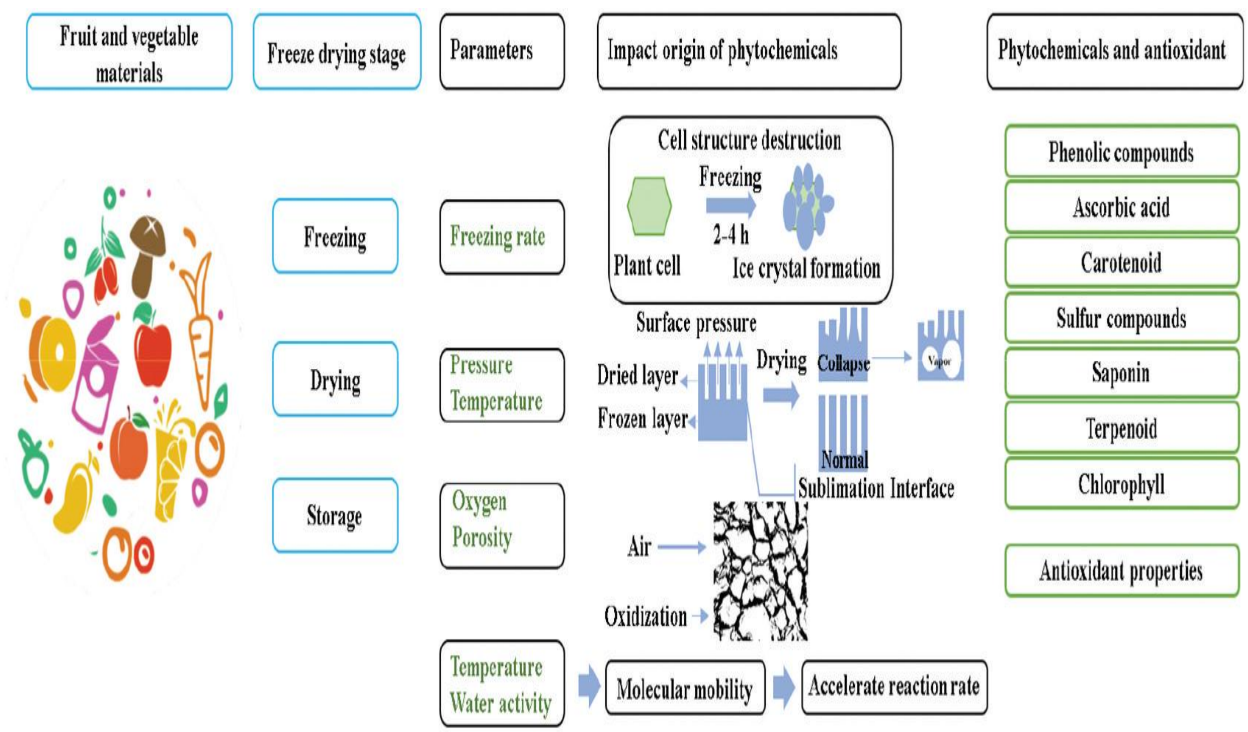

Fig. 1.3. A typical freeze-drying process, including freezing, primary drying, and secondary drying (the data were adopted from a production-type vacuum freeze dryer) (Ma et al. 2023).

Freeze-drying effectively preserves bioactive compounds in fruits and vegetables by reducing thermal and oxidative degradation. The quick-freezing process, elevated drying temperatures, and reduced drying durations aid in preserving phytochemicals and bioactive properties. Moreover, the low-temperature settings of the process avoid the degradation of heat-sensitive nutrients such as phenolics, anthocyanins, and carotenoids, essential for antioxidant function (Ma et al. 2023).

In pomegranates, freeze-drying preserves elevated amounts of phenolic compounds and anthocyanins, enhancing substantial antioxidant capacity, as shown by DPPH and ABTS radical scavenging tests. These substances can neutralize free radicals, thus diminishing oxidative stress. Moreover, extracts from pomegranate have shown antimicrobial properties against different pathogens, in part because of their phenolic compounds (Youssef et al. 2016).

Additionally, the sublimation process of freeze-drying preserves the structural integrity of cellular components, lowering enzyme activity and oxidation that may compromise bioactives. Research indicates that freeze-drying can enhance the extractability of specific phytochemicals by breaking down cell walls, thereby aiding their release and boosting their quantified levels. This structural change may result in increased phenolic and flavonoid levels after drying, aiding in the maintenance or improvement of antioxidant properties (Santos et al. 2025).

1.4.1 Application of Freeze-Dried Powders

Incorporating pomegranate and raspberry powders as functional ingredients in meat products provides significant advantages mainly because of their abundant bioactive compounds like phenolics, anthocyanins, and ellagitannins. Freeze-drying successfully safeguards these heat-sensitive phytochemicals, retaining their antioxidant qualities, which can aid in preventing lipid oxidation in meat items. This decrease in oxidation not only prolongs shelf life but also enhances sensory attributes by averting rancidity and undesirable flavors. Furthermore, the addition of these fruit powders can improve the nutritional value of meat products by introducing natural antioxidants and bioactives that promote health benefits like cardiovascular protection and anti-inflammatory properties.

Moreover, fruit powders can enhance both the functional and visual aspects of meat products. Natural pigments such as anthocyanins can enhance color stability, increasing the visual appeal of the product. Utilizing natural fruit extracts meets consumer preferences for clean-label, minimally processed products, decreasing dependence on artificial preservatives. Incorporating freeze-dried powders of pomegranate and raspberry as functional additives can enhance product quality, prolong shelf life, and provide additional health advantages, making them essential elements in creating healthier, functional meat products (Santos et al. 2025, Ma et al. 2023).

1.5 Problem Statement

This study focuses on enriching the knowledge gap regarding the use of natural food additives to improve the quality and health advantages of processed meat products, such as roast beef. Meat industry heavily depends on artificial antioxidants and preservatives to prolong shelf life and preserve product quality, even though consumers are increasingly demanding "clean label" products with fewer artificial preservatives.

Due to possible health risks, these artificial additives, like BHT and BHA, are coming under more scrutiny, necessitating the development of efficient, natural substitutes. The food industry sector faces a big challenge as a result of this reliance on artificial ingredients: it needs to develop innovative strategies to stop lipid oxidation and microbial spoiling in meat without sacrificing consumer appeal or safety.

Furthermore, roast beef processed and stored using conventional methods frequently results in lipid oxidation, a major cause of quality deterioration that causes rancid flavors, discoloration, and a loss of nutritional value. Although many plant-based extracts have been investigated as natural antioxidants, the use of freeze-dried pomegranate and raspberry powders, which are rich in polyphenols and anthocyanins, in roast beef has not received sufficient attention.

Thus, there are two issues: the need for a natural and safe substitute for artificial antioxidants, and the paucity of studies on the effectiveness of these particular fruit powders in preventing quality deterioration in a complex matrix such as roast beef. To close this knowledge gap, this study will investigate how these powders can enhance the antioxidant properties and overall quality of roast beef. Offering a novel and health-conscious approach to meat preservation.

1.6 Aim of the Thesis

I. **Evaluate the Antioxidant Efficacy**
Assess the effectiveness of FDPP and FDRP in reducing oxidative rancidity and preserving the quality of roast beef during storage.

II. **Analyze Antimicrobial Activity**
Evaluate the antimicrobial efficacy of FDPP and FDRP on the bacterial activity (TPC, *Coliform, and E.coli)* in roast beef

III. **Compare with Synthetic Antioxidants**
Compare the performance of freeze-dried pomegranate and raspberry with conventional synthetic antioxidants (e.g., BHT, BHA, sodium erythorbate) in terms of shelf-life extension and sensory qualities.

IV. **Examine Flavor and Sensory Attributes**

Investigate the influence of these natural additives on roast beef's sensory characteristics, such as taste, aroma, color, and texture.

V. **Study Consumer Acceptability**

Assess consumer acceptance and preferences for roast beef treated with natural antioxidants, including willingness to purchase products enhanced with natural ingredients.

1.7 Hypothesis

- The incorporation of freeze-dried pomegranate and raspberry as natural antioxidants in roast beef will effectively inhibit oxidative methods, thereby improving shelf-life, maintaining sensory features, and improving basic product balance in comparison to conventional synthetic antioxidants.
- Pomegranate and raspberry, rich in polyphenols with potent antioxidant properties, are promising natural candidates.
- This hypothesis is based on the fact that pomegranate and raspberry are rich in natural phenolic compounds, which include flavonoids and tannins, which are regarded for their antioxidant properties. The study focused on probable objectives to test the effectiveness of plant-based alternatives to synthetic additives in meat renovation
- It is hypothesized that the incorporation of FDPP and FDRP into roast beef will significantly extend its shelf life by creating a synergistic 'Hurdle Effect.' Specifically, these fruit powders are expected to act as natural antioxidants and antimicrobial hurdles that, when combined with refrigerated storage, will inhibit lipid oxidation and suppress microbial proliferation more effectively than a negative control. Furthermore, it is hypothesized that at specific concentrations, these natural hurdles will maintain the sensory attributes and quality of the roast beef, providing a viable clean-label alternative to synthetic preservatives like sodium erythorbate.
- The integration of freeze-dried pomegranate and raspberry powders into roast beef preparation is strategically designed to evaluate their efficacy as safer, natural alternatives to synthetic chemical preservatives. This study focuses on assessing the antioxidant and antimicrobial potential of these fruit-based additives, both individually and in combination, to identify the optimal synergy for enhancing product quality. By shifting from synthetic to natural antioxidants, the research aims to meet consumer demand for clean-label products while maintaining the highest standards of food safety and shelf-life stability.
- Freeze-dried pomegranate and raspberry have antimicrobial activity on the growth of the roast beef pathogenic and spoilage bacteria.

Chapter Two

Literature Review

This chapter reviews prior research related to the inclusion of plant-based ingredients to enhance the quality and antioxidant properties of food products. It focuses on the use of freeze-dried pomegranate and raspberry powder due to their rich phytochemical composition and powerful biological activities, including antioxidant and antimicrobial effects.

The extraction and drying techniques of fruit powders, their characterization, and their application as natural antioxidants in meat products are discussed in the relevant literature. Additionally, previous studies on the physicochemical properties, shelf-life, and sensory characteristics of fruit powder-treated meat products have been reviewed to establish the basis for the present study.

2.1 Natural Antioxidants: Sources and Benefits

The review by (Das et al. 2021) emphasizes the promising role of pomegranate by-products as natural additives for improving the quality, safety, and shelf life of muscle foods. It highlights their rich content of bioactive phenolic compounds with antioxidant and antimicrobial properties that help reduce lipid and protein oxidation, enhance color stability, and extend product shelf life. The review also discusses the health benefits associated with pomegranate derivatives, suggesting their role in developing healthier and more sustainable meat products. Overall, it presents pomegranate by-products as valuable natural alternatives to synthetic preservatives.

Similarly, (Noreen et al. 2025; and Gullón et al. 2020) investigate the potential of pomegranate peel as a valuable source of bioactive compounds suitable for meat products. They highlight the growing consumer demand for clean-label foods and the need to replace synthetic preservatives with natural alternatives. Pomegranate peel is recognized for its rich content of phenolics and antioxidants that can enhance the oxidative stability, microbial safety, and nutritional quality of meat commodities, aligning with sustainable practices by valuing agri-food by-products.

