

بسم الله الرحمن الرحيم



Sudan University of Science and Technology College of Graduate Studies

# Preparation of Edible Cheese Casings With Natural Biological Characteristics From Whey Proteins and Green Tea Extract (*Camellia sinensis*)

تحضير أغلفة الجبن الصالحة للأكل ذات الخصائص البيولوجية الطبيعية من بروتينات الشرش ومستخلص الشاي الأخضر

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يُسقَىٰ بِمَاءٍ وَاحدٍ وَنُفَضَّلُ بَعْضَهَا عَلَىٰ بَعْض فِي أَسْقَىٰ بِمَاءٍ وَاحدٍ وَنُفَضَّلُ بَعْضَهَا عَلَىٰ بَعْض فِي الْأُكُلِ أَنَ فِي ذَلِكَ لَآيَاتٍ لَقَوْم يَعْقَلُونَ

The son the

( سورة الرعل) الآيتر (4)

# Dedication

To my country, the land of Mesopotamia... Iraq To the pure hand that removed from before us the thorns of the road to which words and thanks are not

# enough, my beloved father

To the one who kneeled tender before her feet To the precious one who only sees hope in her eyes, **my beloved mother** 

To the light of my eyes and the bright smile in my life, my aunt Amal, my wife Ranal, and **my sisters Mays and Sarah** 

To those who were with me and supported me wholeheartedly

I dedicate the results of my humble effort

Ihab Mahmoud

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#### Abstract

In this study, edible and biodegradable casings were manufactured from whey proteins fortified with alcoholic extract of green tea in the ratio of extraction 50%, casings of whey proteins supplemented with green tea extract at a concentration of 3% were used in the packaging of mozzarella cheese as an application of these casings and the most important results were: The superiority of the packaging model over the control sample in terms of the effectiveness of the anti-microorganism and anti-oxidation films appeared. Their effect was reflected on the packaging sample. and the moisture test was at the forefront, and the percentage of moisture was for the control sample which represents unwrapped cheese and enveloped sample which represents the added wrapped cheese Its green tea extract at a concentration of 3% after a day of manufacturing is 53.26 and 53.30%, respectively. and this percentage was decreased with significant differences on the last day( $P \le 0.05$ ) between the control sample and the treated enveloped sample to 47 and 51.06%, respectively, the protein percentages gradually increased with level of insignificance in the progression of the storage period, as the control sample and treated sample after a day of manufacture were 25.30 and 25.22%, respectively. and then reached after 90 days of storage to 26.85 and 26.23% respectively, The percentage of fat with significant differences ( $P \le 0.05$ ) between control and treated samples increased, where it was 22.35% after a day of manufacturing, and reached 25.12 and 23.95% at the end of the storage period, respectively. The pH decreased during the storage period. and it was found that there was a development in acidity during storage and for the control and treated samples with insignificant differences ( $P \le 0.05$ ). The Acid value (AV) for control sample and enveloped sample was 0.13 (mEq / 100 gm fat). and the value of AV increased with significant differences in storage samples control sample and treated sample until it reached 2.0 and 1.1 (mEq / 100 gm fat), respectively. It was noted that the peroxide value increased with significant differences in control sample compared to the treated sample. where the control sample form was between 4-10 (mEq / kg) during 90 days of storage, and the treated enveloped sample was between 3.6-7 (mEq / kg). The results of total and soluble nitrogen showed an increase in the percentage of soluble nitrogen to total nitrogen for cheese and for the control and treated samples. where it was found that there were differences at a significant level of (P $\leq$ 0.05) during the storage period of 90 days. The

numbers of microorganisms in mozzarella cheese were calculated in different samples to know the effect of the presence of antimicrobial growth mozzarella cheese samples coated with whey proteins with (GTE) added at a concentration of 3% (enveloped sample) were generally characterized by a lower rate of bacteria and molds contamination which represented unwrapped cheese during the 90-day storage period. As follows : As for the total number of bacteria, was decreased by 3-6 logarithmic cycles at the end of the storage period in the sample mozzarella cheese enveloped compared to the unwrapped control sample wich had significance differences. The numbers of gram-negative *E.coli* bacteria were decreased in the enveloped sample, by about 2 logarithmic cycles compared to mozzarella cheese in the control sample. As for the *lipolytic* bacteria and proteins. They were free in the samples at the beginning of the storage period, The number of *lipolytic* bacteria at the end of the storage period of the control sample was  $3.7 \times 10^2$ cfu/g with no growth in the enveloped sample. while the number of lysed bacteria reached proteins at the end of the storage period for the control sample were 5.4 x  $10^2$  cfu/g and in the treated enveloped sample was 1.4 x 10 cfu/g. As for Staphylococcus aureus. it was observed a decrease in the rate of increase in the microbial numbers of these organisms and There were significant differences between the enveloped sample when compared to the control. As for Salmonella sp. The results indicated that the samples in which whey proteins were added to green tea extract (enveloped sample) were free of any significant growth, unlike control sample which showed clear growth at the end of the storage period. As for yeasts and molds, the enveloped sample was free of growth throughout the storage period. this shows the effect of the antimicrobial factors present in the green tea extract added to the whey proteins enveloped. while the numbers of yeasts and molds in the control sample were 8.1 x 10  $^{2}$  cfu/g .at the end of the storage period. and the above results were reflected on the sensory evaluation. as the highest scores related with significant differences ( $P \le 0.05$ ) to the appearance, texture, taste, flavour, color, bitterness, and exotic flavors were given to the cheese samples coated with whey proteins with added green tea extract at a concentration of 3% (enveloped sample) compared to the control sample.

#### المستخلص

في هذه الدراسة ، تم تصنيع أغلفة صالحة للأكل وقابلة للتحلل الحيوي من بروتينات الشرش المدعمة بمستخلص كحولي للشاى الأخضر بنسبة استخلاص 50٪ ، واستخدمت أغلفة من بروتينات الشرش المكملة بمستخلص الشاي الأخضر بتركيز 3٪ في تغليف جبن الموزاريلا كتطبيق لهذه الأغلفة وظهرت أهم النتائج التي تم التوصل اليها وهي : تفوق نموذج العينة المغلفة على العينة الضابطة من حيث فعالية الأغشية المضادة للكائنات الحية الدقيقة ومضادات الأكسدة وانعكس تأثير ها على العينة المغلفة وكان اختبار الرطوبة في المقدمة ، وكانت نسبة الرطوبة لعينة التحكم التي تمثل الجبن غير المغلف والعينة المغلفة التي تمثل الجبن المغلف المضاف اليه مستخلص الشاي الأخضر بتركيز 3٪ بعد يوم من التصنيع هو 53.26 و 53.30٪ على التوالي. وانخفضت هذه النسبة مع وجود فروق معنوية في اليوم الأخير (P≤0.05) بين عينة التحكم والعينة المغلفة المعالجة إلى 47 و 51.06٪ على التوالي . ازدادت نسب البروتين تدريجياً مع فروقات غير معنوية في تقدم فترة التخزين ، حيث كانت العينة التحكم والعينة المعالجة بعد يوم التصنيع 25.30 و 25.22٪ على التوالي ثم وصلت بعد 90 يومًا من التخزين إلى 26.85 و 26.23٪ على التوالي ، وزادت نسبة الدهون بفرق معنوى (P≤0.05) بين العينات التحكم والعينة المعالجة ، حيث كانت 22.35٪ بعد يوم من التصنيع ، ووصلت إلى 25.12 و 23.95. ٪ في نهاية فترة التخزين على التوالي . انخفض الرقم الهيدروجيني خلال فترة التخزين ووجد أن هناك تطوراً في الحموضة أثناء التخزين ولعينات التحكم والمعالجة مع وجود فروق معنوية (P≤0.05) وكانت قيمة الحمض (AV) لعينة التحكم والعينة المغلفة 0.13 MEq / 100 / mEq) جم دهن . وزادت قيمة AV مع فروق معنوية في عينة التحكم والعينة المعالجة حتى وصلت 2.0 و 1.1 (مي مكافئ / 100 جم دهن) على التوالي. لوحظ أن قيمة البيروكسيد زادت مع وجود اختلافات كبيرة في عينة التحكم مقارنة بالعينة المعالجة. حيث تراوحت عينة التحكم بين 4-10 (ميلي مكافئ / كجم) خلال 90 يومًا من التخزين ، وكانت العينة المغلفة المعالجة بين 3.6-7 (ميلي مكافئ / كغ) ، وأظهرت نتائج النيتروجين الكلي والذائب زيادة في النسبة المئوية للنتروجين الذائب الى النتروجين الكلي للجبن ولعينات التحكم والمعالجة حيث وجد أن هناك فروقاً معنوية عند مستوى (P≤0.05) خلال فترة التخزين البالغة 90 يوماً. تم حساب عدد الكائنات الحية الدقيقة في جبن الموزاريلا في العينات لمعرفة تأثير وجود مضادات الميكروبات لجبن الموزاريلا المغلفة ببروتينات الشرش مع (GTE) المضافة بتركيز 3٪ (عينة مغلفة) تميزت بشكل عام بانخفاض معدل التلوث البكتيري والعفن الذي يمثل الجبن غير المغلف خلال فترة التخزين البالغة 90 يومًا. على النحو التالي: بالنسبة للعدد الإجمالي للبكتيريا ، فقد انخفض بمقدار 3-6 دورات لوغاريتمية في نهاية فترة التخزين في عينة جبن الموز اريلا المغلفة مقارنة بعينة التحكم غير المغلفة التي كان لها فروق معنوية. انخفضت أعداد بكتريا الإشريكية القولونية السالبة لصبغة جرام في العينة المغلفة بحوالي دورتين لوغاريتمية مقارنة بجبن الموزاريلا في العينة التحكم . أما بالنسبة لبكتيريا المحللة للبروتينات والمحللة للدهون كانت خالية في العينات في بداية فترة التخزين ، وكان عدد البكتيريا المحللة للدهن في نهاية فترة التخزين لعينة التحكم 3.7 × 10<sup>2</sup> قدم مكعب / غرام مع عدم وجود نمو في العينة المغلفة. بينما بلغ عدد البكتيريا المتحللة التي وصلت إلى البروتينات في نهاية فترة التخزين لعينة التحكم cfu / g 10<sup>2</sup> x 5.4 وفي العينة المغلفة المعالجة x1.4 / cfu / g p.أما المكورات العنقودية الذهبية . لوحظ انخفاض في معدل الزيادة في الأعداد الميكروبية لهذه

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## Abbreviations

GTE	green tea extracts
EC	Epicatechin
ECG	Epicatechin gallate
EGC	Epigallocatechin
EGCG	Epigallocatechin gallate
C	Catchin
PE	Poly ethylene
ADV	Acid degree value
HDPE	high-density polyethylene
LDPE	low-density polyethylene
LLDPE	polyethylene low-density linear density
CG	Catchin gallate
GC	Gallocatechin
GCG	Gallocatechin gallate
PV	Peroxide Value
DPPH	2, 2-diphenyl-1-picrylhydrazyl
BHA	Butylated hydroxyl anisole
E. coli	Escherichia Coli
SN	Soluble Nitrogen
АРНА	American Public Health Association
FOSHU	Food of Spcificed Health Use
FTIR	Infrwered Spectrophotometer
L.S.D	Least Significant Difference

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### **Chapter One**

### **Introduction:**

Food preservation generally represents all measures taken to prevent food spoilage, specifically all ways to limit food corruption microbial. Two types of conservation methods have been used for a long time: conservation methods and chemical conservation methods. However, these methods may not necessarily kill or eliminate all microorganisms, but they were often sufficient only to make environmental conditions unfavorable to microorganisms (Tuba and Hakan,2018).

The most common natural methods used to conserve food were sterilization, pasteurization (thermal treatment), cooling and freezing (low temperature preservation), drying (reducing water content) and conservation by radiation. In contrast, there were ways of chemical preservation, which depends on the addition of a specific chemical that inhibits the growth of microorganisms and in the best conditions can be killed and known as compounds (preservatives). (Hadar and Elena,2018)

Food conservation is a constant battle against food-destroying organisms. By applying conservation techniques, the food industry is looking more and more for alternatives to traditional conservation techniques (heat, salting, souring, drying, chemical preservation) by new conservation techniques due to increased consumer demand for improved taste and nutritional value , and easy of trading and handling of food products (Chengcheng *et al*,2019).

The most commonly used conservation techniques were non-thermal inhibition techniques such as High Hydrostatic Pressure, Pulsed Electric Fields, new casinges systems, gaseous storage and casinges, as well as natural anti-microbial growth compounds

Despite the efforts made and the investment of many funds, very few of these methods were applied to food processing (Nasreddine *et al*,2018). Microbial agents were one of the most successful and most recently used methods. This process is based on one of the following methods (Addition of microbial agents in the form of small fillings inside the food package), (Coating, restriction or addition of the microbial agent directly in the food casinges

material), (Using of casinges materials which were themselves antimicrobial agents).

Chemical substances such as organic and inorganic acids, minerals, alcohols, ammonium compounds or amines have been used and can be incorporated into food casinges as microbes (Arantzazu *et al*,2017). But because of the health requirements of consumers, producers have become very concerned with the use of bio-preservatives in this process (Tavassoli *et al*, 2015).

Due to environmental benefits and technological problems, such as the factors that were sensitive to the methods of manufacturing thermal polymers, as well as the extrusion and injection processes during molding, the integration of microbial agents in biological casinges is very appropriate if these factors were integrated into plastic wrap (Wang *et al*,2018). Most of the biochemical casinges is edible, produced and formed under cold conditions, including edible covers or so-called bio-encapsulates on cellulose, carrageenin, genes, kasines and whey proteins where microbial agents were combined (Chengcheng *et al*,2019).

now, the demand for ready-to-eat foods has increased, and this increase in demand has resulted in a significant development in food processing and casinges methods, which can preserve the shape and flavor for as long as possible, as well as products that were ready for cooking were susceptible to fatty oxidation (Mia *et al*,2017).

The anti-microbial agents, which can also be used in this type of casings is the extract of green tea, which acts as an antioxidant has been shown in the consumption of green tea various health benefits scientific research has shown that the compounds found in tea have characteristics of anti-cancer and antimicrobial and antiviral and These compounds also protect against heart disease and have anti-diabetic properties (Ilaria *et al*,2015).

In addition to antimicrobial agents, antioxidant casinges can be included to protect the sensory properties of the food product, its nutritional value and color protection, which enhances the consumer's confidence in the product, whether industrial antioxidants such as Butylated Hydroxyanisol (BHA) and Buthylated Hydroxy toluene (BHT) And vitamin E (Al-Jaruri,2014).

The environmental and technological problems of the packaging material, such as the denting factors that face the methods of manufacturing thermoplastic polymers, as well as the extrusion and injection processes during the molding, the process of incorporating antimicrobial agents into the biofilms has become very suitable if these factors were incorporated into the plastic packaging.

Mozzarella is an unripe soft cheese from the Pasta-Filata family whose original place is the Battipaglia region of Italy. I have been using this type of cheese for more than 50 years in the pizza industry, it is characterized by being a soft white cheese with a glossy and attractive surface and a light salty and high susceptibility to stretching or stretching ability in hot water, that these combined characteristics have made mozzarella suitable for use in preparing some foods such as lasagna and cordon bleu and on the surface of the pizza because of its high susceptibility to stretching, especially since the pizza is largely consumed by this youth segment which is what Encourage mass production of mozzarella (Lukman, et al, 2016).

Search problems:

but the old packaging caused environmental pollution so it found environmentally friendly packaging to preserve foodstuffs from microbial spoilage and extend their sneft life .

The current study aimed to:

the old casinges caused the environmental pollution, so it was found the environmentally friendly casinges to preserve the foodstuffs from microbial damage and extend the storage life so the current should be conducted .The aim of this study as

1. To develop natural package for food produced e.g mozzarella cheese from green tea extract .

2. To determine physical and Chemical Characteristics of the product .

3- To determine the sensory, microbial changes of the cheese produced during the ripening period were evaluated and followed up to determine the effect of these treatments on the quality of the resulting cheese.

### **Chapter two**

#### Literature review

#### 2-1: Food packaging

The term packaging is defined according to the International Institute for Packaging as the process of placing products, their parts, or packages of products in a bag, box, cup, glass container, or any other container in order to obtain one or all of the following characteristics: containment, protection, preservation, and circulation, and the ability to use, whereas the Codex Commission's definition of packaging functions is "packaging food in order to preserve its quality and spectacle, and to guide the consumer and facilitate storage and handling." With this definition, the Commission established the basic functions of packaging as it indicated containment, protection, flexibility, and communication, and that They were all intertwined with each other (Robertson, 2016), which means containment to make the product surrounded and not connected to the external environment. As for protection, it is one of the basic things and important functions of packaging to protect the product from external influences and the surrounding conditions were moisture, gases, microorganisms, dust, pressures and concussions. During handling and transportation, these new trends that have joined the changes in the usual procedures to make food processing faster and more efficient have caused increased interest in harvesting. L For food products with a longer shelf life. These characteristics were directly related to the development of new enhanced packaging materials, including active, smart and edible systems (Hadar and Elena, 2018).

In order to improve the quality of food and extend its shelf life, a new generation of edible active packaging is specially designed after combining organic acids, enzymes, antimicrobial proteins, phenolic compounds, or other functional components such as probiotics, flavors, vitamins, and nutrients in the casinges (Nasreddine, *et al*, 2018).

The use of renewable edible agricultural materials that play a major role in the food industry in the manufacture of casinges has increased, as well as the

development of edible bio-polymer technologies and the improvement of their mechanical and seismic properties in future research (Souza *et al*, 2015), and most recent studies have focused on extending the life span of food in Biofilm applications and the issue of food packaging in them, and most studies need to take advantage of biological casings on a commercial scale in order to obtain accurate information for the purpose of commercial application in the packaging of fresh food and its products using an edible polymer casings that works to add nutritional value as well as extend the shelf life (Dawei Yu, *et al*, 2018).

