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Effect of vacuum packaging combined with natural additives on the shelf life and quality traits of fresh thyme

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This Thesis was defended successfully on 8/12/2019 and approved by:

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Dedication

This thesis is dedicated to my father, who taught me that the best kind of knowledge to have is that which is learned for its own sake, who taught me that even the largest task can be accomplished if it is done one step at a time.

Acknowledgment

The realization of this work was only possible due to several people's collaboration, to which I desire to express my gratefulness. To Dr. Samer Mudalal, my supervisor, I am grateful for the trust deposited in my work and for the motivation demonstrated along this research journey. His support was without a doubt crucial in my dedication to this investigation. I would like to convey special way to Professor Gianluigi Mauriello, my co-supervisor has been very supportive. I express my gratefulness for his feedback and help in interpretation of some results presented in this thesis.

I would like also to express my appreciation to my friends for their support that gives me enthusiasm and encouragement. Finally, I would like to thank my parents, and my brothers, for their true love which has always been a driving factor towards completing this work.

List of Contents

List of Tables

List of Figures

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Abstract

Green thyme (Za'atar) is one of favorite herbs among Palestinian's garden. It refers to genus Origanum of Lamiaceae family. This study aim to extend the shelf life fresh green thyme by hurdle technology by employing vacuum packaging technique combined natural additives (onion, sumac, lactic acid and oil and salt) then stored at room and refrigerated temperature to study the effect of natural additives combined with vacuum packaging on the physochemical properties of green thyme including color, taste, flavor, pH, chemical compositions and how they change during 42 days. Moreover, Study the probability of growth of Clostridium Botulinum in green thyme samples under anaerobic condition by using microbiological challenge test. Lactic acid was the most effective to keep different quality traits during storage. Refrigerated conditions were the best storage condition to extend the shelf life of thyme products. Green thyme leaves are very valuable agricultural product in Palestine. Until now, this product is not exploited to the export market due to short shelf life; the findings of this study may contribute to increase the potential of this product for export market.

Chapter 1

Introduction

1.1 Background

Mediterranean region is rich in fruits, vegetables, and herbs. Green thyme (Za’atar) is one of the favorite herbs among the Mediterranean garden (Figueredo et al, 2005). It is referred to the genus Origanum of Lamiaceae family. The name Za'atar alone most properly applies to Origanum syriacum (Allen, 2007). Nowadays, it is recognized as one of the most aromatic herbs in the world, because of its fragrance and flavor that adds to the food such as meat, vegetables, and breads (Jain et al, 2017).

Three major types of green thyme were grouped from hundreds of its varieties: tall upright, low creeping and clumping. Green thyme included different types such as Lemon thyme, Silver poise thyme, Golden thyme, French thyme, Pink thyme, Bressingham thyme, and Donny valley thyme (Salehi et al, 2018).

A long time ago, green thyme was used as a traditional medicine for different diseases. For example, it used for the treatment of the respiratory disease whooping cough, bronchitis and asthma (Salehi et al, 2018). It is highly recommended because of its pharmacological and biological properties. Thyme oil plays a major role in the treatment of several diseases because of its therapeutic properties. The most common functional ingredients in green thyme leaves are thymol and carvazole (Haoxing et al,

2006). Green thyme leaves are considered as rich source of vitamins such as folic acid, beta carotene, and vitamin E, K, A, and C. Also, it has several antimicrobial properties such as anti-bacterial, anti-oxidizing and anti-fungus (Eqbal et al, 2017).

Thyme leaves are rich in phenolic compounds such as: caffeic acid, rosmarinic acid, eriodyctiol, luteolin, naringenin and apigenin (Exarchou et al, 2002). Carvacrol and thymol play an important role of antibacterial effects. Essential oils especially those packed in phenolic compounds can change permeability and function of cell membrane protein by binding to protein then blocked their normal function after penetrated bacterial cell wall. Essential oils can affect percentage of unsaturated fatty acids and their structure because of their lipophilic nature. Different antimicrobial mechanisms identified according to the site of outer and inner components of the bacterial cell, cell membrane functions and to their effect. Moreover, cytoplasm, enzymes, proteins, fatty acids, ions, and metabolites functions are antimicrobial mechanisms. (Sakkas et al, 2017).

The main oregano oils which are carvacrol and thymol have wide antimicrobial effects including bacteria such as methicillin-resistant *S. aureus, Listeriainnocua, L. monocytogenes, A.baumannii, K. pneumoniae, Citrobacter freundii, S. enteritidis, S. typhimurium, E.coli, E.coli O157:H7, P.vulgaris, P.aeruginosa, P.fluorescens, Y. enterocolitica, Bacillus subtilis, B. cereus, Serratia liquefaciens, Lactobacillus carvatus,* and *Lactobacillus*

sakes. Moreover, antimicrobials spectrum cover fungi as *Aspergillus spp.* and *Candida spp* (Salgueiro et al, 2003)

European countries (including Spain, France, Italy, Switzerland, Bulgaria, Portugal, and Greece) produced thyme from cultivated and wild-harvested plants. Many factors have a great impact on the yield and quality of essential oil. The most important are the genetic make-up of plant material, crop maturity at harvest, as well as, environment and distillation practice. More than 90% of the thyme oil of world trade is produced in Spain. Climate conditions in Southern European are very suitable for herb growing from the longer growing season. Therefore, most of the thyme produced in Europe comes from there (Terraba, 2004). Table (1) shows the oregano distribution in the world.

Table 1.Oregano distribution in the world (Lopez et al, 2017)

Oregano Species	Origin
***Hamelia* patens**	Mexico
Lippa grandis	Brazil
Lippa Graveolens	Mexico
Lippa Origanoides	Colombia
Lippa Palmeri	Mexico
Oregano Acutidens	Mexico, Turkey
Oregano applii	Argentina
Oregano Ehrenbergii	Lebanon
Oregano Bilgeri	Turkey
Oregano Libanoticum	Lebanon
Oregano majorana	Brazil
Oregano majoricum	Colombia, Turkey, Argentina
Oregano hypericifolium	Turkey
Oregano Onites	Greece, Turkey
Oregano Syriacum	Egypt, Lebanon
Oregano syriacum* ssp. *Syriacum	Jordan
***Oregano vulgare* Lippa**	Argentina, Brazil, Chile, China, Colombia, Greece, India, Iran, Italy, Morocco, Pakistan, Poland, Portugal, Serbia, Spain, USA
Oregano vulgare* Lippa ssp. *Glandulosum	Algeria, Tunisia
Oregano vulgare* Lippa ssp. *Gracile	Iran, Turkey
Oregano vulgare* Lippa ssp. *Hirtum	Argentina, Colombia, Greece, Hungary, Italy, Lithuania, Serbia, Turkey
Oregano vulgare* Lippa ssp. *Virens	Argentina, Iran, Portugal
Oregano vulgare* Lippa ssp. *Vulgare	Argentina, Iran, Italy, Lithuania, Turkey, Poland

Green thyme leaves (*Oregano syriacum*), which called Palestine's green gold. Palestinians have known different types of thyme, especially wild thyme where they used it in their food, and traditional cuisine. Few years ago, Palestinians realized the importance of their cultivation due to their economic feasibility. According to the Palestinian Ministry of agriculture, there are 5500 dunum produce 11000 tons of green thyme per year. In detail, every dunum of thyme costs about 5,000 NIS and this capital can be restored in a period between 9 and 12 months. The production of green thyme is 500-800 kg per dunum. The net profit for this dunum was estimated at 2,000 NIS per month/ dunum. It has been noted that one dunum continues production for seven years (Palestine economy portal, 2015).

Humankind used primitive ways to preserve food from spoilage by microorganisms or enzymatic reactions. Bacteria, yeast, and molds need a sufficient amount of moister content to grow in the food. In recent decades, different techniques have been developed to preserve food such as dehydrating, canning, freezing, etc.

Drying is one of the oldest and easiest methods to get off water or moisture from food, which helps to prevent food spoilage (Esper et al, 1998). The speed of drying of green leaves is usually affected by temperature, airflow, relative humidity, and surface area. The rate of evaporation is a function of temperature and humidity. The optimum temperature drying of leaves is around 55℃. Airflow is considered a

critical factor in this process. Because the dry air replaced by saturated humid air from leaves, airflow plays an important role in both speed drying and prevents overheat preservation. Low relative humidity of air increases the evaporation rate. The time of drying decreases when the surface area increases. Chopping and spreading of leaves more thinly on the drying trays are the traditional methods to increase surface area (Babua et al, 2018). Even there are several common benefits of food drying by solar energy, but at the same time, there is a significant destruction to some of the functional ingredients such as beta carotene and vitamin A. Moreover, the leaves are exposed to contamination by excessive bacteria, yeast, and mold. Furthermore, there is a high potential for further damage of green thymes by microbes and enzymes before they reach the desired degree of drying (Vickie et al, 2013). The canning method is important and safe for preserving food. Leafy greens like collards, chard, and spinach can be canned. The canning is done by placing the food to be fed in the glass jars or metal cans. Then it is heated to a high temperature to kill the microbes that cause food spoilage. During the heating process, the air is removed from the jars by evacuation during sealing followed by cooling, which prevents the air from entering the food (Vickie et al, 2013). Several advantages of the canning method including changing the moisture and pH to avoid microbes growth as well as inactivation of spoilage enzymes (Berry, 2003).

1.2 Problem statement

Green thyme (Za'ater) is a seasonal crop. To cover the off-seasons, the traditional preservation methods such as freezing, drying, and packing in plastic bottles are commonly used in Palestine and the Mediterranean region. The common traditional preservation methods may lead to loss of nutritional values and impair some of sensory properties like color, taste, and flavor. Until now, there is no commercial method available in Palestine to increase the availability of green thyme leaves during off seasons or summer time.

1.3 Research objectives

1.3.1 Overall Goal

Our research aim is to extend the shelf life of fresh green thyme using hurdle technology (employing vacuum packaging technique combined natural additives).

1.3.2 Research tasks

1. Study the effect of natural additives combined with vacuum packaging on the physochemical properties of green thyme including color, taste, flavor, pH, and how the change during the storage period.

2. Study the effect of natural additives combined with vacuum packaging on the microbial growth during the storage of total aerobic bacteria, anaerobic bacteria, psychrotrophic bacteria, mold and yeast.

3. Study the probability of growth of *Clostridium Botulinum* in green thyme samples under anaerobic condition by using microbiological challenge test.

4. Evaluation the effect of storage on sensory perception (taste, flavor, appearance, saltiness, and over all acceptance)

5. Evaluate the chemical compositions of fresh green thyme.

1.4 Scope and Limitation

This research has been carried out on a herb that is very sensitive to enzymatic browning under aerobic conditions. The collection of samples should be very fast to avoid any of the plant deterioration before processing. In addition, the growing and harvesting conditions of this herb are traditional, which make the collection of a homogenous sample was very difficult.

1.5 Research significance

This research will contribute to increase the export-market share for green thyme produced in Palestine by employing hurdle technology to extend the shelf life. This study aims to develop a preservation method of fresh green thyme by using natural and available additives combined with

vacuum packaging. Moreover, developing new product of fresh thyme with extended shelf life can help to open new market for unexploited crop.

Chapter 2

Literature review

2.1 Origanum syriacum

The genus *Origanum* (oregano, Lamiaceae) characterized by essential oils content, mainly Carvacrol and Thymol (Lee S, 2008). It includes annual, biennial medicinal and aromatic plants. It is commonly used in the food, cosmetics, aromatherapy, and the pharmaceutical industries. *Origanum* species have wide bactericidal and fungicidal effects. It includes a wide range of important species such as *O. vulgare*, *O. majorana and O.* syriacum (Lozano et al, 2004).

Origanum syriacum (Zatar) is a perennial plant. It produces white to pink flowers which have aromatic properties. Leaves are used fresh, crushed or dried. It's one of the sources of the antiseptic essential oil as carvacrol, and thymol which can inhibit a wide range of nasty bacteria such as *Pseudomonas aeruginosa* by eroding the bacterial cell wall (Caballero, 2003).

Image 1: Oregano Syracum planted in Palestine (Caballero, 2003).

2.2 Green thyme production and its uses

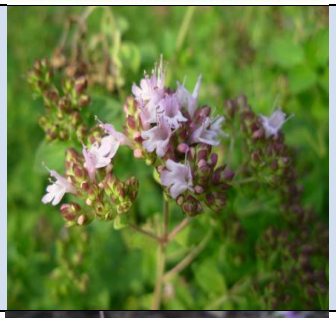
Green thyme is a small shrub, semi-evergreen. Its native name of *Origanum syriacum*, commonly called Syrian oregano or zaatar, it is a bushy perennial herb with highly aromatic foliage. It typically grows in an upright mound to 2 ½ inch tall. It is native to the Middle East (Figueredo, 2004). The genus Origanum is part from a family called Lamiaceae. It is distributed in the Mediterranean area and Northen Africa. Origanum has many species shows in Table (2). The differences between species including: morphological, phenological, genetic and chemical characteristics of the grown and wild species lead to the identification of *Origanum* in the entire world (Ibrahim et al, 2011). Strong therapeutic activity of origanum is attributed due to its constituents which include: phenols, ketones, monoterpenes, alcohol and ester (Santos et al, 2012). High chemical reactive of phenols (thymol and Carvarcol) and ketones

(camphor, and the oxide 1,8-cineole) and light composition of monoterpenes consist of terpinene, limonene, cymene, sabinene, and pinene. Alcohol, and ester which consider as anti-inflammatory, analgesic, anti-spasmodic, and antimicrobial are gently existed and less chemically reactive to use safely with most people (Baser, 2008).

Table 2: Origanum species

(https://nutritiondata.self.com/facts/custom/681598/2))

Origanum Species	
Oregano ehrenbergii	
Oregano syriacum	
Oregano vulgare	
Oregano majorana	

Oregano acutidens			
Oregano glandulosum			
Oregano minutiflourm			
Oregano mychrophyllum			
Oregano libanoticum			
Oregano Compactum			

Farming of green thyme usually starts from the first months of the year (mostly in the period of January to March) because it needs fertile ground and good weather conditions. In summer, the crop needs irrigation around two times per week based on temperature fluctuations (ARIJ, 2015). The diversity of geography and climate in Palestine contributes to farm-wide varieties of Za'atar species including wild and cultivated Za'atar. The average of production of fresh raw green thyme is about 15000 tons/year. Moreover, around 2.5 tons/dunum of fresh-cut Za'atar is annually produced from irrigated lands. Both private and leased lands were used to cultivate Za'atar. Two sources of plant stock including nurseries and wild seeds were used by farmers. Wild plant stock for cultivation should be harvested in the same manner as for production and the un-cleaned dried plant spread over land intended for cultivation. Primary processing was achieved by rural families and small farmers. Shaded and clean location should be offered to dry Za'atar which needs one week to have proper drying. After this processing, the product is beaten to separate leaves from stems by breaking, the separated leaves are subjected to the refining process to remove the undesirable parts. Rudimentary sieves or cleaning machines are used to attain this step (The Palestinian Za'atar Sector, 2017). The crop is usually harvested three to five times per year according to irrigation, rainfall rate, climate conditions, care, etc. The crop rotation period of green thyme may vary from 4 to 7 years according to feasibility.

Image 2: Manual harvesting of green thyme in Palestine (The Palestinian Za'atar Sector, 2017)

However, the lack of propagation, poor raw materials, insufficient processing techniques and lack of quality control lead to poor post-harvest treatment, low yield and quality control which are considered as restrictions for Za’atar’s cultivation and processing. The market of Za’atar contains different channels such as rural families and small farmers who can sell to local trades or small stores. In addition, some companies have private label. There are many challenges that reduce the exporting of Za’atar including: reduction and restrictions of fertilizers on access to fertile land in area C, shortage of quality control on agricultural inputs and practice, limited water for irrigation, lack of international accreditation for food testing laboratories in the State of Palestine resulted in reduced target market confidence, weak cold chain (for fresh and frozen products) which lead to increase volume of waste during transit. But exporting of dried Za’atar’s

exports has rapidly increased because of high demand in the region. Figure (1) shows the Palestinian Za'atar Exports 2009-2015.

Moreover, "Israel" re-exported the Palestinian's herb such as Za'atar under "Isreali" brand to important markets such as the western market which had been bought in high price. "Israeli" exports are able to have high competitive advantage by managing supply consistency of quality herb products (originally supplied by Palestinian exporters) and they have higher chance to face financial challenges in shipping to distant target markets such as the United States (Palestine economy portal, 2015).

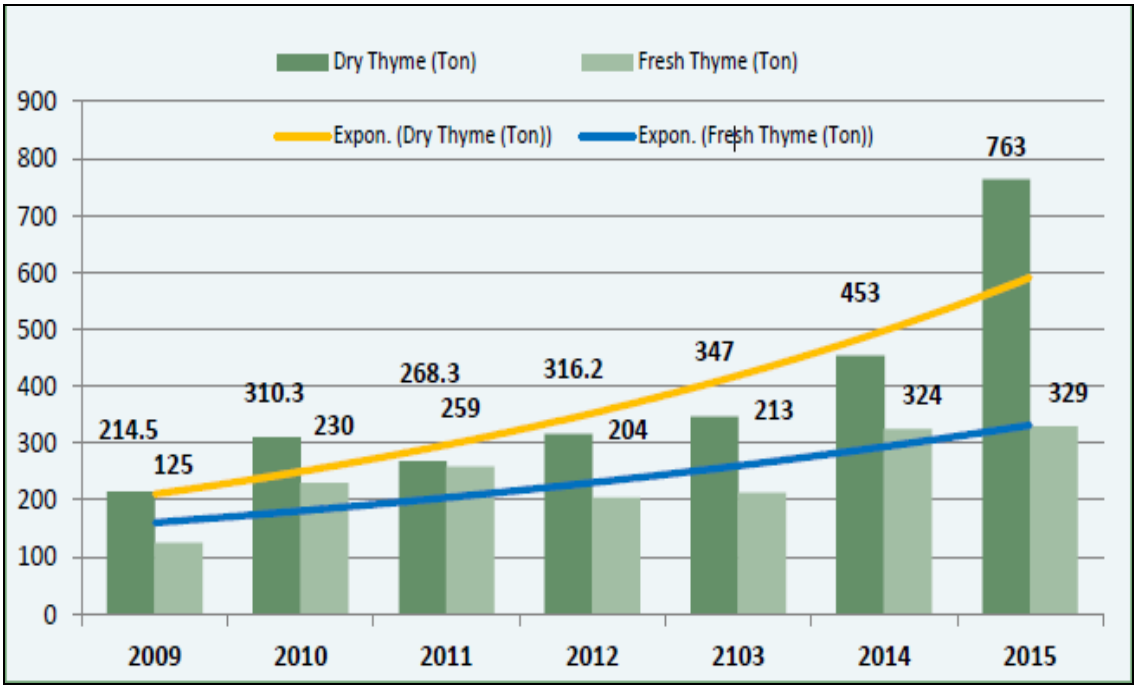


Figure 1: Palestinian Za'atar Export (2009-2015) (The Palestinain Za'atar Sector, 2017).

Green thyme has unique nutritional properties for human health. This aromatic plant is characterized by a high density of nutrients such as minerals, vitamins, and phytonutrients. It is very rich in vitamin A and vitamin C. Vitamin A is an antioxidant, necessary for good vision, and

helps to maintain healthy mucus membranes and skin. Vitamin C is used to fight infections and harmful pro-inflammatory free radicals (Lagouri, 1996). It was found that consumption of 100 gram of thyme provides 0.35 mg of vitamin B6, which contributes to maintain Gamma-aminobutyric acid (GABA), which is a major inhibitory neurotransmitter (Priestly et al, 2003). Moreover, vitamin K, E and folic acid are also found in thyme herbs. On another hand, thyme leaves are rich in minerals such as potassium, calcium, iron, manganese, magnesium, and selenium (Darwish et al, 2009). Tables (3 to 9) shows different nutritional profile of dried Oregano (https://nutritiondata.self.com/facts/custom/681598/2).

Table 3: Minerals content of Oregano per 100 g (https://nutritiondata.self.com/facts/custom/681598/2)

Minerals	
Mineral	**Amount**
Calcium, Ca	1597.00 mg
Copper, Cu	0.633 mg
Iron, Fe	36.80 mg
Magnesium, Mg	270.00 mg
Manganese, Mn	4.990 mg
Phosphorus, P	148.00 mg
Potassium, K	1260.00 mg
Selenium, Se	4.5 mcg
Sodium, Na	25.00 mg
Zinc, Zn	2.69 mg

Table 4: Vitamins content of Oregano per 100 g (https://nutritiondata.self.com/facts/custom/681598/2)

Vitamins	
Nutrient	**Amount**
Betaine	9.8 mg
Choline	32.3 mg
Folate	237.00 mcg
Niacin	4.640 mg
Pantothenic acid	0.921 mg
Riboflavin	0.528 mg
Thiamin	0.177 mg
Vitamin A	1701.00 IU
Vitamin A, RAE	85.00 mcg
Carotene, alpha	20.00 mcg
Carotene, beta	1007.00 mcg
Cryptoxanthin, beta	7.00 mcg
Lutein + zeaxanthin	1895.00 mcg
Vitamin B6	1.044 mg
Vitamin C	2.3 mg
Vitamin E	18.26 mg
Tocopherol, alpha	18.26 mg
Tocopherol, delta	0.92 mg
Tocopherol, gamma	24.42 mg
Vitamin K	621.7 mcg

Table 5: Proteins and Amino acids content of Oregano per 100g (https://nutritiondata.self.com/facts/custom/681598/2)

Proteins and Amino acid	
Nutrient	**Amount**
Protein	9.00 g
Alanine	0.500 g
Arginine	0.449 g
Aspartic acid	1.009 g
Cystine	0.110 g
Glutamic acid	0.975 g
Glycine	0.517 g
Histidine	0.144 g
Isoleucine	0.441 g
Leucine	0.780 g
Lysine	0.500 g
Methionine	0.127 g
Phenylalanine	0.449 g
Proline	1.712 g
Serine	0.314 g
Threonine	0.322 g
Tryptophan	0.203 g
Tyrosine	0.297 g
Valine	0.585 g

Table 6: Carbohydrates content of Oregano per 100g (https://nutritiondata.self.com/facts/custom/681598/2)

Carbohydrates	
Nutrient	**Amount**
Carbohydrate	68.92 g
Fiber	42.5 g
Sugars	4.09 g
Fructose	1.13 g
Galactose	0.15 g
Glucose (dextrose)	1.90 g
Sucrose	0.91 g

Table 7: Sterols content of Oregano per 100g (https://nutritiondata.self.com/facts/custom/681598/2)

Sterols	
Nutrient	**Amount**
Phytosterols	203.00 mg

Table 8: Fats and Fatty acids content of Oregano per 100g (https://nutritiondata.self.com/facts/custom/681598/2)

Fats and Fatty Acids	
Nutrient	**Amount**
Fat	4.28 g
Saturated fatty acids	1.551 g
Decanoic acid	0.004 g
Dodecanoic acid	0.246 g
Hexadecanoic acid	0.792 g
Octadecanoic acid	0.505 g
Tetradecanoic acid	0.004 g
Monounsaturated fatty acids	0.716 g
Cis-octadecenoic acid	0.712 g
Hexadecenoic acid	0.004 g
Octadecenoic acid	0.712 g
Polyunsaturated fatty acids	1.369 g
Cis,cis,cis-octadecatrienoic n-3 acid	0.621 g
Octadecadienoic acid	0.748 g
Octadecatrienoic acid	0.621 g

Table 9: Ash and Water content of Oregano per 100g (https://nutritiondata.self.com/facts/custom/681598/2)

Other	
Nutrient	**Amount**
Ash	7.87 g
Water	9.93 g

Green thyme contains different chemical constituents with biological activity such as: thymol carvacrol, geraneol and borneol. Thymol is the main essential oil in thyme and it has antiseptic and antifungal properties (Dorman et al, 2004). Moreover, thyme had other volatile oils such as carvacrol, geraneol and borneol which have antimicrobial properties. In addition, thyme contains linalool, Apigenin, Eugenol, and rosmarinic acid which are recognized as an antioxidant, anti-inflammatory and antiviral components (Hazzit et al, 2006). It was found that thyme exhibits a significant level of phenolic compounds such as zea-xanthin, pigenin, lutein, luteolin and thymonin (Hazzit et al, 2006) (Salehi et al, 2018).