The review by (Lorenzo et al. 2018) emphasizes that lipid and protein oxidation are the main causes of meat spoilage. While synthetic antioxidants have been widely used, increasing consumer demand for natural additives has shifted attention toward plant-based alternatives. Berries, including blueberries, blackberries, and strawberries, are highlighted as rich sources of phenolic compounds and anthocyanins that exhibit strong antioxidant properties. The review concludes that berry extracts and their by-products have significant potential as natural replacements for synthetic antioxidants.

Further reviews from (Bellucci et al. 2022, Munekata et al.2020, Maneses et al. 2020, Aminazar et al. 2019, Ribeiro et al. 2019, Aziz 2018) collectively confirm that plant extracts obtained from fruits, leaves, spices, and other sources are rich in compounds such as phenolics and carotenoids. These natural compounds effectively inhibit lipid and protein oxidation, delay discoloration, and maintain sensory quality, making them a promising alternative to synthetic antioxidants. These authors also discuss the potential of innovative delivery systems such as microencapsulation and active packaging to increase their efficacy.

(Barbosa et al. 2023), Highlight the increasing trend of using natural antioxidants such as mango peel and pomegranate extracts in chicken meat products due to health concerns over synthetic additives. They noted that these natural alternatives can effectively delay lipid oxidation and maintain meat quality without altering its physical properties. The review by (Efenbarger-Szmechtyk et al. 2021) provides an overview of plant extracts rich in polyphenols, emphasizing their strong antioxidant and antimicrobial properties that can inhibit spoilage and pathogenic microorganisms.

(Petcu et al. 2023) reviewed the replacement of synthetic antioxidants with plant-based alternatives, highlighting how natural compounds can enhance oxidative stability, extend shelf life, and improve the sensory and nutritional properties of meat. The review by (Ahmad et al. 2013) also confirms that fruit-based natural antioxidants are an effective alternative to synthetics, as their high content of phenolic compounds helps protect against oxidative degradation that affects meat quality. (Aziz et al. 2018) further explore the use of natural antimicrobial and antioxidant agents from various plant sources, noting that their strong activity is due to bioactive compounds such as phenolic acids and flavonoids.

2.2. Experimental Studies on Antioxidant Application

An experimental investigation by (Das et al. 2021) was conducted to evaluate the use of pomegranate peel extract (PPE) as a natural antioxidant in lamb patties. The study compared the effects of PPE to a control group (no additive) and a positive control group using a synthetic antioxidant (BHT). The researchers measured several parameters over a storage period of 14 days at 4 °C, including oxidative index (using TBARS and peroxide value), color stability (using a Hunter Lab colorimeter), and shelf life (through microbial analysis). Two different concentrations of PPE were evaluated in the study: a low concentration of 500 ppm and a high concentration of 1000 ppm. The Results section provides detailed data on how each of these concentrations affected the oxidative stability, color, and microbial growth of lamb patties. It was found that 1000 ppm of PPE was the most effective in reducing lipid oxidation and maintaining color stability.

In another original research article, (Chandralekha et al. 2012) described a controlled experiment on the use of pomegranate peel powder extract as a natural antioxidant in chicken meatballs. The methodology involved preparing different batches, including a control group and groups with different additives. Treatments included 2.5% and 5% of pomegranate peel powder with synthetic additives (BHA and BHT) for comparison. Chicken meatballs were stored under refrigeration at 4 ± 1 °C for up to 8 days, with samples analyzed every two days for physicochemical properties, microbial count, and organoleptic quality.

They have a look at offers, particular concentration values, and quantitative effects, focusing on the pomegranate rind powder extract's effect on the meat. The results confirmed that the cooking loss was drastically lower, and the pH values were drastically lower inside the samples with 5% pomegranate rind powder extract. The 2-TBARS values, which imply lipid oxidation, had been additionally extensively decreased inside the meatballs with 5% pomegranate rind powder extract than within the different formulations (0.37 ± 0.04 in comparison to the control's 0.79 ± 0.07).

Additionally, the samples with 5% pomegranate rind powder extract had appreciably decreased population plate counts (4.72 ± 0.12 as compared to the control organization's 5.62 ± 0.17) and better emulsion stability for the duration of refrigerated storage.

2.3 Processing Techniques and Evaluation Metrics

According to (Ma et al. 2023), freeze-drying (FD) is broadly recognized as a superior dehydration technology for fruits and vegetables due to its capability to retain dietary quality, sensory characteristics, and bioactive compounds. The evaluation emphasized that FD parameters together with freezing charge, drying temperature, vacuum degree, and garage conditions play an important role in determining phytochemical retention. While FD regularly leads to minimal structural and nutrient loss, certain compounds continue to be vulnerable to extended drying or expanded temperatures. The assessment additionally describes the three phases of freeze-drying (freezing, primary drying, and secondary drying) and info various processing parameters from the reviewed research that affect the final product's excellence, including freezing rates, drying temperatures, pressures, and times.

The review by (Lorenzo et al. 2018) mentions several methods used by other researchers to assess oxidation in meat, such as measuring the levels of peroxide value (PV) and thiobarbituric acid-reactive substances (TBARS), as well as quantification of sulfhydryl and carbonyl groups. This review focuses on the use of berry extracts in various meat products, including pork/porcine meat and Frankfurter sausages. It also provides specific concentration and quantitative results from the studies it reviews, such as a study on bearberry extract that found that a concentration of 1000 mg/kg prevented the increase in TBARS values, indicating dose-dependent antioxidant potential. Another study cited in the review on blackberry extract found that extract-enriched pork patties had a significant reduction in protein carbonyls during cooking and cold storage, with the enriched samples having 3.23 nmol carbonyls/mg protein after 12 days, while the control group had 9.52 nmol carbonyls/mg.

(Noreen et al. 2025) Additionally, provide examples of concentrations and quantitative consequences from the reviewed research. For instance, pomegranate peel powder was found to have a complete phenol content of 18.75 GAE mg/g and an antioxidant activity of 59.64%. An ethanolic pomegranate peel extract was observed to have an inhibitory effect against five pathogens at a concentration of 15 mg/ml, while an extract with a concentration of 0.33 g/mL had an inhibitory impact on *Staphylococcus aureus*. Additionally, pomegranate powder had a minimal fungicidal awareness (MFC) of one hundred µg/mL in opposition to *Candida albicans*, and an ethanolic extract showed good-sized outcomes in opposition to lipase with a cost of 603.50 µg/ml.

Chapter Three

Materials and Methods

3.1 Materials

3.1.1 Instruments and Tools

IKA T-25 Ultra Turrax Homogenizer, Labnet International C0226R centrifuge, Jenway 74 Series Spectrophotometer, HunterLab colorimeter, Digital thermometer, Hot plate, Water bath from Jouan, Graduated cylinder, Test tubes and Test tubes rack, Micro pipets, Spatula, Thermometer, Funnel, Beaker, colony counter, betri dishes.

3.1.2 Natural Antioxidant Powders

Food-grade freeze-dried raspberry and pomegranate powders were obtained from Diaita Foods (Portugal). These powders served as the primary natural antioxidants in this study.

3.1.3 Chemicals and Microbiological Media

All chemicals used in this investigation were of analytical reagent grade. The chemicals for the oxidative stability assay, including thiobarbituric acid (TBA), trichloroacetic acid (TCA), and hydrochloric acid (HCl), were purchased from Sigma-Aldrich, Germany. Sodium erythorbate will be used as a PC and was also sourced from Siniora Food Industries Co., Jerusalem, Palestine. For microbiological analysis, media such as Plate Count Agar, Violet Red Bile Agar (VRBA), and Eosin Methylene Blue (PCA) were purchased from Oxoid Company.

3.1.4 Meat Product

Boneless beef cuts, specifically from the beef shoulder, suitable for producing roast beef, were obtained from Siniora Food Industries Company, Jerusalem, Palestine. The beef cuts were received frozen and immediately transported to the thawing room until the core temperature was down from -18 to about 0-2 °C, and then kept refrigerated to begin the second step of processing, which is trimming and cutting to remove all unwanted parts from the beef shoulder. Visible impurities, connective tissue, or fat are meticulously removed manually using knives to ensure consistency and quality. laboratory in insulated containers to maintain a temperature of 0-2°C before processing.

3.2 Methods

3.2.1 Preparation of Roast Beef Samples

The beef cuts were prepared for injection with the antioxidant solutions, which contain water and a mixture of seasoning, salt, and synthetic antioxidant (in case of normal application in industry), and this step was substituted with pomegranate and raspberry powder in our study. Separate brine solutions were formulated for each treatment.

The roast beef was processed using a multi-needle injection system. The brine solution, provided by Siniora Food Industries, was incorporated at a 45% injection level relative to the initial raw meat weight. This brine contained a standardized commercial blend of water, salt, soy protein, seasoning, phosphates, and nitrate designed to achieve the desired functional and sensory characteristics of the final product. For the positive control group, sodium erythorbate was added to the formulation, while for the treatment groups, the brine served as the carrier for the freeze-dried pomegranate and raspberry powders.

An NC group was prepared by injecting the brine solution without the addition of any antioxidant. A PC was established by injecting a solution containing 100 ppm of sodium erythorbate. The natural antioxidant treatments were prepared by dissolving the freeze-dried powders in the brine solution at specific concentrations. The beef was divided into multiple treatment groups, with each group receiving a different injection as follows:

- Treatment 1: NC (injected with saline solution only).
- Treatment 2: PC Control (injected with 100 ppm sodium erythorbate solution).
- Treatment 3: Injected with a solution containing 0.3% FDRP.
- Treatment 4: Injected with a solution containing 0.7% FDRP.
- Treatment 5: Injected with a solution containing 1.5% FDRP.

- Treatment 6: Injected with a solution containing 0.3% FDPP.
- Treatment 7: Injected with a solution containing 0.7% FDPP.
- Treatment 8: Injected with a solution containing 1.5% FDPP.

A professional meat injector was used to ensure a uniform distribution of the solution, flavor, and preservative throughout the meat, targeting an injection level of approximately 30% of the meat's raw weight. The injected roast beef is now transported to the tumbler machine to ensure the homogenized contribution of remaining brine solution in the beef shoulder and to keep it under refrigerated and vacuum conditions to avoid oxidation.

3.2.2 Cooking and Storage of Samples

All beef cuts were cooked in a preheated oven until a target internal (core) temperature of 75°C was reached; the cooking time depended on the core temperature and the weight of the pieces. The internal (core) temperature of the meat was monitored by a calibrated digital meat thermometer to ensure uniformity across the sample. After cooking, the roasts were rapidly cooled to a core temperature of 4°C. The samples were then sliced into uniform pieces, vacuum-packed under aseptic conditions to minimize oxidation, and stored in a refrigerated unit at 4°C for the duration of the study period (60 days), which was the shelf life of the product.

3.2.3 Analytical Methods

Samples were analyzed on designated days (day 0, 15, 30, 45, and 60) to monitor their quality parameters.

3.2.3.1 Oxidative Stability (TBARS Assay)

The oxidative stability of the refrigerated roast beef was determined using a Thiobarbituric Acid Reactive Substances (TBARS) assay, described by (Zamel 2022).

1- A 5 g sample from each treatment was homogenized (IKA T-25 Ultra Turrax Homogenizer) with 50 mL of a solution containing 0.38% TBA and 15% TCA in 0.25N HCl at 10,000 rpm for 3 min.
2- Three 15 mL samples taken from the homogenate were then heated in a boiling water bath for 45-60 minutes to allow the pink color to develop.
3- After developing the pink color, the samples were cooled in tap water

4- Then the samples were centrifuged at 5000 rpm for 10 minutes (Labnet International C0226R) to clarify the solution.
5- The absorbance of the supernatant was measured at 532 nm using a spectrophotometer (Jenway 74 Series Spectrophotometer).
6- An average of three absorbance values was used to determine the oxidative stability of the refrigerated samples (Aytul, 2010; Bekhit et al., 2003), with higher absorbance indicating greater oxidation. A calibration curve prepared with malondialdehyde (MDA) standards at various concentrations (1-15 ppm) was used to quantify the results in mg MDA/kg sample.