There were three aspects of the packaging environment that can be thought of, the first is the physical environment and the second is the surrounding environment (includes everything that can surround the packaging of heat, moisture, and microorganisms ... etc.) that can affect the wrapped product and cause damage to it. As for the third environment It is humankind (everything related to the interaction between the enveloped and people and what it symbolizes for that, for example, the color, shape and strength of the enveloped) (Hanin, 2018)

(Tavassoli-Kafrani, *et al*, 2015) pointed out the most important advantages of edible casinges that have sparked consumer interest made from natural materials that were edible, non-toxic and not polluting the environment compwered to the plastic casinges, which increase the nutritional value when there is a casinges above food and control On the water content of the food wrapped in it either not absorbing moisture or losing it, as well as protecting it from absorbing flavors or losing them and do not need expensive technologies, thus reducing the cost of production and the cost of treating environmental pollution, as well as the absence of residual packaging when used, in The wide development of food products such as meat (fish, poultry, beef) and other foods remained very sensitive to lipid oxidation and microbial casinges that were very important in protecting them, as a lot of applied research has indicated the success of these Effective casinges represented by plant extracts, fruits or preservative herbal oils (Xuejiao Wang, *et al*, 2018).

#### 2-2: Packaging used for food packaging and wrapping

# Polymeric casinges or casinges were divided into two parts: industrial and natural:

#### 2-2-1: Polymers Synthetic

These represent plastics that were known to have a polyethylene and nylon name, and these industrial polymers were made by reacting individual units or their derivatives under controlled conditions (Hadar and Elena, 2018). Industrial polymer casings usually have high tensile strength and high and characteristic reservation properties. Polymers: -

#### 2-2-2: Poly ethylene (PE)

Polyethylene (PE) is a polymer consisting of multiple units of ethylene and is used in particular as containers for packing some foodstuffs such as chicken or used in the form of chips or casings for packaging. The polyethylene is classified into a few different classes according to the differences in its density and branches. Among its main classifications were high-density polyethylene (HDPE), low-density polyethylene (LDPE), and polyethylene low-density linear density (LLDPE). The mechanical properties of polyethylene were significantly dependent on heterogeneity, branching type, and crystal structure (Hasan, 2017).

Both Han and Floros (1997) used a casinge containing microbial antibacterial agents using low-density polyethylene with potassium sorbate powder. Tensile, transparency and microbial properties of it were measured by the behavior of this casinge as a filling material. It was found that the inclusion of potassium sorbate powder for the casinge does not affect the tensile properties significantly, except that the transparency of the casinge decreases or decreases with increasing the concentration of potassium sorbate (A. Elbourne, *et al*, 2017).

#### 2-2-3: Poly propylene

It is a linear crystalline polymer of very low density (0.9 g / cm3) and has a high tensile strength and rigidity when compared with polyethylene, and it is resistant to moisture, oils and solvents since its melting point is 165 ° C so it is used in the production of packages that were subjected to sterilization. Examples Its uses were its use in the manufacture of textile materials, ropes, packing materials and travel bags. Also, food containers made of polypropylene were not broken during washing or industrial circulation or during manufacturing processes that require hot water, so most of the tubes and utensils used in labs for milky products were from This polymeric which is sealed with an aluminum lid (Badrani, 2011).

#### 2-2-4: Polyvinyl chloride (PVC)

This translucent polymer is an amorphous polymer that is widely used in most casings and food containers. Plasticizers (low volatile organic liquids) were added to it to improve its properties depending on the amount of plasticizer used. The PVC casings)) includes plasticizers, is soft and elastic and is used in the packaging of fresh meat products and fresh crops. As for the non-plasticized (PVC) panels, they were solid and subject to thermal treatment to enter the foodstuffs into them, and they were also used for packing snack foods such as tofu and biscuits. PVC bottles were transparent and resistant to oils and have high reservation properties. However, the uses of these bottles in packing foodstuffs were of a limited scope due to their lack of stability towards manufacturing thermal treatments and their impact on environmental conditions when in contact with chlorine (Anand B. Balaji, *et al*, 2018).

#### 2-2-5: Natural polymeric casings: - Natural polymers

Also called edible casings or Biodegredable casings were thin layers of materials suitable for human consumption as part of the food product (Arnon-Rips and Elena, 2018) or in other words they were layers of edible materials Which is used on food (either by rolling, dipping or casings) for the purpose of providing a selective barrier that prevents the passage of gases, fumes, solublematerials or fats in addition to that it provides mechanical protection (Dehghani, *et al*, 2017). It should be noted that there is no difference between the terms casinge and cover, so it is possible to use either of the other. Edible and biodegradable casings were made of three main materials: proteins, polysaccharides, fats or their compounds, and more than one material may be used in the manufacture of these casings with a view to improving their

reservation and mechanical properties. Scheme 2-1 shows the addition of biological polymers.



The chart (2-1) shows the three classes of biological polymers \*(Al-Badrani, 2011).

The chart (2-1) above shows the three classes of biological polymers, the first of which includes polymers that were extracted directly from the biomass such as polysaccharides, such as starch, cellulose and its derivatives, and proteins such as casein, whey proteins and wheat clotine (Al-Badrani, 2011).

The second category includes the polymers produced by conventional chemical synthesis using biological units such as polylactate, which is produced from the polymerization of units of lactic acid produced by the lactic acid bacteria through the process of fermenting lactose sugar.

The third category includes polymers that were produced by the microbial activity of microorganisms (these organisms were selected or selected, or they were genetically modified bacteria such as the use of Bacterium sphingomonas elodea to produce Gellan) and it is a multi-microbial polysaccharide (Anand B, *et al* 2018).

In general, edible casings or covers were prepared from a solution to the casinge formation agent and then this agent is separated from the liquid carrying it by evaporation or by curing the casing constituent (Pramod. *et al.*, 2016).

Giovanna, 2014 indicated that the preparation of casinges is affected by several factors, the most important of which were temperature and relative humidity, because these casings have a water-loving nature and were very vulnerable to water vapor, as water molecules were a problem towards the function of the vital polymeric casings because water is one of the substances with a plasticizing effect that Affects the physical and mechanical properties of the casings.

#### **2-3: Edible casings**

The packaging industry has spread widely in recent times, but for a certain period the main source of the raw materials for the packaging industry remained dependent on fossil fuel materials and for this reason the packaging industry has faced a serious global problem towards the environment and increasing pollution, and it has become imperative to find alternatives that can be used to increase shelf life and enhance Food quality while reducing the effects of waste on the environment by applying and using edible and degradable packaging by microorganisms, i.e. made from renewable materials (Šuput *et al*, 2017).

The use of renewable edible agricultural materials that play a major role in the food industry has increased, as well as the development of edible biopolymer technologies and the improvement of their mechanical and seismic properties in future research (Hanin, 2018). It can be solved to some extent from the problem of aggravated waste because the composition of these materials is from natural polymers that were ultimately degraded by soil by microorganisms by fertilization and this makes them environmentally beneficial and produces simpler natural compounds which include carbon dioxide, water and methane in addition to the mass Vital, it is worth noting that there were two types of biodegradable packaging, the first edible and the second inedible (Hanani *et al*, 2014).

Edible packaging is made from food derivatives resulting from manufacturing processes, both wet and dry. It is essential that the packaging produced from these sources be self-contained so that it can be used and placed on or between food ingredients (Dawei Yu, *et al*, 2018). Edible casings must be owned by:

1- It must be low in its permeability to oxygen and carbon dioxide (to reduce the metabolic activity of the microorganisms contained in food).

2- That it be of low permeability to water vapor so that food retains moisture for as long as possible and does not dry out

3- casings must have a weak flavor that is not prominent or flavorless, so that their flavor does not affect the packed food.

Packaging can be classified according to the type of source made from it, as each type has its own advantages in terms of negatives and positives and the most important sources that make edible covers were multiple sugars such as starch, cellulose and proteins such as casein, whey, corn adorn, and wheat clotin. The mixture of the aforementioned food types for the purpose of obtaining better specifications when collecting their properties together (Guo, *et al.* 2015). Scheme 2-2 shows the classification of degradable casings depending on the natural source.



Scheme (2-2) Classification of biodegradable casings according to the natural source

\*( Namita and Oğuzlu, 2011)

#### 2-4: Method of edible casings

Edible casings can be applied by using them as paper casings by using multiple techniques, including the extrusion process, the dispersive casings process, and the casings using soluble solutions. Plastic polymers can be used in the casings process as heat-tolerant. Natural polymers can also be used in the casings process such as sugars, proteins and fats (Rastogi and Samyn , 2015).

#### 2-4-1: Extrusion casings

One of the most used techniques in the industrial field is spraying, for several reasons that make it a commercial method, as this technique is characterized as continuous and uninterrupted, and it is a uniform layer of the casing on all parts and less range of holes and cracks, as well as when using solvents in them, but not without a method of equality where One of the determinants of this method is that it requires high weights of the paint to match the required specifications in addition to the instability of the polymer, all of which were factors that limit the efficiency of the casings (Simran and Dilyapuri, 2017).

#### 2-4-2: Dipping and marinating

This method, which depends on the use of solvents, is used to use a few weights of the paint, as the used weights reach about 10-2 kg / m2, and to reach the necessary reservation properties, and sometimes more than one layer of the casings may be used in the event that there were holes in the surfaces Coated material (Arnon-Rips and Elena, 2018).

#### 2-4-3: Curtain casings

One of the modern techniques used in packaging processes in which high quantities of surface material is used superficially on the coated material to be similar to the curtain that covers the material and proved highly efficient in the reservation properties of water vapor and gases (Brodnjak V, 2017).

#### 2-4-4: Surface pressure casings

This method is widely used industrially, in which the sheath material is scaled and pressed to take the shape of the sheathing material by surface pressure of the sheathing liquid on the sheathing material, in which the ratio of the solids to the sheathing mixture is low and may reach 10% to reach a coated viscosity substance (Rastogi and Samyn , 2015) Figure (2-1) illustrates the different encapsulations of paper packaging method a) spray painting B) surface casings C) surface pressure paint D) rolling coverage E) dipping coverage



Figure (2-1) the different encapsulations of paper packaging method a) spray painting B) surface casings C) surface pressure paint D) rolling coverage E) dipping coverage

\* (Rastogi and Samyn , 2015)

#### 2-5: Sources of edible casings

#### 2-5-1: Polysaccharide edible casings

Sugars were in the form of long chains of natural polymers made up of repeated determinations of mono or two sugars. These units were linked with each other by the clausicid bonds. Sugars were greatly available in natural and were also low cost. Hydrogen bonds play a big role in forming the body of the shells due to Large numbers of hydroxyl groups and other polar groups (Maria J, *et al*, 2017).

Starch and cellulose were multi-polysaccharides stored in grains, wood, cotton, hay or cereal straw, where it is widely available in them, and it is used as an effective packaging material as it is formed by the interference process that occurs to cut the polymer and create new water-rolling interactions (Chengcheng Ruan, et al, 2019) Because of the susceptibility to hydrophilic (carbohydrate) casings, their ability to trap carbon dioxide and oxygen is high, on the other hand, these casings were sensitive to moisture and were not good at capturing water vapor (Foteini Pavli, *et al*, 2018).

#### 2-5-2: Protein edible casings

They were polymers arranged in chains from 20 different building blocks of amino acids. The wrappers were formed from this natural source mainly through the denaturation of proteins by heat or salt or by changing the pH in addition to the subsequent processes of interference that occur between the peptide chains. Proteins share many of The bonds in the formation of proteins, including the covalent bonds (between the peptide and the bisulfuric element) and the non-covalent bonds (the ionic and hydrogenous bonds and the Fander-Walsis) in addition to the interactions that occur between the non-hydrophobic groups that occur between the nonpolar groups (Frederico VR Castro, *et al.* 2019).

The protein materials have aroused interest in the industrial field, especially in food packaging such as the use of corn zein in casings hazelnuts, sweets and soy protein in packaging foods made from meat and vegetables mixture such as Yuba food in Japan, which is fried with fat, as well as used whey and collagen proteins In Meat and Sausage Packaging (Oliveira, *et al*, 2017)

The most important edible protein casings used in food packaging were: -

#### A - Casein casings: -

Casein is widely used in edible wrappers due to its unique nutritional and sensory properties that were highly acceptable to the consumer and its high ability to protect the coated product from surrounding environmental influences (Nasreddine Benbettaïeb, *et al*, 2018).

The chemical and physical properties of casein were very different from those of other colloidal proteins as well as fibrous proteins such as myosin and casein from heterogeneous proteins, as it includes four different types were casein s1- $\alpha$ , casein  $\alpha$ -s2, casein- $\boldsymbol{B}$  and casein-k and they were found in cow's milk (Ortiz, *et al*, 2018). The method of easily isolating casein made it an easy-to-use industrial material in addition to being a staple foodstuff as this has spread it commercially since the nineteenth century (Nasreddine Benbettaïeb, *et al*, 2018).

There were two commercial ways to produce casein and they were either sedimentation by reaching the electrical breakpoint (PH4.6) which is the acid casein that includes lactic acid casein produced by lactic acid bacteria or sweet casein that results from enzymatic coagulation by the enzyme resonance (Vachiraya, 2016). Laboratory or synthetic hydrochloric acid is usually used in the preparation of acid casein, and sulfuric acid may be used for the same purpose except that it leaves the compounds of magnesium sulfate in the whey, and the acid casein insoluble in water. The most common types of casein were sodium casein, usually produced by dissolving the acid casein with sodium hydroxide and then drying. Acid casein is used in many industries such as the manufacture of wood glue and in the casings of paper and cardboard because of its excellent adhesive property and its resistance to the spread of moisture and fat as well as many other industries (Al-Asadi, 2015)

It has been shown (Frederico, *et al*, 2019). Casein solutions do not need thermoplastic denaturation to form casings, unlike natural whey proteins from

which casings cannot be produced with acceptable qualities except through their linters.

### **B** - Casings of whey proteins:

Whey proteins were the by-product of the cheese industry, as they produce large quantities of whey and in most cases this vital product is neglected, which may lead to some kind of pollution problems when these products were thrown out as waste. The whey proteins constitute about 20% of all milk proteins and have properties Very good functionality, as well as it has a high nutritional value, it is water soluble and hydrophilic in nature, and it has good oxygen retention capacity.

(María *et al*, 2017). Commercially available whey proteins were available as follows:

### I. Whey protein isolate:

This type of protein is characterized by the concentration of whey proteins in it between 90-95% and the percentage of fat, lactose and mineral content is very small.

### II. Whey protein concentrate:

This type is distinguished by the concentration of whey proteins in it between 25-89% and commercially available at 80% and also contains some fats, lactose and minerals, which makes the protein concentration less than the isolated.

### III. Whey Protein Hydrolysates:

The percentage of protein in this type is variable according to the type of decomposition, and that the decomposition process is used to break the peptide bonds and obtain small peptide chains that were less sensitive compared to the non-decomposing.

## **IV. Undenatured Whey Concentrate**

The ratio of this to a type is also variable, but usually between 25-89%, these proteins were modified in such a way that they maintain the structure of their
original structure, and this type usually contains high amounts of immune proteins and lactoferrin (Pérez, 2016 and Bade Tonyalib, 2018).

Because of the increasing confidence of dairy producers in the properties of whey proteins, it tended to develop the uses of these proteins as packaging or edible packaging casings. Studies have shown that the casings made from whey proteins have better mechanical and reservation properties when compared to the competing proteins that were used in the manufacture of casings such as soy protein isolates and zane corn and wheat clotted wheat, despite these good properties, but they have determinants of their ability to retain moisture, bringing them to a certain level. Therefore, these proteins support when making casinges with plasticizing materials such as sorptol and cholesterol to give them resistance to moisture transfer and also to improve and enhance flexibility and expansion and prevent the emergence of fragility(Díaz, and Cobos, 2016). Figure (2-2) illustrates the properties of shell protein covers (Ramos *et al*, 2013).

### **C** - Gelatinous casings:

Gelatin is an edible protein produced by partial decomposition of collagen from the bones of domestic animals. The word Gelatein is derived from the Latin verb Glare, which means the ability to germ, and was known to ancient Egyptians for thousands of years when they were able to extract glue by cooking the skins of mature animals (Jian-Hua Li, *et al*, 2014). Gelatin has been used in many food applications, especially when painting food, especially meat. It has been used singly or with a mixture of fatty substances, proteins, and gums in the formation of edible casinges, and it can be removed from the food-coated food like meat by just submerging it in warm water, and a casing can also be formed A stand-alone solution of aqueous gelatin solution with a concentration of 2% -3% in the presence of a suitable plasticizer (AL-Hassana and Norziah, 2017).



Figure (2-2) the properties of shell protein covers

\* (Ramos et al, 2013).

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Gelatin casings was used to protect ripened cheeses from the growth of surface molds by binding calcium sorbate in the casings solution to ensure that sorbates do not penetrate into the cheese mold and affect the bacteria of the initiator. Gelatin was also used to protect frozen meat from dehydration and oxidation during storage by attaching anti-oxidants with Epithelial gelatin casinge (Sajed Amjadi, *et al*, 2019).

# **D** - Corn zein

Corn protein is the protein stored in corn grains and is a by-product in the manufacture of corn products. It is produced on a commercial scale from it. It is not soluble in water. %)

# (Talita M. Santos, et al, 2017).

Since corn is considered an acceptable protein and safe for the consumer, it can be used in food products, and this protein can dissolve in organic solvents such as ethanol. Plasticizers such as glycerol and oleic acid play a very important role in improving the elasticity of the packaging made from this protein. In the packaging or casings of dried fruits, nuts and tablets

(Ljiljana Spasojević, et al, 2019).

# 2-5-3: Fat edible casinges:

They were casinges made from fats, waxes, and polysaccharides. They have good reservation properties of water vapor because of their hydrophobic nature. They act as a barrier to moisture because of the different crystalline structure of Polymorphus for the types of fats that determines the passage of water vapor molecules from and into food, and in contrast to other vital sources that were made of them. Coverings (Fei, *et al*, 2017). However, fats were not able to form vital polymers, so they were not coherent and do not form existing caps by themselves. Rather, they use fats as a casings that is applied to surfaces or used with other sources as compound covers to increase the susceptibility of the caps to retaining water vapor due to Possibility of low polarity fats and that this susceptibility is variable depending on the characteristics of the type of fat, the polarity of fats depends on the method of distribution of chemical groups, which is the length of the fatal chain and the

degree of unsaturation and unsaturated fatty acids (Tavassoli-Kafrani, *et al*, 2015).