Green thyme and thyme oils have been used as antimicrobial components. Thymol had an inhibitory growth effect against *Salmonella* and *Staphylococcus* bacteria (Celike et al, 2008). Moreover, it was found that it had preventive and treatment effect against chest infections such as bronchitis, whooping cough, and pleurisy due to its antiseptic and tonic properties. In addition, fresh leaves of thyme can be chewed to decrease sore throats and it may contribute with other herbs to treat asthma, hay fever, and worms in children. Furthermore, thyme is a folk medicine in treatments of several types of diseases such as gastroenteric and bronchopulmonary disorders (Hayrapetyan et al, 2013).

The fresh green thyme leaves were used in different types of dishes. Bread pastry that can be stuffed with onion, oil, salt, and green thyme

leaves. Sometimes are used other ingredients such as sumac, and lemon juice (Savil, 2005). In addition, a salad made of fresh green thyme leaves. Moreover, it can be used as a seasoning during cooking which can give a good flavor for chicken, meat and vegetables. Moreover, it is used as common beverage in Palestine by boiling water with green thyme to make herbal tea (Hayrapetyan, 2013).

2.3 Risk assessment of *Clostridium botulinum*

Clostridium botulinum is anaerobic, gram-positive, rod shape, and spore forming bacterium. It has ability to produce the neurotoxin botulinum. The toxins of botulinum lead to sever disease as flaccid paralytic disease in humans and other animals (Jawetz et al, 2011).

Four known groups of C. botulinum have the ability to produce botulinum toxin including: *C. botulinum* groups I–IV, as well as some strains of *Clostridium butyricum* and *Clostridium baratii*, are the bacteria responsible for producing botulinum toxin. Moreover, food born botulism, infant botulism, and wound botulism refer to *C. botulinum* (Peck, 2009).

Commonly, *C. botulinum* found in soil, and spores can live in high temperatures, and low acid food (Peck, 2009).

Storage conditions have a huge effect on the decrease growth of the bacterium, including: very low moisture, and temperature below 3 °C. Furthermore, high acidity, high ratio of dissolved sugar, high levels of oxygen leads to prevent the growth of bacterium (Fleming, 2006).

As an example, In green beans; it is difficult to inactivate spores by heating (a pressurized environment) may provide an oxygen-free medium for the spores to grow and produce the toxin. On the other hand, acidic food like pickles are sufficient to prevent growth; even if the spores are present, they pose no danger to the consumer. Spore germination risk increases in low acid food having a pH more than 4.6 (Raatjes & Smelt, 1979).

Thyme is considered as low acid food (pH > 4.6) and it grows near soil. Accordingly, the risk of presence and growth of *C. botulinum* in thyme should be investigated. The common approach to investigate the risk of *C. botulinum* is the microbiological challenge testing using surrogate microbes to assess the probability of *C. botulinum* growth (Raatjes & Smelt, 1979).

Clostridium Sporogenes is considered as surrogate microbe for *C. botulinum*. *Clostridium Sporogenes* is anaerobic, gram-positive, and rod shape bacterium. It is found in the soil, marine or sedimentation of fresh water. It is usually isolated from human faeces. It has potential benefits as bacterial therapy for certain types of cancer. *Clostridium Sporogenes* is so closely related to *Clostridium botulinum* Group I strain. Normally, it used as a non-toxic surrogate for common food-borne pathogens (Poehlein, 2015).

2.4 Microbiology challenge test

Microbiological challenge test is a helpful tool to understand the ability of spoilage organisms or pathogens to grow in food. It is used to

assess processes validation that needs to deliver some degree of lethality against a target microrganism or group of target microrganisms (Notermans, 1994).

In the challenge testing, there is a need for the ideal organism which isolated from similar media or environment of surrogate microbe. Moreover, pathogens from known foodborne outbreaks should be included to ensure the formulation is robust enough to inhibit those organisms as well (Notermans, 1994).

Microorganism selection in challenge testing relies on knowledge, commercial experience, and epidemiological data. Additionally, the intrinsic properties (for example, pH, water activity, and preservatives) and extrinsic properties (for example, atmosphere, temperature, and processing) should be taken into consideration. The hazard characterization of each microorganism may lead to increase varieties of the population (Russell, 2003).

Some of microorganisms that are commonly used in challenge testing are toxigenic molds (such as *Aspergillus, Penicillium, and Fusariumspp.)* as well as bacteria (such as *Bacillus cereus., Campylobacter spp., Clostridium perfringens*) (Russell, 2003).

2.5 Common preservation and storage methods for herbs

In this section, the different preservation techniques for herbs will be discussed. The most common and classical preservation method is heating.

Heat is usually employed to preserve food by inactivation of spoilage microbes, as well as spoilage enzymes. Nevertheless, heat has a detrimental effect on the nutritional value of food due to destruction of some flavor and nutrients such as ascorbic acid and other heat-labile components. (Ferrante et al, 2004).

Solar heating is another way to preserve herbs is to dry. Drying is carried out by diffusing water from inside to the surface where the moisture on the surface is evaporated. Evaporation rate depends on the environmental conditions of air-speed, relative humidity and heat (Leistner, 2000). Different techniques were used to dry green leaves which depend on various sources. Table (10) shows some sources of heat for food dehydrators and their advantages (Kennedy, 2012).

Table 10: The Sources of heat for food dehydrators (Kennedy, 2012).

Sources	Advantages
Electricity	Small device, it can found in the market. Increases air flow because it has electrical resistance heating element. Can dry a kilogram of fresh leaves in 5 or 6 hours. Capable of drying leaves in any sort of weather. Good quality control and convenience.
Wood heat	Can be run at night or cloudy weather. Appropriate for drying large volumes of food. Free, local carbon neutral. Renewable energy source.
Gas heat	It is easier to control than wood heat. Can be used in any weather. Gas burns cleaner than wood.
Solar heat	The only method that uses free non-polluting energy of sunshine.

Hundreds of designs for the solar drying method were used. The glass or plastic was used to absorb heat from the sun. Natural airflow was used to dry food. After sun set, rocks were used as heat sinks to drying. Green leaves were traditionally dried by laying it on mats in the sun shine. Sometimes the green leaves were left on rooftop to allow rapid drying (Tiwari, 2019). There are many benefits of drying food but, unfortunately, some problems have existed which will be explained in chapter 1. Indirect solar drying method was used to resolve those problems. It had been an good concept. It was used sunlight which is collect in channels by trapped the radiation warms through glass or plastic cover. Heat and airflow were brought and left in the drying chamber by warmed air. Then the warmed wet air leaves through the top. This process continues until the leaves completely get dried during sunlight. This design protects leaves from direct sunlight. But the solar-energy collecting area and the arrangement of drying trays doesn't allow for enough air speed are the main problems for this type of solar dryer. Figure (2) was described the design of common indirect solar drying (Kennedy, 2012).

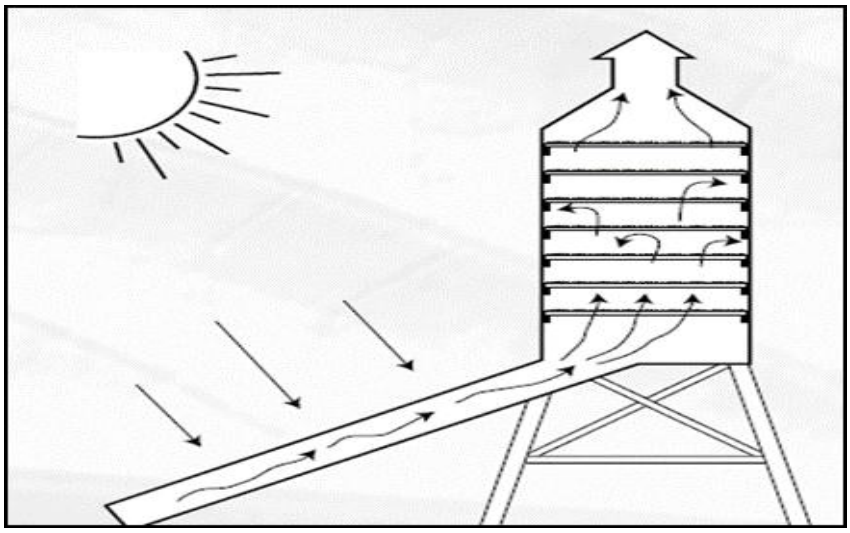


Figure 2: Common indirect solar dryer (Kennedy, 2012).

Freezing is the best way to maintain the quality of food products and their sensory characterizations and shelf life of products. Although freezing causes water loss and reduction in the quality of food, but it is better than other methods (drying, canning) **(**Mallett, 1993).. It has a beneficial effect which leads to limit the growing of microorganisms, reduced the chemical reactions, and delayed the cellular metabolic reactions. The process of freezing consist of two major components which are thermodynamic and kinetic. Thermal events achieved by reducing the heat content from the material during the freezing process are including: cool downs the material that wants to be frozen till reach into nucleation temperature. Before ice can be formed, a nucleus, or a seed, is required upon which the crystal can grow; the production of this seed called nucleation. Phase change from liquid to solid when the first crystal appears in the solution. So the nucleation is considered as the initial process of freezing which leads to the main phase change from liquid to solid with further crystal growth. The definition of a freezing point is the temperature at which the first ice crystal appears and the liquid at that temperature is in equilibrium with the solid **(**Mallett, 1993). The frozen point of pure water occurred at 0 °C (273°K). But more complex process when the food system is frozen because of existence of free bond and bound water; which doesn't freeze even at low temperature because it contains soluble solids which lead to decrease the freezing point of water lower than 0 °C. During the freezing process, the soluble solids concentration had been increased in the unfrozen water, because of freezing temperature variation. Therefore, the temperature of the

first ice crystal appeared considered as initial freezing temperature. Two main parameters were used in freezing system design; freezing time and freezing rate **(**Mallett, 1993).

According to The International Institute of Refrigeration (IIR), the process of freezing divided into three stages based on major temperature changes as demonstrated in figure (3 and 4) for pure water and food respectively (Mallett, 1993).

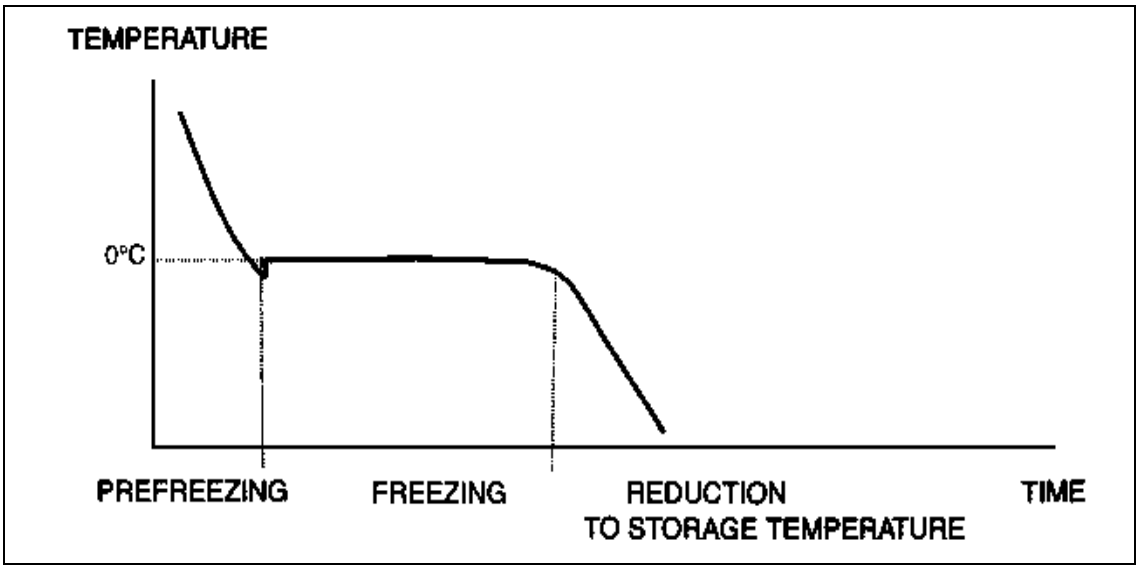


Figure 3: Practical definition of the freezing process for pure water (Mallett, 1993)

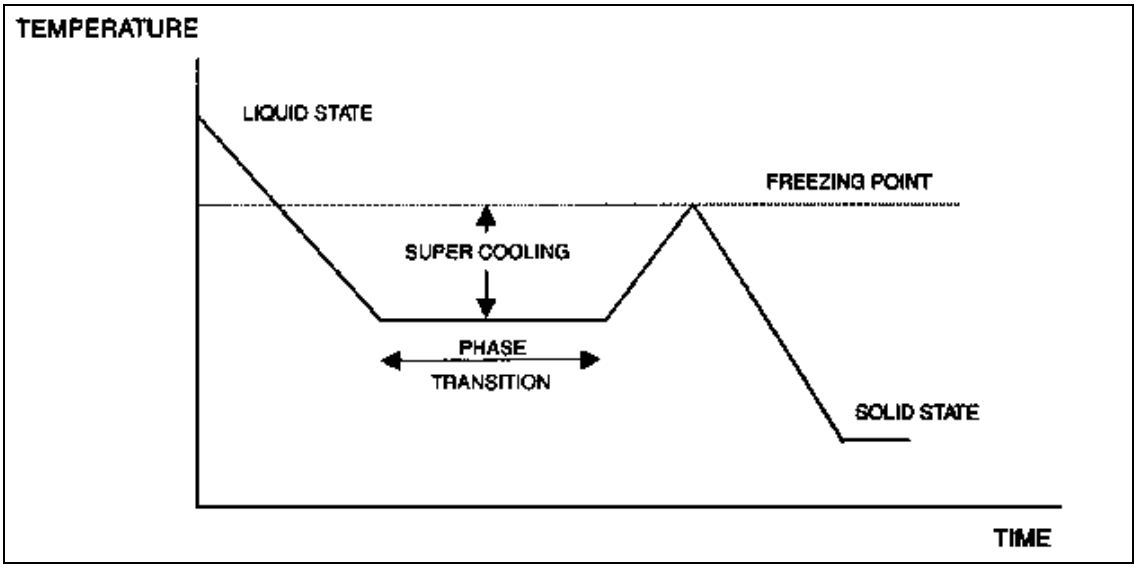


Figure 4: Practical definition of the freezing process for foods (Mallett, 1993)

On another side, even the chemical additives are harmful to human health but they are usually used under certain recommendations to maintain nutrients of preserved food. For example, organic acids such as ascorbic acid and phosphate can be used separately or in combination (Bing et al, 2002).

Packaging of fresh herbs is the main step to reduce water loss, maximize shelf life. Controlled atmosphere (CAP) and modified atmosphere packaging (MAP) have a beneficial effect on herbs during storage which reduces the deterioration. However, a control atmosphere packaging has a high degree of precision more than modified atmosphere packaging. CAP guarantees a specific level of O_2, CO_2, and other gases. In addition, it helps to delay chlorophyll loss and deterioration in green leaves. Therefore, it effectively controls the respiration rate, ethylene production, growth of pathogens, and breaks down undesirable metabolic changes (Tomkins, 1997). For example, Sauerkraut is preserved by adjusting the

gaseous atmosphere that the ratio of O_2 / CO_2 remains constant to reduce breathing and ethylene production which prevents the fermentation of sauerkraut. Therefore, leads to the production of lactic acid. Different types of green leaves preserved under modified packaging such as chives, watercress, sorrel, coriander, dill and parsley (Ball et al, 1957).

Novel technology had been used to improve microbial safety and nutritional quality called hurdle technology. It usually uses several methods of preservation to achieve high quality and sensory characterizations. High demand in fresh and natural food lead to develop this technique and minimize the consumption of processed food. Moreover, it helps to avoid the severity of using one method like heat treatment as it provides synergy of combination. Multi-hurdle was used to determine food stability such as: heat treatment, pH, salt, natural preservatives, packaging, and storage temperature. Table (11) shows the principle hurdles used for food preservation (Leistner, 2000)

Table 11: Principle hurdles technology used for food preservation (Leistner, 2000).

Parameter	Application
High Temperature (F)	Heating
Low Temperature (T)	Chilling, freezing
Water Activity (a_w)	Drying, curing (with add salt), conserving with addition of sugar
Acidity (pH)	Acid addition, formation
Redox Potential (E_h)	Removal of oxygen (by vacuum packaging, Nitrogen packaging) or addition of ascrobate
Bio preservatives	Competitive flora such as microbial fermentation
Preservatives	Sorbates, sulfites, nitrites

The major preservative factors of hurdle technology focused on the physiology and behaviour of microorganisms such as homeostasis, metabolic exhaust, and stress reactions. Homeostasis is the main way to achieve stability and uniformity in the internal status of the organism cell. When the homeostasis of organism changes, microbes cell consume their energy in fixing their physiological status rather than growth and multiplying. Therefore, the microorganisms remain in the lag phase or maybe die before repair their homeostasis (Rostami et al, 2016). Low level of nutrients, low pH, low water activity, low temperature for growth, and raised level of oxygen are some of stress factors that can disturb the homeostasis of microorganisms the in food (Leistner, 2000). Metabolic exhausted and it's called auto-sterilization too. In this factor, the microorganism dies automatically because of deficiency of energy during the struggle of a microorganism in repair mechanism to overcome hostile environment by using their energy. The main limitation for hurdle technology happens when the microorganism becomes more resistant in result of exposure to stress reactions. Microorganism can generate shock proteins which help the organism to face the stress situations. Many factors affect the synthesis of these proteins such as heat, pH, a_w, ethanol as well as starvation. Apple juice, skim milk beverage, pickled fruits and vegetables, and kimchi are some of applications of hurdle technology (Neeha et al, 2014)

2.5.1 Storage at room temperature

Temperature and humidity are the most important factors to have proper storage conditions. To extend storage life and maintain the quality of fresh green leaves, low temperature and high relative humidity can decrease the respiration rate. Accordingly, metabolic processes are alleviated this leads to reduction enzymatic deteriorating reactions (Correa et al, 2010).

Room temperature (25℃) is considered among temperatures that cause an increase in growth of bacteria like *Salmonella, E.coli, and campylobacter* to dangerous levels that can cause illness (Rao et al, 1994). Many studies demonstrated that the sensory properties including (color, taste, flavor, and saltiness) of green leaves changed at room temperature. Stored at room temperature leads to increase the probability of browning which have a direct effect on quality and sensory properties (Lipscomb, 2012). Furthermore, the studies illustrated the effect of temperature on color degradation of leafy green vegetables as well as the chlorophyll content as a factor that contributes to color variations (Manolopoulou, 2016).

2.5.2 Storage at refrigeration temperature

The main purpose of having a refrigerator in households is to keep food cold at a temperature below 4℃ . At this temperature, the food can stay in fresh form longer if it was kept at room temperature. The

fundamental idea behind refrigeration is to decrease the activity of all bacteria that present in food, protecting it from spoiling by bacteria (Winslow et al, 1920).

In danger zone (4.4 °C and 60 °C), the risk of growth of pathogenic bacteria is dramatically increased. Therefore, controlling temperature and refrigerating conditions can contribute significantly to the safety of food products. Because they do not generally affect the taste, smell, or appearance of a food, one cannot tell that a pathogen is present. In another hand, the bacteria that cause foods to deteriorate and develop unpleasant odors, tastes, and textures called spoilage bacteria (Winslow et al, 1920).

Spoilage bacteria can grow at cold temperatures, such as in the refrigerated conditions. they lead to develop off or bad tastes and smells of food. Spoiled food probably would not cause diseases for people who choose to eat spoiled food. However, some bacteria such as *Listeria monocytogenes* thrive at cold temperatures, and if present, will grow in the refrigerator and could cause illness (Winslow, 1997). Psychrotrophic microorganisms grow well in the range of 0℃ to 15 ℃ but it is much slower in this range. The rate of respiration and ripening usually decline as the temperature reduced below 4℃ (Gounot, 1986)

2.6 Enzymatic browning

Herbs have enormous benefits for consumer health, due to their content of fiber, vitamins and antioxidant compounds. But undesirable

reactions may occur, lead to many changes in taste, flavor, and color of fruits and vegetables which lead to quality loss. These reactions called enzymatic browning reaction (Ioannou et al, 2013).

Enzymatic browning reaction may have occurred at any step of preparing fruit and vegetable from harvesting into the final product passing through storage. These changes induce a pronounced loss of the microbiological and antioxidant qualities (Eissa et al, 2009)

Two main oxidoreductases enzymes responsible for enzymatic browning: peroxidase (POD), and polyphenoloxidase (PPO) which catalyze two deteriorative reactions. hydroxylation of monophenols to diphenols, which is relatively slow and results in colorless products. Then the oxidation of diphenols to quinines which is rapid and gives colored products (Eissa et al, 2009) Figure (5) demonstrates the Reactions of enzymatic browning

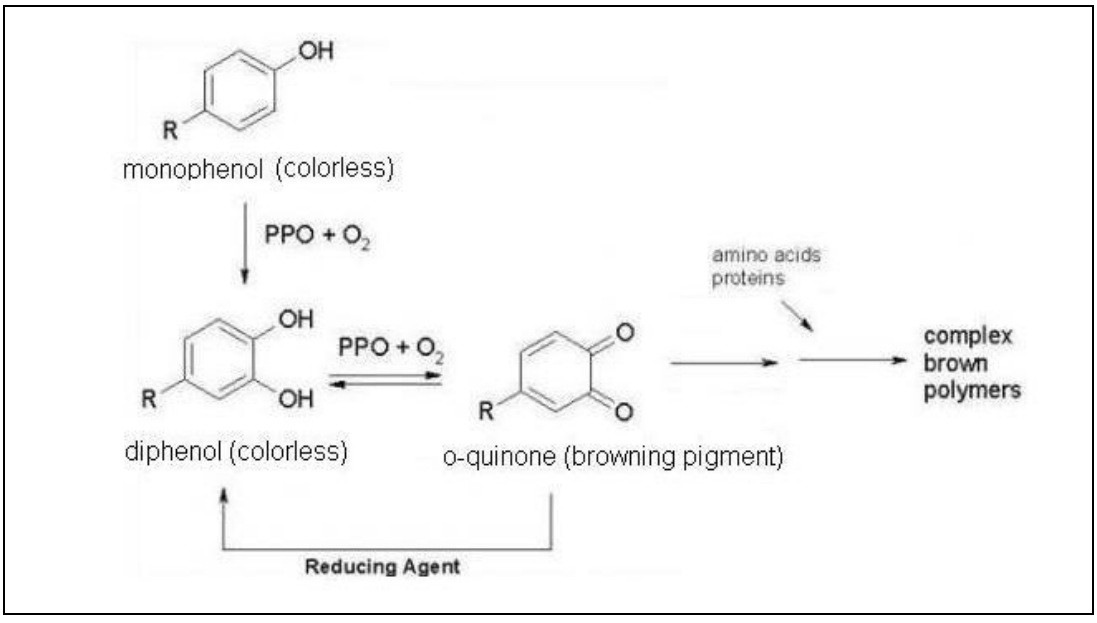


Figure 5: Reactions of enzymatic browning (Ioannou et al, 2013)

Different approaches with various actions where used to limit oxidation phenomena of fruits and vegetables. Then involve, pretreatment of fruit and vegetables by chemical and physical approaches as well as coating. Moreover, storage time, temperature, kind of packaging, and the oxygen content affect oxidation reactions (Ioannou et al, 2013)

2.6.1. Pre-treatment of herbs

Several research suggested strategies to use pre-treatment approach to breakdown the cuticle of fruit and vegetable, which is a natural barrier against external environment. Its help to transport water and solutes into and out of the plant. It represents the main limitation to the diffusion of molecules used in chemical treatments or to the efficiency of physical treatments such as blanching. Pre-treatments techniques involve different permeabilisation methods such as mechanical or chemical pre-treatments (Eissa et al, 2009).