3.2.3.2 Microbiological Analysis

The microbiological quality was assessed by determining the TPC. A 10 g sample from each treatment was homogenized in 90 mL of sterile peptone water to create a 10^{-1} dilution. Appropriate decimal dilutions were then pour-plated with Plate Count Agar (PCA) in triplicate. The plates were incubated at 37°C for 36 hours (Adam et al. 2010), and colonies were counted using a colony counter (Gerber, Colony Star 8500). The results were expressed as CFU per gram of sample.

Total Coliform count. From the same prepared sample for Total Plate Count TPC, appropriate decimal dilutions (10^{-1}-10^{-2}) of roast beef samples were pour-plated on 15 ml VRBA. The medium was allowed to solidify before incubating at 37 °C for 24 h. Typical dark red colonies were considered as coliform colonies (Firstenberg-Eden et al., 2004).

For microbial assay, colony counts between 25 and 250 were used for calculating the number of CFU per gram of sample according to the formula, colony count = n * 1/V * 1/d (Shewail et al., 2018). Where n is the number of colonies counted per plate, V is the volume of inoculum in each plate (ml), and d is the dilution factor used to determine the colony count. The average number of countable colonies after the incubation time of the triplicate plates was used for the calculations.

3.2.3.3 Color Measurement

The coloration stability of the sliced roast beef was measured using a HunterLab colorimeter. L* (lightness), a* (redness), and b* (yellowness) values have been recorded from 3 different locations on every sample slice. The average values for every treatment were used for statistical analysis to assess the effect of the antioxidants on meat coloration (King et al. 2023).

3.2.3.4 ph Measurement

The pH values of the roast beef samples were determined using a digital pH meter (Model Hanna HI 2210, Hanna Instruments, USA) equipped with a glass electrode. The measurements were performed by inserting the probe directly into the core of the roast beef samples at three different locations to ensure a representative and accurate reading. Before each measurement session, the device was calibrated using standard buffer solutions at pH 4.0 and 7.0. The average of the three readings was recorded for each treatment throughout the 60-day refrigerated storage period to monitor any acidity changes (Honikel, K. O. 1998).

3.2.3.5 Sensory Evaluation

Consumer acceptance of the roast beef samples was evaluated by a panel of 50 untrained consumers. Sensory analysis of the roast beef samples was performed through a collaborative effort involving staff members from Siniora Food Industries and external volunteers ,familiar with the product. The sensory attributes assessed included Color, Odor, Texture, Taste, and Overall Acceptability. The evaluation was performed using a 9-point hedonic scale, ranging from 1 (dislike extremely) to 9 (like extremely). Samples from each treatment group (FDPP, FDRP, PC, and NC) were sliced into uniform pieces and served at room temperature in identical containers. Each sample was labeled with a unique three-digit random code to prevent bias. To ensure accuracy, panelists were provided with water to cleanse their palates between tasting different samples. The evaluation was conducted in a controlled environment to minimize external distractions (Civille et al. 2024).

3.3 Data Analysis

For all information, the mean ± standard deviation (SD) was used. Two-way evaluation of variance (ANOVA) was used for statistical analysis to verify whether the remedy companies differed significantly from each other. A post-hoc test, just like the Fisher test for LSD, was then used to decide which precise remedies were substantially distinct from each other. For statistical significance, a p-value of less than 0.05 ($p <$ zero.05) was used. Utilizing the SPSS software program, the statistical analysis was completed

Chapter Four

Results and discussions

The combined findings and analysis of the study assessing the effects of FDPP and FDRP on the oxidative stability, microbial load, physicochemical properties, color attributes, and sensory quality of roast beef during chilled storage are presented in this chapter. Three concentrations of both natural antioxidants (0.3%, 0.7%, and 1.5%) were used, and the results were compared to positive and negative controls. The total efficacy of these natural extracts in enhancing product quality and shelf life is assessed by interpreting the results in light of oxidative and microbiological mechanisms.

4.1 Lipid Oxidation (TBARS Test)

One of the primary reasons meat and meat products lose their quality is lipid oxidation, which leads to rancidity, odd tastes, and texture and color changes. The rate of oxidation, measured in milligrams of malondialdehyde (MDA) per kilogram of roast beef (Fig.4.1), was assessed using the thiobarbituric acid reactive substances (TBARS) test.

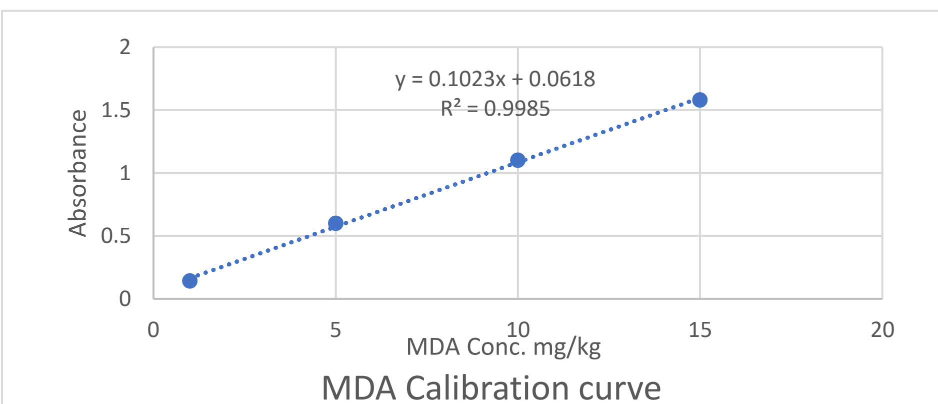

Fig.4.1 Calibration curve of MDA

After 60 days of refrigerated storage, all samples exhibited a marked increase in MDA content (Table 4.1), indicating the ongoing oxidative degradation of lipids. However, the degree of lipid oxidation differed between treatments, emphasizing the significance of antioxidant quantity and type.

Table 4.1 Oxidation products of refrigerated roast beef samples treated with different concentrations of Freeze-dried powder (0.3, 0.7, and 1.5%) determined by the TBARS (mg MDA/kg) method.

Treatment	Day 0	Day 15	Day 30	Day 45	Day 60
PC	3.08±0.11c	3.22±0.12d	3.39±0.13e	3.96±0.14e	4.29±0.15f
NC	3.78±0.13b	4.26±0.15b	4.49±0.16b	5.49±0.18a	9.40±0.22a
FDRP 0.3%	3.76±0.13b	4.16±0.14b	4.25±0.15c	4.68±0.17c	5.61±0.19d
FDRP 0.7%	3.85±0.13b	4.57±0.16b	4.62±0.16b	4.92±0.18c	6.02±0.20c
FDRP 1.5%	4.66±0.15a	6.14±0.19a	6.45±0.20a	6.93±0.21b	7.47±0.23b
FDPP 0.3%	3.61±0.12b	3.74±0.13c	4.02±0.14d	4.23±0.15d	4.99±0.17e
FDPP 0.7%	2.91±0.11c	3.19±0.12d	4.17±0.15c	4.93±0.18c	5.01±0.17e
FDPP 1.5%	4.85±0.16a	5.43±0.18a	5.51±0.19a	6.25±0.20b	6.40±0.21c

Note: small letters (a-f) indicate differences in the amounts of oxidation products for control samples and treated samples (0.3, 0.7, and 1.5%) at each storage time, LSDs=0.42

Because untreated beef is naturally susceptible to oxidation, the NC exhibited the highest TBARS values across all storage periods, reaching approximately 9.40 mg MDA/kg on day 60. Conversely, the sodium erythorbate-treated PC maintained the lowest values (about 4.29 mg/kg), demonstrating its efficacy as a synthetic antioxidant.

According to Table 4.2, FDPP at 0.3% and 1.5% confirmed the strongest antioxidant overall performance among freeze-dried fruits treatments, reducing MDA accumulation by 75.4% and 72.4%, respectively, as compared to the NC. Notably, FDPP 0.3% changed into the most effective 14% more than the PC, indicating close to-equal oxidative stability.

On the other hand, FDRP 1.5% decreased MDA by 50% as compared to NC and increased PC by 132%, suggesting confined efficacy at higher doses. These consequences confirm that mild concentrations of FDPP are more effective than higher ones, probably because of the principal phenolic activity without saturation or interference. Similar trends have been observed in other studies; for instance, (Naveena et al. 2008) demonstrated that pomegranate rind powder significantly lowers TBARS values in meat samples, though the protective effect may vary based on the concentration used. Furthermore, the antioxidant efficacy of berry-derived extracts in meat systems has been attributed to their high phenolic and anthocyanin content, which acts as a potent radical scavenger, as documented by (Özvural and Vural 2011). While these previous studies primarily focused on comminuted or ground meat, the current findings extend this efficacy to whole-muscle roast beef, showcasing FDPP 0.3% as a superior clean-label alternative to synthetic preservatives.

Table 4.2 Percentage Reduction ins MDA Levels by Natural Treatments Compared to Negative and Positive Controls

Treatment	Δ MDA (Day 60 − Day 0)	% Higher than PC Δ (1.21)	Lower than NC (%)
FDPP 0.3%	1.38	14.00%	75.40%
FDPP 1.5%	1.55	28.10%	72.40%
FDRP 0.3%	1.85	52.90%	67.10%
FDPP 0.7%	2.1	73.60%	62.60%
FDRP 0.7%	2.17	79.30%	61.40%
FDRP 1.5%	2.81	132.20%	50.00%

Note: lower than NC reflects the percentage reduction in MDA accumulation compared to the negative control. Higher than PC shows how much each treatment exceeds the synthetic antioxidant's performance

MDA levels were measured using the TBARS assay on days 0, 15, 30, 45, and 60. Treatments included FDPP, FDRP, NC, and PC.

The abundance of phenolic substances, including ellagic acid and punicalagins, which scavenge free radicals and bind transition metals that promote lipid oxidation, is responsible for FDPP's excellent antioxidant efficacy. As reported by (Gil et al. 2000), these specific polyphenols in pomegranate exhibit superior antioxidant activity compared to other fruits due to their unique chemical structure and synergistic effects. These findings suggest that moderate dosages of pomegranate powder are more effective in preserving oxidative stability than higher dosages.

Overall, FDPP and FDRP slow lipid oxidation in roast beef during refrigerated storage, according to TBARS data. The most promising natural antioxidant was FDPP, especially in modest doses, which offered a useful substitute for artificial additions.

4.2 pH Changes During Storage

An important factor affecting meat quality, microbial development, and shelf life is pH. Initial pH values were typical of freshly processed roast beef, ranging from 6.01 to 6.50, which was in the range of accepted results (5.8-6.5), according to the Palestinian Standards Institution.

All treatments exhibited modest declines in pH during the 60 days of storage, although the degree of reduction differed between treatments (Table 4.3). The NC sample showed the largest decrease, from 6.50 to 6.20, indicating microbial metabolic activity and protein breakdown. The PC sample showed the stabilizing impact of sodium erythorbate on meat pH, remaining comparatively stable at around 6.20.