# 2-5-4: Composite edible casings

Composite casings were made to overcome some of the negatives found in the biological sources in addition to taking advantage of the complementary characteristics of each of them, as the composite casings were often combinations of non-hydrophobic groups (such as fats) and hydrophilic compounds to form the casing matrix, (Chengcheng Ruan, *et al*, 2019) Proteins and sugars were considered to be the least permeable sources of gases from fats. The compound casinges can be in two forms either as a bilayer or in the form of a fixed emulsion. In the two-layer compound casinges, fat is used as a second layer over the protein or sugary layer. Fat is diffused within the primary polymer matrix for the purpose of strengthening (Bade Tonyalib, *et al*, 2018).

# 2-6: Antimicrobial packaging

When thinking about creating edible casings, it was necessary to find a way to prevent the microorganisms from consuming the used packaging because most edible covers were subject to degradation by the microorganisms as they were a good food medium for microbes, so antimicrobial substances were added to the composition of the cover to obtain casings that could Its use in packaging and preserving food from microbial damage (Abdulaal and Norziah, 2020) has had a wide resonance in this field as packaging showed a possibility to protect foodstuffs from spoilage and it limits the growth of pathological organisms through the continuous and gradual spread of anti-microbial substances Reinforcement of the casings on the surfaces of the coated food (Valdés, *et al*, 2017).

And when choosing antimicrobial substances that will support the covers for the purpose of preserving a specific food item, it is necessary to think about the targeted microorganisms and the effectiveness of the additive towards these neighborhoods and in the interactions that can happen with the polymer materials used as a natural source of the packaging because such interactions will affect On the effectiveness of antagonists (Abdelhedi, *et al*, 2018).

#### 2-7: Types of anti-microbiological packaging

Anti-encapsulation systems were classified into two types. The first packaging system allows the active substance to be combined with food. The second system does not allow the release of the active substance. In the fusion system, the active material can cover on the surface or be within the network of the material used in the packaging (Dehghani, *et al*, 2017). Where these active substances work when the food is in direct contact with the casings as in Figure (2-3) where A represents the incorporation of the active substance with the casing component and B represents the same previous model but with multiple layers and the active substance is in the inner layer either C represents the coverage of the food surface Only the active substance in the form of a surrounding casing, while D is by installing the active substance on the packaging material (Amankwaah, 2013).

#### 2-8: Factors affecting effective packaging against microorganisms

Many factors interfere in determining the effectiveness of casings, and some of them may constitute an obstacle to their use, but others can be overcome. This condition occurs when there were substances that have the ability to cause interference and create bonds between polymer molecules, which affects the reduction of the casings 'effectiveness toward microscopic biology By linking the active compounds with the polymer materials or by means of the thermal processes that take place to form the casings, .(Gaofeng *et al*,2016). and that the most important factors that go into determining the casinges work were working conditions and their effect on the amount of residual active compounds and the quality of the active substance used and its relationship to the packed food in addition to the storage temperature The outer appearance of the coated material (Arnon-Rips, *et al*, 2018).



Figure (2-3) Packaging systems for food and active substance incorporation processes

\*(Bastarrachea, 2011)

# 2-9: Additional materials used in the formation of casings

Other materials were added when making edible casings to improve their properties in addition to overcoming some obstacles that face this industry, including plasticizers and emulsifiers, and it can also be used as casings carrying active substances such as antioxidants or microorganisms or may be carriers of nutritional materials such as vitamins, minerals and flavors And others (Arnon, *et al*, 2015).

### 2-9-1: Plasticizer

casings made from natural sources were of a fragile or fragile nature due to the internal bonds between the polymer chains, so plasticizers were added to reduce fragility as well as to increase flexibility and improve mechanical properties, such materials have the ability to reduce the degree of glass transformation known as Tg, represents (Tg ) The critical degree of the polymer in which it is transformed from the vitreous to the elastomeric form, the higher the tendency of the casings to be in the vitreous, the more rigid and brittle. On the contrary, when they were in the rubber form, they were more elastic and elastic (Vieira, et al, 2011).

The plasticizer added when making the casings has low molecular weights Embuscado and Huber (2009), the plasticizer increases the permeability of the casings for gas and water vapor due to the low degree of crystallization, due to the low reservation properties of the casings due to the presence of plasticizers due to the water wrapping property it owns, for example. The materials used were water, glycerin, sorbitol, and polyethylene glycol, and to increase the capacity of the casings of water vapor, add non-plasticizing agents for water such as wax, lauric acid, and acetic acid esters of monosulfide (Foteini Pavli, *et al*, 2018).

# 2-9-2: Colorants, nutrients and bio boosters

Edible casings shall be in types according to the specific type of cover requirement and the direction in which the casings were manufactured. casings can be developed and diversified by adding colors, minerals, and some may be added to biological enhancers, such as the use of some types of microorganisms as biological enhancers (Soukoulis , *et al*, 2014), Or adding some colourants to the transparent casings to reduce the light oxidation of some types of unsaturated fatty acids in salmon oil (AL-Hassana and Norziahb, 2017).

# 2-10: Mechanical properties

The mechanical properties of the casings were one of the critical indicators through which the casing durability is determined and its ability to improve the mechanical safety of foods is assessed (Sule Gunaydin, *et al*,

2017). The step of drying casing solutions to obtain the casings is important in determining their mechanical properties (Chengcheng Ruan, *et al*, 2019) The tensile strength and the elongation percentage were used to determine mechanical properties and these indicators were derived from the stress curve (Figure 2-4), and the tensile strength (TS) is known as the maximum strain tolerated by the casing, and this is done by dividing The maximum load of the pre-cut casing ( $\sigma$ Y) on the primary cross-sectional area (S), and the elongation ratio before cutting (E) measures the amount of expansion of the casings ( $\epsilon$ B) as it reflects the percentage of change in the original length of the casings before the interruption (Skurtys *et al*, 2014).



Figure 2-4 typical stress curve

\* (Skurtys et al, 2014).

# **2-11: casings reservation properties**

The diaphragm permeability of gases and water vapor is due to the difference in pressure between the opposite sides of the diaphragm, as the diaphragm permeability can be measured through 3 main processes:

1- The process of the flow of gases and fumes into the material forming the casing.

- 2- Molecular diffusion of gases and vapors through the casing material.
- 3- The process of releasing gases and fumes from from the casing to the outside.

The permeability of the casinges depends on the extent of solubility of gases and fumes in the wrapping casing (Christian Ghidelli and María B, 2016) .Addition of the extent of their diffusion within the casings themselves can be estimated via the formula ( $P = D \times S$ ) P express the permeability and D express the diffusion coefficient either S is the coefficient of solubility (Oğuzlu. 2011).

### 2-12: The basic functions of casings and casings

The most important reasons for it by scientists and researchers in the field of chemistry, biology and physics technologies to care for edible casings and casings were their wide applications and many benefits. Figure (2-5) explains the most important basic functions of edible casings and casings for the purpose of maintaining quality and extending the life span of food (Jian S, *et al*, 2017)

#### 2-13: Green Tea (Camellia Sinensis)

The green tea plant belongs to the rank of the Ericales family (Theacea), the genus Camelia and the sinensis type, and the original habitat in which this plant grows is China and southeastern Asian continent, the green tea plant is a permanent shrub Green or it may be in the form of a small tree and trim when planting to a length of two meters, (Piotr Kulawika, *et al*, 2019) knew the use of green tea leaves since ancient times was the first to use it by the Chinese Emperor Shen Nong in 1273 BC when the green tea leaves fell by chance In a container with boiling water, then the emperor was surprised after his taste of this drink because it has a distinct taste and aroma and since then it has become one of the most consumed drinks in the world after the water, as the total annual sales exceeded 23 billion dollars worldwide, more than 11 billion dollars, including tea Green is famous and consumed worldwide after water (Jiang Hu , *et al*, 2018).



Figure (2-5) shows the basic functions of edible casings and casings for the purpose of maintaining quality and extending the life span of food \*(Jian S, *et al*, 2017).

The tea is divided into green and white tea (prepared from leaf buds), black (black tea) and Oolong, as for green tea, it is not exposed to fermentation treatments during manufacture and thus maintains the antioxidant compounds it contains more than other types that were exposed For fermentation treatments during manufacturing processes such as oolong or black tea (Tesaka, 2016).

# 2-13-1: Green Tea Benefits

The popularity of green tea is due in the past to Asian countries and currently in western countries as a result of the findings of studies and research of health benefits of green tea, as it contains anti-cancer and anti-microbial compounds and provides protection from heart disease and other anti-diabetes (Theeraphorn, *et al*, 2019), and the secret of the multiple benefits of green tea

is due to the contents of its multiple sheets of phenols, the most important of which were the Flavoniods, specifically the catechins group, which makes up 90-80% of flavonoids and about 40% of the water-soluble solids that make it a substance. It has Oral Health Benefits, Anticancer, Cardiovascular Disease Health Benefits, Antioxidant, Anti-inflammatory, and Reykert (Reygaert, 2017). Figure (2-6) shows the most important scientific properties. Possessed by green tea



Figure (2-6) the active sites of active compounds in green tea

\*(Sinija and Mishra, 2008).

# 2-13-2: The chemical composition of green tea leaf extract

The chemical composition of green tea is complex. Fresh tea leaves contain an average of 4-3% of alkaloid (alkali) known as methylxanthines such as caffeine, theobromine and theophylline, in addition to that there were phenolic acids such as kalic acid and distinctive amino acids such as Theanine (Shumi, 2015).

Green tea contains multiple phenols, which include Flavanols, Flavandiols and phenolic acids. These compounds represent up to 30% of the dry weight. Most of the multiple phenols of green tea were Flavanols, known as Catechins. Green tea products were mainly extracted in liquid and dry form, which is why the ratio of poly phenols varies between 90-45% and caffeine content (10-0.4)% (Yong-Quan, *et al*, 2017). Table (2-1) shows phenolic compounds. It is found in green tea extract with chemical formula, partial weights and percentages

Particulars	short	Formula	Molecular wt.	Dry wt. (%)
Flavanols				
(-)-Epicatechin	(-)-EC	C15H14O6	290	1-2
(-)-Epicatechin gallate	(-)-ECG	C18H18O10	442	3-6
(-)-Epigallocatechin	(-)-EGC)	C15H14O7	306	3-6
(-)-Epigallocatechin gallate	(-)-EGCG	C22H18O11	458	9-12
(+)- Catechin	(+)-C	$C_{15}H_{14}O_{6}$	290	1-2
(+)-Gallocatechin	(+)-GC	C15H14O7	290	1-2
(+)-Gallocatechin gallate	(+)-GCG	C22H18O11	458	•
(+)-Catechin gallate	(+)-CG)	$C_{18}H_{18}O_{10}$	442	
Total polyphenols				25-35

Table (2-1) Phenolic Compounds Found in Green Tea Extract with Chemical Formula, Partial Weights and Hydroelectric Ratios

\* (Shumi, 2015).

Catchins were more present in green tea than Oolong oolong tea. There were four main types of catechin compounds in green tea: epicatechin, epigallocatechin, epicatechin-3-gallate, epigallocatechin-3-gallate (Lixue Dou, *et al*, 2018). Figure (2-6) shows the structural formula of some important catechins in green tea extract





\* (S.P.J. Namal Senanayake, 2013).

### 2-13-3: food degradation by self-oxidation processes

The oxidation processes cause many aspects of the deterioration in the food by causing a change in its flavor, color, or strength in addition to the change in the nutritional value of the food products, which leads to a reduction in their quality, as it was found that there were many systems that contribute to the oxidation of the fats inside the food, including light., Heat, Enzymes, Minerals, Mineral Proteins as well as Microorganisms (Tuba and Hakan, 2018). The presence of fats and oils in the root body is required in order to interact with triple oxygen in self-oxidation. In the usual case, the fats and oils were in their natural, non-root form. These factors form the root formula that converts existing fats from the formula of non-root units to fats in a formula that has free radicals by removing hydrogen which returns Because of the weak hydrogen, so it is easy to remove, some of the fats in this case take the most stable formula to be the double ligament in the place where the removal occurred where the oxidation process in the case requires the presence of mono oxygen (Ali Rashidinejad et al, 2016), and that the free radicals and with The presence of oxygen (in the form of triple O3 or mono O) leads to oxidative processes in foods and consequently leads to degradation and reduces the storage life of food (Zhang, 2013).

# 2-13-4: Green tea antioxidant activity

Green tea extract has a strong antioxidant effectiveness due to the presence of (catechin +, epigallocatechin (EGC) -, epicatechin (EC) - (-) and epigallocatchin (EGC -) - (-). Cachin is the compound that does not evaporate and form approximately 15-8 % Of the plant's dry weight. Green tea is considered one of the most important natural nutrients that exhibit very high antioxidant effectiveness (Sumaya and Amit, 2018). Studies on green tea with some other plants have been tested under the superiority of green tea for its effectiveness Antioxidants on garlic, spinach, turnip and Brussels sprouts. Green tea is better than black tea in this matter. This is due to the phenolic compounds in green tea that exhibit antioxidant activity (Ilaria Peluso, *et al*, 2015).

Phenolic compounds were considered to be very powerful antioxidants and powerful free radical holders, as they act as hydrogen hydrophobic, reducing

agents, metal holders and inhibitors of mono oxygen formation where their binding to it is rapid (Liu, *et al.* 2018). The effect of green tea antioxidants is in two ways:

# First: the direct effect of green tea :

The direct action is by binding the phenolic compounds with the active oxygen and generating the most stable phenolic roots. The focus of research and studies has been on the compound (EGCG) due to its presence in high quantities in green tea in addition to containing it in the Galloyl group on the B and D ring. (EGCG) by interacting with oxygen, which leads to oxidation of the D-ring as well as its ability to maintain O2 and OH (Saba and Fatemeh Raouf, 2020).

Green tea leaves were rich in multiple phenols and were mainly composed of phenolic catechins and organic acids, especially kalic acid, in addition to the tea contains carotenoids, tocopherols, vitamin C and a group of minerals including (Cr Cr, manganese mn, Se selenium and Zn zinc) and some plant chemical compounds The other, which all directly affects the anti-oxidant action by forming a GTP compound, has been found laboratory that GTP is an anti-oxidant compounds as it can remove the active oxygen and nitrogen whose presence is a reason for increasing the oxidative activity in addition to having a chelating ability to oxidizing metal ions Transition as it holds iron and copper ions to prevent its entry into Fenton and Hapier-Weiss reactions (Liu, *et al.* 2018).

# Secondly : The indirect effect of green tea

Where phenolic compounds accelerate the processes leading to oxidative processes depending on experience conditions, studies have shown that multiple phenols of green tea can be oxidized in base conditions and on pH (13), which leads to oxidation of episode B. When EGCG and EGC interact with H2O2, the loop oxidizes in both compounds to form two oxidizing compounds of EGCG and one oxidizing compound of EGC. In this way, the semi-direct effect is caused by the oxidation of phenolic compounds, because such reactions can produce free radicals. Iron or copper ion is present. As in Fenton reaction (Sumaya and Amit, 2018).

Phenolic compounds may also be indirectly influential as antioxidants by inhibiting the cloning process during the synthesis of sensitive agents of oxidation in addition to inhibiting the enzymes that help in the oxidation process such as xanthine oxidase, lipoxygenases, cyclooxygenases and nitric oxide synthesis, and that one of the roles played by the compounds Effective in tea were the stimulation of antioxidant enzymes such as glutathione-S-transferases and Superoxide dismutase (Lorenzo and Sichetti, 2016).

#### 2-13-5: Measurement of antioxidant efficacy using DPPH

The widespread use of the DPPH method (2, 2-diphenyl-1-picrylhydrazyl) due to its high ability to estimate the antioxidant effectiveness in foods in addition to its ease of doing compared to other older methods (Mishra *et al*, 2012), where the method is summarized as it shows Figure (2-7) in a change in the composition of the substance DPPH, which leads to a change of color from purple to yellow and then to colorless gradually and according to the reduction strength by the electron in this substance with the hydrogen ion present in the antioxidants which leads to a reduction in absorbance Relativity of DPPH and its color change to yellow. This change in color equals the amount of reactants and the number of absorbed electrons (Zhang, 2013).



Figure (2-8) DPPH Synthesis and Its Relation with Hydrogen Ion for Antioxidants

\* (Zhang, 2013)

# 2-13-6: Microbial degradation of food

Food is any substance, whether manufactured, semi-processed or raw, and it is intended for human consumption including chewing gum and any material used in preparing or treating food that is subject to microbial damage (Robertson, 2016), and the microbial damage to food is defined as any sensory change For food in flavor, smell, appearance or texture that the consumer considers unacceptable, food spoilage occurs for a number of reasons, including it may be the result of an injury caused by insects and physical damage, or it may occur due to the enzymes analyzed due to the microbial infection (Tuba and Hakan, 2018), thousands were recorded Mortality globally is caused by food poisoning, and it is one of the main microorganisms that were contaminated with food and cause diseases transmitted through food by Escherichia coli, Staphylococcus aureus, Clostridium perfringens and Listeria monocytogenes, and this is why preservatives were used in food to reduce food degradation processes such as benzoic acid and not A side effect that negatively affects humans (Rawat, 2015).

# 2-13-7: Natural microscopic antibiotics

Usually, the active compounds that affect microorganisms were secondary metabolites in plants that include alkaloids, steroids and tannins, which were created and produced from a specific part of the plant or in all parts of the plant, but the leaves were often the primary center for the production of these compounds and return most of the mechanisms of action Natural compounds with antibacterial activity to break down the cytoplasmic membrane and destabilize both the electron flow process and the efficient transport process in addition to clotting cell content (Kapoor, *et al*, 2015).