2.6.2. Chemical treatments

Various chemical treatments reported to limit oxidation phenomena by using different chemical agent: antioxidant agent, chelating agent, firmness agent, and acidifying agent (Artes et al, 1998)

2.6.2.1. Treatment with antioxidant agent

Antioxidants react with oxygen and the intermediate of product to prevent formation of melanin and browning by breaking the chain

reactions. Several factors affect the effectiveness of this technique such as pH, water activity (aw), temperature, light and composition of the atmosphere. Ascorbic acid is a traditional agent, which is used in the industry, with other main agents like hexylresorcinol E586, erythorbic acid E315, N-acetyl cysteine E920, cysteine hydrochloride E920, and glutathione (Ioannou et al, 2013)

2.6.2.2. Treatment with chelating agent

There are several chelators used to reduce the enzymatic activity of PPO. PPO is active in presence of copper ions, by binding divalent cations in the medium. kojic acid, citric acid E330 and EDTA E385 are proposed principal chelating agents (Albanese et al, 2007)

2.6.2.3. Treatment with firmness agent

Firmness agents is usually used to increase strength of cell wall by prevent destruction of cell compartments also the contact of PPO with polyphenols in the vacuole. Calcium salts are the main agents of firmness such as calcium lactate E327, calcium propionate E282, calcium chloride E509, calcium ascorbate E302 and sodium chloride (Amodio et al, 2011)

2.6.2.4. Treatment with acidifying agent

The activity of PPO activity has been controlled by adjusting of pH value to acidic conditions (sometimes pH reached to less than 3). Citric

acid E330, erythorbic acid E315, ascorbic acid E300 and glutathione are used as acidifying agents for this purpose (Dea et al, 2010).

2.6.3. Physical treatments

According to the Arrhenius law, the reduction of temperature leads to a decrease in browning reaction. PPO is sensitive to temperature variation, which is inactive at 80 °C or above. Freezing helps to reduce activity of PPO by decreasing the available water for enzymatic reactions. Moreover, preservation under a modified atmosphere reduces oxygen content and avoids the reaction of enzymatic browning (Di Matteo et al, 2000)

2.6.3.1. Blanching

Blanching is a heat treatment used to preserve fruit and vegetable by inactivation of spoilage enzymes. There are different methods to employ blanching commercially: water, steam, and microwave blanching. The effectiveness of blanching is commonly affected by several factors such as: the type of product, size or maturity status, as well the blanching time varies depending on the technique used. This process is responsible for the inactivation of oxidative enzymes such as PPO (Lin et al, 2005)

on the other hand, blanching can lead to nutrients loss, and decrease the weight product. Thus, it alters the consistency of the product and its flavor (Oboh et al, 2004).

2.6.3.2. Freezing

Freezing is the most widely used technique to preserve fruit and vegetable and increase shelf life. It is used to inhibit browning reaction, by decreasing the availability of water for enzymatic reaction. Many studies showed that water activity decreased with temperature, when the storage temperature reached -24°C, water activity was 0.3.

However, freezing leads to irreversible changes in the food product such as firmness loss during thawing, thus, food quality is often altered and enzymatic reactions take place very rapidly in the product. Therefore, the freezing method can be useful by combination with other conservation methods such as dipping or blanching (Pimia et al, 2003)

2.6.3.3. Modified atmosphere packaging

The main element of oxidation and PPO activity is oxygen. Adjusting the oxygen content of the storage atmosphere leads to control enzymatic-browning reaction. Modified atmosphere packaging was found that reduction of the browning reaction was associated with a decrease in nutrient loss, in particular when nitrogen (N_2) replaced by nitrogen dioxide (NO_2) and argon. The combination of chemical treatment and packaging was considered a successful way to improve the shelf life of fruits and vegetables (Marrero et al, 2006).

2.6.4. Coating

Many studies demonstrated that applying an edible layer on the surface of fruits and vegetables was companied by decrease of moisture and aroma losses. the delaying of color changes and gas transfer, and the improvement of the general appearance of the product through storage are considered as benefits of coating. Moreover, enzymatic browning was delayed by isolating the coated product from the environment. (Debeaufort, 2010).

2.7 Packaging

Packaging is one of the most critical steps in the post-harvesting operations. It plays an important role in processing and preservation of food in respect to physical and chemical changes. In addition, it contributed to expand the export market for food products. Packaging protects the contents from the external environmental conditions and ensures full retention of the utility value of the product as well as prevents loss and damage of product quality. Some plastic materials were used very widely for food packaging because of their obvious advantages of being light in weight, having good productivity, can be manufactured into a number of forms and shapes and being recyclable (Ahvenainen, 1999).

More demand for advanced and creative packaging has led to create several innovative active and intelligent packaging materials. Different purposes for each type of packaging. Active packaging is used to protect

against oxygen and moisture. On other hand, intelligent packaging was used to provide information about aspects of the history of the package and/or the quality of the food (Gaikwad, 2018).

The goal of active packaging is to extend the shelf life of food and quality of food for long time. Some materials were used to change interactions between packaging and product and headspace including: physical, chemical and biological actions. Two types of active packaging system; sachets and pads which were placed inside packages then active ingredients were incorporated directly with packaging materials (De Jong et al, 2005).

Sachets system contains oxygen scavengers which uses the process of rusting or iron oxidation compound in the presence of water and oxygen. Enzyme technology was used to make oxygen scavengers. Iron and ascorbic acid were mainly used to produce oxygen absorber powder which commonly used in coffee, pizzas, baked goods and dried foods. Moreover, there were carbon dioxide absorbers which usually found in roasted and ground coffee packages. Furthermore, some sachets can emit ethanol as an antimicrobial agent to increase shelf life of high moisture products such as bakery products (Gander, 2007).

Pads system was used in packages of meats that were likely to leak after temperature fluctuations. This system was based on water absorption by super absorbent polymer granules placed between two layers of

microporous non-woven polymer. This type of pads contributed to prevent the growth of mold and bacteria (Hurme et al, 2002)

Some active packaging techniques contain active components which used as scavengers of oxygen. Polyethylene terephthalate (PET); was one of the materials that used in bottles and many plastic containers, can use in this method. Adding scavengers to plastic can solve many problems such as packaging film which is tight fitting like cheese pack (Robertson, 2006).

BioSwitch system has been used to reduce bacterial growth. This system was designed to activate antimicrobial respond when environmental changes such as UV light, pH, and temperature occur. Inclusion polysaccharide particles that encapsulated antimicrobial components are the common example of BioSwitch system. Polysaccharide is considered as "food" to many bacteria which can digest it when they grow. So if a bacterial contamination occurs, the antimicrobial compounds released when the bacteria grow which lead to inhibit subsequent microbial growth (Yam, 2000).

The importance of monitoring different quality defects of food product and providing information related to food safety to consumer have led to intelligent packaging world. Intelligent packaging aimed to improve the quality of food product and make it more convenience as well as provide limitations for tamper. In addition, Intelligent packaging has the ability to report the conditions outside of packaging as well as measuring the quality of product directly by direct contact between product and

packaging. Intelligent packaging is a tool for increasing shelf life, enhances safety and quality of product as well as gives information and warns about potential problems that may happen. Many indicators were used to achieve intelligent packaging such as time-temperature indicators (TTIs), gas indicator, thermochromic inks (Huff, 2017).

Vacuum packaging is usually carried out by removing air from the product pouch and hermetically sealing it. This technique contributs to increase shelf life of a product, inhibits microorganism growth, and improves hygiene. Moreover, it preservesflavor, and protects against dehydration and weight loss (Gorris, 1992).

Many studies showed that vacuum packaging had a positive influence on properties of food. It played an important role to prolonged maintenance of original flavor of the herbs. In addition, it contributed to preserve flavor and color of the sliced celeriac for one month (Gorris, 1992).

Furthermore, combination between vacuum packaging and chemical treatment using ascorbic acid based antioxidant solutions and storing at 5ºC contributed to reduce enzymatic discoloration in potato strips (Ferrer et al, 2006).

2.8 Respiration

Reduction of the respiration rate has contributed to maintain the quality of the product for a longer period, which increased the shelf life of

the food products. An increased respiratory rate leads to the product being unable to repel the microbial attack, causing damage. The temperature and atmosphere surrounding the product affect the respiratory rate. Ambient atmosphere lowers oxygen concentration and increases carbon dioxide from the respiratory rate of most products. Basters et al. (1993) showed that the atmosphere comprised 8% of carbon dioxide and 3% oxygen, extended the shelf life of the broccoli to seven weeks. The same authors observed that there was a delay in the yellowing of the florets and reducing the number of infected sites. Burton et al, (1987) found that 5 % O2 is suitable for slowing the development of fresh mushrooms. Sveine et al, (1967) found similar observations. It was found that 5 % CO2 led to delay in the opening of mushroom caps. Similarly, it was found that atmosphere composed of 2.5 to 5 % O2 improved the storage period of tomatoes, provided that CO2 concentration must not exceed 5 %. The respiration of strawberries was strongly decreased when stored under an atmosphere of decreased oxygen content (5 - 6% O2) but rich in CO2 (15 to 20 % CO2) (Tano et al, 2009).

2.9 Functional and technical properties of onion, sumac, lactic acid and oil

In this study, onion, sumac, lactic acid and oil were used as additives to improve the sensory properties and the stability of green thyme leaves products. From the view point of hurdle technology, vacuum packaging with temperature control in presence of previously mentioned additives

were employed to extend the shelf life the products. In this section, the preservative effect of onion, sumac, lactic acid, and oil will be discussed

2.9.1 Onion

Onion (*Allium cepal*) is one of the oldest crops in the world. There are more than 500 species that differ in shape, taste, and color. Onion was used for multiple purposes. It was also considered to be a rich antimicrobial agent. It contains phenol, which has been shown to be effective against a wide range of microorganisms. Onion prevents the development of bacteria such as *Bacillus cereus, Staphylococcus aureus*, *Bacillus cereus*, *Staphylococcus aureus, Micrococcus luteus, Listeria monocytogenes*, and all microorganisms typically associated with the deterioration of foods by inhibiting of DNA gyrase of microorganism (Kabrah et al, 2016)

The suggested mechanism of antimicrobial action of onion includes: distribution of membrane function and structure by altering cell membrane. Moreover, an antimicrobial agent may interrupt DNA/RNA synthesis and function and interfere with metabolic enzyme which leads to both cell and its metabolism alteration. Furthermore, induce coagulation of cytoplasmic constituents which altered the environment of cytosolic. Moreover, it can interfere with intercellular communication and disturb of quorum sensing (Asad Ullah et al, 2019)

Onion is one of the products that rich in phenolic compounds such as flavones, flavanones, flavonols, isoflavones, flavanonols, flavanols,

chalcones, and anthocyanins. Moreover, products such 2-(3,4-dihydroxyphenyl)-4, 6-dihydroxy-2-methoxybenzofuran-3- derived from querectin which the main component of onion had activity against Helicobacter pylori strains.

Furthermore, sulfur compounds were considered as antimicrobial agents in onion which used as a natural preservatives. Organic acid and sugars can shape the sensory profile of onions. Organic acids play an important role in the acidity and pH of the onion juice. Soluble sugars have given the sweetness of onions which affect on the acceptability of onions by consumers (Liguori et al, 2017) Onion contains glutathione which promotes the ability of liver to metabolize fat. In addition, onion is rich in minerals such as calcium, magnesium, sodium, potassium, selenium, and phosphorus where they help to improve the texture of skin by inhibiting melanin formation (Kabrah et al, 2016). It was found that allicin in onion was effective against *Candida*, *Cryptococcus*, *Trichophyton*, *Epidermophyton* and *Microsporum*. In addition, Allicin that found in Allium has an antifungal and antibacterial activity such as *Staphylococcus epidermidis* and methicillin-resistant *Staphylococcus aureus*. Also, it reduced the production of aflatoxin production by *Asparguillus flavus* and *Asparguillus parasiticus* (Yuniarti, 2018).

The effect of three types of onion (red, green and white) on 6 species of bacteria (methicillin-resistant *Staphylococcus aureus (MRSA)*, methicillin-sensitive *Staphylococcus aureus(MSSA)*, *Escherichia coli*,

Pseudomonas aeruginosa, Klebsiella species, and Salmonella species) were investigated. Three types of onion exhibited antibacterial effect on all tested microbes. Moreover, the degree of an antibacterial activity depends on the concentration and type of onions. Hence, the red onion had an effect on the most tested microbes (Kabrah et al, 2016)

2.9.2 Sumac

Somac is the common name of the genus (Rhus). Several studies showed that somac increased the microbiological stability of some food products (Rayne, 2007). Somac had antimicrobial compounds. Other studies found that Rhus, such as Rhuscoriaria L. and Rhus Coriaria, had antimicrobial effects on *B. cereus, L. monocytogenes, E. coli and S. typhimurium* (Rayne, 2007).

Furthermore, a research was done to study the relationship between antioxidant and antibacterial activity of R. coriaria and its active constituents (Phenols, glycosides, Alkaloids, and terpenoids) against six microorganisms (*E.coli, S.aureus, P.vulgaris, Shigella SPP., Staph. aureus, and P. aerugenisis*). The study showed that total R. coriaria extract and its active constituents (phenols, and glycosides) were the most effective as antioxidant and antibacterial agent appeared against all studied bacteria compared to Alkaloids, and terpenoids. The antibacterial activity of these compounds may relate to its total antioxidant activity. Therefore, R. coriaria extracts and its active constituents could act as bactericidal agents

against bacterial infection and as a natural preservative in food against food borne disease (Rayne, 2007)

Sumac contains phenolic acids and quinines which are known as antimicrobial substances among herbal second metabolites. Phenolic acids are one of the simplest bioactive phytochemicals which consists of a single substituted phenolic ring. Because of oxidizing ability, it inhibits enzymes possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins.

Quinones are aromatic rings with two ketone substitutions. They are ubiquitous in nature and are highly reactive. These compounds are colourful and responsible for the browning reaction in cut or injured fruits and vegetables. They provide a source of stable free radicals and can form irreversible complexes with nucleophilic amino acids in proteins that often cause their function loss and subsequent cell death. Surface-exposed adhesions, cell wall polypeptides, and membrane-bound enzymes are probable targets of quinone oxidization (Attari, 2016).

Sumac contains a representative for each of the mentioned phytochemicals. 1,2-dioxo-6-hydroxycyclohexadiene-4-carboxilic acid and gallic acid are phenolic acids. 1,2-dioxo-6-hydroxycyclohexadiene-4-carboxilic acid also belongs to quinones (Saxena G, 2008).

They have different spectra of antibacterial activity of these compounds which related to their chemical structures. 1,2-dioxo-6-

hydroxycyclohexadiene-4-carboxilic acid is more polar than gallic acid and therefore, it cannot pass through gram negatives' cell walls; so its antibacterial effect is limited to gram-positive bacteria. Gallic acid affects both gram-positive and gram-negative bacteria, but because of its relatively weak oxidizing activity, its antibacterial activity is not so strong. Gallic acid has been found in another species of genus *Rhus*, namely, *R. glabra* L. (Cowan, 1999).

2.9.3 Lactic Acid

Lactic acid is an organic acid($CH_3CH(OH)CO_2H$), has white color in solid-state, extremely soluble in water and colorless in liquid state. It is produced synthetically and naturally (Benninga, 1990).

Lactic acid is one of the earliest acids to be used in foods. It was first commercially produced about 60 years ago. Within the past two decades, it became an important ingredient. The mild taste characteristics of the acid do not mask weaker aromatic flavors. Lactic acid has several functions in food such as pH reduction, flavor enhancement, and microbial inhibition (Zeitoun, 2002)**.**

Lactic acid is considered as a traditional substance to improve food safety and extend the shelf life of products, and at the same time it has low toxicity to humans. Recently, lactic acid was employed to reduce the microbial load on meat carcasses, and in fermented food products such as soups, dairy products, beer, and jam. The production of lactic acid is

focuses on bacterial fermentation by *Lactobacillus*, *Streptococcus*, *Leuconostoc*, and *Entrococcus* species (Sowinski et al, 2005).

Lactic acid is microbiologically generated *in situ* or exogenously. Its antimicrobial action appears to be the result of the ability of the lipophilic, undissociated acid molecules to penetrate the bacterial plasma membrane. At a high pH environment of the cytoplasm, the acid dissociates to release protons and conjugate bases; this disrupts the membrane proton-motive force, thus disabling the energy-yielding and transport process dependent upon it. The antimicrobial effect of organic acid will therefore depend upon its pK- value and the pH of the external medium. Lactic acid has a very broad mode of action and inhibits gram-positive and gram-negative bacteria as well as yeast and molds (Adams et al, 1998)

2.9.4 Vegetable Oils

Vegetable oils are derived from different sources such as seeds, nut, fruit, and grains of cereal. Canola, corn, olive, palm, kernel, cottonseed, peanut, soya bean, and sunflower are considered as major food oils which are used in cooking and salad preparation (J. Dupont, 2003). Complex mixtures of triglyceride which usually $> 95\%$ and $< 5\%$ of diacylglycerols. In addition, vegetable oils are comprised of a minor amount of tocopherols/tocotrienols (up to 900mg kg^{-1}) and phytosterol esters/phytosterols (up to 1%) (Hammond, 2003). Mainly, vegetable oils are extracted from oilseeds by using solvent extraction. But some oils like palm and olive oil were recovered by separating it from the aqueous phase present in the fruit after crushing. Refined and modified oils were the most

steps before consumption which occurred to change the physical properties of oils (Hamm, 2003). Partial hydrogenation used to reduce the content of the highly unsaturated linolenic and linoleic acids. Therefore, flavor and oxidative stability of vegetable oils were developed. Moreover, vegetable oil increased the stability of storage or cooking. Vegetable oils are sensitive to oxygen, heat and light exposure. Cool and dark are the best stored for vegetable oils (Talbot, 2016). Different fatty acid contents of common oils that were used in cooking and bakery which were led to determine the shelf life of vegetable oils. Canola, olive and peanut may have a long shelf life more than corn and safflower oils due to different percentages of monounsaturated fatty acid and polyunsaturated fatty acid. Table (12) demonstrates some of the fatty acid contents of common oils and their uses (Talbot, 2016).

Table 12: Fatty acid contents of common oils and their uses.

Vegetable oil	%SFA	%MUFA	%PUFA	Uses
Canola oil	6%	62%	32%	baking, frying and salad dressings.
Coconut oil	92%	6%	2%	baking, confections, frying, nondairy coffee creamers, salad dressings, shortening and whipped toppings
Corn oil	13%	25%	62%	baking, frying, margarine, salad dressings and shortening.
Cotton seed oil	24%	26%	50%	frying, margarine, salad dressings, and shortening.
Grape seed oil	12%	17%	71%	all-purpose cooking, margarine, and salad dressings.
Olive oil	14%	73%	11%	all-purpose cooking, margarine and salad dressing.

Vegetable oils are a major source of vitamin E which is considered as an antioxidant agent. Linoleic acid, α-linolenic acid and γ-linolenic that found in vegetable oils contributed to cholesterol-lowering properties, heart health and treat breast pain and atopic eczema. Castor oil has a ricinoleic acid that powerful stimulant laxative (Riechart RD, 2002). By product of refining vegetable oils were plenty of beneficial compounds as ß-carotene, Vitamin K, phosphatidylcholine linked to treatment of liver conditions as well prevent brain deterioration (Rios, 2014)

Various methods have been used to preserve food which designed to attack the pathogens such as bacteria and molds and prevents oxidation that causes the destruction of essential biochemical compounds. Vegetable oils

produced to reduce or eliminate one or the other (or both) of these causative agents (Aminzare, 2016)

One of the major problems related to vegetable oils is rancidity. Many factors were responsible for rancidity including: Time, temperature, light, air, exposed surface, moisture, nitrogenous organic material, and traces of metals (Wallace et al, 2011). Rancidity refers to oxidative state of oils which affect on nutritional values and their applications (Okogbenin, 2014). It led to form undesirable effect such as: bad aroma and off-flavors change in color including: color fading, browning, or degradation (Embuscado, 2015). Oxidation of lipids proceeds through three different stages: initiation, propagation, and termination Figure (6). During the initiation stage, the lipid (RH) through action of catalysts which broke down to produce free radicals that react with other food components. More free radicals are formed during the propagation phase, resulting in rapid degradation of food. These free radicals react with oxygen to produce more free radicals to quickly oxidize lipid molecules. This rapid degradation of oils or lipids is because of the ease of oxidation of free radicals to yield hydroperoxides and their breakdown compounds. Hydroperoxides and secondary oxidation products (aldehydes, ketones, acids, etc.) are responsible for the rancid aroma and off-notes in foods. Different factors affecting lipid oxidation are the presence of oxygen and transition metal ions, moisture, heat, and light. To prevent, minimize, or slow down the rate of lipid oxidation, oxygen and metal catalysts must be removed or

sequestered to render them un-reactive. The food prone to oxidation must be stored at low temperatures and/or shielded from light (Talbot, 2016).

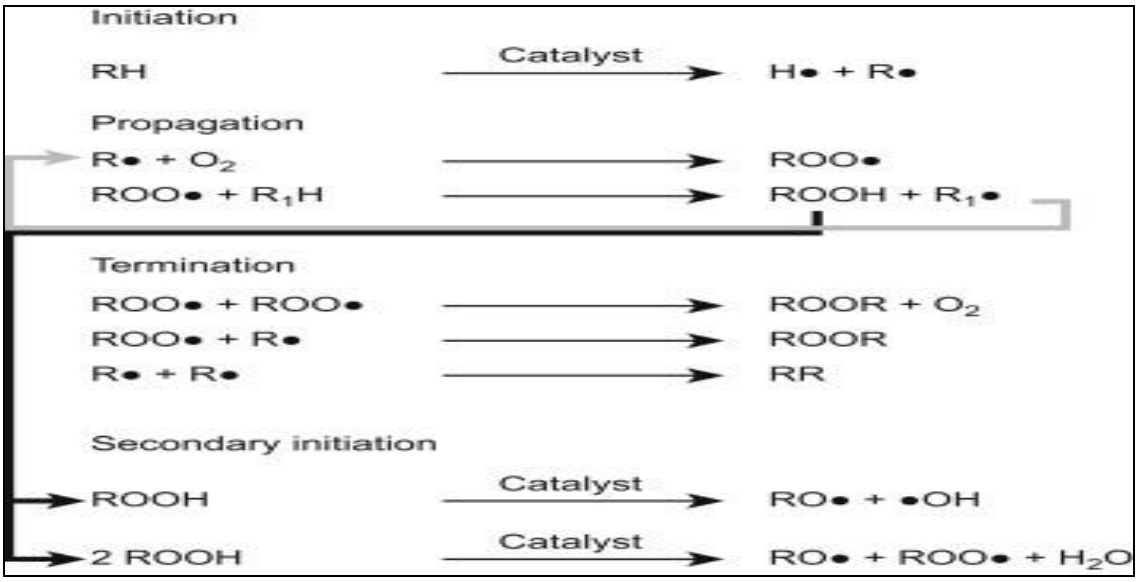


Figure 6: Mechanism of oxidation of fats and oils. Lipid substrates = RH, R1H; Free radicals = R, RO, ROO; Hydroperoxide = ROOH; Secondary oxidation products (aldehydes, ketones, acids, polymers) = ROOR; RR (Talbot, 2016).