The most stable samples were those treated with FDPP, which maintained the pH between 6.10 and 6.31 for 0.3% and around 6.00 for 0.7% and 1.5%. This slight decrease suggests that FDPP successfully inhibited oxidative processes and microbiological activity. This stability is consistent with the findings of (Devatkal et al. 2010), who observed that pomegranate extract effectively limits the pH fluctuations in meat samples by controlling the microbial growth and enzymatic activities during refrigerated storage

On the other hand, FDRP samples, particularly at 1.5%, showed a more pronounced pH drop (6.01 to 5.80), probably as a result of organic acid generation during anthocyanin degradation .As noted by (Ganhão et al. 2010), the addition of fruit extracts rich in organic acids can lead to a significant initial reduction and subsequent changes in meat pH, which may also be linked to the degradation of phenolic compounds over time.

Table 4.3 pH of refrigerated roast beef samples (controls and treated samples)

Treatment	Day 0	Day 15	Day 30	Day 45	Day 60
PC	3.08±0.11c	3.22±0.12e	3.39±0.13e	3.96±0.14f	4.29±0.15f
NC	3.78±0.13b	4.26±0.15b	4.49±0.16b	5.49±0.18b	9.40±0.22a
FDRP 0.3%	3.76±0.13b	4.16±0.14b	4.25±0.15b	4.68±0.17c	5.61±0.19c
FDRP 0.7%	3.85±0.13b	4.57±0.16b	4.62±0.16b	4.92±0.18c	6.02±0.20c
FDRP 1.5%	4.66±0.15a	6.14±0.19a	6.45±0.20a	6.93±0.21a	7.47±0.23b
FDPP 0.3%	3.61±0.12b	3.74±0.13d	4.02±0.14c	4.23±0.15d	4.99±0.17d
FDPP 0.7%	2.91±0.11d	3.19±0.12e	4.17±0.15c	4.93±0.18c	5.01±0.17d
FDPP 1.5%	4.85±0.16a	5.43±0.18a	5.51±0.19a	6.25±0.20a	6.40±0.21c

Note: different lowercase letters within each column indicate statistically significant differences between treatments (LSD = 0.12, $p < 0.05$). Shared letters mean no significant difference between those treatments.

4.3 Microbiological Activity

The main indicator of meat deterioration and storage safety is microbial growth. Microbial quality was assessed over 60 days using TPC, *coliforms*, and *E. coli* levels.

4.3.1 Total Plat Count

Microbiological Evaluation of Roast Beef Samples Treated with Natural Antioxidants

Throughout the 60-day refrigerated storage period at 4°C, all roast beef samples exhibited a progressive increase in microbial counts. The NC, which lacked both synthetic preservatives and natural antioxidant treatments, consistently showed the highest bacterial loads, reaching 320 cfu/g by day 60. This group served as a baseline for evaluating the antimicrobial efficacy of all treatments (Table 4.4)

In contrast, the PC, which contained sodium erythorbate and sodium nitrite, maintained the lowest microbial counts across all time points, ending at 80 cfu/g. This confirms the strong antibacterial effect of conventional preservatives and provides a reference point for assessing the performance of natural alternatives.

Table 4.4 Total Microbial Count of Roast Beef Samples at 10^{-1} Dilution (cfu/g)

Treatment	Day 0	Day 15	Day 30	Day 45	Day 60
PC	50±4 d	60±5 e	60±5 e	70±5 e	80±6 e
NC	90±6 a	130±8 a	200±10 a	260±12 a	320±15 a
FDRP 0.3%	65±5 c	80±6 c	100±7 c	130±8 c	160±9 c
FDRP 0.7%	60±5 c	70±5 d	85±6 d	110±7 d	130±8 d
FDRP 1.5%	55±4 d	65±5 e	75±6 e	90±6 e	110±7 e
FDPP 0.3%	60±5 c	70±5 d	90±6 d	110±7 d	130±8 d
FDPP 0.7%	55±4 d	65±5 e	75±6 e	90±6 e	110±7 e
FDPP 1.5%	50±4 d	60±5 e	65±5 e	75±6 e	90±6 e

Note: Microbial counts were determined using serial dilution and expressed as colony-forming units per gram (cfu/g). Different lowercase letters within each column indicate statistically significant differences among treatments on the same day (LSD ≈ 25 cfu/g, $p < 0.05$).

Treatment with FDRP at 1.5% followed, presenting a final count of 110 cfu/g, which corresponds to a 65.6% reduction in relation to NC and 37.5% greater than PC. This inhibitory effect is supported by (Lacombe et al. 2012), who demonstrated that berry phenolic fractions possess potent antimicrobial properties capable of reducing aerobic counts in food systems by inhibiting bacterial enzyme activity and disrupting cell membranes. (Table 4.5).

At the intermediate concentration of 0.7%, both fruit powders exhibited moderate microbial suppression. Treatment with 0.7% FDPP reached 110 cfu/g, representing a 65.6% decrease in relation to NC. These results are in agreement with (Al-Zoreky 2009), who confirmed that pomegranate peel extract is highly effective in inhibiting the total aerobic counts in various meat types due to its high content of phenolic compounds like tannins.

At the lowest concentration (0.3%), the antibacterial effects were weaker. Treatment with 0.3% FDPP yielded 130 cfu/g, while 0.3% FDRP showed the lowest inhibitory effect with 160 cfu/g. As noted by (Tajkarimi et al. 2010), the antimicrobial efficacy of plant extracts in food is strictly dose-dependent; lower concentrations often provide insufficient active molecules to neutralize the microbial load, especially in complex food matrices like meat.

Table 4.5 Antibacterial Effectiveness of Natural Treatments Based on Microbial Reduction Compared to Controls (Day 60)

Treatment	Final Count (cfu/g)	Increase vs PC (%)	Reduction vs NC (%)
PC	80	0%	300%
NC	320	300%	0%
FDRP 0.3%	160	100.00%	50.00%
FDRP 0.7%	130	62.50%	59.40%
FDRP 1.5%	110	37.50%	65.60%
FDPP 0.3%	130	62.50%	59.40%
FDPP 0.7%	110	37.50%	65.60%
FDPP 1.5%	90	12.50%	71.90%

Among the natural treatments, FDPP at 1.5% demonstrated the most potent antibacterial activity, achieving a final microbial count of 90 cfu/g. This represents a 71.9% reduction compared to the NC and was only 12.5% higher than the PC, indicating near-equivalent antimicrobial effectiveness to the synthetic antioxidant treatment.

These results clearly demonstrate a dose-dependent antibacterial effect for both FDPP and FDRP. FDPP's superior performance at all concentrations can be attributed to its higher ellagic acid content and broader polyphenolic profile, which improve its antimicrobial mechanisms. Specifically, FDPP 1.5% likely exerts its effect through radical scavenging and metal ion chelation, disrupting microbial membranes and enzyme systems essential for bacterial survival. This mechanism is consistent with (Al-Zoreky 2009), who highlighted that pomegranate phenolic compounds effectively inhibit a wide range of foodborne pathogens by altering cell membrane permeability.

Interestingly, FDPP 0.3% outperformed FDRP 0.3%, maintaining microbial counts closer to PC despite the low concentration. However, increasing FDPP concentration beyond 0.3% did not yield proportional improvements, possibly due to saturation effects or interactions with meat matrix components that limit bioavailability. Similar non-linear responses have been reported by (Tajkarimi et al. 2010), suggesting that the complex interaction between plant polyphenols and meat proteins can sometimes mask the active sites of the antimicrobial agents at higher concentrations.

Overall, the inclusion of FDPP, especially at 1.5%, offers a promising clean-label alternative to synthetic preservatives in roast beef formulations. While FDRP also shows potential, its efficacy at lower concentrations is limited. These findings align with the work of (Lacombe et al. 2012), who observed that while berry extracts are effective, their antimicrobial potency in meat systems is highly dependent on the concentration and the specific phenolic profile of the extract.

4.3.2 *Coliform* and *E. coli* Counts

***Coliform* inhibition**

The antimicrobial efficacy of FDRP was evaluated at three concentrations (0.3%, 0.7%, and 1.5%) in roast beef samples during a 60-day refrigerated storage period. While sodium nitrite was incorporated across all treatments to provide a foundational antimicrobial barrier, the Positive Control (PC) was further stabilized with sodium erythorbate. In contrast, the Negative Control (NC) relied solely on nitrite, lacking any additional antioxidant or antimicrobial reinforcement. The study specifically focused on the synergistic potential of FDRP and FDPP as potent bio-preservatives; these natural extracts were expected to enhance the microbial stability of the meat by acting as secondary antimicrobial hurdles against spoilage and pathogenic bacteria, thereby serving as functional alternatives to synthetic preservatives.

Over the 60-day refrigerated garage duration, *coliform* counts revealed clear differences in microbial manipulation between all designed treatments. NC, which lacked any antioxidant or antimicrobial components, exhibited the highest bacterial proliferation, recording 80 cfu/g by day 60. In the opposition, the PC, containing sodium erythorbate and sodium nitrite, maintained the lowest count number at 14 cfu/g, confirming the efficacy of artificial preservatives (Table 4.6)

Table 4.6 (a) Total *coliform* counts across treatment

Treatment	Day 0	Day 15	Day 30	Day 45	Day 60
PC	<10a	<10b	<10b	<10c	14±2c
NC	<10a	22±3a	38±4a	60±5a	80±6a

Table 4.6 (b) Total *coliform* counts across treatment

FDRP 0.3%	<10a	<10b	<10b	18±3b	35±4b
FDRP 0.7%	<10a	<10b	<10b	12±2b	22±3bc
FDRP 1.5%	<10a	<10b	<10b	<10c	16±2c
FDPP 0.3%	<10a	<10b	<10b	16±2b	28±3b
FDPP 0.7%	<10a	<10b	<10b	<10c	20±3bc
FDPP 1.5%	<10a	<10b	<10b	<10c	14±2c

Note: microbial counts for coliforms were determined using serial dilution and expressed as cfu/g. Values below the detection limit (<10 cfu/g) were considered non-detectable. Different lowercase letters within each column indicate statistically significant differences among treatments on the same day (LSD ≈ 10 cfu/g, $p < 0.05$).

Among the natural treatments, FDPP at 1.5% showed the strongest antibacterial effect, with a final microbial count of 14 cfu/g, which was statistically similar to the PC and represented an 82.5% reduction compared to the NC. The FDPP 0.7% treatment came next, reaching 20 cfu/g, corresponding to a 75% reduction relative to NC and only 42.9% higher than the PC. Meanwhile, FDPP at 0.3% reached 28 cfu/g, resulting in a 65% reduction compared to NC and a microbial count that was 100% higher than the PC, indicating a weaker antibacterial response at the lowest concentration (Table 4.7).

For the raspberry applications, FDRP 1.5% confirmed promising activity with a final coliform count of 16 cfu/g, which was only 14.3% higher than PC and 80% decrease than NC. The FDRP 0.7% category recorded 22 cfu/g, reflecting a 72.5% decrease as compared to NC and 57.1% higher than PC at the same time, as the FDRP 0.3% treatment exhibited the least efficacy, accomplishing 35 cfu/g, equivalent to a 56.25% reduction versus NC and 150% higher microbial load than PC.

These results verify a dose-based antibacterial impact for each FDPP and FDRP. However, FDPP always outperformed FDRP at all equal concentrations, probably due to its better ellagic acid content and broader polyphenolic spectrum. Notably, FDPP at 1.5% matched the synthetic PC in microbial suppression, reinforcing its ability as a clean-label natural preservative for improving the microbiological stability of roast beef for the duration of refrigerated storage.