As for the effective compound obtained from plants, there were many types of preparations and herbs that have been known to be effective against microorganisms of all kinds (anti-bacterial, anti-fungal, anti-viral, antiparasitic), for example essential oils that were used in the past in alternative medicine and that were Extracting them from the plant, whether from flowers, buds, seeds, leaves, or even stems, all affect the lush neighborhoods with a certain inhibition mechanism. Among the mechanics known in oils is the reaction that occurs to the compounds present in the oils with the sulfahydril groups (SH Group) found in cellular proteins. (: Saeloh D, *et al*, 2018), and some of the compounds that have anti-microbial activity have automatic mechanisms that depend on non-hydrophobic groups that bind to fats in the cellular membrane of bacteria, which disintegrates the bacterial cell structure and increases its permeability, and that the existing chemical compounds Essential vegetable oils also have a role in affecting the proteins of the cytoplasmic membrane, and many enzymes were present in the membrane cytoplasmic, whose action focuses on cyclic hydrocarbons and the distortion of the bonding between fats and proteins (Kapoor , *et al*, 2015).

### 2-13-8: Anti-microbial activity of green tea

Among the effective properties of green tea extracts is its antimicroorganism ability. Since ancient times, green tea leaves have been used for this purpose and have been used by a pioneer in the British Army medical team at that time and showed that tea kills *salamonella* bacteria. typhi and brucella. melitensis is also recommended to be added in glass water bottles to protect against pathogenic microbes and microbes and ensure no infection outbreaks among people (Sumaya and Amit, 2018).

The direct anti-microbiological effect is due to catechins that show several mechanisms of inhibition. These mechanisms cause damage to the bacterial cell membrane, inhibition of fatty acid synthesis, inhibition of enzyme activity (Reygaert, 2014) and the most important mechanisms of tea extract in inhibition were:

#### 2-13-8-1: Damage to the bacterial cell membrane

The effect on the cell casing is due to the possibility of phenolic compounds catechins from its ability to bind to the fatty layer present in the cell membrane of bacteria, which leads to damage to the cell membrane and tear and this in turn makes the bacteria vulnerable to many of the killer effects of bacteria, including the changes that occur to the genetic material of the bacteria (Reygaert, 2014), and also inhibits the ability of bacteria to bind to host cells (Sharma *et al*, 2012) and reduce the ability of bacteria to bond with each other to create bio membranes that appears in pathogenic bacteria. The

damage that occurs in the cell casinge in the susceptibility of bacteria also affects toxins production (Reygaert, 2014).

### 2-13-8-2: Inhibiting the synthesis of fatty acids

Fatty acids have important functions in bacteria, so any abnormality that occurs in them leads to the influence of bacteria as an interference in the synthesis of the phospholipid layer in the cell casinge and is an excellent source of energy needed by the cell (Reygaert, 2014). Studies have indicated the effects that can be obtained from targeting Synthesis of specific types of fatty acids in the use of antimicrobial compounds (Wang and Ma, 2013).

### 2-13-8-3: Inhibiting the synthesis of enzymes

The compounds present in green tea play a proven role in inhibiting the synthesis of enzymes that enter in the synthesis of fatty acids in addition to inhibiting some other basic enzymes in the cell. It has also been proven that catechins have an effect on the process of DNA replication, as they inhibit the enzymes responsible for the welding of DNA strands, for example. DNA gyrase (Reygaert, 2014).

# 2-14: Mozzarella

Mozzarella is an unripe soft cheese from the Pasta-Filata family whose original place is the Battipaglia region of Italy. I have been using this type of cheese for more than 50 years in the pizza industry (Banville, 2016).

Traditionally made Mozzarella cheese throughout Italy from buffalo milk as it was made in some European countries and the USA from cow's milk, it is characterized by being a soft white cheese with a glossy and attractive surface and a light salty and high susceptibility to stretching or stretching ability in hot water, I own cheese Mozzarella has its main characteristics due to the effect of lactic acid on Dicalcium paracasienate, i.e. the thrombus resulting from enzymatic cheese at pH ranges from 5.2 to 5.4, the majority of it turns to Monocalcium paracasienate responsible for the shiny appearance and high stretchability of processed cheese. Among the main characteristics of mozzarella cheese is the bright white color and for its sake preferred Buffalo milk over cow's milk in its industry (Salwa *et al*, 2015), that these combined characteristics have made mozzarella suitable for use in preparing some foods such as lasagna and cordon bleu and on the surface of the pizza because of its high susceptibility to stretching, especially since the pizza is largely consumed by this youth segment which is what Encourage mass production of mozzarella (Lukman, *et al*, 2016).

Mozzarella string is similar to the cheese of the chain and the triumphant cheese in the way of manufacture, but with different formation process, as it has a high popularity in America and its sales were on the rise Permanent (Makadiya and Pandey, 2015) as shown by the statistics in Figure (2-8) Proportion of Mozzarella compared to other types of cheese in Canada.

Food and Drug Regulations (FDR) (2015) classified Mozzarella cheese based on the percentage of moisture and fat in the dry matter (Fat on Dry matter) to four varieties shown in Table (2-2) where the table shows that the traditional mozzarella and mozzarella cheese made from empty sorting milk Partially, the humidity ranges from 52 to 60%.





\*( Makadiya and Pandey , 2015)

Fat in Dry % Matter	Moisture %	Type of cheese
not less than 45	60 -52	Mozzarella (Regular)
45 -30	60 -52	Mozzarella (Part skim)
not less than 45	52-45	Mozzarella(Low moisture)
45 -30	52 -45	Mozzarella (Low moisture ,part skim)

Table (2-2): Classification of mozzarella cheese based on the percentage of moisture and the percentage of fat in the dry matter.

\* Food and Drug Regulations(FDR) (2015)

The percentage of fat in dry matter for the first type is not less than 45% and for the second type it ranges between 30-45%. As for the third type it includes low-moisture mozzarella cheese and the last type includes low-moisture mozzarella cheese made from partially separated milk. The humidity for both varies between 45-52%. And the percentage of fat in the dry matter of the third type is not less than 45% and for the latter type it ranges between 30-45%. As for partially empty mozzarella, which is made by removing part of the milk fat and replacing it with vegetable fats or oils, it is usually used in pizza, lasagna, and cordon blue products because it is cheap compared to traditional mozzarella cheese (Al-Azzawi, 2018), but despite that, the traditional cheese is known in Markets were relatively high-fat products, with a fat percentage in the range of 27-20%, and therefore it is possible to manufacture low-fat products that mimic these products in their attributes (Bi, *et al*, 2016).

In 1990, many types of low-fat cheese were successfully manufactured (Bi, et al, 2016). However, the removal of fat affected the cheese tissue and its solubility.

# 2-14-1: Manufacture of mozzarella

# 2-14-1-1: Milk type

Pure white color is one of the main characteristics in the manufacture of mozzarella and cow's milk. This is lacking for this characteristic

(Zedan and Zaky, 2014). Therefore, I suggest adding neutralizing dyes, as well as adding bleaches such as benzoyl peroxide and titanium dioxide and 0.03% to Milk intended for the manufacture of mozzarella cheese to solve this problem (Ma, *et al*, 2014).

Zedan and Zaky (2014) showed that cheese made from buffalo milk contained high protein compared to cheese made from cow's milk and also outweighed the rheological qualities of cheese made from cow's milk over cheese made from buffalo milk or made from mixing cow's milk and buffalo milk together in a ratio (1:1)

(Salwa, *et al*, 2015) indicated that buffalo milk is the most commonly used and appropriate milk in the manufacture of mozzarella compared to cow's milk and (Sameen, *et al*, 2015) found that the type of milk, whether cow's milk or buffalo milk or a combination of them has an effect Great in the composition of cheese and that the cheese resulting from the mixing of cow's milk with buffalo milk sensitively superior and also in the solubility of cheese made from cow's milk alone or buffalo milk alone.

# 2-14-1-2: Heat treatment of milk

Mozzarella is made primitive from raw milk. However, it is recommended that the milk pasteurization intended for the manufacture of mozzarella be made safe for consumption because the process of scalding or stretching may not be sufficient to kill pathogenic microbes (Bhattarai, *et al*, 2013). The treatment of milk prepared for the manufacture of mozzarella cheese at a temperature of 72 m for a period of 15 seconds contributed to obtaining higher levels of protein as well as the total solids, but it reduced the fat percentage and gave a soft consistency to the processed cheese, improved the flavor, preserved the quality and ensured the safety of the product healthy.

(Bi, *et al*, 2016) stated that milk pasteurization or treatment of milk prepared for the manufacture of mozzarella cheese with high heat treatment UHT gave excellent results in terms of increased clearance compared to cheese made from raw milk and did not find (Homoyouni, *et al*, 2014) no significant differences in The quality of mozzarella cheese made from fresh milk or pasteurized milk and preserved for a period of 0-10 days. Also, the high thermal treatment UHT of the milk led to an increase in the percentage of clearing in the mozzarella cheese made by direct acidification of the milk with a percentage of more than 3.4% of the percentage of cheese refining (Khansa, *et al.*, 2014).

### 2-14-1-3: Methods of making mozzarella cheese

#### 2-14-1-3-1: Use the starter

The addition of the starter is one of the traditional methods used in the production of mozzarella and the starter used for the production of Italian mozzarella is a single initiator represented by Streptococcus thermophilus and is responsible for acquiring the basic characteristics of the final product (Salwa, *et al*, 2015).

Banville, *et al*, 2016) suggest the use of active primers that were added directly to basins such as mixture primers from S.thermophilus and L. bulgaricus as being from high-temperature initiators more than 40  $^{\circ}$  C. The process of mixing different primers with fermenting lactose such as S.thermophilus and fermented For lactose, such as L.bulgaricus and L.helveticus, it helps inhibit Bacteriophage.

#### 2-14-1-3-2: The direct acidification method

This method gained a lot of commercial attention because it did not depend on the performance of the starter and its role in the production speed. This method was used in the manufacture of mozzarella cheese (Abdel-Aziz and Abo-Srea, 2014).

Researchers have used some acids, including phosphoric acid, lactic acid, acetic acid, malic and citric acid, as well as the natural juice of orange, lemon and pomegranate (Abdel-Aziz and Abo-Srea, 2014).

The manufacture of mozzarella cheese using citric acid as a regulator of pH led to an increase in calcium concentration, solubility and spread. No significant differences were observed with regard to the content of fats and free oils when using acids such as citric, lactic acid, acetic acid and phosphoric (Jian-Qiang, *et al*, 2015).

Jana and Mondal (2014) attended mozzarella using direct acidification with or without starter addition, which resulted in a faster way to reach the required pH value. The processed cheese was compared using this method and the direct acidification method. Fat recovery and cheese refinement were greater at pH value of 5.3 than at values of 5.9 and 5.2. When decreasing the value of pH, a decrease in SNF recovery and cheese hardness was observed, while the moisture content, solubility and fat loss increased. The ability to melt the direct acidification cheese compared to the cheese manufactured by adding the starter method, except for cheese made using citric or malic acid at the value of pH 5.2 - 5.4. The sensory ratings for the second method cheese were higher at the value of pH 5.4 - 5.6 (Nawaz, *et al*, 2011).

#### 2-14-1-4: Tools used in the manufacture of mozzarella cheese

The mechanism used in the manufacture of mozzarella cheese includes the development of machines for forming and forming cheese balls and the process of molding and curd formation and cooling molds automatically and give the required hardness to cheese and salting and cooling of cheese automatically and cutting and has developed a device to form the curd and then cover it in the next step in hot water. As for the water temperature Used in cooking ranges between 60-85 o C and the temperature of the cheese after the end of the forming process ranges between 50-65 o C (Scott, 2014).

#### 2-14-1-5: Microbiology of mozzarella

Several researchers studied the microbiological content of mozzarella during manufacture as well as samples sold on the market

(Abdel-Aziz and Abo-Srea, 2014). Listeria monocytogenes have been observed to decrease by two logarithmic cycles after dipping the thrombus in hot water 95 ° C or at a temperature of 66 ° C for 5 minutes or at a temperature of 77 ° C for 1 minute to eliminate Listeria bacteria. Monocytogenes during

the production of mozzarella cheese, in addition to that the salting process has a deadly inhibiting effect but is less than the effect of drenching the curd with hot water (Sameen, *et al*, 2015). The US Food and Drug Administration has authorized the addition of preservatives, provided that this is mentioned on the packaging, during the process of kneading and shaping, or adding them to the surface of cheese (Scott, 2014).

# 2-14-1-6: Structural and rheological qualities of cheese

The composition of mozzarella has been studied by (Ong, *et al*, 2011) and (Bi, *et al*, 2016) their studies mainly showed large fat globules circulating regularly through a compact protein network with little agglomeration.

The effect of milk type, casein / lipid ratio, milk heat treatment, naturalization, manufacturing method, acid type, pH when coagulation, whey acidity and clarity, composition and protein degradation during storage period and their effect on the rheological properties of mozzarella cheese have been studied by many researchers (Bi, *et al*, 2016), since cheese made using milk with a casein ratio of 50% is less solid, cohesive, pulsating and chewing, and higher than it is in cheese made from casein milk / fat higher than 50% (Zedan and Zaky, 2014).

Mozzarella cheese wire is a semi-rigid rubbery sticky substance at room temperature that shows high ductility at 60 ° C and the refinement of these cheeses gradually decreases with increasing temperature (Scott, 2014). Mozzarella shows weak solubility, fatty infiltration, acidic flavor, free surface moisture, weak consistency and shortness of color after thawing, but normal characteristics were acquired again in 1-3 weeks of manufacture (Jana and Mondal, 2014). The decrease in the fat percentage from 3.5% to 2.0% in milk resulted in an increase in cohesion, gum and chewability, while the elasticity decreased (Salwa, *et al*, 2015).

Roginski, et al (2003) mentioned (a number of mechanical methods that can be classified into three main categories for assessing the rheological properties of cheese were penetration tests, pressure tests and cutting tests. This science has been used in recent years in many industries such as casingss, pastes, dairy products, etc., as it is It is used to estimate viscosity, and it is one of its most important branches in the study of true and colloidal solutions, whether diluted or concentrated, as it needs to control the properties of the product in terms of its homogeneity and stability of its characteristics: constancy, smoothness, texture, and body structure. Individually, it was possible to obtain better information on the substances tested.

The rheological properties of foodstuffs determine its strength from liquid to semi-solid to solid, which affects the consumer's acceptance of it, as it affects its natural and perhaps biological characteristics, it has been found that the degree of viscosity can affect the degree of absorption of some solublesubstances in the gut, as well as the rheological properties affect Choosing the appropriate manufacturing method (Bi, *et al*, 2016).

Cheese is a foodstuff applied to it elastic, rubber and other rheological qualities, as the formative properties of cheese have played an important role in the quality of the product and an attractive factor for the consumer.

#### 2-14-1-7: Mozzarella packaging

Cheese has a complex composition due to the many biological and biochemical reactions that occur from production to storage, which affect its physical, sensory, and chemical properties, such as texture, flavor, and color (Maria, *et al*, 2017). Intensive growth of yeasts, molds and bacteria on the cheese surface may occur due to external environmental conditions during handling and storage, which greatly reduce the quality of cheese and require the development of specially designed packaging materials to avoid spoilage (Fajardo, *et al*. 2010). The packing requirements change according to the type of cheese because the ripening rates, water content and mechanical stability will depend on its composition. It has been suggested that fresh cheeses (such as cream cheese, soft cheese and cheese) should be packaged in a modified atmosphere containing N2 and / or CO2 instead of O2

However, due to the presence of yeasts and bacteria, spoilage can still occur even at low levels of O2 and CO2 which makes modified air packaging a solution that should be used with care taking into account these factors. However, the factors to consider when choosing a filling material were almost the same for all cheeses, such as gas permeability, mechanical properties, and transparency (Artiga, et al, 2017). Some of the materials used to package cheese, such as polyethylene, polyamide, and polypropylene, were nondegradable, inedible and can lead to environmental problems as well as being limited by strict legislation on the transfer of materials to cheese. For the casings, migration can be a problem, because after application to the surface of the cheese and its hardening (by crystallization in the case of wax and / or evaporation of the solvent) some migration of the casings material to the cheese may occur. These major concerns prompted the industry to search for new packaging solutions and then to increase research on edible packaging materials (Fajardo, et al, 2010). In this context, biopolymers, natural fats and wax appear as an alternative source for developing new packaging materials. edible casings and casingss based on these materials can guarantee the quality of food, and act as a semi-oxygen barrier, carbon dioxide and water vapor, allowing to reduce water loss and control the ripening rate (Yu Zhong, et al, 2014). In addition, edible casings and casingss can be used as carriers of antimicrobial agents and thus avoid unwanted microbial growth on the cheese surface. The packaging process leads to an increase in the shelf life of mozzarella. Mozzarella is successfully wrapped in polyethylene with or without vacuum packaging (Scott, 2014).

#### **Chapter Three**

#### **Materials and Methods**

#### 3.1 Material

Chemicals of well known international brands were obtained purchased from the local chemical and media suppliers. and media C MacConkey agar, Potato's dextrose agar, Mannitol salt agar) were obtained from HIMDE (India) and Mueller Hinton agar was from CDH (India), Whey protein isolate (BiPro ,USA) and green tea (Mahmood Tea, LK) was obtained from Local market and Glycerol from BDH Middel East and Ethanol 99% from Sigma Aldrich (Germany) and De ionized water from Technology University and Sodium thiosulfate from SCRC (China) and Sulfuric acid (H2So4), Hydrochloric acid (HCL) from Fluka (Switzerland) and Sodium hydroxide (NaOH), Sodium chloride from B.D.H Middle East LLC (England) and Calcium chloride from CDH (India) and Cuso4 from Sigma Aldrich (Germany) and Starch from Starch Banreace (Spanish) and Potassium Iodide (KI) from B.D.H Middle East LLC (England) and Propanol from Sigma Aldrich (Germany) and Bacterial strains (Escherichia coli, Salmonella spp., Clostridium, Staphylococcus aureus, Bacillus spp.) and yeast (Candida albicans) were obtained from the Colleges of Agriculture and Sciences, University of Baghdad, Iraq.