Chapter 3

Materials and Methods

3.1 Samples collection and preparation

About 10 kg of thyme stems were harvested from local farms in a village (Kufur Jammal) near Tulkarem city /Palestine. The harvested area was carefully selected to be free from plant and any abnormalities. The leaves were manually separated from the stems. At the end of the separation, the net weight of the leaves was about 5 kg. The impurities such as weeds, straw, stems, gravels, etc were separated manually. Ultimately, the thyme leaves were cleaned under running tap water until the output water got clear. The leaves were left to dry on paper towels. The whole quantities of leaves were mixed throughly to obtain a homogenous mixture.

3.2 Treatments

The study was split into two experiments. For each experiment, about 2500 g of green thyme leaves was used. The first experiment was carried out at room temperature while the second experiment was carried out at refrigerated conditions (1-4 °C). Each experiment was divided into four groups. In each group, there were 33 samples (weight about 60 g). Accordingly, the total number of samples for each experiment was 132 samples Figure (7).

In each group, different natural ingredients were added to green thyme leaves, then the ingredients were mixed and packaged under vacuum. The groups were as following:

Group A: (100% green thyme leaves, Control)

Group B: (15% Fresh onion, 20% oil, 1.8% salt)

Group C: (15% Fresh onion, 20% oil, 1.8% salt, 1.29% Sumac)

Group D: (15% Fresh onion, 20% oil, 1.8% salt, 4% Lactic Acid, ultimate pH 4.4)

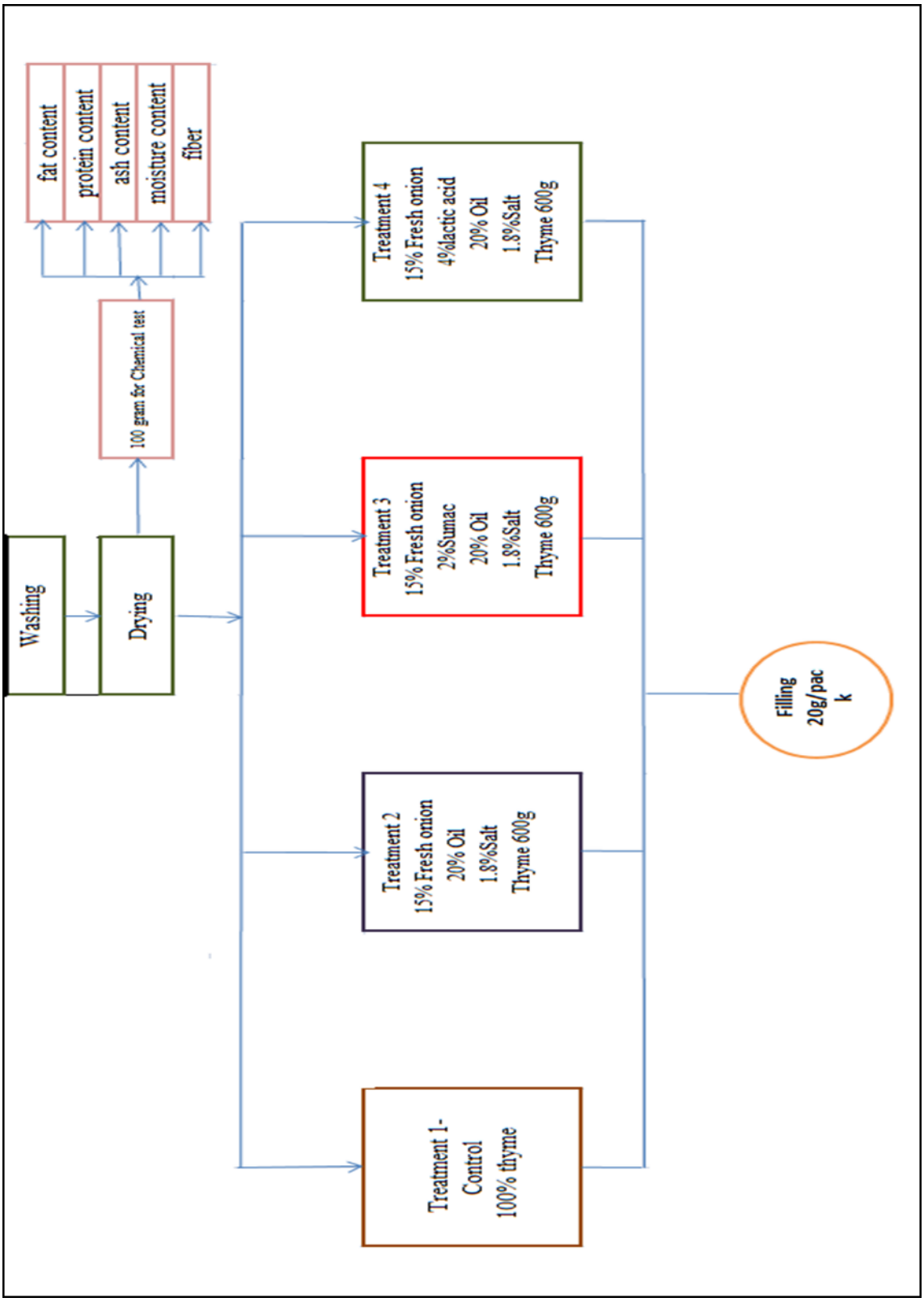


Figure 7: Flow diagram of sample treatments

3.3 Chemical analysis

About 100g of raw green thyme leaves was dedicated for proximate chemical analysis (Moisture, fat content, protein content, fiber content, and ash content).

3.3.1 Determination of moisture content

Aluminium dishes were dried in air oven (Blnder, Germany) at 105°C for 2 hours. After that, they left to cool inside desiccators for cooling at room temperature for 15-20 min. The dishes were weighed [W1]. About 5 g of the sample was accurately weighted and recorded as W2. The samples have been dried at 105°C for 16 h. After that, the samples were left for cooling in desiccators until they reached the room temperature. Eventually, the samples were weighted on analytical balance and the weights were recorded as W3. Moisture content was calculated according to the following equation:

$$Moisture\ content\% = \left\{\frac{\{(W2-(W3-W1\}}{W2}\right\} * 100\% \quad \text{.....(1)}$$

3.3.2 Determination of ash contents

The crucibles were pre-cleaned with 2M HCl and rinsed with distilled water then placed in the muffle furnace (Raypa, Spain) for one hour at 600°C. After that, the muffle furnace was turned off and crucibles were left in the furnace until the temperature has reached 250°C. The

crucibles were transferred to the desiccators to cool at room temperature, and then crucibles were weighed [W_1]. About 2 g grams of green leaves of thyme was weighed in the crucibles [W_2] and incinerated in the muffle furnace for 5 hours at 550°C. Crucibles were left in the furnace until the temperature reached 250°C, then crucibles were transferred to the desiccators to cool at room temperature, and then crucibles were weighed [W_3]. Ash content was calculated as follows:

$$Ash\ content\% = \left\{\frac{\{W3-W1\}}{W2}\right\} * 100\% \quad \text{.....(2)}$$

3.3.3 Determination of lipid content

The filter bags (ANKOM, USA) were weighed [W1]. About 1.5 gram of green leaves of thyme was placed in the filter bags and re-weighed [W2]. After that, the filter bags were sealed to encapsulate the sample and were placed in the extraction vessel (ANKOM, USA). Petroleum ether (350 ml) was added to the extraction vessel and the extractor was run for 30 min. At the end of the process, the samples were dried at 102°C for few minutes to remove the extraction solvent and the weight of samples was taken [W3g]. Lipid content was calculated as follows:

$$Lipid\ content\% = \left\{\frac{\{W2-(W3-W1)\}}{W2}\right\}.100\% \quad \text{.....(3)}$$

3.3.4 Determination of fiber content

The filter bags were weighed [W1]. Then approximately 0.5 gram of dried thyme leaves was placed in the filter bags and re-weighed [W2].

After that, the filter bags were sealed to encapsulate the sample and placed in a fiber analyzer (ANKOM, USA) in the suspender tray. The suspender tray was lowered in the acid detergent solution; a lightweight was placed on suspender tray to make sure the samples were immersed in the solution. Time was set for one hour and the heat was turned on. The samples were weighed after extraction [W3]. Fiber content was calculated as:

$$Fibre\ content\% = \left\{\frac{\{W3-W1\}}{W2}\right\} * \ 100\% \quad \text{.....(4)}$$

3.3.5 Determination of protein content

protein content was determined according to the procedure reported by Kjeldahl method (AOAC, 1990). A digestion tube, which contains 0.5 gram of green leaves of thyme. Catalyst (copper sulphate) and boiling chips, were added to the digestion unit (Gerhardt, Germany). The samples were digested for 3 hr at 420 C. In case if the sample was not clear, the tube was returned to the digestion unit for additional one hour. After completing the process, the sample was left to cool at room temperature. A 250ml flask containing 25.5ml of boric acid in addition to digestion tube was placed in the distillation unit (Gerhardt, Germany). The solution was titrated with 0.1M HCl. Protein content was calculated as:

$$Protein\ content\% = \left(\left[\frac{\{(ml\ standard\ acid - ml\ blank) \times N.acid \times 1.4007\}}{weight\ of\ sample\ (g)}\right] \times 6.25\right)$$

.....(5)

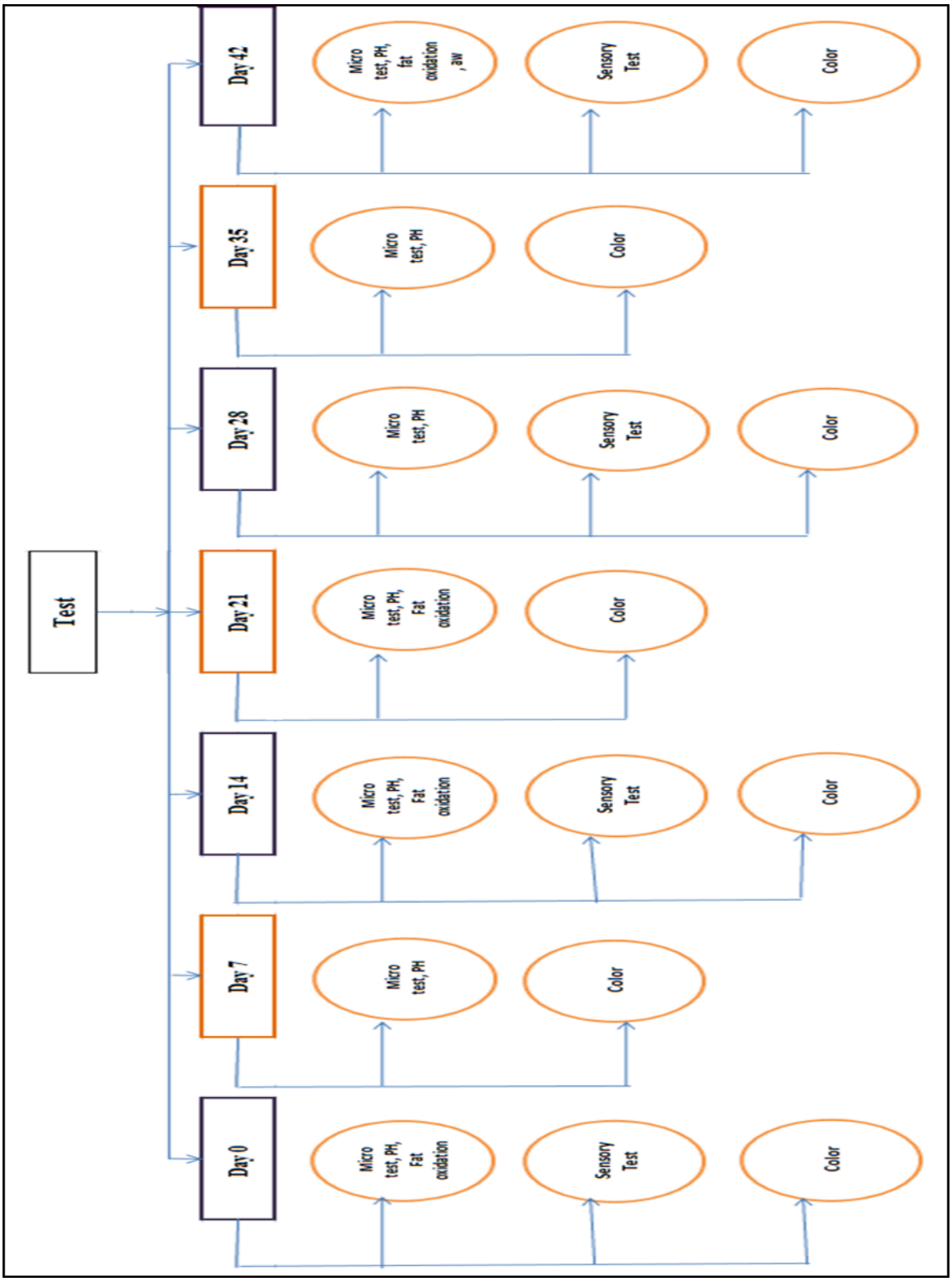


Figure 8: Quality traits analysis during storage period (42 days)

3.4 Physical analysis

3.4.1 Color measurement

CIE system (Commission Internationale de l'Eclairage) was used to measure color parameters according to standard values that are used in the world. The values used in CIE are called: L*: (Lightness) which represents the difference between light (where L*= 100) and dark (where L*= 0), a*: represents the difference between green (-a*) and red (+a*), and b* represents the difference between yellow (+b*) and blue (-b*).

In each group, six different areas were highlighted with black circles over each pack as shown in image 3. The colour indexes were measured in triplicates for each area and the mean value was determined. Reflectance colorimeter (Minolta Chroma Meter CR-400) with Control (C) as illuminant source was used to carry out the measurements. The colorimeter was calibrated with a reference white ceramic tile (Y = 93.9, x = 0.3130 and y = 0.3190) before measurement

Image 2: Measurement of color indexes of green thyme samples by chromo meter

3.4.2 pH measurement

Each treatment about 2.5g of green thyme collected and homogenized with ultra-turrax in 25 mL of Distilled water to measure pH value.

3.5 Microbiological analysis

3.5.1 Aerobic and Anaerobic Bacteria

In this study, a total of 12 replicates for each group were separated to estimate total aerobic, anaerobic, and psychrotrophic bacteria growth for 42 days. four samples are used for aerobic, anaerobic, and psychrotrophic bacteria enumeration. 10 gram of green thyme samples are took with 90 ml ringer solution. Seven dilutions (10^{-1} to 10^{-7}) are used to bacteria, mold and yeast growth. Plate Count Agar (PCA) was used as microbiological growth medium to assess total viable bacterial growth. The incubation temperature for total bacteria is 37 °C and anaerobic bacteria tested on anaerobic jar at 37 °C for 48 hrs. Four different samples were taken to check bacterial growth (Psychrotrophic Bacteria) at 4 °C.

Image 3: Enumeration of aerobic and anaerobic bacteria of green thyme samples

3.5.2 Yeast and Mold

In this study, four different samples were selected from each group. Potato Dextrose Agar (PDA) was used to enumerate molds and yeasts. The plates were incubated for 4-5 days at room temperature.

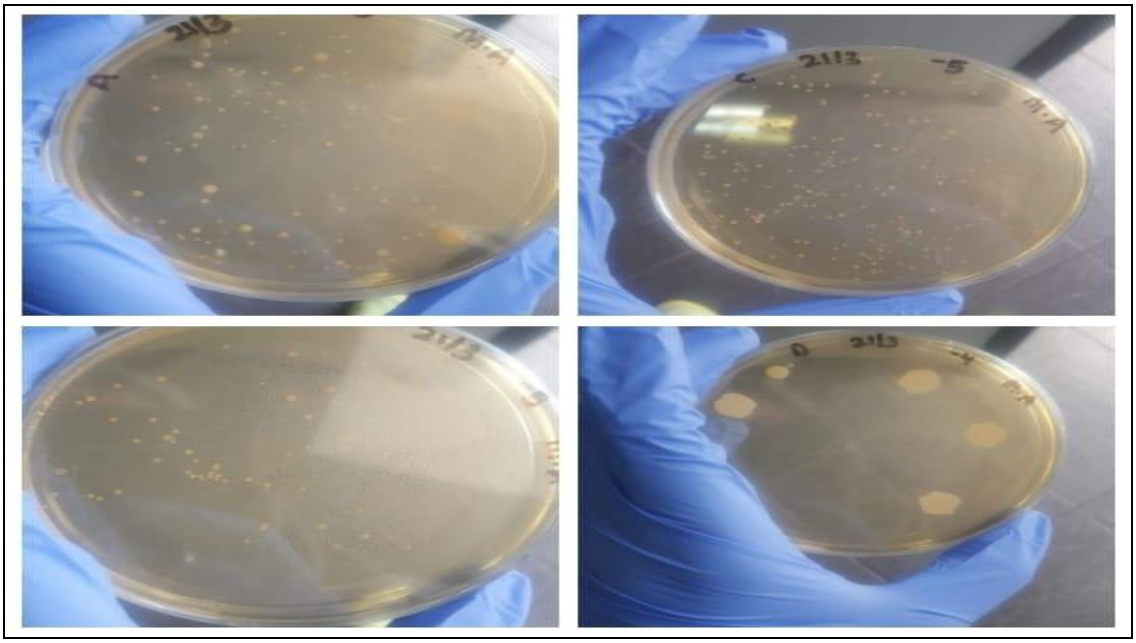


Image 4: mold and yeast of green thyme samples

3.4.3 Microbiological Challenge Testing

In this study, *Clostridium sporogenes* DSM 795 (Bactiva GmbH Co., Germany) used to simulate *Clostridium botulinum* growth in green thyme samples. This test divided was into 2 tasks:

First task: Test the effect of each treatment (group) on the growth of *Clostridium sporogenes* DSM 795 by using Agar journey and sensitivity test Methods.

I. **Pour technique:** Solution recipes for treatment B (onion, salt, oil) were prepared by adding the same percentage of natural additives in 600 ml of distilled water for each treatment. 40 ml of previously prepared of TSA culture was put in falcon tube. After that, 0.5 ml of broth *C. sporogenes* added and well-mixed. The mixture TSA culture and broth of *C. sporogenes* was poured into plates and solidity. After drying, 10 µl of recipe solution were transferred and performed in plates as spot for each group. Moreover, the spreading method was used by transfer 100 µl to TSA plates. Finally, all plates were stored under vacuum condition at 37℃ for 24 hr.

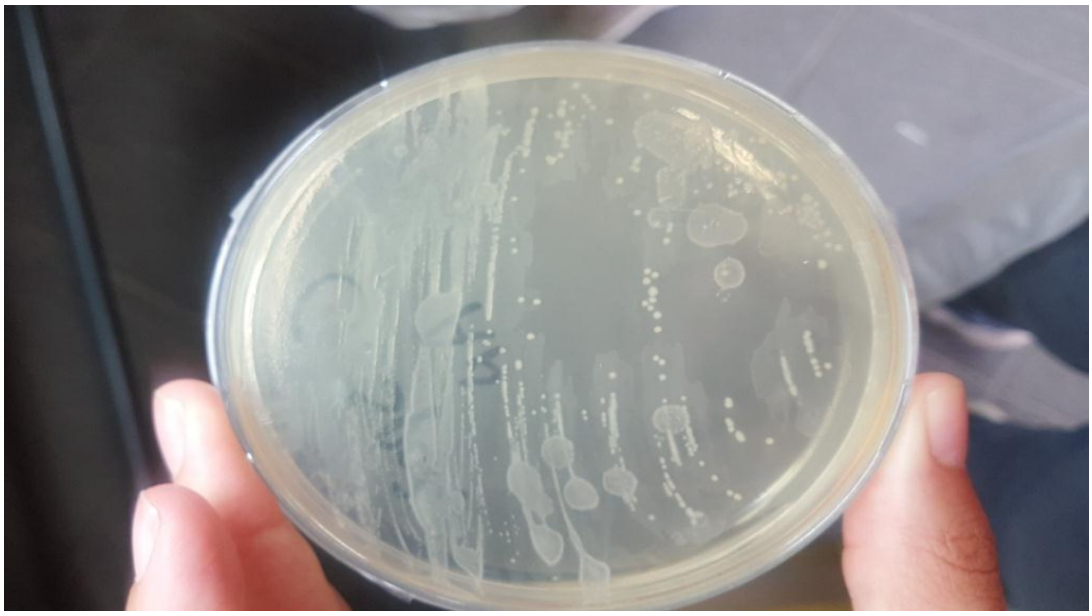


Image 5: Preparation *C. sporogenes* broth and spores form

Sensitivity test Method: 100 µl of broth *C. sporogenes* was drawn and added into previously prepared TSA plates then speared on the surface. Then, prepared plates stored in the fridge for 30 min. After that, 10 µl and 20 µl of recipe solutions spotted in paper disk, then transferred to TSA plates. Finally, all plates were stored under vacuum condition at 37 ℃ for 24 hr.

Second task: test the ability of *C. spergenes* growth in the green thyme samples (green thyme, recopies, vacuum packaging) during 7 days storage period divide into (0, 1, 3, 7 day).

Procedure of test: 10 gram of green thyme leaves weighted. Then washed and dried well. *C. sporogenes* broth was diluted into 10^{-4}. Five ml of diluted *C. sporogenes* sprayed on the 10 gram of green thyme leaves which previously weighed. Then sprayed leaves are left for almost 15 min at room temperature to dry. After drying, the ingredient for each groups were added

to prepared green thyme leaves and gently homogenized. Vacuum packaging applied to the samples, and stored at room temperature. 10 gram of samples taken and 90 ml of ringer solution was added then homogenized by stomacher machine for 3 min at 400 p/min speed. stock sample 10^{-1} was taken and put in water bath at 80 ℃ for 10 min. Then the mother sample was diluted till 10^{-4}. The diluted samples TSA plates were inculcated. Finally, the samples were incubated under vacuum condition by jars at 37 ℃ for 24 hr.

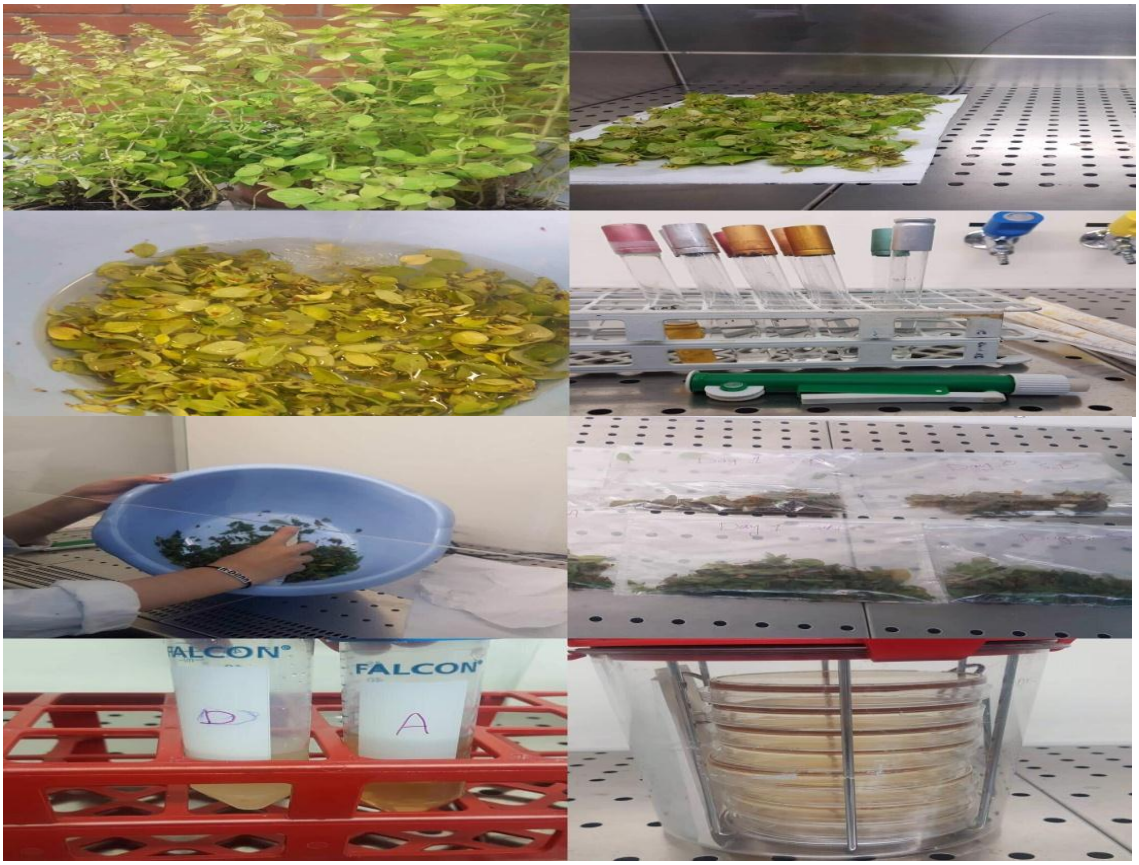


Image 7: preparation and application of microbiological challenge testing on green thyme samples

3.5 Sensory analysis

Three green thyme samples were selected from each group to prepare bread with thyme as normally prepared in bakeries that already present in Palestine. Bread with thyme (Qraas Za'atar) were cut into small pieces. These pieces were subjected to the sensory analysis performed by descriptive panel consisting 30 persons (trained) to evaluate 5 descriptors including taste, flavor, appearance, saltiness, and over all acceptance. Each four persons are trained before they started the sensory analysis by explain the scale hedonic test (in Arabic). The tasters expressed the intensity of each attribute with a mark on a 9-point [9= Like Extremely; 1= Dislike Extremely]. The samples presented to the assessors coded and in random order. The a scale hedonic test is shown in table (13).