Table 4.7 (a) Comparison between treatments in the Total *Coliform* test

Treatment	Final Count (cfu/g)	Increase vs PC (%)	Reduction vs NC (%)
PC	14	0%	471.40%
NC	80	471.40%	0%
FDRP 0.3%	35	150.00%	56.30%

Table 4.7 (b) Comparison between treatments in the Total *Coliform* test

FDRP 0.7%	22	57.10%	72.50%
FDRP 1.5%	16	14.30%	80.00%
FDPP 0.3%	28	100.00%	65.00%
FDPP 0.7%	20	42.90%	75.00%
FDPP 1.5%	14	0.00%	82.50%

***E. coli* Inhibition**

The presence or absence of *Escherichia coli* in meat products is a critical sentinel indicator of fecal contamination and the overall hygienic quality of processing and handling protocols. Throughout the 60-day refrigerated storage period*, E. coli* counts remained undetectable across all treatment groups, including both control and antioxidant-enriched samples. This consistent absence confirms that the thermal processing effectively minimized the initial microbial load and that the sanitary standards maintained during raw material selection and production were sufficient to prevent contamination. According to (Al-Zoreky 2009), maintaining high sanitary standards, combined with the use of natural preservatives, is essential for ensuring that ready-to-eat meat products remain within safe regulatory limits.

Beyond baseline hygiene, the incorporation of natural antioxidants such as (FDRP) and (FDPP) may have provided an additional hurdle against microbial survival. While these extracts were primarily utilized for oxidative stability and color retention, they contribute to the product's safety by creating an environment less conducive to bacterial proliferation. This aligns with the findings of (Lacombe et al. 2012), who demonstrated that phenolic compounds in berry and fruit extracts can disrupt the cell membranes of Gram-negative bacteria like *E. coli*, thereby preventing microbial revival or recontamination during extended storage. These findings reinforce consumer confidence in using natural bio-preservatives as a viable 'clean-label' alternative to synthetic additives without compromising the microbiological safety of the roast beef.

4.4 Color Evaluation

Color plays a vital role in consumer perception and the acceptability of meat. To evaluate the visual attributes of the treated samples of roast beef with FDRP and FDPP, color parameters (L*, a*, and b*) were evaluated over a 60-day refrigeration period. Sodium nitrite was present in all treated samples, but sodium erythorbate, as a synthetic antioxidant that is known to stabilize the color of roast beef, was present in the PC sample. Erythorbate and natural antioxidants were absent from the NC, which included only nitrite.

4.4.1 Lightness (L*)

The NC sample showed the most noticeable darkening by Day 60 (L* = 34.2), indicating oxidative deterioration and the formation of metmyoglobin. Lightness (L*) values gradually decreased throughout storage in all treatments (Table 4.8); however, the PC sample remained significantly lighter (L* = 39.8), demonstrating the stabilizing impact of sodium erythorbate. Notably, the treatments with 1.5% FDRP and 1.5% FDPP exhibited the most effective surface brightness preservation, maintaining L* values of 40.2 and 39.5, respectively. These values were significantly higher than NC (LSD = 1.5, $p < 0.05$) and statistically comparable to PC. The efficacy of pomegranate extract in stabilizing L* values and delaying meat discoloration is well-documented by (Devatkal et al. 2010), who found that its potent antioxidant components inhibit the oxidation of pigments and lipids. Similarly, the protective role of berry extracts against oxidative browning has been confirmed by (Ganhão et al. 2010), who attributed the maintenance of lightness to the high radical-scavenging activity of anthocyanins, which protects the muscle proteins from degradation.

Table 4.8 Lightness (L) values of roast beef samples during refrigerated storage (n=3)

Treatment	Day 0	Day 15	Day 30	Day 45	Day 60
NC	42.0±1.2b	40.5±1.3b	38.8±1.4c	36.5±1.5d	34.2±1.6d
PC	42.5±1.2b	41.8±1.3b	41.2±1.4b	40.5±1.5b	39.8±1.6a
FDRP 0.3%	43.0±1.1b	42.2±1.2b	41.5±1.3b	40.0±1.4b	38.5±1.5ab
FDRP 0.7%	44.2±1.0ab	43.0±1.1ab	42.0±1.2ab	40.8±1.3b	39.0±1.4a
FDRP 1.5%	45.5±0.9a	44.0±1.0a	42.8±1.1a	41.5±1.2a	40.2±1.3a
FDPP 0.3%	42.8±1.1b	41.5±1.2b	40.2±1.3b	39.0±1.4c	37.5±1.5b
FDPP 0.7%	43.5±1.0ab	42.0±1.1b	40.8±1.2b	39.5±1.3bc	38.0±1.4b
FDPP 1.5%	44.8±0.9a	43.5±1.0a	42.2±1.1ab	40.8±1.2b	39.5±1.3a

Note: different lowercase letters within each column indicate significant differences among treatments (LSD ≈ 1.5, $p < 0.05$).

4.4.2 Redness (a*)

"The visual freshness of roast beef is directly reflected in its redness (a*), which serves as a crucial indicator of oxymyoglobin stability. Throughout the 60-day refrigerated storage, all treatments showed a decrease in a* values due to pigment oxidation; however, the rate of decline varied significantly between groups. Among the samples, the NC displayed the largest fall, decreasing from 13.5 to 7.5. While this drop is numerically substantial, it is important to note that the statistical significance of such changes depends on the specific sampling point, yet it clearly indicates remarkable metmyoglobin formation and pigment loss. In contrast, the PC confirmed the stabilizing effect of sodium erythorbate, maintaining a greater redness (a* = 11.2).

Table 4.9 shows that FDRP 1.5% and FDPP 1.5% achieved the highest redness values (13.2 and 12.5, respectively), exceeding both NC and PC (LSD ≈ 0.8, $p < 0.05$). This suggests that elevated levels of powders loaded with polyphenols, specifically the anthocyanins in raspberries and the ellagic acid in pomegranates, can enhance color stability enough to outperform artificial antioxidants. Notably, the intense redness observed in these treatments may be partly attributed to the natural pigments present in the fruit powders themselves, which can impart a supplementary reddish hue to the meat matrix, thereby masking oxidation-induced browning. This dual effect of antioxidant protection and natural pigmentation aligns with the findings of (Devatkal et al. 2010), who noted that pomegranate peel extracts not only inhibit lipid oxidation but also contribute to the retention of the red hue in meat products.

FDPP's enhanced effectiveness is associated with its ability to chelate iron ions and eliminate free radicals, preventing the heme iron in oxymyoglobin from being oxidized to metmyoglobin. While the anthocyanins in FDRP assist in pigment preservation, their natural coloring properties may impart a subtle purplish shade, contributing to a distinct visual intensity. This phenomenon of natural color enhancement by berry extracts is consistent with the observations of (Ganhão et al. 2010), who suggested that fruit polyphenols act as a protective shield for meat pigments while simultaneously providing a stable natural color source. In general, the cured meat color was effectively maintained by both FDPP and FDRP at 1.5%, with FDPP offering a more uniform visual appearance and FDRP contributing to a heightened intensity of redness.

Table 4.9 Redness (a) values of roast beef samples during refrigerated storage (n=3)

Treatment	Day 0	Day 15	Day 30	Day 45	Day 60
NC	13.5±0.6d	12.0±0.7d	10.5±0.8d	9.0±0.9d	7.5±1.0e
PC	14.2±0.6cd	13.5±0.7cd	12.8±0.8c	12.0±0.9c	11.2±1.0d
FDRP 0.3%	14.5±0.5bc	13.8±0.6cd	13.0±0.7c	12.2±0.8c	11.5±0.9cd
FDRP 0.7%	15.0±0.5b	14.2±0.6bc	13.5±0.7b	12.8±0.8b	12.0±0.9bc

Table 4.9 Redness (b) values of roast beef samples during refrigerated storage (n=3)

FDRP 1.5%	16.2±0.4a	15.5±0.5a	14.8±0.6a	14.0±0.7a	13.2±0.8a
FDPP 0.3%	14.0±0.6cd	13.2±0.7cd	12.5±0.8c	11.8±0.9c	11.0±1.0d
FDPP 0.7%	14.8±0.5b	14.0±0.6bc	13.2±0.7b	12.5±0.8b	11.8±0.9bc
FDPP 1.5%	15.5±0.4ab	14.8±0.5ab	14.0±0.6ab	13.2±0.7ab	12.5±0.8b

Note: different lowercase letters within each column indicate statistically significant differences among treatments, based on LSD analysis (≈ 0.8 units, $p < 0.05$).

4.4.3 Yellowness (b*)

Throughout the 60-day refrigerated storage period, yellowness (b*) consistently declined across all treatments, reflecting similar patterns to lightness (L*) and redness (a*). The NC decreased to 6.0, indicating significant pigment oxidation and surface browning. On the other hand, the PC settled at 7.2, demonstrating the protective role of sodium erythorbate (Table 4.10).

The two natural antioxidant treatments that exhibited the highest b* values (7.6 and 7.8) were FDPP 1.5% and FDRP 1.5%, respectively, reflecting improved surface freshness and reduced browning. This retention of yellowness in treated samples is consistent with the findings of (Devatkal et al. 2010), who observed that pomegranate peel extract effectively stabilizes b* values in cooked meat by inhibiting lipid oxidation products that lead to yellow-brown discoloration. Specifically at elevated concentrations, these variations reached statistical significance (LSD ≈ 0.6, $p < 0.05$).

Even with the presence of nitrite, color deterioration in the NC group was hastened due to the absence of sodium erythorbate, which typically acts as a color stabilizer. In every color parameter, natural antioxidants at 1.5% (especially FDRP and FDPP) demonstrated superior color retention, often matching or exceeding the PC group. This stabilization of yellowness (b*) is supported by (Devatkal et al. 2010), who indicated that the decline in b* values during storage is often linked to the progression of oxidative reactions, which natural polyphenols in fruit extracts can effectively mitigate by acting as potent free radical scavengers. These findings confirm that fruit powders rich in polyphenols can serve as a viable clean-label alternative to synthetic antioxidants in roast beef products.

These findings indicate that fruit powders rich in polyphenols can serve as effective substitutes for artificial additives in roast beef products, providing clean-label options while maintaining visual attractiveness.

Table 4.10 Yellowness (b) values of roast beef samples during refrigerated storage (n=3)

Treatment	Day 0	Day 15	Day 30	Day 45	Day 60
NC	8.0±0.4c	7.5±0.5c	7.0±0.6c	6.5±0.6d	6.0±0.7d
PC	8.5±0.4bc	8.2±0.5bc	7.8±0.6b	7.5±0.6c	7.2±0.7c
FDRP 0.3%	8.8±0.4bc	8.5±0.5bc	8.0±0.6b	7.6±0.6bc	7.3±0.7bc
FDRP 0.7%	9.2±0.3ab	8.8±0.4ab	8.3±0.5ab	7.8±0.6b	7.5±0.6bc
FDRP 1.5%	9.8±0.3a	9.2±0.4a	8.7±0.5a	8.2±0.6a	7.8±0.6a
FDPP 0.3%	8.6±0.4bc	8.3±0.5bc	7.9±0.6b	7.5±0.6c	7.2±0.7c
FDPP 0.7%	9.0±0.3ab	8.6±0.4bc	8.2±0.5ab	7.7±0.6b	7.4±0.6bc
FDPP 1.5%	9.5±0.3a	9.0±0.4ab	8.5±0.5a	8.0±0.6ab	7.6±0.6ab

Note: different lowercase letters within each column indicate significant differences (LSD ≈ 0.6, $p < 0.05$).