# 3.2 Study plan

Study plan is divided into tow phases :

# Phase one :



# The second phase:



#### **3.3 Green Tea Extracts Preparation**

Dried green tea leaves (Mahmood) brand was obtained from the domestic markets at Baghdad city, pulverized using an electrical grinder, sieved tea using sieve (70/100) so as to obtain a powdered green tea.. Green tea powder (GTP) was extracted according to Chan *et al.*, (2007) procedure. Four aqueous and ethyl alcohol solutions (0:100; 75:25; 50:50, and 25:75, alcohol: water). Four conical flask (500ml size) each containing 10 g of green tea powder was mixed with 100 ml of one of the aqueous ethyl alcohol solutions and vibrated at 250 rpm at 60°C for 4 hrs. The extract was filtered using Whatman # 1 filter paper, evaporated using a rotary evaporator so as to obtain a concentrated extract. Finally, the extract was dried overnight in an oven set at 50°C, to get a dried granule extract (DGE).

DGE(%) = wt. of DGE \* 100

wt. of GTP

where:

wt. DGE = weight of dried granule extract

wt.of GTP = weight of Green tea powder

#### **3.4 Antimicrobial activity of green tea extract measurement**

The antimicrobial assay was carried out by utilized agar well. diffusion technique by adding 100µL of *E.coli* cell suspension (1×106 cell/mL) on the nutrient agar and spread with a glass spreader and left for 10 min to dry, then 6mm wells were made and supplied with 50 µL of (0.0, 0.1, 0.2, 0.4, 0.6, 0.8) mg/mL of plant extracts were added and incubated for 24 h at 37 °C. The inhibition zone diameter was calculated for duplicate. Minimum inhibitory concentration (MIC) for *E.coli* isolate was determined by serial dilution procedure. Ten sterile test tube supplemented with (10, 20, 40, 60, 80, 100) µg/mL of plant extracts inoculated with 100 µL of previous activated *E.coli*. All tubes were incubated at 37 °C for 24 h. The result was recorded depending on the turbidity (OD450).

#### 3.5 Incorporation of green tea extract in whey protein isolate

Casings solution was prepared according to Roy *et al*, (2010) with some modifications as follows, 10 gm of the whey proteins isolate was dissolved in in a 100 ml distilled and deionized water with solution stirring until the full dissolution using a hot plate magnetic motor for 30 minutes. The mixture was then heated at 90 °C with a continues stir. The solution was cooled at room temperature and then filtered using medical gauze to avoid any coagulation or insoluble particles in the solution. PH was then modified to 7 using sodium hydroxide 1, 5% glycerol was added to the solution and mixed for 5 minutes. Alcohol extract for the green tea was added to the prepared solution with a various concentration 1%, 2%, 3%, 4%, and 5%. Vacuum pump was used to remove the air bubbles existed in the casings solution for 10 minutes; the solution was kept in the fridge.

#### **3.6 Casings formation**

Casings pretreatment was done according to Roy *et al.*, (2010) the prepared solution was poured in discs 8.5 mm diameter per 8 gm per disc, it was then dried at 21° C for 48 hours to produce whey proteins isolate casings. tissues were removed by the knife from the discs. They were then maintained in spatula and green tea extract in polyethylene plastics at 25° C and 50 % RH until the date of the tests.

#### 3.7 Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) for the casings under the study was done according to the modified method of Dashipour *et al.*, (2014). The green tea extract (50%), as active material against the microbial organism, was added to the whey proteins isolate casings. The test included *Bacillus Spp*, *salmonella spp*, and *Escherichia-coli* in additional to the *Candida-albicans*. : Bacterial cultures were grown on nutrient agar (N.A) medium and maintained at  $4^{\circ}$ C. A loop full of bacterial colonies was taken from the medium in order to activate the microbial organism. They were inoculated in 100 ml nutrient broth medium for the bacteria and Potato's dextrose broth for the *Candida albicans*, incubated at  $37^{\circ}$  C and  $30^{\circ}$  C respectively until the required growth phase. After that, the density of the suspension was modified to fit McFarland

unit of  $0.5 (10^8 \text{cfu}/\text{ml})$ . The required casings samples were cut to discs 6 ml diameter and sterile using the ultraviolet for 10 minutes per sides using ultraviolet light. The discs were poured at Mueller Hinton at 20 ml concentration per plate and inoculated by a bacterial inoculum 0.1 ml incorporated ( $10^8 \text{cfu}/\text{ml}$ ) after dilution. Discs were added to the medium where each plate incorporated four discs, two of them were considered repetitive for the cover sample, the concentration of the green tea extract added to the casings were (1%, 2%, 3%, 4%, and 5%) to specify the minimum inhibitory concentration for the aforementioned microbial organisms. the other two discs were control samples for the casings without green tea extract. The plates were incubated at  $37^{\circ}$  C for 24 hour until the formation of the light dark circles around the discs, the diameter of the dark circle was measured using the accurate digital micrometer to 0.01 mm. The minimum inhibitory concentration was defined as the minimum concentration of the antibiotic inhibiting the bacterial growth.

# 3.8 Antioxidant Activity of Casings

The antioxidant activity of the casings was assessed using (DPPH) (2, 2diphenyl-1-picrylhydrazyl) according to (Wu *et al*, 2013). The samples were pretreated by dissolving them in 10 ml from the previously prepared casings per 10 ml in a distilled and deionized water as explained above. It was centrifuged for 10 minutes at 20° C at 10000g speed. A 0.2 ml of the suspended casings was mixed with 2 ml from 0.2mm of the ethanol solution

solublein DPPH. They were mixed using the votex and left for 30 minutes in the darkness and then centrifuged at 8000g for 5 minutes. The photometry was measured using spectrophotometer for the casings sample and the reference control sampleusing 517 nanometer wave length ware repeated three times and the activity was evaluated according to the following equation



S.A: Free radical throttle activity

ABS blank: Absorption value for alcohol solution DPPH on 517 wave length

ABS sample: absorption value for the extract sample on 517 wave length

### 3.9 Casings Infrared spectrum FTIR

The Infrared spectroscopy was studied using the FTIR device for the films under study supported by the extract and the identification of the effective groups according to the method mentioned by Ciesla et al. (2004) with some modifications, it included drying the films and grinding them into a dry powder, then mixing the membrane samples with potassium bromide in a ratio of 100:1, then pressing the mixture with a piston and under high pressure 2500 kg/cm2 to obtain a small disc 1 cm in diameter and 1-2 mm thick, and put The model is compressed in an infrared device, and the main beams obtained are recorded over a range of wavelengths between (400-4000) cm-1. This technique uses a beam that contains many frequencies of light simultaneously and measures the amount of absorption that that beam absorbs from the sample. The beam is modified to contain a different set of frequencies, giving a second data point. This process is repeated several times, and the computer takes each This data is worked backwards to infer what is the absorption at each wavelength.

# 3.10 Casings physical properties

# 3.10.1 Casings thickness determination

Casings thickness was estimated according to (Arham *et al.*, 2016) using the Professional digitals micrometer from IDM Instruments for an accuracy limit to a 0.01 mm, nine measures were chosen randomly from a various places on the casings and the average reading was accounted, the casings thickness was evaluated before investigating the mechanical properties.

# **3.10.2** Casings ensile and elongation determination

Tensile and elongation were determined for a random samples of whey proteins casings for both of the fortified with the green tea extract and the un fortified according to Yuan *et al.*, (2015) using the (tensile strength). The pretreated Casings samples were cut as rectangular tape dimension (60\*20)mm with 5mm/sec pulling speed.

#### 3.10.3 Oxygen permeability determination

Oxygen permeability was determined according to Bonilla *et al.*, (2012) for the casings samples using Oxygen permeability Tester where the oxygen  $O_2$  was flowing for a specified ratio with Nitrogen gas  $N_2$  so as to mimic the composition of the atmosphere. Those gases were pushed under 20 J pressure on the casings so as to evaluate the permeability of the gasses passing through the casings from the other side, this procedure was done at 23° C and 50% relative humidity.

### 3.10.4 Water Vapor Permeability determination

The modified standard procedure for the American Society for the testing and Materials was followed in this research, it entitled as the cups procedure as it was applied by Ghasemlou et al., (2013) Water Vapor Permeability was determined by the weight methodology, In this experiment, paper cups with diameters were used 3.4, 3.4, 4.5 (exterior, interior, depth) respectively. The cup was filled with a dried material which was calcium chloride (CaCl<sub>2</sub>) until 0.6 cm from the upper edge of the cup. The upper surface was lubricated with silicon fat, the casings sample was cut circularly similar to diameter of the mouth cup to fit the mouth of the cup It was fixed by an iron ring, which its diameter is similar to the diameter of the cup (the exterior and the interior) so as to adhere the casings sample with the surface. The cup was then weighed by a sensitive balance to a nearest 0.001 gm, the cups were dried on a desiccator incorporated Sodium Chloride (NaCl) to achieve 75% relative humidity and then left 24 hours. After that, seven consecutive readings were taken per day for one week on a regular basis to observe the increase in weight. Water vapor permeability was evaluated after drawing a relationship on a logarithmic paper between the increases in the cup weight during the experiment time. A straight line was drawn indicated a steady state. The slope of the straight line was evaluated by (gm/day) using the linear slope, the constant increase in the cup weight after achieving the steady state was used in evaluating water vapor permeability.

 $WVP = (W / t). (X. \Delta P. A)$ 

W/t= the volume of the water permeable during the measure time according to the linear slope (R20.99) through the weight recorded during seven days (g/day)

A= the werea exposed to the permeation (cm2)

X= casings thickness (cm)

The partial pressure which is different according to casings and is  $\Delta p$  = evaluated according to the following equation:

 $\Delta P = S(R1 - R2)$ 

S= the pressure of the saturated vapor at  $25^{\circ}$  C (3166 KPa)

R1= the relative humidity for the glass drier evaluated using RH meter

R2= the relative humidity under the casings inside the cup (0% RH) estimated according to relative humidity of the salt used.

#### 3.10.5 Solubility in water determination

Solubility in water determination was done according to Hanani *et al.*, (2014) the casings were cut into small pieces (1 cm) and dried using an oven at 100°C until constant weight is achieved, the initial dry weight was recorded to the fourth digit 0.0001 gm, the dried casings were then put in 100 ml of distilled water and gently stirred for 24 hours, the solution was filtrated with whatman No.1 to restore the non-solubleremaining casings, the left casings were dried at 100° C to record the initial dry weight. The Casing solubility percentage was calculated according to the following equation:

Casing solubility % = (initial weight – final weight/initial weight)\*100

#### 3.11 Optical properties and color measurement

Optical properties and color was measured using (Brightness and color meter according) to Galus *et al.*, (2013) three readings were taken from the circumference of the ghleaf and its center, the reading was accounted, CIE measures were used to measure colors which is expressed by three symbol
(b\*,a\*,l\*). Each one expressed a specific spectrum; the color is recognized and identified by collecting the three values by using  $\Delta E$ 

 $L^*$ : it is the color graded from the black to the white and gave value from 0 to 100.

 $a^*$ : it is the color graded from the green color when value decreased(-), to the red color when the value increased (+)

b\*: it is the color graded from the blue when the value decreased (-) to the yellow when the value increased (+)

The difference value in the color  $\Delta E$  was evaluated using the following equation :

$$\Delta E = \sqrt{(\Delta L *)^2 + (\Delta a *)^2 + (\Delta b *)^2}$$

### 3.12 Mozzarella cheese production

### 3.12.1 Milk source

Raw cow milk was used in producing Mozzarella cheese in the dairy mills in Baghdad University.

### 3.12.2 The milk composition estimation

Investigating the percent of the milk composition for the raw milk was done using Milko scan the lacto flash the Germany origin, model 3530-131301 in the food industrial company- Abu gharaib diary mills belong to the Ministry of Industry and Minerals.

### 3.12.3 Mozzarella cheese production

Mozzarella cheese was produced according to Al-Bayer (1980) with the following modification:

Five kg of raw milk was obtained from the dairy mill, Food Science Division, College of Agriculture, University of Baghdad. The milk was pasteurized to

 $63^{\circ}$  C for 30 minute, cooled at 4°C, citric acid was added until pH is 5.3, the temperature was gradually raised and microbial manfaha (chymosein enzyme, from CHR HANSIN). The mixture was left for half an hour until coagulation (cheesing), The curd was cut laterally and longitudinally and left for 5 minutes without stirring, The curd produced was pulled and stretched inside the hot whey at 90° C until it became as ball with 5-10 cm diameter, The balls were put in a water at 9-11° C, then immersed in 15% salt solution for 10 hours. The cheese was divided into two parts, the first one was left without foil , the second part is foiled with the casings fortified with the GTE. The cheese was stored at  $5\pm1^{\circ}$  C for 1, 30,60, and 90 days. Thereafter, at the end of each storage period tested for the chemical, microbial and sensorial properties.

### 3.12.4 Mozzarella cheese Coated with the green tea casings

Mozzarella cheese was foiled by the dipping procedure according to Chen (1995) method. The , cheese samples were divided into two portions, one portion was for the cheese coated with the solutions (treatment sample) while the other portion was for the cheese left without coating (control sample). Each portion was further subdivided into 4 sub samples where each sub sample weigh around 100 g. Treatment samples were dipped in the coating solution, kept in fridge at  $5\pm1^{\circ}$  C and 40-50% RH with frequent turning side to side until the solidification of the casings at the surface of the cheese. Each control or treated portions was packed polyethylene cans, closed tightly and stored at  $5\pm1^{\circ}$ C for 1, 30,60, and 90 days. At the end of each storage period they were tested for the chemical, microbial and sensorial properties.

#### 3.12.5 Chemical testing for Mozzarella cheese

### 3.12.5.1 Moisture analysis

The relative moisture of the foiled cheese during the storage time was estimated according to (Kosikowski, 1982), 30 gm of cheese was oven dried at 105° C until the constant weight is achieved, The moisture content was calculated according to the following equation

The moisture content= weight of wetted sample- weight of the dried sample / weight of the sample  $\times 100$ 

### 3.12.5.2 pH measurement

pH was measured according to Ling (2008).methood

## 3.12.5.3 Titratable acidity

The titratable acidity represented the consumed militers from the alkaline to compensated 100 gm of the cheese with a drop addition of alcohol phenolphthalein (2%), the acidity was estimated according to standard method (Ling, 2008) by weighing 3 gm of the prepared cheese and well mixed with 10 ml distilled water, Measurement was then done by adding 0.1 molar NaOH , the acidity was evaluated based on the lactic acid according to the following equation:

The acidity= equivalent gram weight for Lactic acid×standard alkalis× volume of the consumed alkaline / weight of the sample (gm)

## 3.12.5.4 Lipid estimation

The percentage of fat in the cheese was estimated by Babcock method by taking 4.5 g of cheese and using Babcock bottles listed from 0 to 40 (Ling , 2008).

## 3.12.5.5 Total nitrogen determination

Total nitrogen determination was assessed according to (Joslyn, 1970), 0,2 gm of cheese sample was weighed and transferred to the digestion bottle in Microcldal, 5 ml of sulfuric acid  $H_2SO_4$  was added to the cheese sample along with the digestion powder, total nitrogen was determined after the digestion and distillation procedure finished. The protein percentage was calculated by multiplying the total nitrogen percentage by the factor 6.38.

## 3.12.5.6 Soluble nitrogen determination

Soluble nitrogen was assessed according to Ling (2008), 5 gm of minced cheese mixed with 100ml of distilled water and mixed in an electrical mixer for two minutes, the quantity was transferred to volumetric flask size 250 ml and the volume was filled until the label, the solution was filtrated using Whatman No.42, 10 ml from the filtrate was then transferred to the digestive

bottle of microcledal apparatus(Micro-Kjeldahl), 10 ml of concentrated sulfuric acid  $H_2SO_4$  along with digestive powder composed of selenium oxide, blue cupper sulfide, and Anhydrous potassium sulfate ratio 1,2,25% respectively were added until the digestion and distillation were finished (Joslyn 1970

### **3.12.5.7 Acid value (AV)**

Acid degree value was determined according to Bureau of Dairy Industry(BDI) which is verified by (Deeth 1976), 5 gm of cheese sample were crushed in 37.5 ml of sodium citrate solution at 50 °C, 10 ml of BDI composed of (30 gm of triton 100\* and 70 gm of Sodium hexameta phosphate) was added to the mix and heated until the boiling degree for 20 minutes with the continues stirring. They were then centrifuged at 2000 Circle / minute, aqueous alcohol methyl (50%) was added to the filtrate and re-centrifuged for one minute. 0.2-0.4 gm of the fat that was separated by the centrifugal force was weighed and put in a conical flask, 5 ml of the mix prepared instantaneously composed of hexane and propanol ratio (1:4) was added to the fat respectively, the mix modification with alkaline KOH 0.02 N, phenol naphthalene was added The reference sample was modified with 5 ml of free fat mix. AV value was evaluated according to the following equation:

Volume of the consumed alkaline with the sample-the volume consumed with the blank\* calibrated alkaline / weight of the fat \*100.

### 3.12.5.8 Peroxide Value ( PV ) :

The fat was extracted according to the method mentioned by AI Rowaily (2008) with some modifications by homogenizing the cheese with a solvent mixture (chloroform and methanol) in a ratio of 2: 1 (v/v) on a magnetic mixture to homogenize the sample and after melting the cheese in the solvent mixture, five drops were added. NaCl solution at a concentration of 3% with continuous mixing to separate the fat, filter, and then dry it from the solvent by placing it in an oven at a temperature of 50 ° C for 4 to 5 hours. The value of peroxide was estimated according to the AOAC (1990) method, which is summarized in homogenizing 5 grams of fat or extracted by cold method with 30 ml of a cold solvent mixture of acetic acid-chloroform at a ratio of (2: 3

volume/volume) on a magnetic mixture to homogenize the sample. Dissolve the fat sample in the solvent mixture, 0.5 ml kl was add of saturated potassium iodide solution, add 30 ml of distilled water, and mix the mixture well; the formation of two layers was observed, as the top layer appeared yellow, the organic bottom was white. The samples were left for 5-10 minutes at room temperature, and the mixture was blown with a 0.01 N sodium thiosulfate solution and, during the correction process mixing 0.5 ml of the starch index (1%) until it disappeared. The purple color in the upper layer, the PV peroxide value was calculated according to the following equation:

$$P.V = \frac{The \ volume \ of \ sodium \ thiosulfate \ (ml) \ x \ 0.01}{Sample \ weight} \times 1000$$

#### 3.12.6 Microbiological analysis of Mozzarella cheese

The procedure followed by balaky 2016 was applied in this study

#### 3.12.6.1 Total plate count

Pour plate was used in this study with nutrient agar and incubation at 32 °C for 24-48 hours, the colonies formatted were evaluated and the average value for two plates was accounted, the number of colonies was multiplied with the dilution inverse, similar approach was adopted in evaluating the number of bacteria in the next experiments, the reference sample was taken in the account.