Table 13: Scale hedonic for sensory test

Scale	Taste	Flavor	Appearance	Saltiness	Overall acceptance	
9						Like extremely
8						Like very much
7						Like Moderately
6						Like Slightly
5						Neither like nor dislike
4						Dislike slightly
3						Dislike Moderately
2						Dislike very much
1						Dislike Extremely

3.6 Statistical Analysis

The differences in quality traits (chemical, physical, and microbiological properties) between different treatments have been evaluated by ANOVA. The model investigated the main effects of treatments (both at refrigerated and room conditions) as well as the interaction effect (during the time of storage) using the general linear model (GLM)

GLM= $\frac{M1-M2}{\sqrt{(Msw/n)}}$ where (M1-M2): means differences, Msw: Mean square.SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) on the main quality traits of green fresh thyme. Means were separated using Tukey's (Qs)honestly significant difference multiple range test with $P \leq 0.05$ considered as significant.

Qs = $\frac{Ya-Yb}{SE}$, where Y_A is the larger of the two means being compared, Y_B is the smaller of the two means being compared, and SE is the standard error of the sum of the means.

Chapter 4

Results and Discussion

4.1 Chemical Compositions of green thyme

An important indication of the quality of green thyme is the compositions. Data of 100 grams of fresh green thyme as moisture, ash, fat, and fiber constituents that investigated in this research are shown in Table (14). The proximate chemical composition of thyme is usually affected by different factors such as species, breed, season, and farming conditions (Alanis et al, 2019). Our findings were in agreement with previous studies (Sadowska et al, 2017). This aromatic plant is characterized by a high density of nutrients such as minerals, vitamins, and phytonutrients (Hels et al, 2004). Wide variations in the chemical compositions have the potential of contributing to the nutritional and health needs to consumer (Hels et al, 2004).

Table 14:The proximate chemical composition of fresh thyme leaves.

	Composition	**Mean ± SD (g/100g)**
1	Moisture	44.30±0.42
2	Fiber	20.51±1.32
3	Proteins	0.71±0.05
4	Ash	5.50±0.22
5	Fat	6.33±0.22

4.2 Experiment 1: Room Temperature 25 ℃

The result of pH has been measured at an interval 7 days during the whole storage period (42 days) and it was shown in Figure(9). The pH is considered one of the most important factors that affect the survival and growth of microbes during processing, storage, and distribution. Measuring pH during storage can give an indication of microbial growth and thus the spoilage. At the starting point (day 0), group A had the highest value of pH and group D exhibited the lowest value. Group B and C had similar pH values. The main differences in pH values at starting point may be attributed to the additives in each group. Group A was the higher value because there were no additives; it was contained just fresh green leaves of thyme. The lowest pH value for group D can be attributed due to the addition of lactic acid. During storage period (42 days), pH was moderately decreased in all groups and this can be attributed due to microbial growth which can be observed in the results of the microbiological part and particular due to lactic acid bacteria. At the end of the storage (day 42), all groups exhibited the moderate changes in pH value as at starting point.

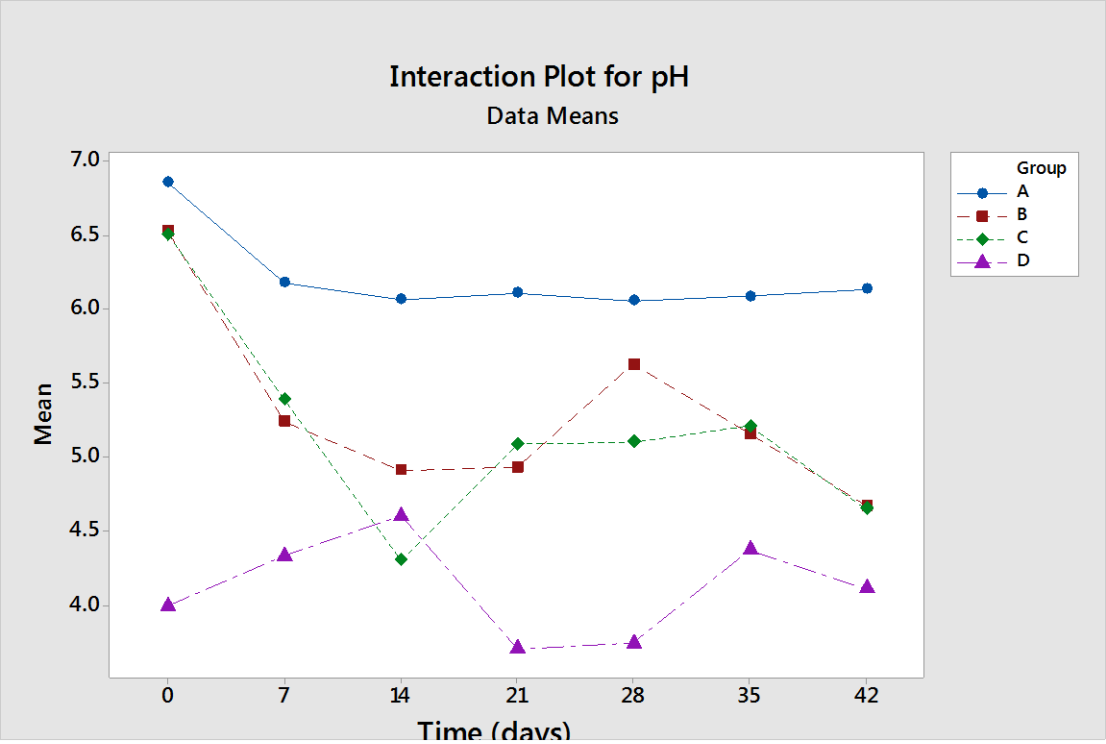


Figure 9: pH of green thyme samples at room temperature 25 ℃

It was found that the pH of green thyme in all groups at room temperature was affected by three main factors: additives, storage time, and storage conditions (vacuum packaging and temperatures). It was observed that there was a sharp drop down in pH for groups B and C at the first two weeks. The changes that occurred in pH for each treatment during the storage period were affected clearly by natural additives. Lactic acid was an effective substance among other additives in pH reduction. Lactic acid can control the pH of group D between 3.5 and 4.5 which had an effect on microbial growth, color, and shelf life described in the former sections. There were significant differences in pH- values between group A and group B and C. The addition of sumac in group C led to a reduction in pH which may be attributed to the fact that sumac is rich in phenolic acid, and quinines which help to decrease pH (Rayne, 2007).

4.2.1 Microbiological Analysis

For experiment 1 (room temperature): total count bacteria, anaerobic bacteria, and yeast and mold have been evaluated during the storage period (42 days) while in the experiment 2 (refrigerated); Psychrotrophic bacteria count was added as test beside to other previously mentioned tests. Moreover, the challenge test was employed to evaluate the risk of *clostridium botulinum* growth. The total aerobic bacterial count was shown in Figure (10).

4.2.1.1 Total aerobic bacteria analysis (PCA)

At the start point (Day 0), groups A and C had the same initial microbial load and they are the highest between four groups while group D had the lowest load. But they have different behavior during the storage period. Treatment A has dropped constantly. In contrast to group C which exhibited mild significant differences during the whole period of storage. In other hands, the initial aerobic bacterial load was significantly higher in groups A and C compared to group B. Group D had the lowest initial aerobic bacterial load. Even the whole quantity of green thymes leaves which were used to prepare all treatments, was mixed thoroughly but it was difficult to obtain homogeneous bacterial load for all groups. These differences may be attribute to the ingredients that were added for each group. Group B and C exhibited similar changes in aerobic bacterial growth during storage (Day 28,35).

In our finding, it was clear that the addition of lactic acid was the most effective ingredients against aerobic bacterial growth as was shown in group D. In the second order, sumac exhibited good preservative effect as shown in group C. Sumac contains bioactive substance because it has a phenolic acid, and quinones which worked as antimicrobial agent by decrease the pH and it can attack gram positive and negative bacteria. However this effect didn't appear in our experiment and it can be attributed due to contamination of sumac itself and lack of homogeneity (Saxena et al, 2008).

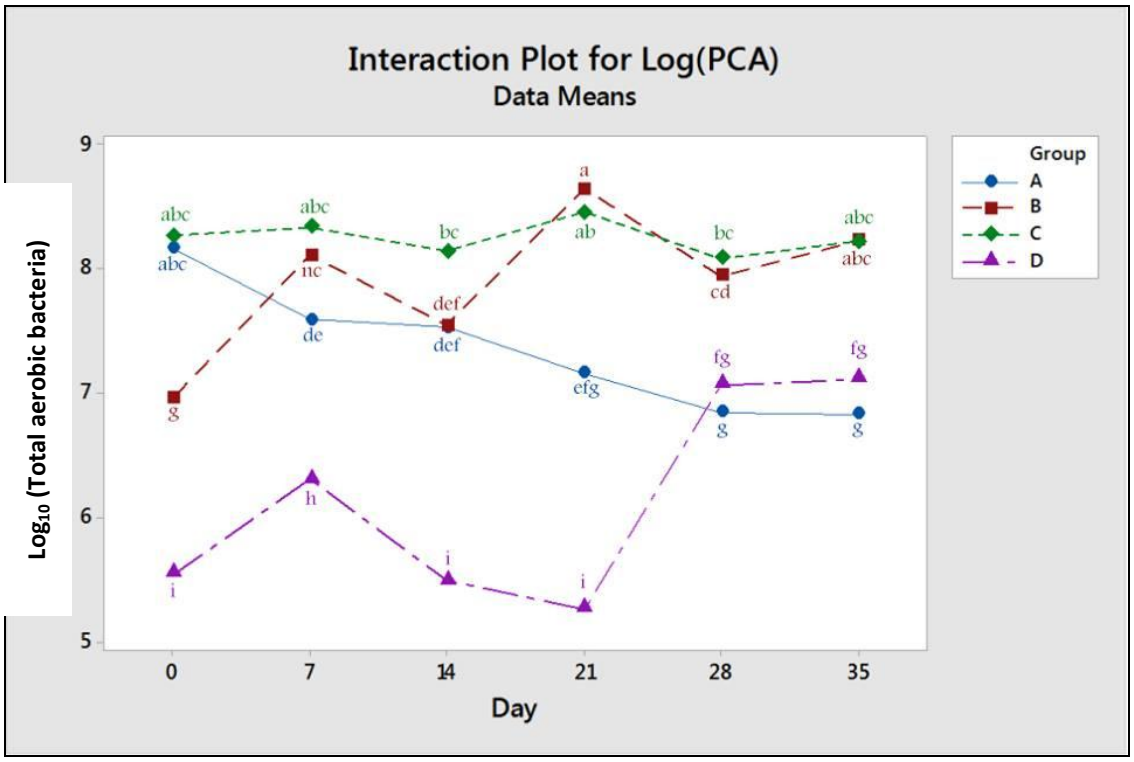


Figure 10: Total Count Bacteria of green thyme samples during 35 days at room temperature

4.2.1.2 Anaerobic bacterial analysis (PCN)

The anaerobic bacteria growth for four groups during the storage period was illustrated in Figure (11). Different initial microbial loads for each group have been observed. All groups have the significant differences in the first 14 days. Group D has the lowest growth which may be attributed to lactic acid as the main component in this group. On the other hand, the beginning points in each group (A, B, C) almost similar. During the storage period for group A, the anaerobic bacteria growth was constant after the first 14 days (Day 21, 28, 35), But the experiment has shown mild growth in groups B, C, and D in the same period.

The main point in Figure 11 is that the behavior of anaerobic bacteria is similar to behavior found in total bacteria count which means the bacteria that found in green thyme samples can grow in anaerobic and aerobic conditions. Moreover, lactic acid was the most effective ingredient against anaerobic bacterial growth as was shown in group D.

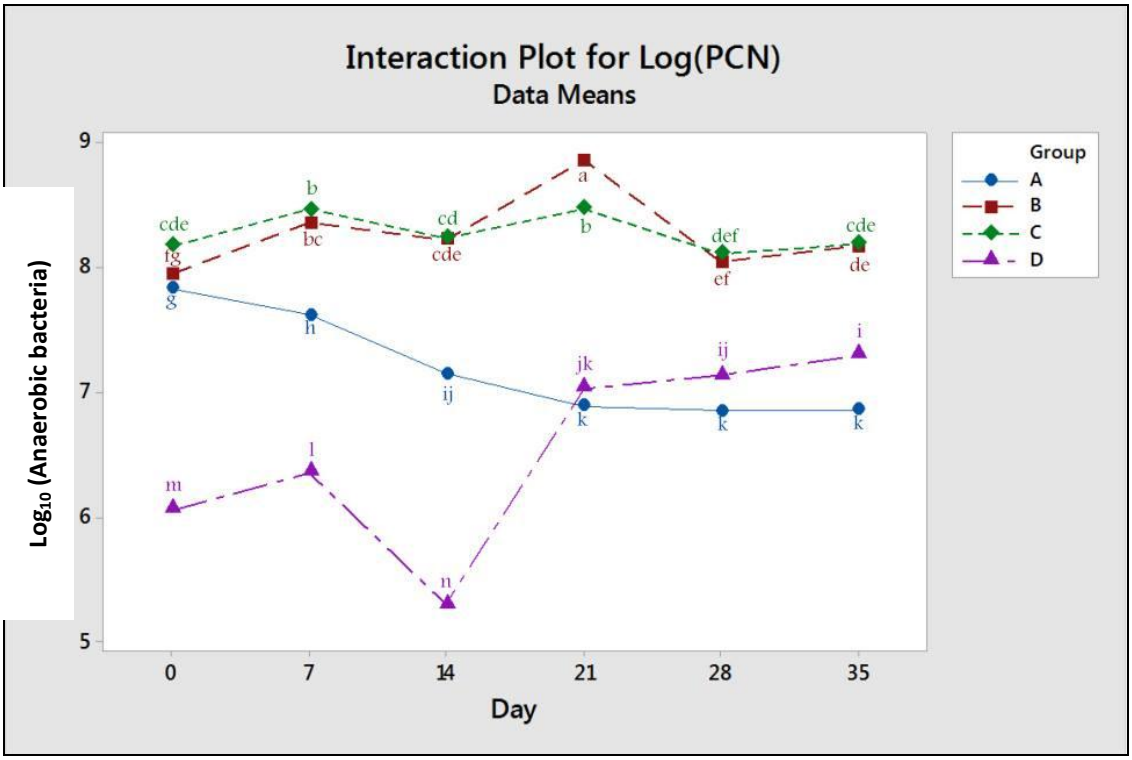


Figure 11: Anaerobic bacteria growth of green thyme samples during 42 days at room temperature

4.2.1.3 Psychrotrophic bacteria analysis (PCF)

The fresh green thyme leaves for each group had shown significant differences in psychrotrophic bacteria during the storage period (35 days) which are shown in Figure (12). Group D had the lowest and strongest an effect on different types of bacteria which appeared in previous figures of total count, anaerobic as well as Psychrotrophic bacteria. Lactic acid can control the pH of group D between 3.5 and 4.5 which had effect on microbial growth was probably caused by physiological and morphological changes in bacterial cells. Wang et al. (2015) had found that lactic acid could completely prevent the growth of *Salmonella enteritidis, Escherichia coli and Listeria monocytogenes* by leakage of protein of these pathogens

which led to disruptive action on content and activity of bacterial proteins. Moreover, cytoplasmic membrane, membrane structure and intracellular structures were damaged by lactic acid (Chenjie Wang et al., 2015). In second-order, it was clear at the end of the storage period that there was a significant decrease in Psychrotrophic bacteria count in group C compared to group A and B. This effect may be attributed due to the addition of sumac. Nimri et al. (1999) showed that there was strong effect of sumac on gram positive and gram negative bacteria including *Bacillus cereus*, *Escherichia coli* strains (B, 01111, 2759, and 25922), *Klebsiella*, *pneumonia, Proteus vulgaris*, *Pseudomonas aeruginosa, Shigella dysentariae, Staphylococcus aureus, S. Epidermidis, Streptococcus pyogenes, Enterococcus*, *faecalis* and *Yersinia enterocoltic*. In addition, group B had significant differences in Psychrotrophic bacterial counts during whole storage period if compared to group A. The control group (A) exhibited significantly the highest Psychrotrophic bacterial count at the end of storage period if compared to other groups. As general observations, there was significant interaction effect where the effects of treatments for all groups during storage period were not similar. The combination between natural additives and vacuum packaging may contribute to decrease in Psychrotrophic bacteria growth. Similar findings were documented by Bhat et al. (2010) in which the vacuum packaging of herbs resulted in lowering the Psychrotrophic bacterial count during storage as compared to aerobic packaged. In addition, phenolic acids and flavonoids present in oregano exhibited low Psychrotrophic bacterial count as well as

natural additives like onion and sumac. As a general conclusion, it was clear that the most effective ingredients against aerobic, anaerobic, and Psychrotrophic bacteria were lactic acid. Moreover, the growth patterns for aerobic, anaerobic, and psychrotrophic bacteria was quite similar during the whole storage period with mild differences in some points of storage.

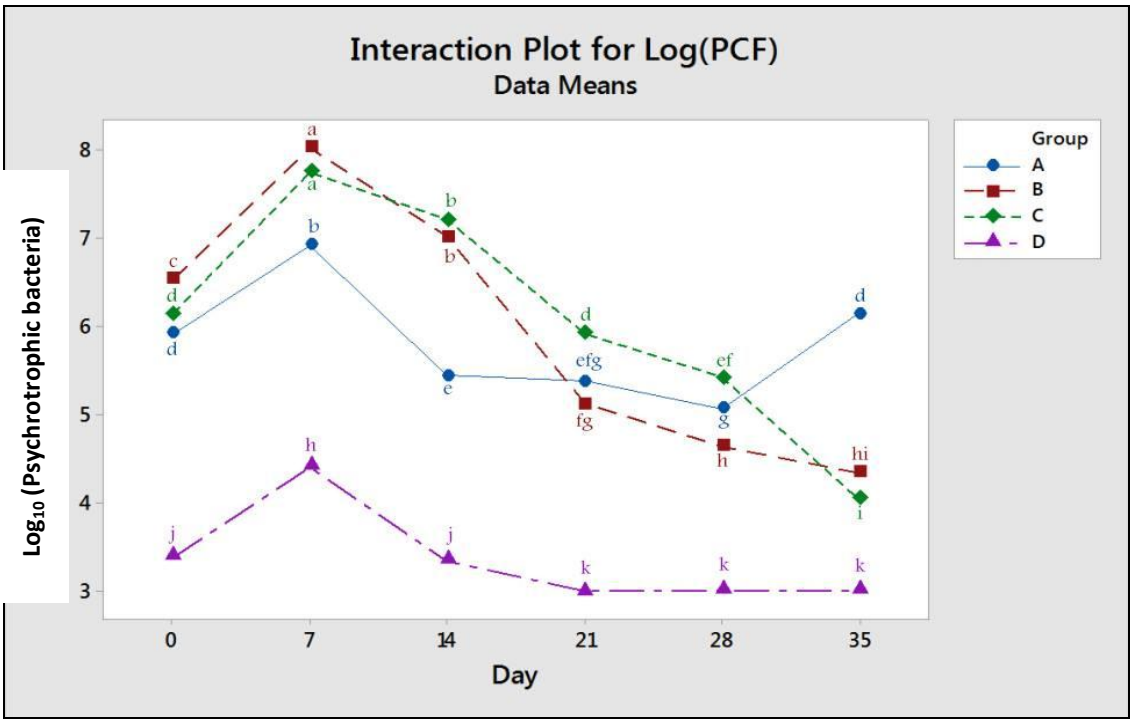


Figure 12: Psychrotrophic bacteria growth of green thyme samples during 35 days at 4℃

4.2.1.4 Yeast and mold analysis (Y&M)

The growth behavior of yeast and mold during the period of storage (42 days) of green thyme was shown in Figure (13). There were no significant differences in the initial microbial load for all four groups. In general, there was an interaction between the effect of treatments and the effect of storage time. At the end of storage, group C exhibited significantly the lowest count of yeasts and molds compared to other

groups while group A, B, D exhibited moderate values. Even oregano has high antifungal properties due to high content of phenolic derivatives (such as carvacrol and thymol) but this effect didn't appear in our experiment which may relate to ability of various yeasts and molds to grow in a wide range of pH (around pH 2 up to pH 9) and a broad temperature range (5 to 35°C). As well as, some genera can grow at reduced water activities (aw≤0.85) (Parra et al, 2004).

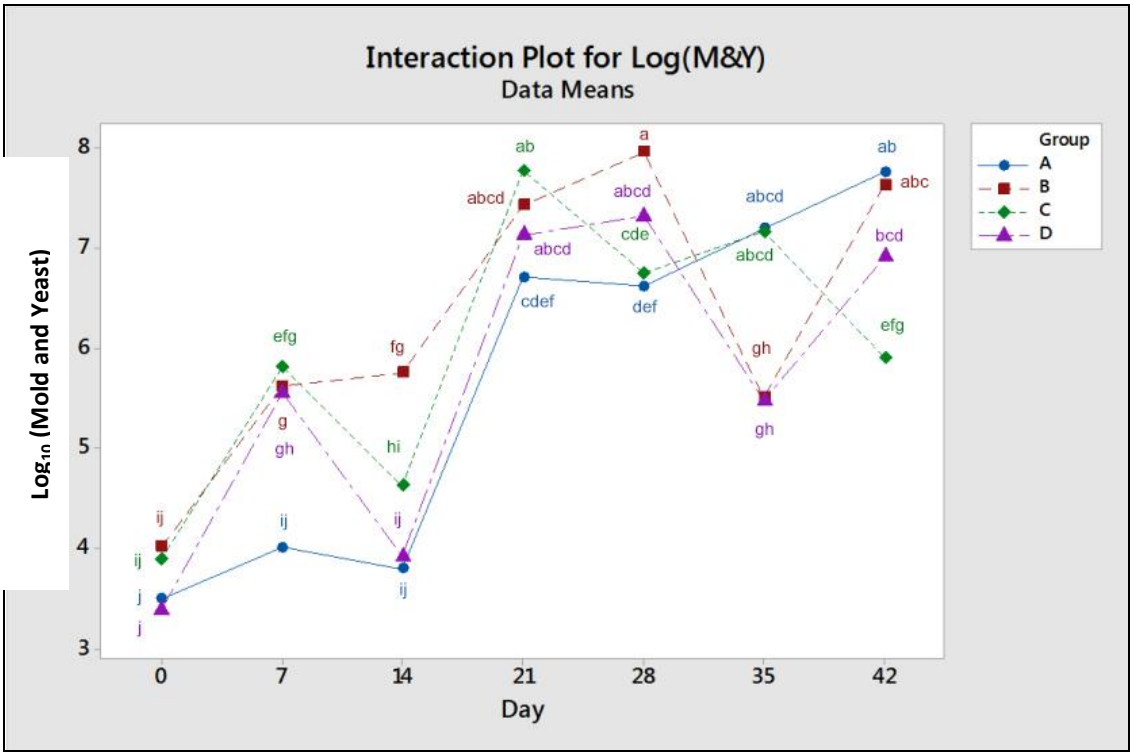


Figure 13: Mold and Yeast growth in green thyme during 42 days

4.2.2 Color measurements

During the whole period of storage (42 days), color indexes (L*a*b*) have been evaluated to investigate the effect of treatments on color traits.