4.5 Sensory Evaluation

Fifty participants conducted a sensory evaluation to determine the effects of FDPP and FDRP on the sensory quality of roast beef samples. The negative control (NC) contained only sodium nitrite, while the positive control (PC) included sodium erythorbate; all treatments incorporated sodium nitrite. Sensory attributes assessed included color, flavor, texture, aroma, mouthfeel, overall acceptability, and preference. (Table 4.11).

In each sensory category, FDPP 1.5% and the PC achieved the highest scores, showing excellent preservation and enhancement of color, flavor, texture, and acceptability. Like PC and FDPP 0.3%, FDRP 0.3% also showed effective performance while maintaining satisfactory sensory characteristics.

Table 4.11 (a) Sensory scores of roast beef samples (Mean ± SD, n = 50 panelists)

Treatment	Color	Flavor	Texture	Aroma	Mouthfeel	Overall Acceptability	Preference
NC	5.8±0.6c	6.0±0.7c	5.5±0.8c	5.9±0.6c	5.6±0.7c	5.7±0.6c	5.5±0.7c
PC	7.8±0.5a	7.5±0.6a	7.2±0.6a	7.6±0.5a	7.3±0.6a	7.5±0.5a	7.4±0.6a
FDRP 0.3%	7.5±0.5ab	7.2±0.6ab	7.0±0.6ab	7.4±0.5ab	7.1±0.6ab	7.3±0.5ab	7.2±0.6ab
FDRP 0.7%	6.5±0.6b	6.8±0.6b	6.0±0.7b	6.9±0.6b	6.2±0.7b	6.5±0.6b	6.0±0.7b

Table 4.11 (b) Sensory scores of roast beef samples (Mean ± SD, n = 50 panelists)

FDRP 1.5%	6.0±0.7b	6.5±0.7b	5.8±0.8b	6.7±0.6b	6.0±0.7b	6.2±0.6b	5.8±0.7b
FDPP 0.3%	7.6±0.5ab	7.3±0.6ab	7.1±0.6ab	7.5±0.5ab	7.2±0.6ab	7.4±0.5ab	7.3±0.6ab
FDPP 0.7%	7.7±0.5a	7.4±0.6a	7.2±0.6a	7.6±0.5a	7.3±0.6a	7.5±0.5a	7.4±0.6a
FDPP 1.5%	7.8±0.5a	7.5±0.6a	7.3±0.6a	7.7±0.5a	7.4±0.6a	7.6±0.5a	7.5±0.6a

Note: different lowercase letters within each column indicate statistically significant differences among treatments (LSD ≈ 0.5, $p < 0.05$).

FDRP 0.7% and 1.5%, conversely, earned lower ratings for taste, consistency, and preference. Natural fruit colors and gentle acidity, which can alter flavor perception and reduce juiciness, were cited as the reasons for these decreases. Consistent with the raspberry's anthocyanin-rich characteristics, panelists noted a darker appearance and a drier mouthfeel when tasting these samples. This reduction in sensory scores at higher concentrations is consistent with the findings of (Ganhão et al. 2010), who observed that high levels of fruit polyphenols can interact with muscle proteins, potentially affecting water-holding capacity and resulting in a tougher texture or drier mouthfeel.

Samples treated with FDPP showed a harmonious flavor and juiciness, likely due to reduced lipid oxidation and enhanced moisture retention. The polyphenolic compounds found in pomegranate, especially ellagic acid and punicalagins, enhanced oxidative stability while maintaining sensory quality. As documented by (Devatkal et al. 2010), pomegranate extracts effectively preserve the desirable sensory attributes and red hue of meat products without imparting off-flavors, making them highly acceptable to consumers even after extended storage.

FDPP 1.5% often matched or slightly surpassed PC in overall acceptability, indicating its promise as a clean-label substitute for synthetic antioxidants. Conversely, the NC sample consistently garnered the lowest scores across all categories. This reinforces the necessity of antioxidant supplementation to prevent the development of rancid odors and pigment loss, as both (Devatkal et al. 2010 and Ganhão et al. 2010) emphasize that natural plant extracts are essential for maintaining the visual and eating quality of processed meats in the absence of synthetic additives.

4.6 Comparison with literature review

A thorough evaluation of the literature shows that previous studies on natural antioxidants in meat systems have predominantly centered on plant extracts, including rosemary, sage, green tea, and grape seed, as well as pomegranate peel extracts, all of which are recognized for their polyphenolic richness and radical-scavenging properties (Das et al. 2021; Gullón et al. 2020). Although those studies continuously reported reductions in lipid oxidation, they also noted limitations in sensory acceptability due to robust herbal notes, color changes, or phenolic harshness (Lorenzo et al. 2018;

Bellucci et al. 2022). Moreover, most earlier investigations have been performed on minced meat products, patties, or sausages, where antioxidant incorporation is technically easier and flavor deviations are more tolerated (Chandralekha et al. 2012). In comparison, the present study affords a complete and methodologically distinct evaluation via making use of freeze-dried pomegranate powder (FDPP) and freeze-dried raspberry powder (FDRP) via brine injection into roast beef (a whole-muscle), cooked, and cured meat system with greater stringent sensory expectancies and higher susceptibility to oxidative rancidity for the duration of extended storage.

This injection-based technique, which has not been previously documented for those fruit powders, enabled deeper integration into the meat matrix, resulting in significantly superior oxidative stability. This aligns with the necessity of replacing synthetic preservatives with natural alternatives to meet clean-label demands (Noreen et al. 2025). Specifically, FDPP at 1.5% performed a 75% reduction in malondialdehyde (MDA) relative to the NC and produced TBARS values comparable to synthetic antioxidants. This advanced overall performance exceeds the antioxidant effects generally suggested in research using pomegranate derivatives or raspberry extracts, such as the findings of (Chandralekha et al.2012 and Das et al. 2021), who reported significant reductions in lipid oxidation but in simpler meat matrices. By using freeze-drying (FD), which is recognized by (Ma et al. 2023) as a superior technology for retaining bioactive phytochemicals and sensory quality, this research ensured the maximum protection of the meat matrix.

Beyond oxidative stability, this study advances the literature by presenting a multidimensional assessment encompassing antimicrobial activity, color preservation, and sensory quality parameters that prior studies regularly evaluated one after the other (Petku et al. 2023; Ahmad et al., 2013). While earlier studies have proven the antimicrobial and color-stabilizing capability of fruit-derived phenolics (Noreen et al. 2025), applications were in large part constrained to surface treatments or mixing into ground meat. The 1.5% FDPP treatment resulted in a 71.9% decrease in total plate count, which approached sodium erythorbate performance and exceeded the microbial reductions observed in previous studies using pomegranate rind powder (Chandralekha et al. 2012). This antimicrobial efficacy is further supported by (Effenbarger-Szmechtyk et al. 2020), who emphasized the role of polyphenols in inhibiting spoilage microorganisms. The research established that FDPP and FDRP at elevated concentrations maintained redness (a*) values and enhanced visual appearance, consistent with the role of anthocyanins and phenolics in delaying discoloration as highlighted by (Lorenzo et al. 2019). Furthermore, the methodology used to assess these changes, including TBARS and colorimetric analysis, aligns with the evaluation metrics described by (Lorenzo et al. 2018). The sensory tests confirmed that FDPP maintained or enhanced flavor and juiciness, whereas high phenolic levels in other plant extracts typically cause sensory problems (Munekata et al. 2020; Barbosa et al. 2023). The commercial value of FDPP as a natural preservative becomes evident because it demonstrates dual antioxidant and antimicrobial properties (Aziz et al. 2018). This research establishes itself as a groundbreaking study because it uses fruit-based bioactive powders to improve roast beef storage duration and product quality.

Chapter Five

Conclusions and recommendations

This research efficaciously illustrated the efficacy of freeze-dried fruit powders, in particular pomegranate (FDPP) and raspberry (FDRP), as natural antioxidants and antimicrobial treatments in roast beef at some stage in refrigerated garage conditions. The findings substantiated the research hypothesis positing that polyphenol-rich fruit powders possess the ability to mitigate lipid oxidation and microbial proliferation, thereby presenting a clean-label opportunity to synthetic antioxidants, including BHT and BHA

Across all concentrations evaluated, each FDPP and FDRP notably diminished the formation of secondary lipid oxidation products, as evidenced by reduced thiobarbituric acid reactive substances (TBARS) values for the duration of the storage period. Nonetheless, FDPP always exhibited superior overall performance relative to FDRP in terms of oxidative and microbial stability. At the treatment of 1.5%, FDPP afforded oxidative balance that became statistically significant compared to the PC and decreased malondialdehyde (MDA) accumulation by means of over 75% in terms of the NC, thereby corroborating its powerful antioxidative capacity.

Microbiological tests corroborated these consequences, demonstrating that FDPP at 1.5% attained a reduction of up to 71.9% in TPC, while in comparison to NC, it displayed overall performance almost equal to that of PC. This antibacterial activity can be attributed to the extended phenolic content of pomegranate, especially punicalagins and ellagic acid, recognized for their radical-scavenging and metal-chelating properties. Although FDRP manifested considerable antioxidant and antimicrobial outcomes, its performance became incredibly mild in comparison to FDPP, with the most efficient efficacy determined at 0.3%. Elevated concentrations of FDRP (0.7% and 1.5%) verified dwindling effectiveness, probably due to interactions between pigments and lipids or the degradation of anthocyanins

Physicochemical investigations indicated that the incorporation of these powders preserved strong pH stability and chromatic attributes without adversely influencing the beef matrix. Samples treated with FDPP exhibited more advantageous lightness and redness, signifying advanced oxidative stability and normal product aesthetics.

From a sensory standpoint, FDPP exhibited exceptional consumer acceptance. Formulations incorporating 0.7% and 1.5% FDPP completed elevated ratings for taste, texture, and overall acceptability, suggesting considerable commercial viability. Conversely, samples treated with FDRP received marginally decreased sensory reviews, predominantly attributed to color alterations and the emergence of a faint fruity taste at high concentrations.

In summary, the application of FDPP at a concentration of 1.5% demonstrated substantial efficacy as a dual natural antioxidant and antimicrobial agent, efficiently rivaling the performance of synthetic preservatives. Furthermore, lower concentrations, specifically 0.3%, attained the most effective equilibrium between preservative effectiveness and sensory quality. These outcomes robustly support the use of FDPP as a clean-label, natural alternative for enhancing the quality and safety of roast beef. Specifically, under refrigerated storage at 4°C, FDPP treatments successfully extended the product's shelf life to 60 days, significantly delaying oxidative rancidity and microbial spoilage compared to untreated samples. These findings confirm the potential of FDPP for the preservation of roast beef and analogous heat-treated meat products throughout extended cold storage chains.

Recommendations

In light of the findings derived from this investigation, a series of recommendations is posited for subsequent practical implementation and research advancement.

1. Industrial Application: The application of FDPP at a concentration of 1.5% is encouraged for tremendous usage within the meat processing industry as a clean-label preservative. It is imperative to conduct industrial-scale trials to verify its efficacy under commercial situations, compare its processing stability, and ascertain the economic viability of the ingredient of elements in relation to the extension of shelf-life.

2. Formulation Optimization: Although high concentrations can augment antioxidant efficacy, immoderate stages exceeding 1.5% may not yield extra advantages and will probably modify sensory attributes. Hence, slight incorporation levels starting from 0.3% to at least 1.5% are deemed ideal to sustain product acceptability and practical effectiveness

3. Advanced Research on Active Compounds: Subsequent investigations have to prioritize the isolation and concentrations of the bioactive phenolic compounds discovered in pomegranate, especially ellagitannins and ellagic acid, to facilitate lower inclusion rates without compromising antioxidant effectiveness. Furthermore, the exploration of encapsulation technology is suggested to enhance the stability and controlled release of those bioactive compounds through storage and culinary applications.