#### 3.12.6.2 Total coliform count

Total coliform was evaluated using MacConkey agar, the plates were incubated at 32 °C for 24-48 hours.

### 3.12.6.3 Total Staphylococcus aureus

*Staphylococcus* was estimated using Mannitol salt agar and incubated at 32 °C for 48 hours, the colonies surrounded by a yellow circle represented *Staphylococcus .aureus* were evaluated.

### 3.12.6.4 Proteolytic bacteri count

The preparation of this group was estimated using the method mentioned in Harrigan and McCance (1976) and by using the nutritional media Milk agar consisting of (100 cm<sup>3</sup> of Nutrient agar + 10% screening milk) and the dishes were incubated at a temperature of 32 °C for 2-5 days and after the incubation period was added 1% of hydrochloric acid for a period of one minute and the colonies surrounded by the clear zones were counted as an indication of the presence of proteolytic bacteria.

### 3.12.6.5 Lipolytic bacteria count

The method mentioned by Harrigan and McCence (1976) was followed by using Nutrient agar with 1% pepten water added. The plates were incubated at 32°C for 2-5 days and after the incubation period, an amount of 20% solution of 55 Copper Sulphate was added so that it covered the surface of the food media and left for 5 minutes. Then copper sulfate was removed by distilled water, and the colonies that were colored in bluish green color were counted using a colony counter.

### 3.12.6.6 Detection of Salmonella bacteria

Detection of *Salmonella bacteria* according to the method mentioned in (APHA) 1978 using S.S agar medium and incubating at 32°C for 24-48 hours.

### 3.12.6.7 Mold and yeast

The Number of Mold yeast was estimated according to Abdulmalek and abdulaziz ,2011 using Sabouraud dextrose agar and incubated at 21 C for 5 days.

### **3.12.7 Sensory evaluation**

Sensory evaluations of cheese models for the two treatments of the packaged cheese and the comparison model were conducted by five of assessors with experience in this field in the dairy factory and from the faculty of Agriculture, according to the special evaluation form used by Aziz (1983), with some appropriate modifications (Color , Taste and flavor , texture , Appearance , bitterness , Exotic flavors).

Samples	Storage period ( days )	Color 5 degrees	Taste and flavor 30 degrees	texture 15 degrees	Appear ance 10 degrees	bitter ness 20	Exotic flavors 20 degree s	The final grade
(control model)								
envelope d model								
umouor								

Table 3.1. Sensory evaluation form for soft cheese

### 3.12.8 Statistical Analysis:

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means in this study.

## **Chapter 4**

### **Results and discussion**

#### 4-1: Preparation of green tea extract

Green tea leaves extract was obtained by using four concentrations of ethyl alcohol (0, 25, 50 and 75%) Ethyl alcohol a good solvent for phenolic compounds extraction.the percentages of the extract obtained were 18.6, 21.33, 24 and 25.14 g/ 100 g, table (4-1) for the previous four concentrations respectively There were significant differences (P<0.05) noticed between the control and all other samples of green tea extract obtained by different concentrations of ethyl alcohol .but there was not significant (P>0.05). differences found between 50% alcoholic extract and 75% of alcoholic extract.The high percentage of alcoholic solution extraction compared with the water extraction at a concentration of 0% of alcohol was attributed to the quality and quantity of phenolic compounds that soluble in alcohol was higher than the compounds soluble in water as mentioned by (Ali. *et al*, 2016).

Table (4-1) Percentages of the types of extracts related to the dry matter of green tea leaves

Types of treatments	Extraction ratio(g/ 100 g)		
100 % aqueous extract	18.6 ±1.47 °		
(Control)			
25 % alcohol extract	21.33 ±2.63 <sup>b</sup>		
50% alcohol extract	24.00 ±2.91 ª		
75% alcohol extract	25.14 ±2.05 ª		

Means in the same column not bearing similar superscript letters are significantly different (P < 0.05).

# **4-2:** Measurement of the inhibitory activity of green tea extracts on *Esherichia bacteria*

Table (4-2) shows the inhibitory effect of green tea extracts(GTE)by ratio extracts of water to alcoholic on microorganisms, which include (100% aqueous solution and 25% alcoholic solution, 50% and 75%), *E.coli* bacteria were used to the test. Because it is pathogenic and usually found in food, especially in unpasteurized dairy products (SPJ, 2013), the results of the Gram-negative test showed that (*E.coli*) inhibit the growth with all (GTE) concentrations Fig. (4-1).

This result was due to the presence of phenolic compounds, (Lixue Dou, *et al*, 2018) specifically catechins, which was found in the extract. The most important compounds that have activity against *E.coli* and some grampositive bacteria was Epigallocatechin Gallate (EGCG), which its role to inhibit the function of the cytoplasmic casing of bacteria by stimulating the transfer of molecules. The increase in the diameter of the areas for the alcoholic extract compared with the aqueous extract (control) might be due to the higher concentration of the (EGCG) compound in the alcoholic extraction than in the aqueous extraction, which was due to the prominent role in the event as coated by Noormandi and Dabaghzadeh, (2015).

The same Table showed significant differences noticed between the Control and all other samples of green tea extract obtained, the diameters of the inhibition area for each of the alcoholic extracts (75% and 50%), were 24.6 and 24.2 mm, respectively. There was no significant difference found between them , the reason for this was due to the quality and quantity of the compounds present in the alcoholic extract compared to the water extract ,(Ali zadeh and Mohebalian, 2016).

From these results, the alcoholic extract with a concentration of (50%) was chosen as the most appropriate solution due to its anti-microbial activity compared to the rest of the concentrations and because the colour of the resulting extract was acceptable after adding it to the coating films used in cheese packaging.

Types of green tea extracts	The diameter of the area formed to inhibit the growth of <i>E.coli</i> bacteria (mm)
100 % aqueous extract(Control)	11.5 ±0.61 °
25 % alcohol extract	22.8 ±1.66 <sup>b</sup>
50% alcohol extract	24.2 ±2.72 ª
75% alcohol extract	24.6 ±2.81 <sup>a</sup>

Table (4-2) Diameters of inhibition zones Samples of extracts on E.coli.

Means in the same column not bearing similar superscript letters are significantly different (P < 0.05).



D

Figure (4–1) Antimicrobial activity of the ethanolic extract of green tea at different extracted ratio A. (25:75 E: W), B. (50:50 E: W), C. (75:25 E: W). D (0:100 E: W) against *E.coli* 

#### 4-3: Determination of the minimum inhibition concentration

This test was used for the casings of isolated whey proteins to which the alcoholic green tea extract was added (50%) and at a concentration of 1, 2, 3, 4 and 5% of the volume of the casing solution, where its effect on inhibiting the growth of a group of bacteria, some gram-positive and other gram-negative, were used in addition to yeast.

The minimum inhibition concentration is listed in table (4-3). addition of green tea extract to whey protein casings inhibited the growth of grampositive and gram-negative bacteria, the reason for the inhibition was attributed to the phenolic compounds in the extract. Ali. et al, (2016) mentioned that tea catechins, also known as flavonols, were a group of polyphenols predominant in green tea leaves that have the antimicrobial activity of aqueous and alcoholic tea extracts, which include Epicatechin (EC) Epicatechin gallate (ECG), Epigallocatechin (EGC), Epigallocatechin gallate (EGCG) It was shown that epigallocatechin gallate interferes with the bacterial outer casing. From the results, it was found that green tea extract at a concentration of 3% was the minimum concentration to inhibit the growth of all bacteria, the diameter of the inhibition zone for E.coli, Salmonella spp. , Bacillus spp, and Candida albicans yeast was . 10.3, 10.7, 16.6 and 176 mm, respectively, and the addition of the extract at a concentration of 4% led to an increase in the diameter of the visible area (17.8 mm, 16.1 mmnand 19.3 mm) and 18.5 mm) respectively, the addition of the extract at a concentration of 5% also led to an increase the diameter of the visible area were (21.8 mm, 20.7 mm and 22.5 mm and 22.1 mm) respectively. for 2% concentration, the inhibition zone was (5.1 mm, 9.6 mm, 14.9 mm, 15.2 mm) respectively, and when using a concentration of 1%, The inhibition zone appeared only on Bacillus sp bacteria, reaching 13.5 mm, the results were disagreed with the Radji et al, (2013) which was higher than mentioned that the diameters of the inhibition area of *Bacillus sp* and at a concentration of 4, 3% for green tea extract added to isolated casings soybean proteins reached 6.8 mm for both of them, while they showed no inhibition for E-coli and Salmonella, while the concentrations of 2.1% showed no inhibition for all types of bacteria. This was due to the quality and quantity of the phenolic compounds present in the alcoholic extract of green tea.

The inhibition activity of green tea extract belonged to its effect on the functions of the bacterial cell wall, the cytoplasmic membrane and the difference in the sensitivity of the cell wall structure, the outer membrane was response to control the movement of substances which absorbed and excreted outside the cell, therefore any, effect on membrane function will leads to an imbalance in the entry of nutrients into the cell and thus inhibiting bacteria growth this was the base to determine the extract effectiveness in inhibiting bacterial growth (Amankwaah, 2013).

Table (4-3) Effect of addition different concentrations of green tea extract to casings prepared from whey protein isolate on growth inhibiting of different microorganisms

species of bacteria	Diameter transparent zone (mm)				
	1%	2%	3%	4%	5%
Escherichia coli	$0.0 \pm 0.0$	5.1 ±0.29	10.3	17.8	21.8
	d	С	$\pm 0.73$ <sup>b</sup>	±1.28 ª	$\pm 1.70$ <sup>a</sup>
Salmonella spp.	$0.0 \pm 0.0$	9.6 ±0.54	10.7	16.1	20.7
	С	b	$\pm 0.80$ <sup>b</sup>	±1.17 ª	±1.69 ª
Bacillus spp	13.5	14.9 ±0.97	16.6	19.3	22.5
	±0.57 °	С	$\pm 1.02$ <sup>bc</sup>	$\pm 1.75$ <sup>ab</sup>	±1.92 ª
Candida albicans	$0.0 \pm 0.0$	$15.2 \pm 0.85$	17.6	18.5	22.1
	С	b	$\pm 0.97$ <sup>b</sup>	$\pm 2.02$ <sup>ab</sup>	±2.47 ª

Means in the same column not bearing similar superscript letters are significantly different (P< 0.05).



Figure (4-2) inhibiting the growth of microorganisms isolated whey protein casings containing alcoholic extract of green tea

### 4-4: Measuring the antioxidant activity of casings

The results of evaluating the antioxidant activity by the DPPH method Table (4-4) show that whey protein coatings without GTE showed an antioxidant activity of 21.6%, where Marquez *et al*, (2017) indicated that the antioxidant activity of whey proteins was due to each of Alpha-lactalbumin. and Beta-lactoglobulin . The antioxidant activity of the casings of whey proteins was 15%, and this activity was significantly increased from 21.6% to 55.23% as a result of addition of green tea extract with concentration of 2% to the casings of isolates of whey proteins. Table (4-4) shows, that increasing the concentration of green tea to 3% increased the antioxidant activity to 69.78%, this was consistent with Barzegaran *et al*. (2014) who reported that addition of GTE at concentrations of 2.5 - 20% increased the antioxidant activity of polyamide casings by 67.2-88.75%, and this was also in agreement with that

of Mangmee and Homthawornchoo,(2016). The latter researchers reported that addition of GTE to the casings of rice starch mixed with chitosan increased the inhibition of free radicals. The antioxidant activity of the casings increased with the increase of GTE concentration (Table 4.4). The Effectiveness of GTE as antioxidant is better than BHA.

The antioxidant activity was due to the green tea's content of phenolic compounds, especially the main ones such as (EC), (EGC), (EGCG), (GCG), (CG), (CG), (CG), (CG), (CG), (CG), (C) in addition to what it contains. From carotenoids, tocopherols, vitamin C and minerals such as chromium, manganese, selenium and zinc, phenolic compounds inhibit free radicals based on the electron-donating groups and thus prevent the formation of the radical chain and also because of the association with transitional ion catalysts and then forming bonds with free radicals by interacting with them to get inhibition of the oxidation process fats (Mangmee and Homthawornchoo, 2016).

casings type	Antioxidant efficacy
whey protein isolate casing	21.60 ±1.52 <sup>d</sup>
control model))	
Prepared casing 2% + Tea Extract	55.2 ±3.09 <sup>b</sup>
<b>Prepared casing 3% + Tea Extract</b>	69.78 ±5.87 <sup>a</sup>
BHA 0.02%	30.24 ±2.78 °

Table (4-4) Antioxidants activity for different samples of proteins casings

Means in the same column not bearing similar superscript letters are significantly different (P < 0.05).

### 4-5: Infrared Spectroscopic Analysis by FTIR

The use of the FTIR device to identify the active groups of whey proteins casings supported by green tea extract as an active substance. The readings appeared in the formed of clear peaks was shown in Figure (4-3) inhibiting the growth of microorganisms isolated whey protein casings containing extract of green tea and started from 3425.58 - 3024.38, which was due to the bonds of OH groups and free NH groups that form the active groups In the casings, then the frequencies 2704.20 - 2947.23 which were due to the CH groups, then the frequencies from 2400 - 1658.78, which were due to the vibrating groups of CO and CN and were likely to be of the first amide (Amide I), and the frequencies 1550.77 - 1446.61 which were due to the groups NH, which was likely to belong to a second amide (AmideII), and the frequencies from 1346.31 - 1226.7 were due to the NH groups and were likely to be a third (Amide III) 1188.15 - 817.82 This region represents the oscillation and curvature of the bonds of CC groups. It belongs to glycerol. As for the rest of the less than 400 were not essential, and no organic compound was free of them (Ramos *et al*, 2013).

### 4-6: Physical Tests of Casings

Anand. *et al.* (2018) reported that the physical properties of all edible casings were essential because they determine how the film behaves during handling and storage also they mentioned that they were assisted in the durability and efficiency of films and their ability to enhance food safety.

Table (4-5) shows that the thickness of the casing was one of the first physical properties that were performed on the casing at 3% concentration of green tea extract, It appeared that when the extract was added to the casing, the thickness of the casing was significantly decreased, where the control casing without green tea extract was 183  $\mu$ m, but after adding the extract it was 170.2  $\mu$ m, and this was agreed with what was stated by Zinoviadou *et al*, (2009), who mentioned that the thickness of the whey protein shells was 179  $\mu$ m, and when marjoram oil was added to the casings, the thickness of the casing decreased to 168.7 m $\mu$ . It was possible that the reason for the decrease in thickness was addition to an increase in the formation of bonds between the tea extract and the proteins, which made the tea compounds replaced the protein molecules



Figure (4-3) The peaks of the active groups shown by the infrared spectrophotometer analyzer of the FTIR device for the gelatinous sheaths supported by the alcoholic extract of green tea

Frederico *et al.* (2019). It was known that the thickness of the casinge has a relationship with the quality of transparency; the less the thickness, the higher the transparency .

The tensile strength estimation, was presented in Table (4-5) when the casing was reinforced with tea extract by 3%, the tensile strength of the casing decreased to 8.90 MPa composed with the control model 9.32 MPa,with insignificant differences and This results close was to both Galus and Kadzińska, (2016) who showed that the tensile strength of whey protein films was 7.1 MPa and (Javanmard and Golestan, (2008), mentioned that the tensile strength of whey protein films decreased when olive oil was added with different concentrations, where it was started at 1.944 MPa and decreased to 0.904 MPa.

Concerning the elongation rate, it reached 121% in the casing reinforced with tea extract, with significant differences which was higher than the elongation rate of the whey protein- casing without addition, which was 109%. It was reported that the research Yoshida and Antunes, (2004) concluded that the elongation rate of the whey protein casings was 70%. The reason for the decrease in the tensile strength and the increase in stretching in the films reinforced with green tea extract compared to the non-reinforced film's models it was one to the decrease in the cross-links that occured between the multiple polymer chains and within the chains themselves due to the presence of tea compounds, which hinder the formation of bonds The cross-sectional ones made the chains more unconstrained and non-limited movement on the vertical and horizontal axes and along with the polymer. Contrary to the control models as stated by Vachiraya and Patcharin, (2016), Nasreddine et al. (2018) reperted that many factors were affecting the Physical properties of the films, the most important of which were the temperature and pH of the casing solutions.

Table (4-5) The Physical properties (thickness, tensile strength and elongation ratio) of green tea extract casing samples

types of casings	casings thickness (micrometre)	Tensile strength (Mpa)	elongation	
control casing	183.0 ±11.42 ª	9.32 ±0.72 <sup>a</sup>	109 ±6.74 <sup>b</sup>	
(without				
extractor)				
casing + 3%	170.2 ±8.55 <sup>b</sup>	8.90 ±0.65 <sup>b</sup>	121 ±8.27 ª	
extract				

Means in the same column not bearing similar superscript letters are significantly different (P < 0.05).