4.2.2.1 Colour indexes: a*-values for all groups (A, B, C, D) during storage period.

a*values for all groups during the storage period are shown in figure (14). In general, there were slight significant increases in a*-values during storage for all groups except in some cases (such as: Day 28 in group A and Day 21 in group D). In addition, there was an interaction between the effect of treatments and the effect of storage time, and this was clear after 14 days of storage. Group B exhibited the lowest change in redness values during the storage period if compared to other groups except on day 28. The increase in redness values may be attributed to chlorophyll degradation which is a symptom of transition of chloroplasts to gerontoplasts, score characteristics of plastids of aging (Sitte et al, 1980). Degradation of chlorophyll brings to the surface pre-existed colors, in this case carotenoids (Gross, 1991)

Green thyme is a non-durable leafy herbs, in which degradation of leaf pigments (chlorophylls and carotenoids) or tissue browning occurs during storage due to oxidative reactions of phenolic compounds by polyphenol oxidase, which produces quinones to various polymerized products (Ferrante et al, 2004). The degradation of green leaves pigment depends on many factors including: storage environment, pH, duration of the storage period, and the concentration of natural additives that applied after harvesting (Garcia, 2002) as well as chlorophyll content of leaves (Kaur et al, 2011). Chlorophyll located in the intercellular lamella organelle

called a chloroplast. Its existence is protected by proteins that form a protein-chlorophyll complex. The complex is surrounded by protein-lipid bilayer thus making chlorophyll stable.

The content of chlorophyll in green leaf was affected by growth location, leaf age in one tree, and leaf position. The age of leaf can determine from the position of leaf out on the stem from the shoot (Indrasti et al, 2018). A research on spinach plant found that the end portion of a leaf far from the stem also has higher chlorophyll and phytochemical content than the base of the leaf attached to the stem (Ozgen and Sekerci, 2011).

Tan and Francis (2006) found that the effect color changes were attributed due to storage conditions in which chlorophyll (a) degraded to pheophytin (a), and the second degradation is transformation of chlorophyll (b) to pheophytin (b). In addition, it was found that chlorophyll (a) degraded faster than chlorophyll (b) at pH 5.5, 6.5 and 7.5. Moreover, It was found that adjusting of temperature and pH had led to color improvements (Garcia, 2002)

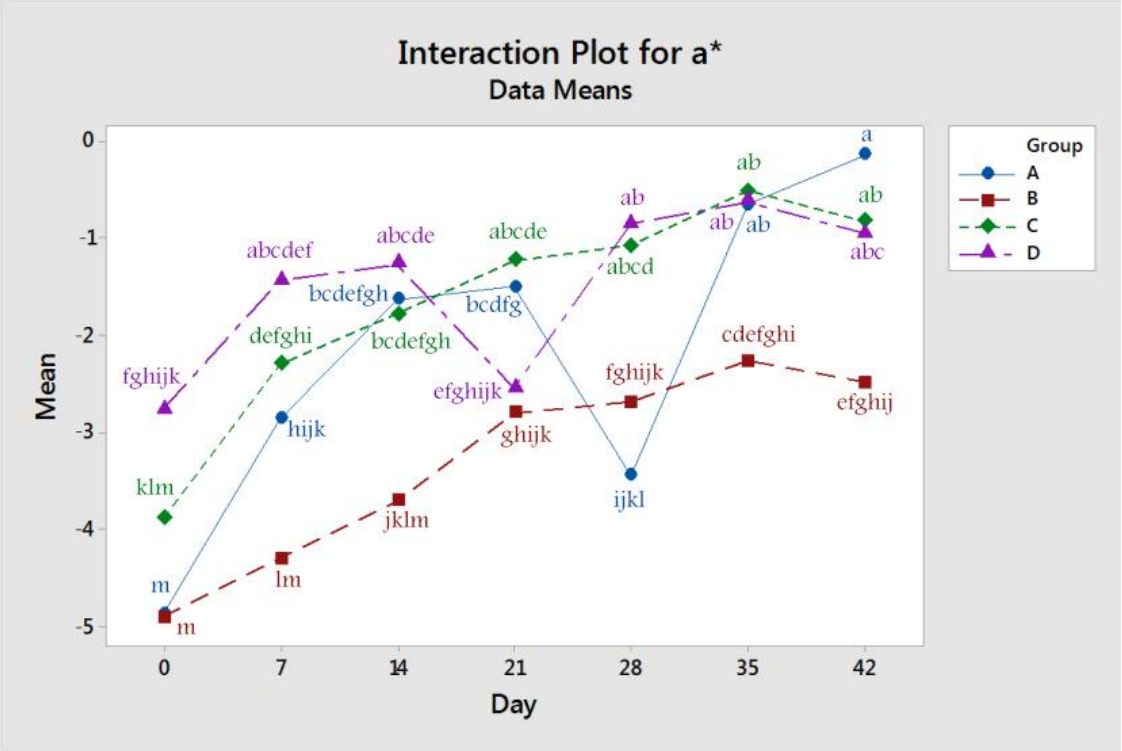


Figure 14: a* values of fresh green thyme during storage

4.2.2.2 Color indexes: b*- values for all groups during storage period

b* values for all groups during the storage period are shown in Figure (15). In general, there were slight significant increases in b*-values during storage for all groups in particular in the first week of storage. In particular, Group C exhibited significantly lower b-values during the whole period of storage if compared to other groups (A, B, D). It was clear that the addition of sumac in group C had a preservative effect on b*-value. Senescence usually leads to leaf yellowing. Ferrante et al, (2004) examined the effect of citric acid on the color of fresh-cut rocket and swiss during 12 days of storage in both dark and light conditions. It was found that the treated samples with citric acid did not show any effect in preventing chlorophyll degradation during light storage. On the contrary, the samples

treated or untreated with citric acid stored in darkness did not show any symptom of chlorophyll degradation.

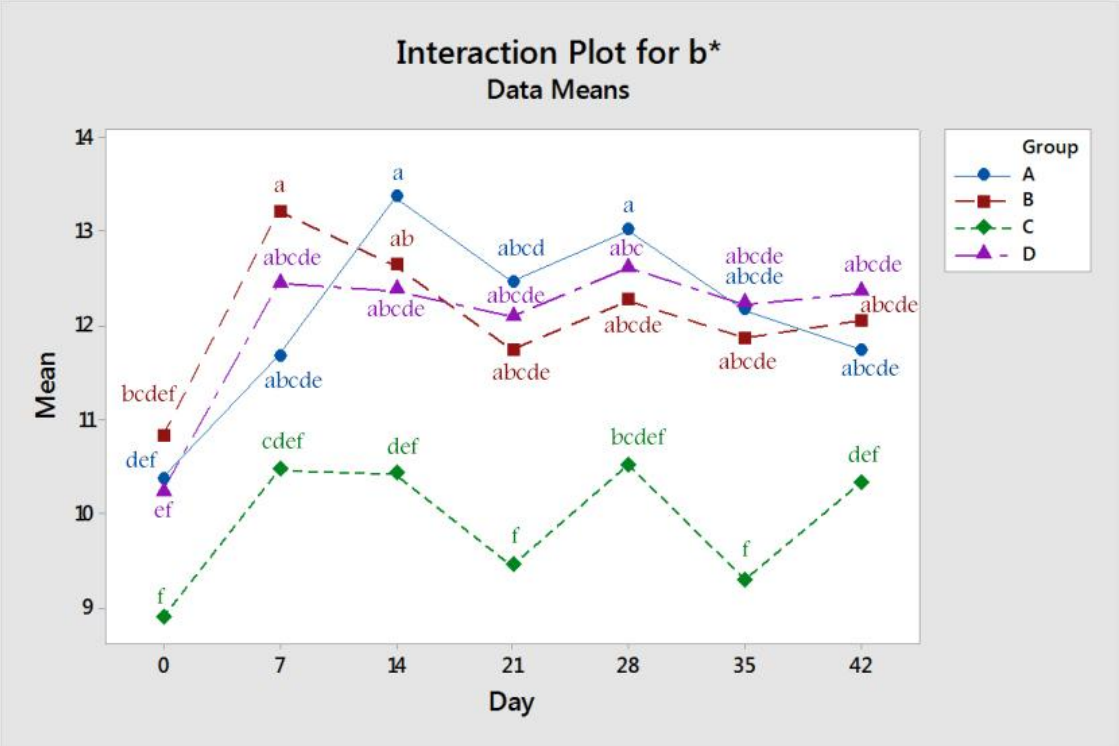


Figure 15: b* values of fresh green thyme during storage

4.2.2.3 Color indexes: L*- values for all groups during storage period

In the present work, L*- values for all groups during the storage period are shown in figure (16). In general, there were slight significant decrease in L*-values during storage for all groups. The interactions between the effect of treatments and storage time was significant after 28 days of storage. It was found that the lowest L*-values during the whole period of storage were observed in group C if compared to other groups. This can be attributed due to the addition of sumac which contains a high level of natural pigments that may be contributed to darken the color of thyme leaves.

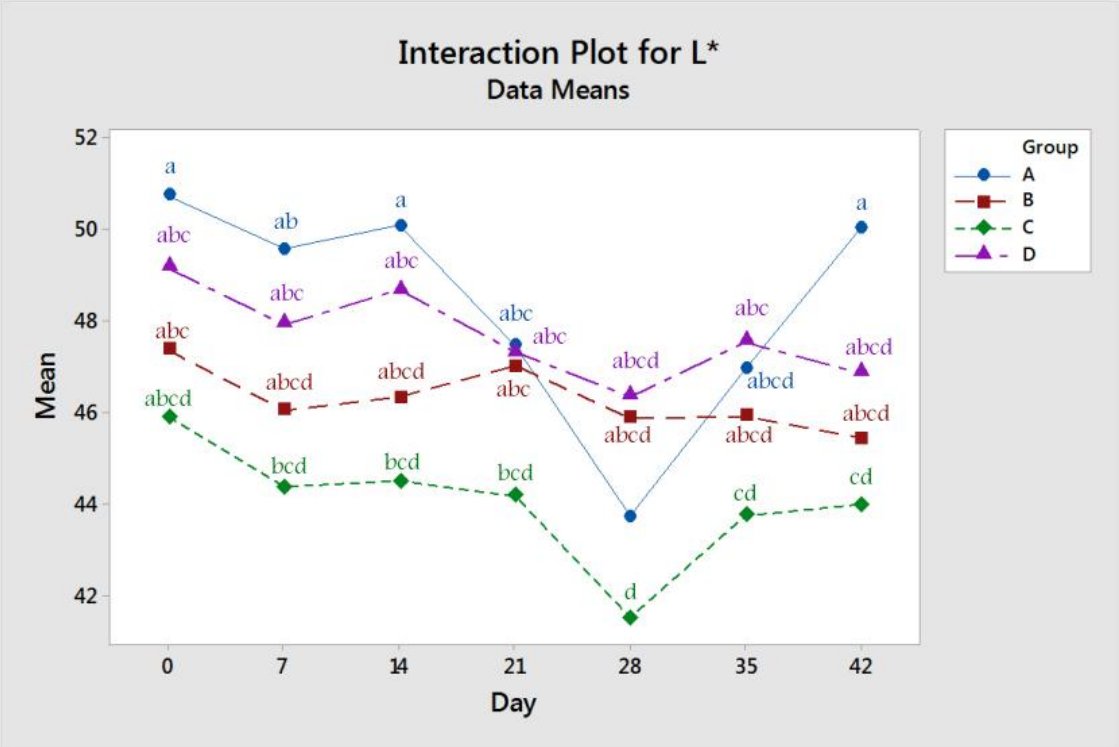


Figure 16: L* values of fresh green thyme during storage at room temperature

4.3 Experiment 2: Refrigerator temperature 4℃

4.3.1 pH measurement analysis

pH- values were measured during the refrigerated storage period Figure (17) to understand the effect of microbial growth on pH-values. Initial pH for group A was significantly higher than other groups which represented that natural pH of thyme leaves (there were no additives). Group D exhibited significantly the lowest value when compared to other groups. There was no significant difference between group B and C. the addition of sumac had led to reduction about 0.3 degree of pH while the addition of lactic acid to group D caused reduction in pH about 2.8 degrees. In general, there were no significant changes in pH during the storage period for groups A, B, C. On another hand, there was a significant drop

down in pH values in group D appeared on day 28. This may be due to the growth of lactic acid bacteria. In general, there were similarities in the patterns of changes in pH between groups stored at room and refrigerated conditions.

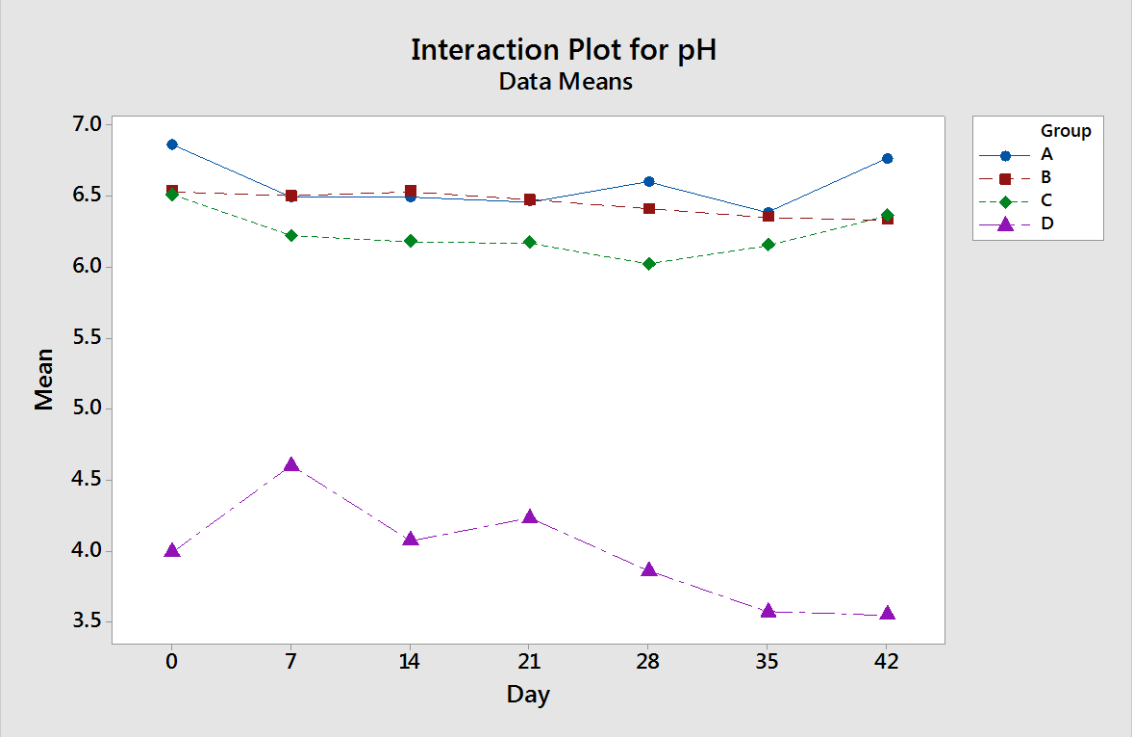


Figure 17: pH of green thyme at Refrigerator Temperature 4℃

4.3.2 Microbiological analysis

4.3.2.1Total aerobic bacteria analysis (PCA)

PCA was used as an indicator of bacterial aerobic populations in different groups. It based on an assumption that each cell will form a visible colony when mixed with agar containing the appropriate nutrients. It is not a measure of the entire bacterial population; it is a generic test for organisms that grow aerobically at mesophilic temperatures (25 to 40°C).

Figure (18) demonstrated the growth of total bacteria during the storage period (42 days). There was an interaction between the effect of treatments and the effect of storage time. Group A had significant differences in the whole period. In general, moderate differences had shown in groups B, C and D during the experiment period but significant differences shown in days (21,42 B, 28 C, 35 D). Our finding clearly related to pH measurements found in figure (17). The pH of treatment A is the highest between other groups which explained the highest growth of bacteria. On other hands lowest growth of bacteria had shown in group D.

Moreover, the results achieved in this experiment were better than experiment 1. The combination of natural additives, good packaging and cold environment to improve performance of bacteria growth elimination. Growth of Psychrophiles, mesophiles and thermophiles had decreased consistently as the temperature drops below the optimum temperature that is because of many reasons including: membrane lipids were stiffening below the optimum temperature which leads to decreased efficiency of transport proteins embedded in the membrane. Growth of bacteria was reduced at low temperature which means the organism was not able to supply the maintenance requirement of the growth rate-limiting nutrient because of loss of affinity for that substrate. The relation between temperature and affinity for substrates had taken up by active transport which uptake organic and inorganic substrates. This effect of decreased substrate affinity at low temperatures may have profound implications on the availability of substrates in the natural environment as environmental

temperatures change. At temperatures below their optimum for growth microorganisms will become increasingly unable to sequester substrates from their environment because of lowered affinity, exacerbating the anyway near-starvation conditions in many natural environments (Nedwell, 1999).

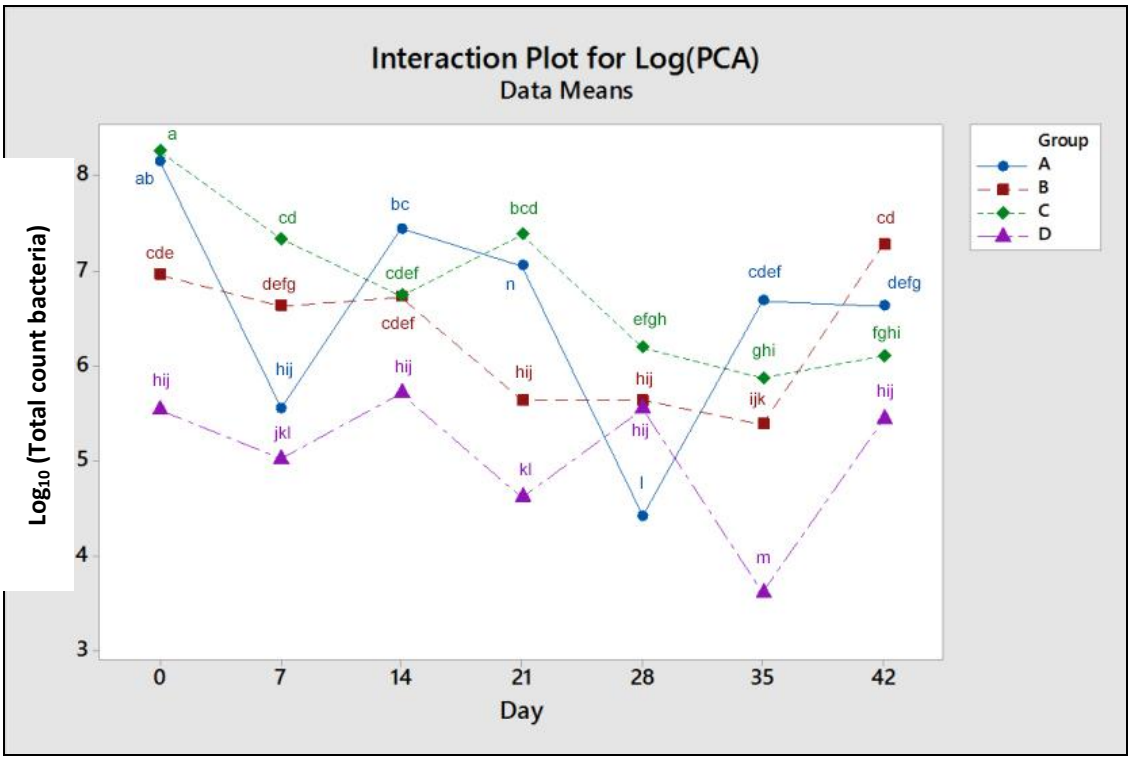


Figure 18: Total count bacteria of green thyme at refrigerator temperature 4℃

4.3.2.2 Anaerobic bacteria analysis (PCN)

Figure (19) explained the anaerobic bacteria growth during the experimental period (42 days) at refrigerator temperature. The interaction between the effect of treatments and the effect of storage time was significant. Group A had shown significant differences in the whole storage period. In general, a moderate significant decrease appeared in the other three groups. This finding may be attributed to a combination of

refrigerator temperature and natural additives. Low temperature slowed the metabolic process of microorganisms (Russell et al, 2002). Low temperature lead to decrease bacterial growth .Cold environments led to the slow rate of growth and bacteria are not able to maintain homeostasis or the maintenance of a constant internal state, to survive and grow (Sarrau et al, 2012). It was obvious that group D exhibited significantly the lowest anaerobic bacteria counts during the whole period of storage if compared to other groups. This effect may be due to the addition of lactic acid that led to a sharp drop in pH.

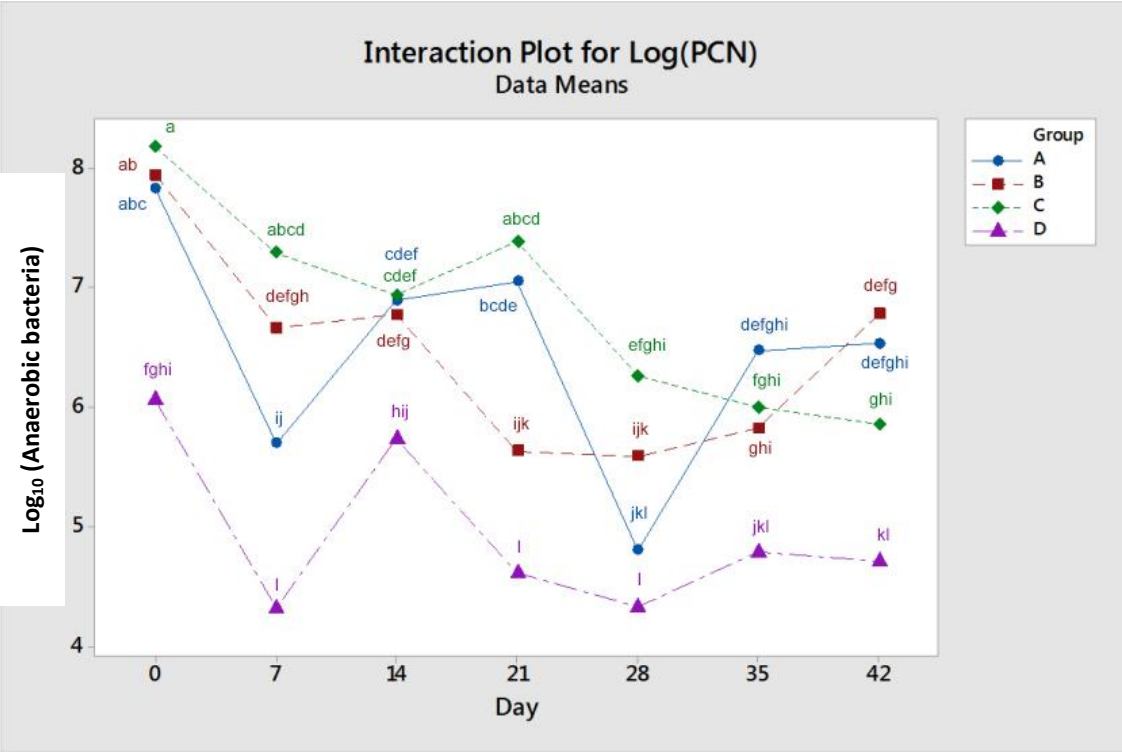


Figure 19: Anaerobic bacteria growth of green thyme at refrigerator temperature 4 ℃

4.3.2.3 Psychrotrophic bacteria count (PCF)

The growth of Psychrotrophic bacteria in green thyme leaves at refrigerator temperature 4℃ was shown in Figure (20). It was obvious that

group D exhibited significantly the lowest anaerobic bacteria counts during the whole period of storage compared to other groups. This effect may be due to the addition of lactic acid that led to a sharp drop in pH.