4. Synergistic Formulations: Exploring the interactive outcomes of FDPP in conjunction with FDRP may additionally yield an optimized formulation that harnesses the antioxidant properties of pomegranate alongside the sensory attributes of raspberry. Such combinations could probably provide customizable maintenance structures tailored for numerous meat products or regional flavor tendencies.

5. Predictive Shelf-Life Modeling: Future studies endeavors should include oxidative kinetic modeling and complex analytical methodologies (inclusive of volatile compound profiling) to predict shelf-life with precision and establish standardized exceptional quality control metrics for both regulatory endorsement and commercial scalability.

6. Consumer and Regulatory Considerations: Emphasizing the incorporation of natural antioxidants like FDPP on product labeling may resonate with health-aware purchasers in pursuit of clean-label alternatives. Moreover, adherence to food safety and labeling guidelines and regulations should be ensured prior to the commercial implementation of those practices.

References

- Abd El-Khalek, H. H., & Zahran, D. A. (2013). Utilization of fruit by-product in ground meat preservation. *Food Science and Quality Management*, 11, 49-60.

- Adam, Y. S. I., & Abugroun, H. A. (2010). Effect of Sudanese marketing condition on quality attributes of meat products. *Pakistan Journal of Nutrition*, *9*(12), 1149-1156.

- Ahmad, S. R., Gokulakrishnan, P., Giriprasad, R., & Yatoo, M. A. (2015). Fruit-based natural antioxidants in meat and meat products: A review. *Critical reviews in food science and nutrition*, *55*(11), 1503-1513.

- Al-Shibli, M. A., Al-Ali, R. M., Hashim, A. Z., Altemimi, A. B., Elsayed, N., & Abedelmaksoud, T. G. (2023). Evaluation of meat and meat product oxidation and off-flavor formation: Managing oxidative changes. *Теория и практика переработки мяса*, *8*(4), 302-315.

- Al-Zoreky, N. S. (2009). Antimicrobial activity of pomegranate (*Punica granatum L.*) fruit peels. *International Journal of Food Microbiology*, 134(3), 244-248.

- Aminzare, M., Hashemi, M., Ansarian, E., Bimakr, M., Hassanzad Azar, H., Mehrasbi, M. R., ... & Afshari, A. (2019). Using natural antioxidants in meat and meat products as preservatives: A review. *Advances in Animal and Veterinary Sciences*, *7*(5), 417-426.

- Aytul, K. K. (2010). *Antimicrobial and Antioxidant Activities of Olive Leafextract and Its Food Applications* (Master's thesis, Izmir Institute of Technology (Turkey)).

- Aziz, M., & Karboune, S. (2018). Natural antimicrobial/antioxidant agents in meat and poultry products as well as fruits and vegetables: A review. *Critical reviews in food science and nutrition*, *58*(3), 486-511.

- Baenas, N., Abellán, Á., Rivera, S., Moreno, D. A., García-Viguera, C., & Domínguez-Perles, R. (2018). Foods and supplements. In *Polyphenols: Properties, Recovery, and Applications* (pp. 327-362). Woodhead Publishing.

- Barbosa, A. C. S., Mendes, P. S., Mattos, G., Fuchs, R. H. B., Marques, L. L. M., Beneti, S. C., ... & Cardoso, F. A. R. (2023). Comparative analysis of the use of natural and synthetic antioxidants in chicken meat: an update review. *Brazilian Journal of Biology*, *83*, e275539.

- Bariya, A. R., Patel, A. S., & Joshi, A. B. (2020). Application of natural antioxidants from vegetables, plant and fruits origin into meat and meat products-A review. *Journal of Meat Science and Technology| January-March*, *8*(1), 12-22.

- Bekhit, A. E. D., Geesink, G. H., Ilian, M. A., Morton, J. D., & Bickerstaffe, R. (2003). The effects of natural antioxidants on oxidative processes and metmyoglobin reducing activity in beef patties. *Food Chemistry*, *81*(2), 175-187.

- Bellucci, E. R. B., Bis-Souza, C. V., Domínguez, R., Bermúdez, R., & Barretto, A. C. D. S. (2022). Addition of natural extracts with antioxidant function to preserve the quality of meat products. *Biomolecules*, *12*(10), 1506.

- Belwal, T., Cravotto, C., Prieto, M. A., Venskutonis, P. R., Daglia, M., Devkota, H. P., ... & Cravotto, G. (2022). Effects of different drying techniques on the quality and bioactive compounds of plant-based products: A critical review on current trends. *Drying Technology*, *40*(8), 1539-1561.

- Brandolini, A., Glorio-Paulet, P., Estivi, L., Locatelli, N., Cordova-Ramos, J. S., & Hidalgo, A. (2022). Tocopherols, carotenoids and phenolics changes during Andean lupin (Lupinus mutabilis Sweet) seeds processing. Journal of Food Composition and Analysis, 106, 104335.

- Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology*, 94(3), 223-253.

- Celiksoy, V., & Heard, C. M. (2021). Antimicrobial potential of pomegranate extracts. In *Pomegranate*. Intechopen.

- Chandralekha, S., Babu, A. J., Moorthy, P. S., & Karthikeyan, B. (2012). Studies on the effect of pomegranate rind powder extract as natural antioxidant in chicken meat balls during refrigerated storage. *Journal of Advanced Veterinary Research*, *2*(2), 107-112.

- Civille, G. V., Carr, B. T., & Osdoba, K. E. (2024). *Sensory Evaluation Techniques*. CRC Press.

- Dahham, S. S., Ali, M. N., Tabassum, H., & Khan, M. (2010). Studies on antibacterial and antifungal activity of pomegranate (*Punica granatum L.*). *Am. Eurasian J. Agric. Environ. Sci*, 9(3), 273-281.

- Das, A. K., Nanda, P. K., Chowdhury, N. R., Dandapat, P., Gagaoua, M., Chauhan, P., ... & Lorenzo, J. M. (2021). Application of pomegranate by-products in muscle foods: oxidative indices, colour stability, shelf life and health benefits. *Molecules*, *26*(2), 467.

- Dave, D., & Ghaly, A. E. (2011). Meat spoilage mechanisms and preservation techniques: a critical review. *Am J Agric Biol Sci*, 6(4), 486-510.

- Deighton, N., Brennan, R., Finn, C., & Davies, H. V. (2000). Antioxidant properties of domesticated and wild Rubus species. *Journal of the Science of Food and Agriculture*, 80(9), 1307-1313.

- Devatkal, S. K., & Naveena, B. M. (2010). Effect of salt, kinnow and pomegranate fruit by-product powders on color and oxidative stability of raw ground goat meat during refrigerated storage. *Meat Science*, 85(2), 306-311.

- Devatkal, S. K., Narsaiah, K., & Borah, A. (2010). Anti-oxidant effect of extracts of kinnow rind, pomegranate rind and seed powders in cooked goat meat patties. *Meat Science*, 85(1), 155-159.

- Dhineshkumar, V., & Ramasamy, D. (2016). Pomegranate processing and value addition: A review. *Magnesium*, *44*, 200.

- Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W., & Lorenzo, J. M. (2019). A comprehensive review on lipid oxidation in meat and meat products. Antioxidants, 8(10), 429.

- Efenberger-Szmechtyk, M., Nowak, A., & Czyzowska, A. (2021). Plant extracts rich in polyphenols: Antibacterial agents and natural preservatives for meat and meat products. *Critical reviews in food science and nutrition*, *61*(1), 149-178.

- Falowo, A. B., Fayemi, P. O., & Muchenje, V. (2014). Natural antioxidants against lipid–protein oxidative deterioration in meat and meat products: A review. *Food research international*, *64*, 171-181.

- Firstenberg-Eden, R., Foti, D., McDougal, S., & Beck, S. (2004). Performance comparison of the BioSys optical assay and the violet red bile agar method for detecting coliforms in food products. *Journal of food protection*, *67*(12), 2760-2766.

- Ganhao, R., Estévez, M., Kylli, P., Heinonen, M., & Morcuende, D. (2010). Characterization of selected wild Mediterranean fruits and comparative efficacy as inhibitors of oxidative reactions in emulsified raw pork burger patties. *Journal of Agricultural and Food Chemistry*, *58*(15), 8854-8861.

- Ganhão, R., Morcuende, D., & Estévez, M. (2010). Protein oxidation in emulsified cooked burger patties with added fruit extracts: Influence on colour and texture deterioration during chill storage. *Meat science*, *85*(3), 402-409.

- Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., Holcroft, D. M., & Kader, A. A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agricultural and Food chemistry*, *48*(10), 4581-4589.

- Graham, J., Hein, I., & Powell, W. (2007). Raspberry. In *Fruits and Nuts* (pp. 207-216). Berlin, Heidelberg: Springer Berlin Heidelberg.

- Gülçin, İ., Elias, R., Gepdiremen, A., & Boyer, L. (2006). Antioxidant activity of lignans from fringe tree (Chionanthus virginicus L.). *European Food Research and Technology*, *223*(6), 759-767.

- Gullón, P., Astray, G., Gullón, B., Tomasevic, I., & Lorenzo, J. M. (2020). Pomegranate peel as suitable source of high-added value bioactives: Tailored functionalized meat products. *Molecules*, *25*(12), 2859.

- Guyon, C., Meynier, A., & de Lamballerie, M. (2016). Protein and lipid oxidation in meat: A review with emphasis on high-pressure treatments. *Trends in Food Science & Technology*, *50*, 131-143.

- Hadidi, M., Orellana-Palacios, J. C., Aghababaei, F., Gonzalez-Serrano, D. J., Moreno, A., & Lorenzo, J. M. (2022). Plant by-product antioxidants: Control of protein-lipid oxidation in meat and meat products. *Lwt*, *169*, 114003.

- Halliwell, B. (1996). Commentary: vitamin C: antioxidant or pro-oxidant in vivo?. *Free radical research*, *25*(5), 439-454.

- Honikel, K. O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat science*, *49*(4), 447-457.

- Horbańczuk, O. K., Kurek, M. A., Atanasov, A. G., Brnčić, M., & Brnčić, S. R. (2019). The effect of natural antioxidants on quality and shelf life of beef and beef products. *Food technology and biotechnology*, *57*(4), 439.4.

- Kamal-Eldin, A., & Appelqvist, L. Å. (1996). The chemistry and antioxidant properties of tocopherols and tocotrienols. *Lipids*, *31*(7), 671-701.

- King, D. A., Hunt, M. C., Barbut, S., Claus, J. R., Cornforth, D. P., Joseph, P., ... & Weber, M. (2023). American Meat Science Association guidelines for meat color measurement. *Meat and Muscle Biology*, *6*(4).

- Lacombe, A., Wu, V. C., White, J., Tadepalli, S., & Andre, E. E. (2012). The antimicrobial properties of the lowbush blueberry (Vaccinium angustifolium) fractional components against foodborne pathogens and the conservation of probiotic Lactobacillus rhamnosus. *Food microbiology*, *30*(1), 124-131.

- Linster, C. L., & Van Schaftingen, E. (2007). Vitamin C: Biosynthesis, recycling and degradation in mammals. *The FEBS journal*, *274*(1), 1-22.

- Lorenzo, J. M., Pateiro, M., Domínguez, R., Barba, F. J., Putnik, P., Kovačević, D. B., ... & Franco, D. (2018). Berries extracts as natural antioxidants in meat products: A review. *Food Research International*, *106*, 1095-1104.