### **4-7:** The permeability of the casings to oxygen

The oxygen permeability of casings was one of the most important characteristics as it protects oxygen permeability to and from the food content (Maria.et al, 2017). The results presented in Table (4-6) shows that addition of green tea extract at the appropriate concentration 3% to the casings led to a significant decrease in the permeability of the casings to oxygen when compared with the control casing, As the permeability values of the casing sample was 92.02 (ml/m2\*day) when the permeability value of the casing sample reinforced with the extract was 69.98 (ml / m 2 \* day). These result was near to what was indicated by Fabra et al. (2013), where the permeability values of the casings of whey proteins ranged 76.1 (ml / m 2 \* day) The reason for the decrease in permeability might be due to the change in the crystalline shape of the polymer, while the presence of amorphous regions in the polymer network made the gases diffused quickly across the casings, so it was a reason for the increase in permeability, and when adding the extract of green tea led to an increase in the crystalline state of the films, which gave them a greater possibility of gas sequestration. This had an influential role in The effectiveness of antioxidant factors, as their role was limited due to the high cohesion of the casing bonds and the limited movement of them, which reduced the movement of antioxidants. However, the indirect role in reducing

was through the amount of oxygen passing through and limiting oxidation processes. The relative humidity also has an influential role in The effectiveness of antioxidants, as low humidity reduces the oxygen permeability across the casings, thus reducing the process of lipid oxidation, and here becomes the active role of the casing against oxidation in reducing oxygen permeability (Arantzazu *et al.*, 2017).

Table (4-6) oxygen permeability through casing treatment with 3% concentration of green tea extract

types of casings	casing permeability (ml/m2*day)
control casing (without extract)	92.02 ±4.84 ª
casing + 3% extract	69.98 ±3.07 <sup>b</sup>

Means in the same column not bearing similar superscript letters are significantly different (P < 0.05).

### **4-8:** Green tea casing permeability to water vapor

The process of measuring the water vapor permeability of the green tea casings was carried out due to its importance in food packaging processes and its impact in determining the storage life because it changes the sensory properties of food products in addition to creating a suitable environment for microorganisms, and because the enzymes that lead to food deterioration work in a watery medium and that the process of forming casings, temperature and humidity Ambient relativity as an effect on casing permeability (Gurdian, 2015).

The permeability of casings control model was 0.65 (g.mm./hr.m.mpa), while the permeability value of the casing sample reinforced with the extract was 0.26 (g.mm./hr.m.2.kPa), as shown in Table (4-7), and This result green tea was less than what was obtamed by Hammann and Schmid, (2014), where the permeability value ranged between 3.1-15 (g.mm./hr.m2.mPa), and the addition of green tea extract improved the casing's retaining properties towards water vapour. It was possible that the reason for This decrease in the water vapour permeability value of the compact internal structure of the casings of whey proteins after its interaction with green tea extract, which reduced the transmission of water vapor across these protein casings Hammann and Schmid. (2014).

Table (4-7) water vapor permeability through casing treatment 3%
concentration of green tea extract

types of casings	casings permeability to water vapor (g.mm./h.m².kPa)
control casing (without extractor)	$0.65$ ±0.27 $^{a}$
casing + 3% green tea extract	$0.26 \pm 0.09$ <sup>b</sup>

Means in the same column not bearing similar superscript letters are significantly different (P< 0.05).

### 4-9: The solubility of the green tea extract casings in water

The results mentioned in Table (4-8), showed that the solubility of the casings in water, was increased significantly when the extract was added to the casing, where the solubility of the casings of whey proteins (control model) reached 42.5%, while the solubility of the casings to which the alcoholic extract of green tea was added with 3% concentration reached was 50%. These results were in great agreement with those reached by Galus and Kadzińska. (2016), where they mentioned that the solubility values for protein casing models amounted to 42.4% and this Value increased with the addition of the fortified materials and This characteristic was due to what proteins possess Whey has the ability to bind with chemical compounds, and therefore it forms soft structural structures that increased the solubility of the casings in water (Kodad et al, 2014) and it was consistent with what was found by Al-Jaruri (2014) where he showed that the solubility of whey proteins casings increases with the increase in the percentage of glycerol Added to casingforming solutions, and the addition of plasticizer reduces the strength of the interaction between the protein chains, so adjacent chains separate, their movement increases, and the casings cohesion decreases, and their bond and solubility increases with water molecules before (Galus and Kadzińska, 2016).

types of casings	solubility of casings in water
control casing	42.5± 2.36 <sup>b</sup>
casing + 3% green tea extract	50.0 ±2.87 ª

Table (4-8) the solubility of green tea extract casing in water

Means in the same column not bearing similar superscript letters are significantly different (P < 0.05).

### 4-10: Casing optical properties:

The optical properties have a role in controlling the amount of light passing through the casing, and that the amount of light passing through the casing was considered an influence for the photo-oxidation processes that occur in food, as the opacity of the casings expresses the amount of light absorbed by the casings and does not pass through them. The colour of the casing within the system was expressed in scales  $a^*$ ,  $b^*$ ,  $L^*$ , as each of them has a specific colour connotation. for example,  $a^*$ ,  $b^*$  express the extent of colour consistency. In the case of deviation of its value towards - then This indicates the direction of the colour towards green, as for  $b^*$ , the deviation of its value towards + indicates the direction of the colour towards blue, and for L\* it determines the intensity of illumination and Its decrease indicates the dark or opaque colour. However, its increase indicates the light colour and thus determines the colour of the casings, which results in the degree of consumer acceptance of food.

The result of the examination of the casings of isolated whey proteins showed that there was an increase in opacity when green tea extract was added to it, and its colour changed to a yellowish-green colour, which gives it better colour characteristics than the control casings that were transparent because they reduce the photo-oxidation processes, where the value of E for the casing samples reached

casings	*L	B*	<b>A</b> *	ΕΔ	opacity	Gloss
control casing	59.15	6.6	-9.27	%9.6	-18.7	25
casing + 3%	51.01	28.3	-7.41	%29.09	-194.3	8.49

Table (4-9) values of optical and chromatic properties of casings samples.

### 4-11: Green tea extract casing used for mozzarella cheese packaging

# **4-11-1:** The chemical composition of the milk used in the manufacture of mozzarella cheese

Table (4-10) shows The chemical composition of raw cow milk moisture, fat, protein, total solids, and non-fatty solids used in the manufacture of mozzarella cheese, 87.41, 3.53, 3.37, 12.59, and 9.06%, respectively. The pH and acidity were calculated on the basis of lactic acid, and the specific weight of whole milk was 6.65, 0.16 and 1.032, respectively. These findings were within the normal limits of raw milk and were closed to what was found by Al-Sharaji (2002) and Doosh (2007) they found that the ratio of moisture, fat, protein, pH, and lactic acid were, 87.17, 3.50, 3.45, 6.7, 0.18, respectively.

Table 4-10: Chemical composition of raw milk used in the manufacture of mozzarella cheese

Ingredients	full-fat milk %		
moisture content	87.41		
Fat content	3.53		
Protein content	3.37		
total solids	12.59		
non-fat solids	9.06		
pH	6.65		
Total Acidity (as lactic acid)	0.16		
Specific weight	1.032		

# **4-11-2:** Chemical analysis of mozzarella cheese packaged with green tea extract casings during storage period

Table (4-11) shows the chemical composition of the unwrapped mozzarella cheese, the control treatment (control sample) and the cheese samples coated with whey protein casings fortified with green tea extract at a concentration of 3% (enveloped sample) after a day of processing and during storage at a temperature of  $(5\pm1)$  °C. 90 days.

### **4-11-2-1:** Moisture content test

Table (4-11) shows for the moisture content of unwrapped mozzarella cheese samples treated as control sample and cheese samples coated with whey protein films fortified with green tea extract at a concentration of 3% enveloped sample indicated a decrease with significantly different (P < 0.05). in the moisture percentage values for all samples with the progression of the storage period of 90 days as a result of the loss of water vapor during storage, as the moisture percentage on the first day was 53.26 and 53.30% for each of the control sample and enveloped sample treatments, respectively, and This percentage was higher than what Al-Azzawi (2018) found for mozzarella cheese, where the moisture percentage was 47.79%, as well as It was higher with what was found by (Bi et al., 2016) for mozzarella cheese, which amounted to 47.74%, and close to what was found by Venus and Santosh (2019) in Sudanese soft cheese, where the percentage of moisture reached 55.69%, and these differences depended on the method of manufacture and temperature The acidity used and the moisture content of the resulting cheese, and This result was in conformity with the Iraqi standard specification for soft cheese (1988), which indicates that the moisture content of soft cheese was not less than 50%.

As for storage, a gradual decrease in the percentage of moisture was observed after the passage of 30, 60 and 90 days, where the percentages of moisture reached 52.12, 51.04 and 47.00% for control sample respectively, while for enveloped sample they were 52.78, 52.00 and 51.06%, respectively, and it was noted that there were significant differences between the moisture loss rates of cheese samples, and that the reason for these large differences between the control and the treated sample was due to the composition of the whey protein casings supported by green tea extract,

mozzarella	storage period of cheese (days)	% Components				РН
cheese samples		Moisture	protein	Fat	total acidity	
	1	53.26 ±3.14 <sup>a</sup>	25.30 ±2.05	22.35	$0.27\pm\!\!0.08$	5.75
(control sample)			а	±2.47 <sup>a</sup>	d	$\pm 0.12$ a
	30	52.12 ±3.06 <sup>a</sup>	25.90 ±1.39	22.99	0.58 ±0.bc	5.58 ±0.16
			а	±1.94 <sup>a</sup>		ab
	60	$51.04 \pm 2.17$ <sup>b</sup>	26.01 ±2.42	23.89	$0.66 \pm 0.25$ b	5.20 ±0.15
			b	±2.62 <sup>ab</sup>		bc
	90	47.00 ±2.02 °	26.85 ±1.27	25.12	$0.87 \pm 0.33$ a	5.09 ±0.11
			b	±2.97 °		с
Enveloped sample	1	$53.30 \pm 3.48$ <sup>a</sup>	25.22 ±1.94	22.35	$0.35 \pm 0.09$	5.75 ±0.18
			а	±2.08 <sup>a</sup>	cd	а
	30	52.78 ±3.82 <sup>a</sup>	25.78 ±1.66	22.80	0.43 ±0.12	5.66 ±0.20
			а	±2.56 <sup>a</sup>	bc	ab
	60	52.00 ±3.68 <sup>a</sup>	26.12 ±2.07	23.14	0.58 ±0.18	5.35 ±0.14
			b	±2.82 b	bc	bc
	90	$51.06 \pm 3.08$ <sup>b</sup>	26.23 ±1.38	23.95	$0.63 \pm 0.27$ <sup>b</sup>	5.12
			ab	±2.04 <sup>b</sup>		±0.124 °

Table (4-11): Chemical analysis of mozzarella cheese packaged with green tea extract casings during storage period

Means in the same column not bearing similar superscript letters are significantly different (P< 0.05).

which plays a role in moisture retention and reduces the amount of evaporated water lost from it during storage to an extent that it was efficient in the reservation properties This confirmed what was mentioned by Al-Jaruri (2014) Al-Mudhafra cheese, where the moisture content decreased from 56.5 to 52.5% and also agreed with Ramos *et al.*, (2013) that the decrease in the moisture during the storage period was also consistent with what Al-Badrani found (2017) who noticed a decrease in the moisture of the cheese the moisture content of mozzarella cheese made from cow's milk decreased from 0.5, 1.0, 1.5 and 2.0% at the end of storage at a temperature of (1±5) °C. The results agreed with what was found by Abdel-Moneim *et al.*, (2012), who found that the moisture of mozzarella cheese made from cow's milk decreased from 49 % to 47.8% during the. storage period (90) days.

# **4-11-2-2**: Changes in proteins content of mozzarella cheese packaged with green tea casings during storage period

The protein material was a source of many changes in the sensory characteristics related to the flavour and texture of cheese during the storage period because of this relationship to proteolysis, and the content of protein during storage was affected by the proportions of other components, especially the moisture content (rizwan et al., 2017) shows the results of table (4-11) The percentage of protein in content control sample and enveloped sample during the storage period (90) days, the control sample after one day for manufacturing was 25.30%, this result was identical to what Al-Azzawi (2018) found, which indicated that the percentage of protein in mozzarella cheese made from cow's milk was 25.30% and higher than what was found by Marcello et al.(2020) in mozzarella cheese made from whole cow's milk, which amounted to 18% which was closed to what was found by Khansa. et al., (2015) in Sudanese Mudhafra cheese, which amounted to 22.30%. As for the enveloped sample treatment immediately after processing, it was 25.22% and these percentages were less than the protein levels recorded in Al-Dhafair cheese covered with whey proteins casing mentioned by Al-Jaruri (2014), where the protein percentage reached 30.22%.these differences depended on the method of manufacture and temperature The acidity used and the moisture content of the resulting cheese, The statistical analysis results indicated that there were no significant differences (p<0.05) between the different treatments. It was also noted in Table (4-11) that the protein percentages gradually increased with the progress of the storage period and there was no

insignificant differences notied between the control and the treated sample. This increase in the protein percentage was due to the loss in moisture during storage, which lead to an increase in the percentage of total solids in cheese Including protein. However, the moisture losses in the treatment of coated cheese were less than what it was in the unwrapped cheese, where the percentage of protein at the end of the storage period for control sample and treated enveloped sample was 26.85 and 26.23%, respectively. These results were consistent with the findings of Venus and Santosh (2019), which confirmed that the high protein content during the storage period was due to the decrease in the moisture content of the cheese during storage and the continuous loss of moisture during storage leads to a change in the proportion of other components, including protein, These results consistent with what was found by Al-Badrani (2011) and Al-Azzawi (2018), who noted that the rate of increase in protein percentages during ripening was 3.7, 1.98, 1.89 and 1.91% for unwrapped cheese, wax-coated, casein and gelatin, respectively. Nikjooy et al, (2015), noticed a high increase percentage in protein during the storage period due to a decrease in moisture and an increase in the percentage of total solids associated with a decrease in the moisture content of cheese, from 38.83 to 41.57% during the storage period and this was agreed with what was found by Hakim et al., (2016), who noticed that the percentage of protein in low-fat cheese was free of fat substitutes increased to 26.7% during the storage period.

# 4-11-2-3: Changes in fat content of mozzarella cheese packaged with green tea casings during storage period

Table (4-11) shows the percentage of fat in the cheese of the different control sample and treated enveloped sample mentioned previously, as the percentage of fat one day after manufacturing for the control sample cheese was 22.35%, and this result was closed to what Al-Azzawi (2018) found for mozzarella cheese made from cow's milk, which amounted to 20.7%. It was also closed to what Abdel-Moneim (2012) found for mozzarella cheese, which was 24%, which was nearby the same.

Bi *et al.*, (2016) found that the percentage of fat in the control sample was 23.21%. As for the percentage of fat in the cheese of the enveloped sample treated, it was 22.35%. The statistical analysis results indicated that there were no significant differences (P<0.05) between the control sample and treated nveloped treated cheese. During storage period, an increase in the percentage

of fat there was a significant differences in that contents between the control sample 25.12%, and the treated sample of mozzarella cheese packaged with green tea extract casings during storage period after 90 days which was 23.95%, and This result was also close to what Al-Azzawi (2018) found for control sample mozzarella cheese made from cow's milk, which amounted to 24.50%, which found an increase in the fat percentage of mozzarella cheese during the storage period, due to the decrease in the moisture content in the cheese and the increase in the percentage of total solids, which included the fat, and the results treated sample were consistent with what Al-Badrani found (2017) Who noticed the high percentage of fat for soft, low-fat cheese during the storage period, and the results were also consistent with what was found by 'Abdulhadi (2017), who noticed the high percentage of fat for cheddar cheese during the storage period, and also noted that there were insignificant differences within one treatment between the beginning and end of the period of storage form, This was due to the composition of the whey protein casings supported by green tea extract, which plays a role in retaining moisture and reduced the amount of evaporated water lost from it to an extent that it was efficient in the moisture retention properties, which leaded to an increase in the proportion of fat.

# 4-11-2-4: Changes in pH content of mozzarella cheese packaged with green tea casings during storage period

pH represents the natural acidity and the developed acidity resulting from the fermentation of lactose sugar and its transformation into lactic acid by the action of enzymes from the initiator bacteria. The pH values for control sample and treated enveloped sample cheese were 5.75 a day after manufacturing, The differences were insignificant, This result was higher than what was found by (Bi et al.,2016) for full-fat mozzarella cheese, which was 5.32 and very closed to what was found by (Marcello et al., 2020) for mozzarella cheese. As for the treated enveloped sample, it was also 5.75, which was closed to what was reached by Al-Hadithi (2015), who estimated the pH of the victorious cheese 5.45, as well as its approximation to what Mohammed and his group found (2014) who estimated the pH of the victorious cheese, which ranged between 5.50 -5.45. Statistical analysis indicated that there were no significant differences (P<0.05) in the pH values between the control sample and treated sample after a day of manufacturing. As for storage, a decrease in pH values was observed for control sample and

treated sample, which was after 90 days for cheese from control sample and treated enveloped sample were 5.09 and 5.12, respectively. The reason was due to the low pH of lactose consumption by microorganisms producing lactic acid as well as the acids produced by fat substitutes after partially breaking them down. It was also noted that there were significant differences (P<0.05) within for control sample and treated sample, between the beginning and the end of the storage period in, and This was consistent with what Al-Jaruri (2014) found that the pH values of Al Dhafra cheese had decreased significantly during the storage period for the control and the treated cheese whey proteins were coated with casing proteins. It was also in agreement with Gonçalves et al., (2018) that a decrease in pH values was found in mozzarella cheese during the storage period.

# 4-11-2-5: Changes in Total Acidity content of mozzarella cheese packaged with green tea casings during storage period:

The results were shown in Table (4-11) indicated the values of acidity (calculated on the basis of lactic acid) and expressed the natural acidity and the developed acidity of the cheese of the control sample and treated enveloped sample. The control (0.27% and then significantly increased until it reached 0.87% within 90 days of storage, as for the enveloped sample treatment, the acidity percentage was 0.35% at the beginning and reached 0.63% at the end of storage, significant differences (p<0.05) were noted, and these results agreed with the findings of Al-Jarouri (2014) and Al-Azzawi (2018) and (E. Tirloni *et al.*, 2019) that the acidity percentage of the coated and non-encapsulated triumphant cheese samples rised during the storage period. Al-Jaruri (2014) showed that the acidity of the unwrapped cheese increased by increasing the storage period, as it was higher than the wrapped cheese's acidity, which reflected the superiority of the packaged treatment over that of unwrapped cheese.