In general, group A had moderate significant differences during the storage period but it had significant differences in days (21, 28). Slight differences had observed in the first 21 days in group D which had significant differences on day 28. A significant decrease had found in group B during the experiment period. Finally, group C had shown moderate significant differences during the storage period.

This result may be attributed to the combination of natural additives, vacuum packaging, and cold environment lead to improve the reduction performance in the growth of bacteria (Nimri et al,1999).

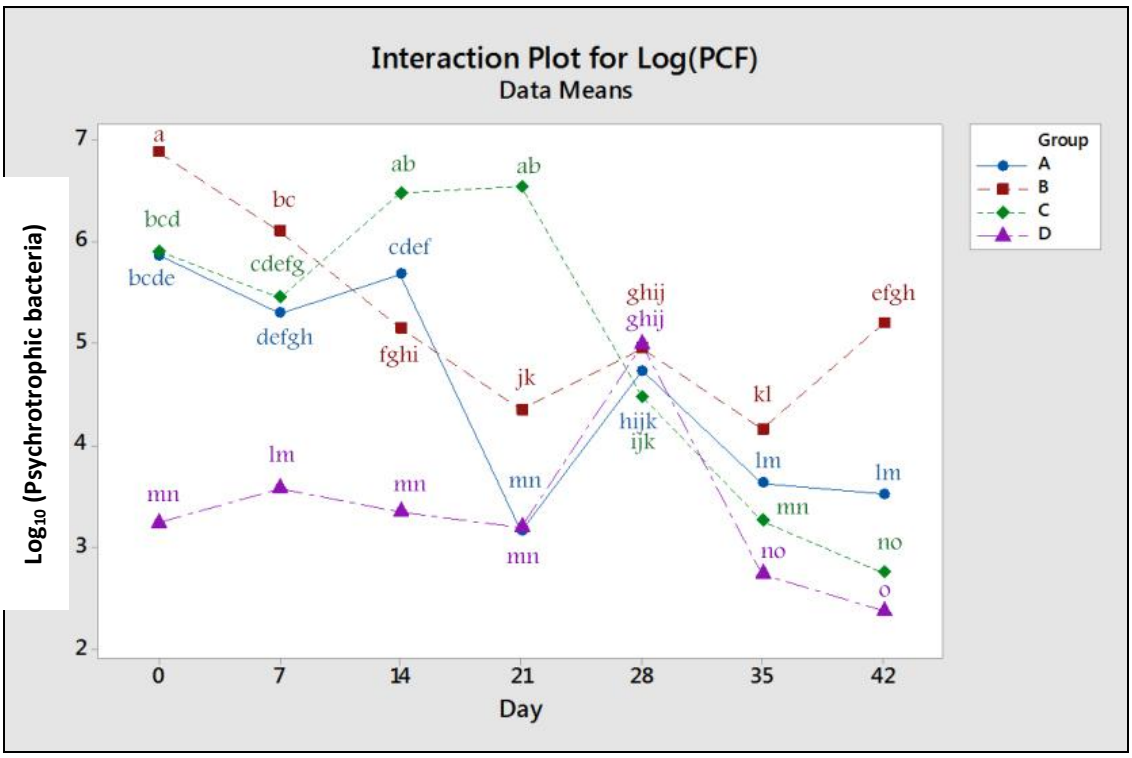


Figure 20: Psychrotrophic bacteria of green thyme samples at refrigerator temperature 4°C

4.3.2.4 Yeast and Mold analysis (Y&M)

Figure (21) showed the growth of yeast and mold in green thyme leaves at refrigerator temperature 4℃. In general, convergent growth of Psychrotrophic bacteria appeared for four groups during the storage period. Moderate significant differences had prevailed during the storage period. A significant increase and decrease had shown at last four times continuously. Our finding may relate to a temperature which is one of the most influencing factors on yeast and mold growth (Ayerst, 1969). Fungi can't control their internal temperature which governed by the ambient climate. It has the ability to live in a relatively large range of temperatures, but growth and metabolism rate change at different temperatures even when other conditions, e.g. nutrient and water activity are constant (Dales et al, 1994). Moreover, temperature affect on fungal physiology (Adan, 1994). Li et al, (2009) found low growth of yeast and mold at temperature 10 ℃ comparing to 15, 20, 25 and 30°C.

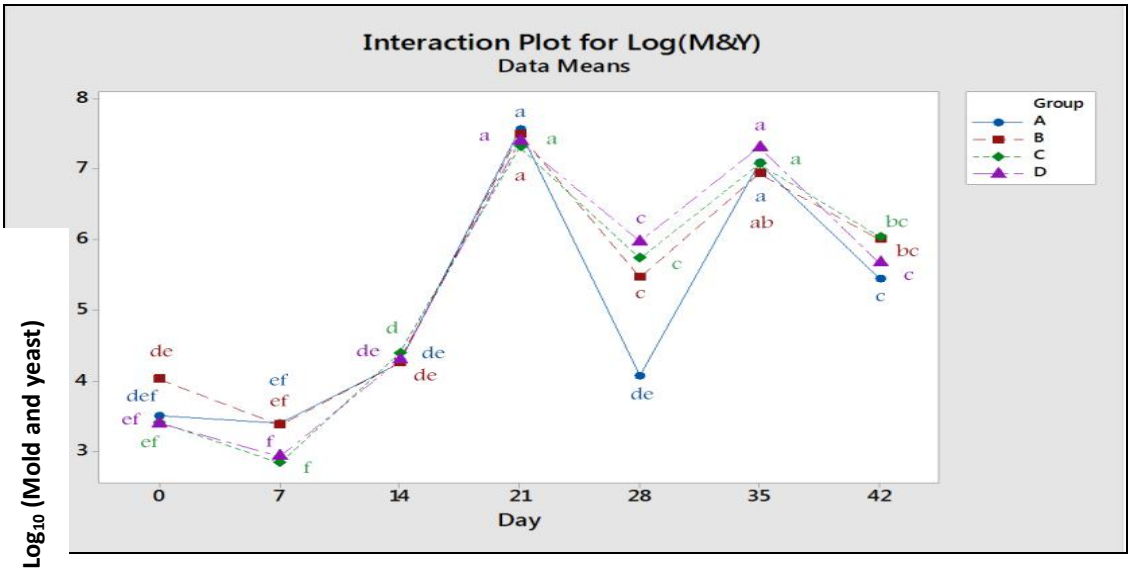


Figure 21: Mold and yeast growth of green thyme at refrigerator temperature 4℃

4.3.3 Color a*, b* and L* values of green thyme at refrigerator temperature

The effect of treatments on a*-values during storage is shown in figure (22). There were moderate significant increases in a*-values during the storage period in all groups. Degradation of chlorophyll may explain these differences. Degradation of chlorophyll is a complex multi-pathway as the biosynthesis of which may be summed in two basic groups of reactions; the first one produces greenish derivatives while the second one, colorless compounds by an oxidative ring-opening. Degradation of chlorophyll reduced the intensity of the green color and led to yellowing. It was that yellowing of leafy vegetables and broccoli was attributed to the action of the enzymes peroxidase and lipoxygenase. The reduction of the intensity of green vegetables was associated with aging, reduction of the nutritional value and in general their quality (Shewfelt, 2000)

The color a* values had slight on significant changes compared with samples that stored at room temperature 25℃ .On other hand, figures 23and 24 have shown that no significant differences between 4 groups in each b* and L* color values.

Manolopoulou et al. (2016) studied the effect of temperature on color degradation of leafy vegetables such as lettuce and broccoli and the chlorophyll content. In this study, it was found that the direct effect of temperature on chlorophyll content in both subjects.

The same result was reported by Page et al, (2001) that broccoli presented a significant reduction in chlorophyll after 11 days at 4 °C. Refrigerated temperature reduced the respiratory activity, the rate of metabolism and the degradation of chlorophyll (Pogson et al, 1997).

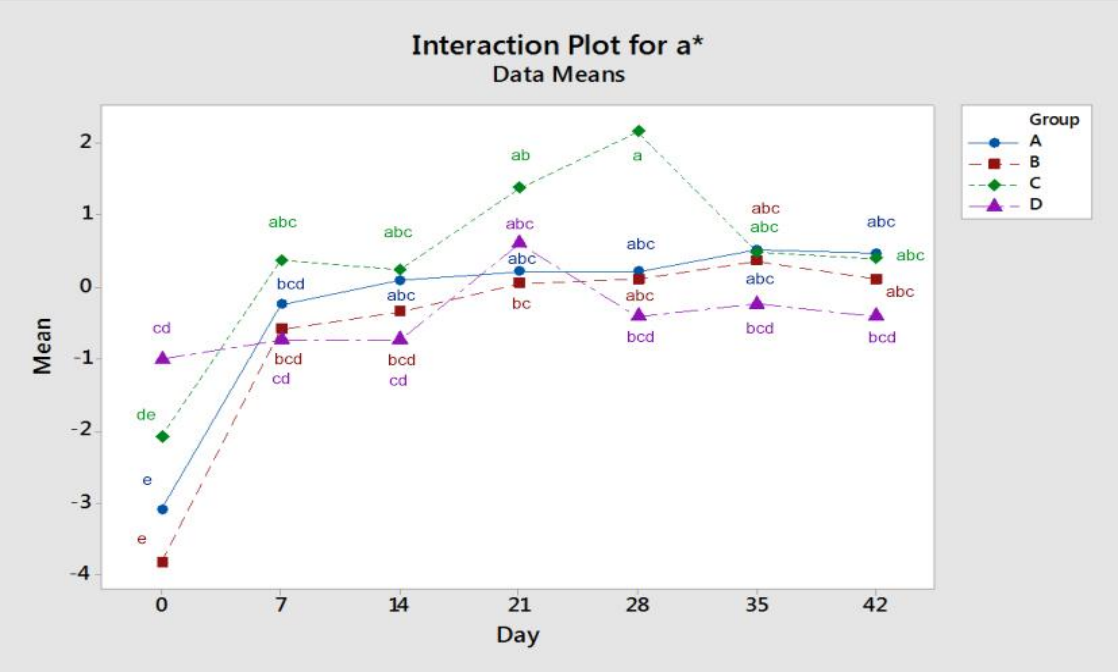


Figure 22: Color a* values of green thyme at refrigerated temperature 4 ℃

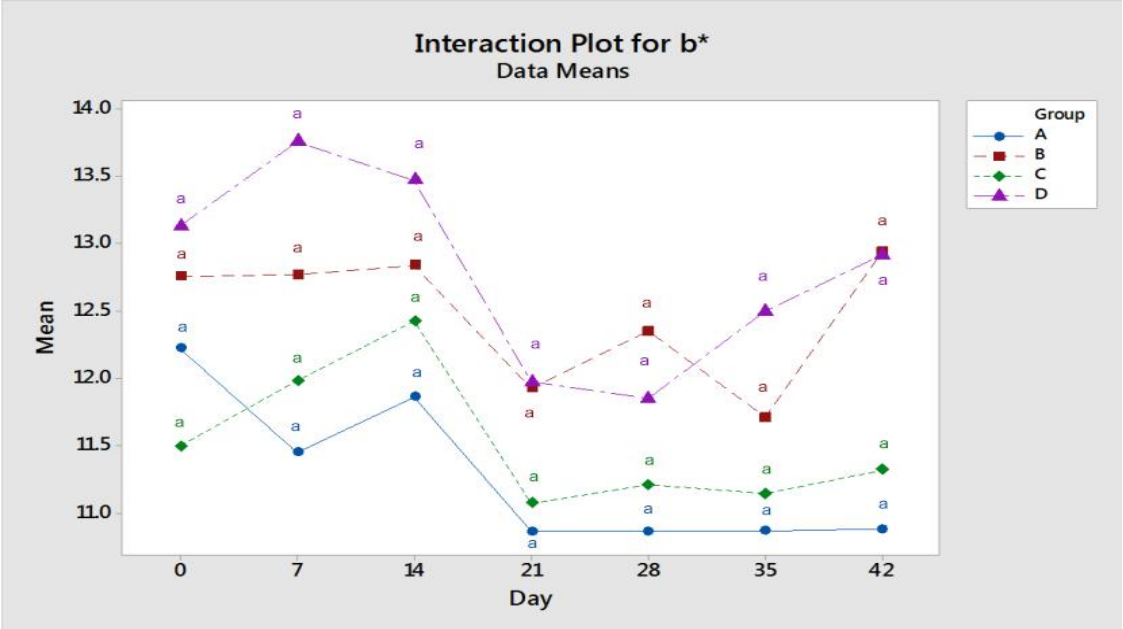


Figure 23: Color b* values of green thyme at refrigerated temperature 4 ℃

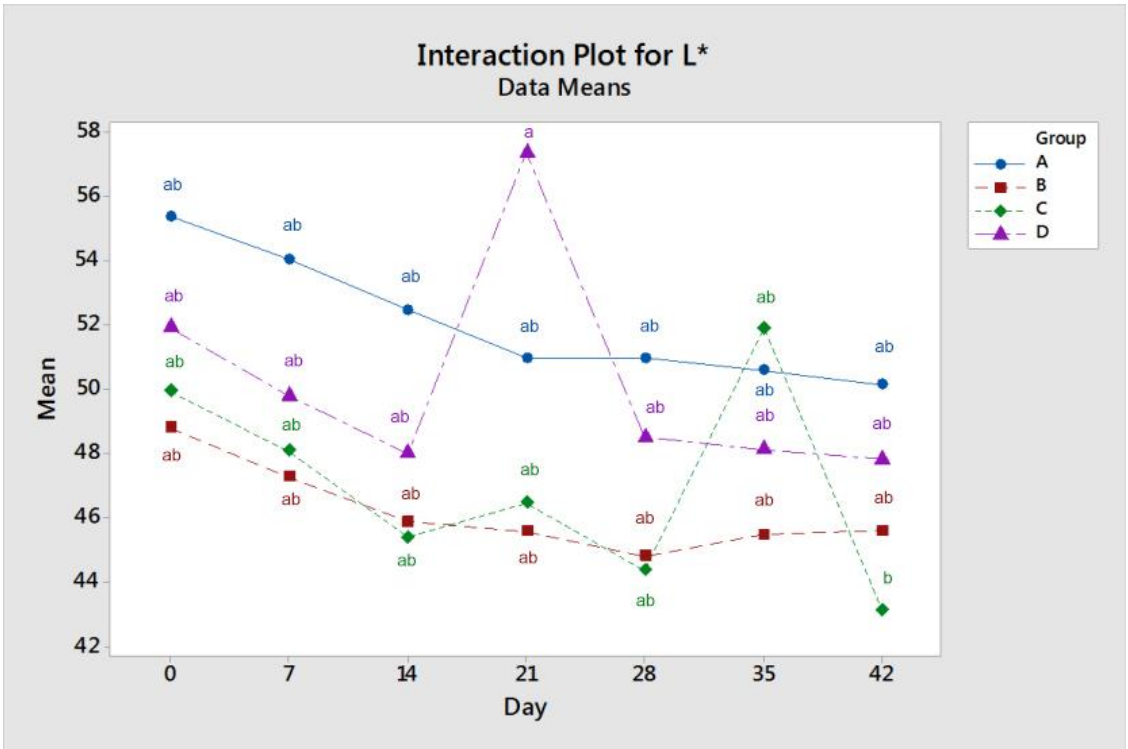


Figure 24: Color L* values of green thyme at refrigerated temperature 4 ℃

4.4 Sensory analysis

The results of the sensory tests of the green thyme samples were reported in different figures (25, 26, 27, 28, 29) for three different groups which stored at room temperature 25℃. Image 8 has shown the way by which samples were presented to panelists. Breaded thyme (Qrass Za'atar) obtained by using the traditional dough was almost identical to those obtained by local baker. The two types of green thyme in group C and D were proved to have the same characterizations, taste, flavor, saltiness, appearance, compared to green thyme leaves that produced with traditional bakery (group B). In figure (25), no difference was recorded to the taste between groups B, C and D. Only in group B, the taste was changed in the last day of the experiment.

Flavor and appearance were affected by several factors such as baking temperature of thyme pastry, which was provided to the testers and method of kneading and baking pastries. However, for all the studied recipes, there were significant differences in the flavor and appearance of the green thyme pastries produced by using different ingredients Figure (26 and 27).

All samples were judged to be satisfactory for salt. Group D had lactic acid showed significant differences only at day 42 shown in figure (28). Breads produced by traditional baker who works and knows the details about making green thyme pastries. This trait is appreciated by Palestinian consumers (students, teachers, and public people) since associated with traditional green thyme pastries. According to the evaluation of the overall acceptance that was seen in figure (29), the pastries produced by using the different recipes were the most appreciated by panelists, However, some complains during testing group C where some of taster indicated a bitter taste.