- Lorenzo, J. M., Trindade, M. A., Ahn, D. U., & Barba, F. J. (2019). Natural antioxidants to reduce the oxidation process of meat and meat products. *Food research international*, *115*, 377-378.

- Lourenço, S. C., Moldão-Martins, M., & Alves, V. D. (2019). Antioxidants of natural plant origins: From sources to food industry applications. *Molecules*, *24*(22), 4132.

- Ma, Y., Yi, J., Jin, X., Li, X., Feng, S., & Bi, J. (2023). Freeze-drying of fruits and vegetables in food industry: Effects on phytochemicals and bioactive properties attributes-a comprehensive review. *Food Reviews International*, *39*(9), 6611-6629.

- Malik, A., Afaq, F., Sarfaraz, S., Adhami, V. M., Syed, D. N., & Mukhtar, H. (2005). Pomegranate fruit juice for chemoprevention and chemotherapy of prostate cancer. *Proceedings of the National Academy of Sciences*, *102*(41), 14813-14818.

- Manessis, G., Kalogianni, A. I., Lazou, T., Moschovas, M., Bossis, I., & Gelasakis, A. I. (2020). Plant-derived natural antioxidants in meat and meat products. *Antioxidants*, *9*(12), 1215.

- Mishra, J., Mishra, B. P., Rath, P. K., Mishra, P. S., Reddy, B. V., Mounika, T., ... & Vitthal, M. S. (2023). Natural Fruit based antioxidants: A novel strategy for use in meat and meat products

- Moylan, S., Berk, M., Dean, O. M., Samuni, Y., Williams, L. J., O'Neil, A., ... & Maes, M. (2014). Oxidative & nitrosative stress in depression: why so much stress?. *Neuroscience & Biobehavioral Reviews*, *45*, 46-62.

- Munekata, P. E. S., Rocchetti, G., Pateiro, M., Lucini, L., Domínguez, R., & Lorenzo, J. M. (2020). Addition of plant extracts to meat and meat products to extend shelf-life and health-promoting attributes: An overview. *Current Opinion in Food Science*, *31*, 81-87.

- Munné-Bosch, S., & Alegre, L. (2002). The function of tocopherols and tocotrienols in plants. *Critical Reviews in Plant Sciences*, *21*(1), 31-57.

- Naveena, B. M., Sen, A. R., Kingsly, R. P., Singh, D. B., & Kondaiah, N. (2008). Antioxidant activity of pomegranate rind powder extract in cooked chicken patties. International journal of food science and technology, 43(10), 1807-1812.

- Noreen, S., Hashmi, B., Aja, P. M., & Atoki, A. V. (2025). Phytochemicals and pharmacology of pomegranate (*Punica granatum L.*): nutraceutical benefits and industrial applications: a review. *Frontiers in Nutrition*, *12*, 1528897.

- Özvural, E. B., & Vural, H. (2011). Grape seed flour is a viable ingredient to improve the nutritional profile and reduce lipid oxidation of frankfurters. *Meat Science*, 88(1), 179-183.

- Pabón-Baquero, L. C., Otálvaro-Álvarez, Á. M., Fernández, M. R. R., & Chaparro-González, M. P. (2018). Plant extracts as antioxidant additives for food industry. In *Antioxidants in Foods and its Applications*. IntechOpen.

- Padayatty, S. J., Katz, A., Wang, Y., Eck, P., Kwon, O., Lee, J. H., ... & Levine, M. (2003). Vitamin C as an antioxidant: evaluation of its role in disease prevention. *Journal of the American college of Nutrition*, *22*(1), 18-35.

- Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. *Journal of nutritional science*, *5*, e47.

- Pateiro, M., Barba, F. J., Domínguez, R., Sant'Ana, A. S., Khaneghah, A. M., Gavahian, M., ... & Lorenzo, J. M. (2018). Essential oils as natural additives to prevent oxidation reactions in meat and meat products: A review. *Food research international*, *113*, 156-166.

- Peiretti, P. G., & Gai, F. (2015). Fruit and pomace extracts: applications to improve the safety and quality of meat products. *Fruit and pomace extract biological activity, potential applications and beneficial health effects*, 1-28.

- Petcu, C. D., Mihai, O. D., Tăpăloagă, D., Gheorghe-Irimia, R. A., Pogurschi, E. N., Militaru, M., ... & Ghimpețeanu, O. M. (2023). Effects of plant-based antioxidants in animal diets and meat products: A review. *Foods*, *12*(6), 1334.

- Rao, A. V., & Rao, L. G. (2007). Carotenoids and human health. *Pharmacological research*, *55*(3), 207-216.

- Ribeiro, J. S., Santos, M. J. M. C., Silva, L. K. R., Pereira, L. C. L., Santos, I. A., da Silva Lannes, S. C., & da Silva, M. V. (2019). Natural antioxidants used in meat products: A brief review. *Meat science*, *148*, 181-188.

- Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free radical biology and medicine*, *20*(7), 933-956.

- Rodionova, K. (2022). Efficiency of using plant antioxidants in the meat processing industry.

- Santos, A. A. D. L., Leal, G. F., Marques, M. R., Reis, L. C. C., Junqueira, J. R. D. J., Macedo, L. L., & Corrêa, J. L. G. (2025). Emerging Drying Technologies and Their Impact on Bioactive Compounds: A Systematic and Bibliometric Review. *Applied Sciences*, *15*(12), 6653.

- Santos-Sánchez, N. F., Salas-Coronado, R., Villanueva-Cañongo, C., & Hernández-Carlos, B. (2019). *Antioxidant compounds and their antioxidant mechanism*. IntechOpen.

- Shah, M. A., Bosco, S. J. D., & Mir, S. A. (2014). Plant extracts as natural antioxidants in meat and meat products. *Meat science*, *98*(1), 21-33.
- Shen, T., Wang, X. N., & Lou, H. X. (2009). Natural stilbenes: an overview. *Natural product reports*, *26*(7), 916-935.
- Shewail, A., SHALTOUT, F., & GERGES, T. (2018). Impact of some organic acids and their salts on microbial quality and shelf life of beef. *Assiut veterinary medical journal*, *64*(159), 164-177.
- Shukla, S. (2011). Freeze drying process: A review. *International journal of pharmaceutical sciences and research*, *2*(12), 3061.
- Stahl, W., & Sies, H. (2003). Antioxidant activity of carotenoids. *Molecular aspects of medicine*, *24*(6), 345-351.
- Stahl, W., & Sies, H. (2005). Bioactivity and protective effects of natural carotenoids. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1740*(2), 101-107.
- Sumner, M. D., Elliott-Eller, M., Weidner, G., Daubenmier, J. J., Chew, M. H., Marlin, R., ... & Ornish, D. (2005). Effects of pomegranate juice consumption on myocardial perfusion in patients with coronary heart disease. *The American journal of cardiology*, *96*(6), 810-814.
- Tajkarimi, M. M., Ibrahim, S. A., & Cliver, D. O. (2010). Antimicrobial herb and spice compounds in food. *Food control*, *21*(9), 1199-1218.
- Traber, M. G., & Atkinson, J. (2007). Vitamin E, antioxidant and nothing more. *Free radical biology and medicine*, *43*(1), 4-15.
- Varnam, A., & Sutherland, J. P. (1995). *Meat and meat products: technology, chemistry and microbiology* (Vol. 3). Springer Science & Business Media.
- Venkitasamy, C., Zhao, L., Zhang, R., & Pan, Z. (2019). Pomegranate. In *Integrated processing technologies for food and agricultural by-products* (pp. 181-216). Academic Press.
- Vinson, J. A. (1998). Flavonoids in foods as in vitro and in vivo antioxidants. *Flavonoids in the living system*, 151-164.
- Vulić, J. J., Velićanski, A. S., Četojević-Simin, D. D., Tumbas-Šaponjac, V. T., Đilas, S. M., Cvetković, D. D., & Markov, S. L. (2014). Antioxidant, antiproliferative and antimicrobial activity of freeze-dried raspberry. *Acta periodica technologica*, (45), 99-116.

- Youssef, K., Shatta, A., & El-Samahy, S. (2016). Impact of freezing and freeze-drying processes on color, phytochemical contents and antioxidant capacity of pomegranate seeds. *Suez Canal University Journal of Food Sciences*, *3*(1), 27-38.

- Zamel, S. A. N. M. (2022). *Antimicrobial and Antioxidant Effect of Essential Oil in Refrigerated Beef Burger* (Doctoral dissertation, Al-Quds University).

أثر مسحوق الرمان والتوت المجفف بالتجميد على الخصائص المضادة للأكسدة وجودة لحم البقر المشوي

اعداد: أمير كمال السكافي

إشراف: الدكتور فؤاد الريماوي

الملخص:

أظهرت نتائج هذه الدراسة أن استخدام مسحوق الرمان المجفف بالتجميد (FDPP) ومسحوق التوت الأحمر المجفف(FDRP) كبديل طبيعي لمضادات الاكسدة الصناعية يُعدّ وسيلة فعّالة وطبيعية لتحسين جودة لحوم الروست بيف أثناء التخزين المبرد لمدة 60 يوما على درجة حرارة 4 درجات مئوية. فقد أثبت FDPP، خصوصاً بتركيز 1.5%، كفاءة عالية في تثبيط الأكسدة الدهنية وتقليل النمو الميكروبي، حيث خفّض قيم TBARSبنسبة تصل الى 75% والعدّ البكتيريا الكلي بنسبة قاربت 72% مقارنة بالعينة الضابطة السالبة، وحقق أداءً مقاربًا للعينة الضابطة الايجابية القياسية المحتوية على مضاد أكسدة صناعي (صوديوم ايريثوربات). ويرتبط هذا التأثير بمحتواه المرتفع من المركبات الفينولية مثل الإيلاجيك أسيد والبونيكاليجن ذات الخصائص المضادة للأكسدة والبكتيريا. كما حافظت العينات المعاملة بـ FDPP على مستويات مرتفعة من القبول الحسي من حيث الطعم واللون والملمس، مما يعزز إمكانية اعتماده كمكوّن نظيف وآمن في صناعة اللحوم.

في المقابل، أظهر FDRP فعالية متوسطة، وكانت أفضل نتائجه عند تركيز 0.3%، بينما أدّت التركيزات الأعلى إلى تراجع طفيف في القبول الحسي نتيجة تغير اللون والقوام وظهور نكهة فاكهية خفيفة. لذلك، يُوصى باعتماد FDPP بتركيز 1.5% كمضاد أكسدة ومضاد ميكروبي طبيعي بديل للمواد الاصطناعية مثل صوديوم ايرثوربيت وBHT وBHA، مع إمكانية استخدام تراكيز معتدلة (0.3–1.5%) لتحقيق توازن بين الفعالية والجودة الحسية. كما يُنصح بإجراء دراسات تطبيقية صناعية لتقييم ثبات الأداء في خطوط الإنتاج الفعلية وتحديد الأثر الاقتصادي والعملي على مدة الصلاحية.

وتقترح الدراسة مستقبلاً التركيز على عزل المركبات الفينولية الفعالة أو تغليفها بتقنيات دقيقة لزيادة ثباتها خلال التخزين والمعالجة الحرارية، إضافةً إلى دراسة التفاعل التكاملي بين FDPP وFDRP للحصول على تركيبات طبيعية متوازنة تجمع بين الفعالية العالية والمظهر الحسي الجيد. وأخيراً، يُستحسن تسويق هذه المنتجات بوصفها أغذية "امنة صحيا" لإبراز قيمتها الصحية وتعزيز ثقة المستهلكين باللحوم الطبيعية الخالية من الإضافات الصناعية.