Image 8: Sensory test

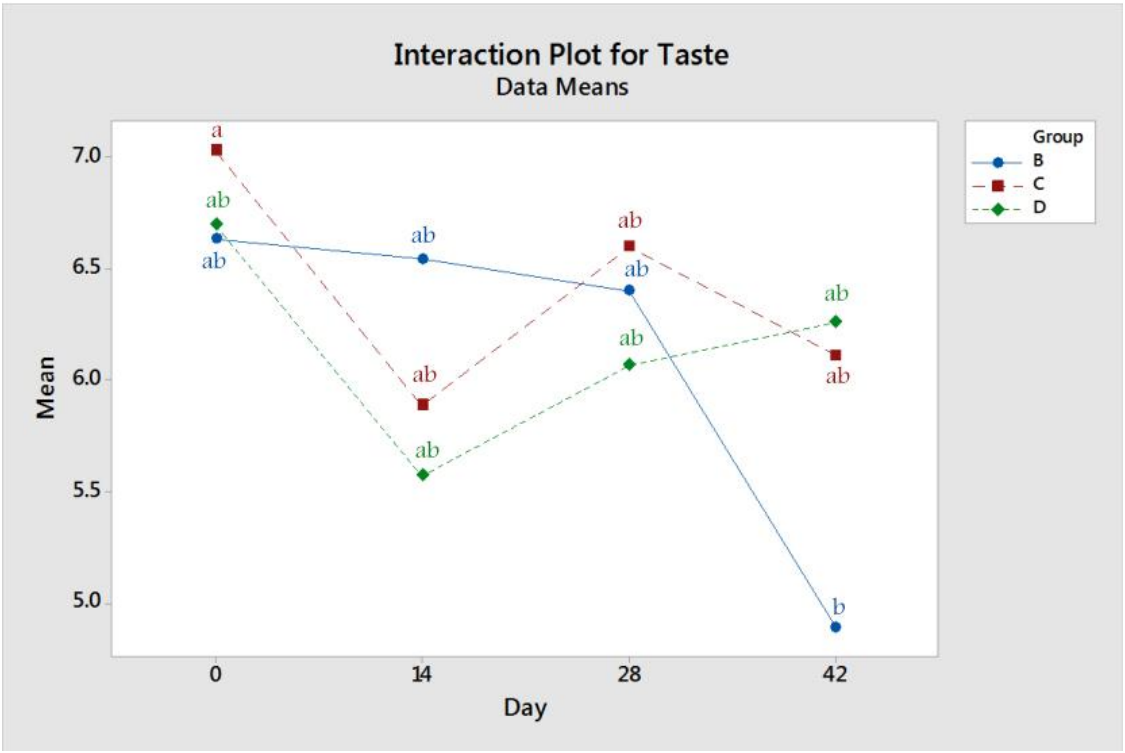


Figure 25: Taste analysis for green thyme at room temperature

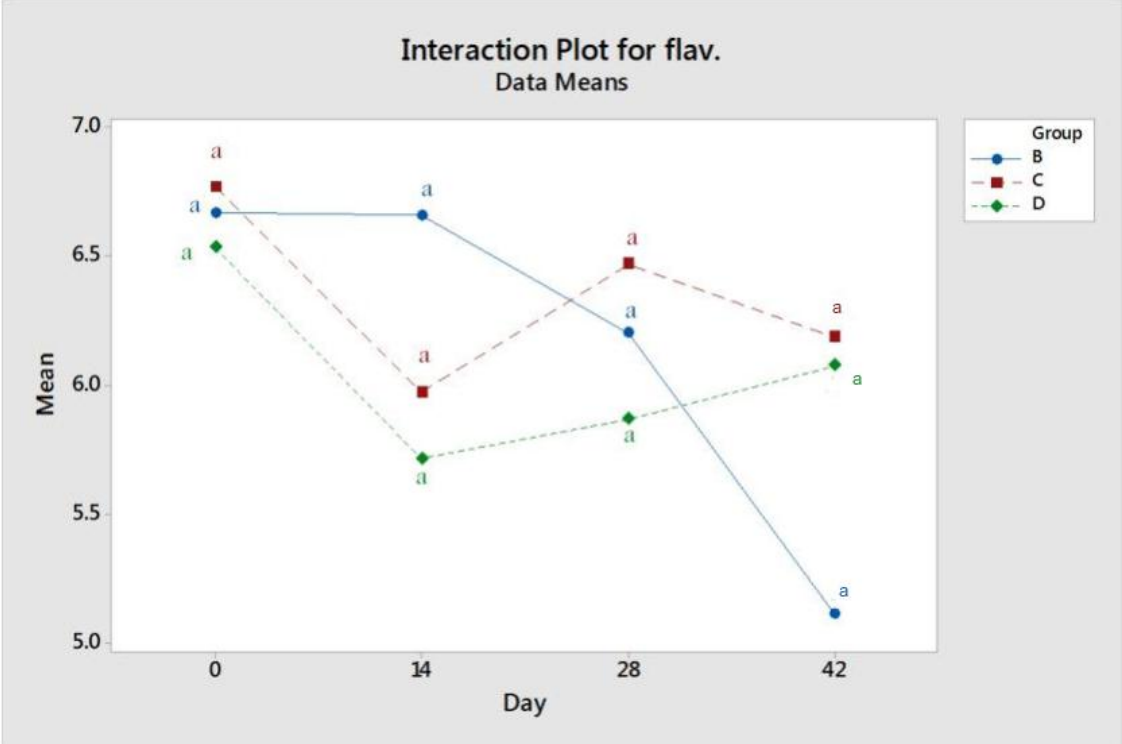


Figure 26:Flavor analysis for green thyme at room temperature

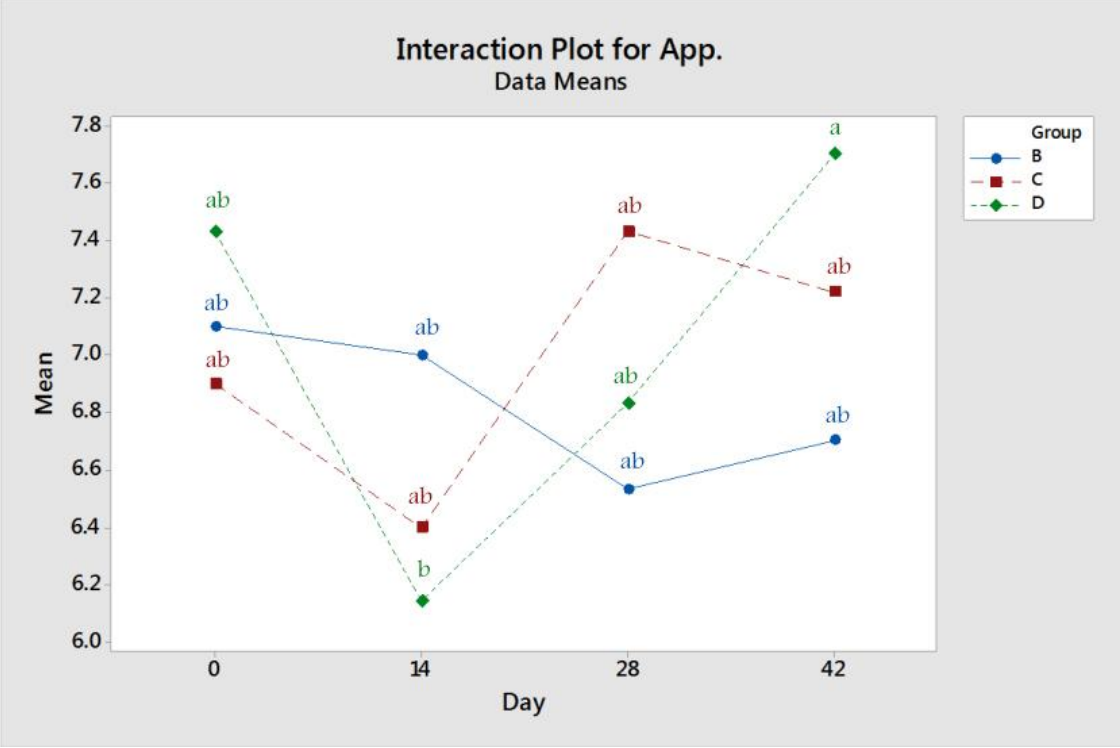


Figure 27: Appearance analysis for green thyme at room temperature

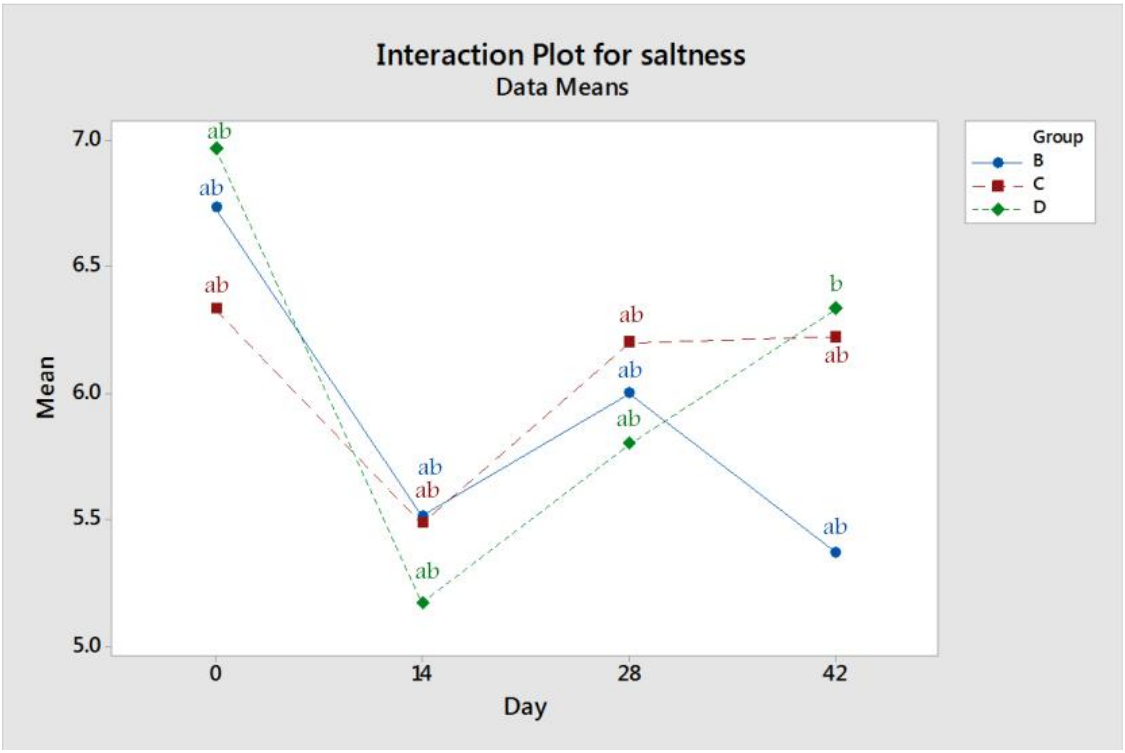


Figure 28: Saltiness analysis for green thyme at room temperature

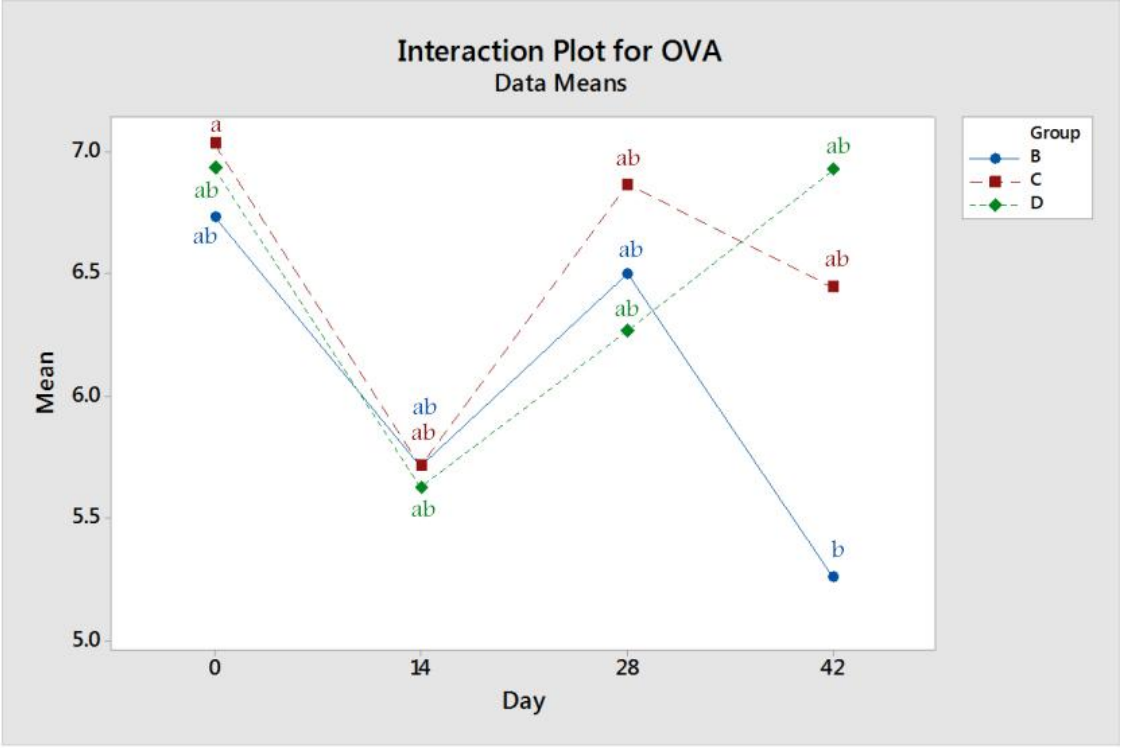


Figure 29: Over all acceptance analysis for green thyme at room temperature

4.5 Microbiological Challenge Testing

In our study, microbiological challenge testing was applied to study the ability of growth of *Clostridium sporogenes* DSM 795. It is used to mimic *clostridium botulinum* growth, in both the recipes that used in B and D treatments using agar journey (spot, spreading methods) and sensitivity methods.

In both methods, the result demonstrated that the recipe of treatment D (containing onion, oil, salt, and lactic acid) had the ability to resist the growth of *clostridium sporogenes*. This result may be due to the presence of lactic acid that reduced pH to 4.4. It has been traditionally accepted that spores of *Clostridium botulinum* will not germinate and grow at pH 4.8 or below (Wong,2012) On another hand, the components of the group B containing only onion, salt and oil, were unable to prevent bacterial growth as shown in images(9, 10, 11).

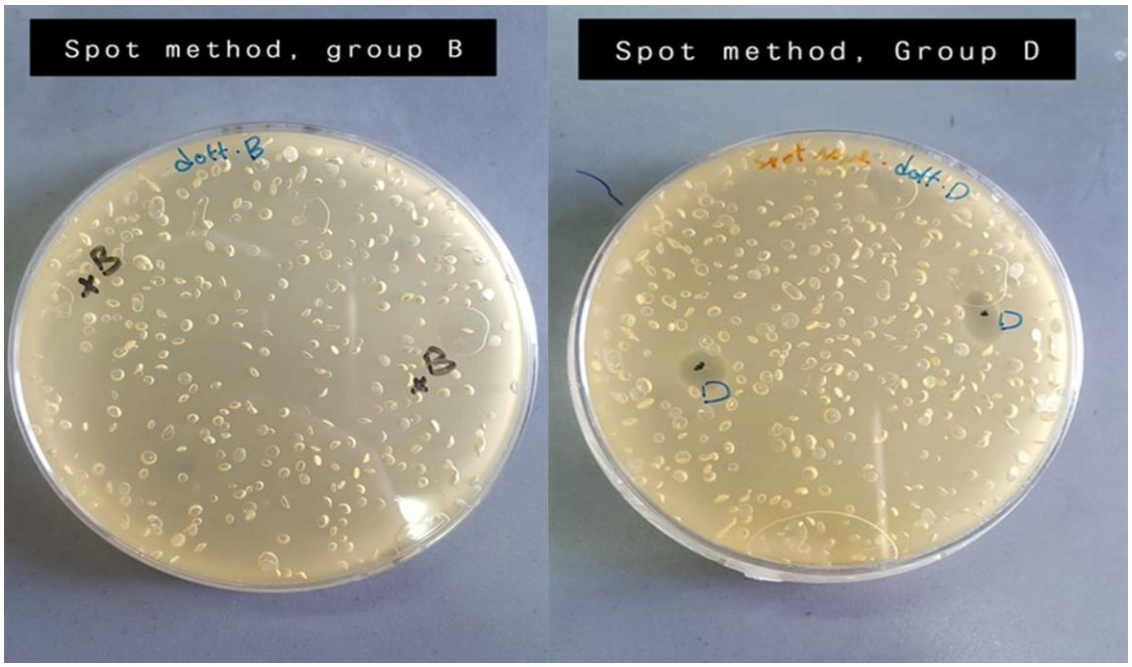


Image9: growth of C. Sporogenes in group B and D recipes by using spot method.

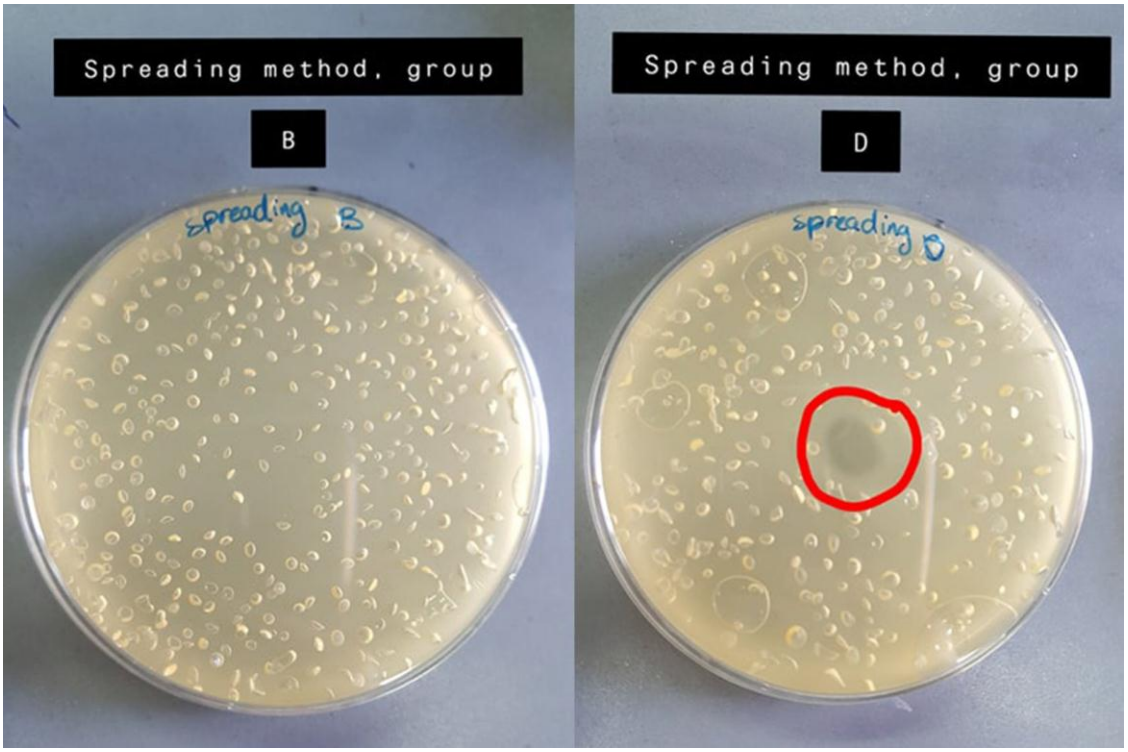


Image10: growth of C. Sporogenes in group B and D recipes by using spreading method.

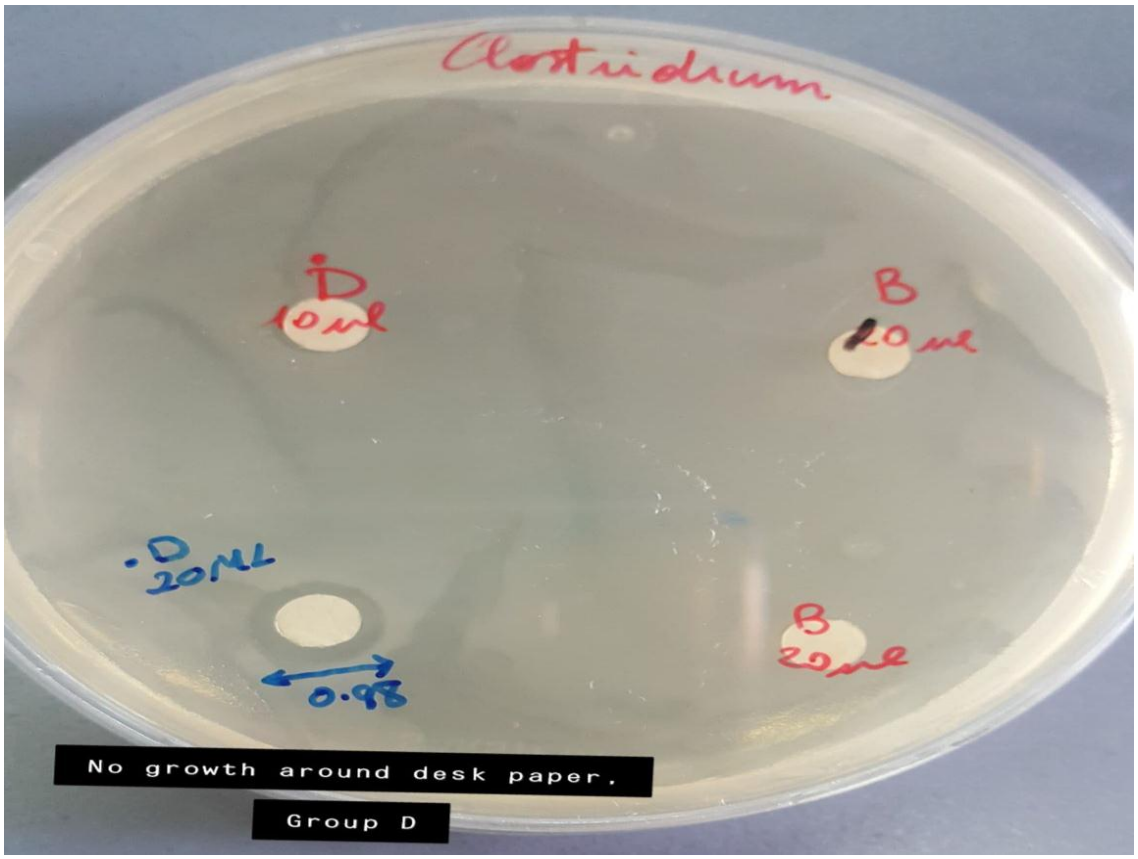


Image11: Growth of C. Sporogenes in group B and D recipes by using sensitive test.

Figure (30) demonstrated the growth of *Clostridium sporogenes* in green thyme leaves for 7 days. Two groups were selected, A (Control) and D (Lactic acid), to study the ability of growth of *C. Sporogenes* in green thyme leaves which were stored at room temperature 25 ℃ for 7 days. The experiment had shown the ability of green thyme leaves in group A to decrease growth of *C. Sporogenes* during storage period comparable with lactic acid group D which hadn't decrease during the same period. Green thyme is rich in essential oils and antimicrobial agents such as thymol, carvacrol, geraneol and borneol which may affect the growth of bacteria such as *C. Sporogenes*. On another hand, lactic acid is common preservatives substance that used to increase the shelf life of products

(Benninga, 1990). However, in this study there was no strong effect on the growth of *C. Sporogenes* that maybe due to lack of homogeneity of lactic acid with green thyme leaves or potency of antimicrobial agents that exist in green thyme leaves were decreased in presence of lactic acid in group D.

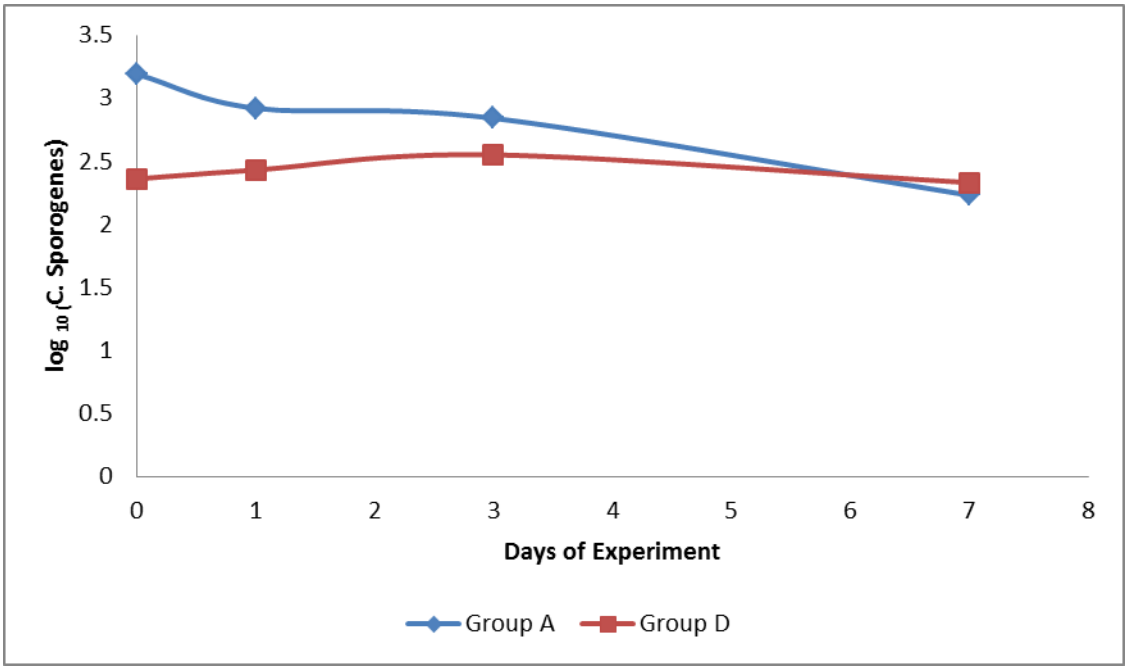


Figure 30: Microbiological Challenge test in group A and D green thyme leaves during 7 days.

Chapter 5

Conclusion and Recommendation

Conclusions

As a main conclusion of this study, it was possible to employ hurdle technology to obtain fresh green thyme products that were stable for a reasonable shelf life. Different ingredients were employed during this, but it was found that lactic acid was the most effective to keep different quality traits during storage. Refrigerated conditions were the best storage condition to extend the shelf life of thyme products. Green thyme leaves are very valuable agricultural product in Palestine. Until now, this product is not exploited to the export market due to short shelf life; the findings of this study may contribute to increase the potential of this product for export market.

Groups that were preserved at refrigerated temperature have shown the abilities to maintain an acceptable quality. However, based on previous findings in the literature that color gives a perception of good nutritional value to the consumer, the refrigerated temperature fulfilled this requirement. The green thyme samples preserved at refrigerator temperature were more acceptable than the room temperature samples. As stated earlier, the green thyme samples preserved at refrigerated temperatures had a brighter green color than other experiment.

The findings of this study indicate that preservation of green thyme at refrigerated temperature could be easy to find in resource-poor seasons and leave traditional practices such as drying and freezing. Over and above that, in the case of refrigerated temperature, the green thyme products would be economically accessible due to low cost, nutritious due to retention of nutritional value and provide food safety-nets due to prolonged shelf-life.

Recommendations

1. Ensure that the green thyme is clean and free from dust and other plants that may be present during harvest.

2. Ensure homogeneity of additives with green thyme and use other non-manual methods in mixing such as spraying method.

3. Adjust the temperature used to store green thyme and choose a suitable place for non-interference factors affecting color change such as light.

4. Use special jars to make an anaerobic environment.

5. Make sure the quality of additives, such as sumac, to avoid cross contamination and quality deterioration.

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جامعة النجاح الوطنية
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تأثير التغليف بتفريغ الهواء مع الإضافات الطبيعية على جودة ومدة صلاحية الزعتر الاخضر الطازج

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قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في تكنولوجيا التغذية والغذاء بكلية الدراسات العليا في جامعة النجاح الوطنية، نابلس، فلسطين.
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الملخص

الزعتر الأخضر (الزعتر) هو أحد الأعشاب المفضلة في حديقة الفلسطينيين. ينتمي الزعتر الاخضر الى جنس الاوريجانو من العائلة الشفوية تهدف هذه الدراسة إلى إطالة مدة صلاحية الزعتر الأخضر الطازج بواسطة تقنية العوائق وذلك من خلال استخدام تقنية تعبئة الفراغ مع المضافات الطبيعية (البصل والسماق وحامض اللاكتيك والزيت والملح) ثم تخزينها في الغرفة ودرجة حرارة التبريد لدراسة تأثير المضافات الطبيعية مع التعبئة بتفريغ الهواء على الخصائص الفيزيائية للزعتر الأخضر بما في ذلك اللون والذوق والنكهة، ودرجة الحموضة، والتركيبات الكيميائية وكيف تتغير خلال 42 يوما. وعلاوة على ذلك، دراسة احتمال نمو كلوستريديوم البوتولينوم في عينات الزعتر الأخضر تحت ظروف اللاهوائية. كان حمض اللبنيك هو الأكثر فعالية للحفاظ على سمات الجودة المختلفة أثناء التخزين. كانت الظروف المبردة أفضل حالة تخزين لإطالة العمر الافتراضي للمنتجات الزعتر. أوراق الزعتر الأخضر هي منتج زراعي ذو قيمة كبيرة في فلسطين. حتى الآن، لا يتم استغلال هذا المنتج في سوق التصدير بسبب قصر مدة الصلاحية؛ قد تسهم نتائج هذه الدراسة في زيادة إمكانات هذا المنتج لسوق التصدير