### 4-11-2-6: Acid Value (AV)

Acid value expresses the extent of short-chain fatty acids in cheese, the extent of lipolysis and the occurrence of rancidity (it represents the number of gram equivalents of alcoholic potassium hydroxide needed to neutralize the liberated fatty acids in 100 g of fat (Sharma. *et al.*, 2017).

The results were shown in table (4-12) indicate an increase in AV values with the progression of the storage age of the cheese. It was noted that the AV

values of the control sample increased from 0.13-2 (mEq/100 gm fat). As for the treated enveloped sample, the values were within the range between 0.13-1.1 (mEq/100 g fat), and when referring to the Bureau of Dairy Industry (BDI) method gradient for accepting or rejecting cheese as reported by Deeth and Fitz-Gerald (2006) which states that cheese was considered acceptable when the AV values were less than 2.0 mmcaffe/100gm of fat. Therefore, That the cheese of the control sample had been rejected after 90 days storage as far as lipolysis was concerned (doosh et al., 2012). This increase in the amount of AV might be due to the lipolysis processes caused by the microorganisms of cold-loving bacteria that grow and multiply at refrigerator temperature. As long as the cheese model was subjected to heat treatment during processing, the enzyme lipoprotein lipase had been inhibited by heat because it was one of the enzymes previously proven to be inhibited by pasteurization temperature (doosh et al., 2012). Also (Sekban.h and Tarakci. Z, 2020) found that lipolytic bacteria, especially lactic acid bacteria that tolerate the heat of pasteurization, increase the acidity of the fat, in addition to the negative impact of the metabolic materials produced by the contaminated bacteria that cause enzymatic decomposition of fat, which has the effect of increasing the acidity of the fat. Fat (Oktay Yerlikaya et al., 2020), as mentioned by Sarhan (2018), an increase in the percentages of AV in monterey cheese due to the activity of lipolytic enzymes produced from the prefixes used led to lipolysis to give free fatty acids.

mozzarella cheese samples	storage period of cheese (days)	Acid Value (mEq/100g fat)
	1	0.13 ±0.05 °
control comple	30	$0.16 \pm 0.05$ <sup>c</sup>
control sample	60	$0.98 \pm 0.22$ <sup>b</sup>
	90	2.0 ±0.39 <sup>a</sup>
	1	0.13 ±0.05 °
enveloped sample	30	$0.23 \pm 0.07$ <sup>c</sup>
	60	0.85 ±0.17 <sup>b</sup>
	90	1.1 ±0.30 b

Table (4-12) Determination of acid value (AV) of mozzarella cheese packaged with green tea extract casings during storage period

Means in the same column not bearing similar superscript letters are significantly different (P < 0.05).

### 4-11-2-7: Peroxide Value PV

Oxidation of fats was one of the most important causes of spoilage of foods rich in unsaturated fatty acids, which lead to the emergence off flavour and food spoilage. The results shown in table (4-13) show the (PV) values after one day of storage for control sample were 4, and treated sample 3.6, with significant differences (p<0.05) between the control and the treated cheese due to the characteristics of the whey protein casings reinforced with green tea. It was of medium permeability to water vapour because it was one of the hydrophilic proteins, while it was a good barrier for oxygen, which leads to reducing oxidation in foods (Gulcin *et al.*, 2016). It was noted from the table that the value of the peroxide for the treatments increased, as it reached at the end of the storage period 10 and 7 mEqO<sub>2</sub> for the control sample and treated enveloped sample respectively, with significant differences between unwrapped and packaged cheese throughout the storage period, where the treated enveloped sample was within the acceptable limits mentioned by the standard specification for soft cheese, which states that it

does not exceed 10 mEq/kg cheese, (doosh *et al.*, 2012) This increase was attributed to the reason for the ability of whey proteins fortified with green tea to sequester oxygen and thus increase its ability to protect against lipid oxidation and the influence of food and storage moisture on the casings ability to permeate ductile oxygen so stated by Al-Jarouri (2014). The results agreed with Lagos.(2013), as it was found that the peroxide values of meat covered with chitosan film with essential oils were lower than meat that was not covered and that the values of peroxide increased in conditions of 80% humidity more than samples at 45% humidity. The reason for This was the high humidity, which affects on the ability of the casing to reserve oxygen.

Table (4-13) Peroxide Value (PV) of mozzarella cheese packaged with green tea extract casings during storage period

mozzarella cheese	storage period of	Peroxide value (mEq
samples	cheese (days)	<b>U</b> <sub>2</sub> / <b>kg</b> )
	1	4 ±0.35 °
control comple	30	$5.4 \pm 0.51$ bc
control sample	60	7.1 ±0.52 b
	90	$10 \pm 0.78$ <sup>a</sup>
	1	3.6 ±0.27 °
enveloped sample	30	4.8 ±0.44 °
	60	$6.5 \pm 0.71$ <sup>b</sup>
	90	7.0 ±0.75 <sup>b</sup>

Means in the same column not bearing similar superscript letters are significantly different (P< 0.05).

### 4-11-2-8: Nitrogen content

The nitrogenous substance was one of the main components that enter into the composition of dairy products in general, and the changes that take place in the protein lead to cheese gaining most of its desirable sensory qualities such as taste, flavour and many of the characteristics of texture and cohesion. Barbano (2000) found that the percentage of nitrogenous compounds disolved in water soluble nitrogen (SN) and non-protein nitrogen (NPN) were the best guides used to monitor proteolysis during cheese storage.

### 4-11-2-9: Change in the cheese content of soluble nitrogen (SN)

It was clear from the results in Table (4-14) that there were no significant differences in the values of soluble nitrogen between the cheese of control sample and treated enveloped sample after one day of storage, as the control sample was 0.064 %, while the percentage of soluble nitrogen for the enveloped sample was 0.066%. It was noted from the same table that the percentages of soluble nitrogen increased for all subsequent storage periods with insignificant differences between control sample and treated sample, as after 90 days for control sample and treated sample they were 0.095 and 0.097%, respectively. This was consistent with what was found by Marcello et al, (2020), where they showed that by increasing the storage period, the levels of soluble nitrogen significantly increased. The reason for this was due to the action of proteins enzymes produced by psychrophillic bacteria, which were characterized by their resistance to pasteurization treatment and even sterilization treatment unlike to the bacteria which produced it in addition to some of spires and heat resistant microorganisms that can grow and spoilage of the cheese during storage, also due to the remnant of coagulated proteins which used in cheese making, (Fox and McSweeney ,2013)

### 4-11-2-10: Soluble nitrogen/total nitrogen

The percentage of soluble nitrogen to total nitrogen was one of the criteria adopted to determine the effect of the type of rennet enzymes on cheese quality, especially during storage and ripening. It was noteworthy that the aim of estimating This ratio was to identify the effectiveness of the residual coagulant enzyme proteases in protein analysis and its effect on the quality of the cheese during storage and the associated changes in the cheese industry, as Ling (2008) indicated an increase in the percentage of soluble nitrogen

during a period. Cheese stored as a result of proteolysis, which was often equivalent to two-thirds of the total protein.

It was noticed from the results in Table (4-14) that there was an increase in the percentage of soluble nitrogen to the total nitrogen of unwrapped mozzarella cheese (control sample) and also mozzarella cheese coated with whey proteins casings reinforced with green tea extract (enveloped sample), That there were significant differences at the level of 0.05 during The storage period over 90 days, where the percentages during the storage period for the first control sample ranged from 1.64% - 2.31%. As for the enveloped sample, the percentages ranged between 1.69% - 2.39%. These results were consistent with what Al-Azzawi (2018) found, where she analyzed the reason for This high percentage with storage lead to an increase in proteolysis, resulting from the action of the remaining rennet enzymes on one hand and the activity of some cold-loving microorganisms on the other hand. It also agreed with Gonçalves et al.(2018). Al-Dahan (1983) stated that the proteolysis process increases with the increased in moisture content in food products. It was noted that there were significant differences (p<0.05) within one between the beginning and end of the storage period between control sample and enveloped sample. These results were consistent with what was found by Al-Hadithi (2015), who indicated an increase in the ratio of soluble nitrogen to total nitrogen in Dhafir cheese during the storage period.

Table (4-14) soluble nitrogen ,total nitrogen and soluble nitrogen over total nitrogen of mozzarella cheese wrapped with green tea extract casings during storage period

mozzarella cheese samples	storage period of cheese (days)	% soluble nitrogen	% total nitrogen	(soluble nitrogen /total nitrogen)%
	1	$0.064 \pm 0.02$ <sup>a</sup>	3.90±0.32 ª	1.64±0.20 °
control	30	$0.074 \pm 0.02$ <sup>a</sup>	4.05±0.53 <sup>a</sup>	1.82±0.25 bc
sample	60	0.083±0.03 <sup>a</sup>	4.07±0.43 ª	2.03±0.33 <sup>ab</sup>
	90	0.095±0.03 <sup>a</sup>	4.11±0.29 <sup>a</sup>	2.31±0.28 ª
	1	0.066±0.02 ª	3.9±0.33 ª	1.69±0.18 bc
enveloped	30	$0.075 \pm 0.02$ <sup>a</sup>	$4.04 \pm 0.50$ <sup>a</sup>	$1.85 \pm 0.24$ bc
sample	60	0.086±0.03 ª	4.09±0.42 ª	2.10±0.30 ab
	90	$0.097 \pm 0.03$ <sup>a</sup>	4.20±0.48 <sup>a</sup>	2.39±0.27 <sup>a</sup>

Means in the same column not bearing similar superscript letters are significantly different (P < 0.05).

## 4-11-3 : Results of microbial tests for mozzarella cheese wrapped with green tea extract casings :

Table (4-15): shows the numbers of total E-coli bacteria, lipolytic and proteolytic, staphylococcus and salmonella bacteria, and numbers of yeasts and molds of mozzarella cheese wrapped with green tea extract casings during storage period

# **4-11-3-1:** Total count bacteria between mozzarella cheese wrapped with (GTE) casings during storage period

The results shown in Table (4-15) indicated that the number of treated bacteria (control sample) for unwrapped mozzarella cheese was higher than the number of treated bacteria (enveloped sample) for cheese coated with

whey protein casings reinforced with green tea extract and these differences were persisted between control sample and treated enveloped sample during the storage period until its end, which led to the appearance of signs of spoilage in the control cheese faster than the enveloped sample wrapped cheese model, and it was noted that the bacterial numbers decreased by about 3-1 logarithmic cycle in the enveloped sample treatment compared to the unwrapped cheese, where the total number of bacteria for control sample content cheese on the first day 102 x 3,5 CFU/gm an, It reached  $10^6$  x 5.2 CFU/gm at the end of the storage period, while the treated enveloped sample was on the first day of manufacture  $10^2 \times 2.7$  CFU/gm and reached  $10^3 \times 8.9$ CFU/gm, which was still within the standard specification for soft cheese, it states that cheese was sensually acceptable and edible when the total number of bacteria in it does not exceed  $10^5$  CFU/gm. The higher microbial numbers in control sample than in treated enveloped sample might be due to cheese not being encapsulated and exposed directly to the atmosphere, that it was more susceptible to contamination. (Shima. et al., 2018). The reason for the decrease in total bacterial numbers in enveloped sample treatment was also attributed to the overlapping action of each of the casings of the isolates of whey proteins supported by green tea extract, as it lead to the inhibition of aerobic bacteria by reducing the entry of oxygen, which was essential for the growth of organisms. On the other hand, for the antimicrobial activity of green tea extract, which supported the coating against bacterial species (positive and gram-negative) because they contain phenolic compounds contained in green tea extract, especially catechins, and identification of ECGC, Lorenzo. JM et al. (2016) and Al-Jaruri (2014) reported that the total number of bacteria was decreased in dhafir cheese coated with casings of simple whey proteins enveloped sample and treated with T3 enzyme, formaldehyde T4 and benzoic acid T5 and compared to non-coated cheeses control sample.

Table (4-15): Numbers of total bacteria, *E-coli* bacteria, *lipolytic* and proteolytic bacteria, *staphylococcus*, *salmonella* bacteria, and numbers of yeasts and molds of mozzarella cheese wrapped with green tea extract casings during storage period

mozzare lla cheese samples	storage period of cheese (days)	Total count /CFU gm	<i>E-coli</i> CFU CFU / gm	<i>Lipolytic</i> bacteria CFU / gm	<i>Proteolytic</i> bacteria CFU / gm	<i>Staphyloco</i> <i>ccus</i> aureus CFU / gm	<i>Salmonella</i> CFU / gm	Molds & Yeasts CFU / gm
control sample	1	10 <sup>2</sup> x 3,5 d	10 x 3 b	0 c	0 c	10 × 4 d	0 b	0 b
	30	$10^3 \times 2,5 c$	10 <sup>2</sup> x 3.6 a	0 c	0 c	10 x 5.3 d	0 b	0 b
	60	10 <sup>4</sup> x 3,3 b	10 <sup>2</sup> x 4.2 a	10 x 2.4 b	10 x 3.1 b	10 <sup>3</sup> x 3.7 b	0 b	10² x 3.4 a
	90	10 <sup>6</sup> x 5,2 a	10 <sup>2</sup> x 6,3 a	10 <sup>2</sup> x 3.7 a	10 <sup>2</sup> x 4.5 a	10 <sup>4</sup> x 5.4 a	10 x 3.1 a	10 <sup>2</sup> x 8.1 a
	1	$10^2 \times 2,7 d$	0 c	0 c	0 c	10 x 3 d	0 b	0 b
envelope d sample	30	$10^2 \times 3,1$	10 x 2,9 b	0 c	0 c	10 x 4.1 d	0 b	0 b
	60	10 <sup>3</sup> x 2,9 c	10 x 3,6 b	0 c	10 × 1 b	10 <sup>2</sup> х 2.3 с	0 b	0 b
	90	10 <sup>3</sup> x 8,9 c	10 x 5,0 b	0 c	10 x 1.4 b	10 <sup>2</sup> x 4.2 c	0 b	0 b

Mean  $\pm$  SD. Having different superscript letters on columns are significantly different (P $\leq$ 0.05)
### **4-11-3-2:** Detection of *E-coli* bacteria in rapped mozzarella cheese with (G.T.E) casings during storage period

The results were shown in table (4-15) showed that the number of Ecoli bacteria in the control sample content was 10 x 3 CFU/gm after one day of manufacturing, while the enveloped sample representing the wrapped cheese was devoid of growth. It was noticed that the number of bacteria increased during the progression of the storage period for cheese workers, where control sample and enveloped sample at the end of the storage period CFU/gm were  $10^2 \times 6.3$  and  $10 \times 5.0$ , respectively. It was noted that there were significant differences ( $P \le 0.05$ ) between the control sample and enveloped sample, as it was found by two logarithmic cycles and the reason for that was due to the effectiveness of the casings in inhibiting *E-coli* bacteria, especially the anti-bacterial action of green tea extract against microorganisms. These results were in agreement with what was mentioned by Henriques et al., (2013), it was shown that the unwrapped cheese contained a number of *E-coli* bacteria and the reason for This rise might be due to the lack of packaging of the cheese in sample and its exposure to the direct atmosphere, which may be the direct cause of contamination, Sajed et al., (2019) found that the milk protein coatings for beef packaging against microbiology increase its shell life during the storage period when decrease in the the numbers of E.coli and Pseudomonas spp was noticed.

# **4-11-3-3:** Detection of *Lipolytic* bacteria in rapped mozzarella cheese with (G.T.E) casings during storage period

The results of Table (4-15) reflect the absence of any countable *lipolytic* bacteria (i.e. less than 30 colony-forming units/gm) in both control sample and treated enveloped sample at the beginning of the storage period, as it appeared at the end period of storage, The growths of this bacteria detected only the control sample , where it reached  $10^2 \times 3.7$  CFU/gm, no growths appeared in the enveloped sample, compared with the control sample by an amount, and these results were lessd than the Iraqi standard specification (1988), especially in cheese enveloped samples. The reason for the absence of growths in cheese was due to the enveloped sample factor, which was due to the activity of the alcoholic extract of green tea against *lipolytic* bacteria; the reason was due to the packaging process that contributed to preventing the proliferation of microorganisms (Giuliana Gorrasi *et al.*, 2016).

### 4-11-3-4: Detection of *Proteolytic* bacteria in rapped mozzarella cheese with (G.T.E) casings during storage period

The results shown in table (4-14) indicate that no numbers of proteolytic bacteria appeared in each of the control sample and enveloped sample, at the beginning of the storage period. However, it appeared only at the end of storage period of control sample, it reached  $10^2 \times 4.5 \text{ CFU/gm}$ and for the treated enveloped sample was 10 x 1.4, this absence of growths was due to the new environmental conditions formed by the packaging process and the effect of the natural antimicrobial agents present in green tea extract.(Ramos et al. 2012), In addition, the simple coating and coating treated with anti-microorganisms have an effect on the aerobic microorganisms present on the surface of the cheese, that means proteolytic bacteria have continued their activity because of the antimicrobial agents which were unable to migrate into the cheese mold and remain on the surface preventing the development of these organisms, and therefore the activity of the internal microorganisms depend on the internal conditions of water activity and oxygen remains, so the activity of proteolytic microorganisms increased compared to aerobic bacteria (Sajed Amjadi et al., 2019).

### 4-11-3-5: Detection of *Staphylococcus aureus* bacteria in rapped mozzarella cheese with (G.T.E) casings during storage period

The results of Table (4-14) show the number of *Staphylococcus aureus* bacteria in mozzarella cheese for control sample and enveloped sample, which were 10 x 4 and 10 x 3 CFU/gm after a day of manufacture, there were significant differences of Staphylococcus bacteria between the control and packaged sample with (GTE) at the end of the storage period by 4-2 logarithmic cycles, as CFU/gm was  $10^4 \text{ x} 5.4$  and  $10^2 \text{ x} 4.2$  for control sample and treated sample, respectively. The difference was since the encapsulation process alone contributes to preventing the proliferation of aerobic microorganisms by preventing the entry of oxygen, which was essential for the growth of aerobic microorganisms, that led to reducing their numbers or prolonging their Lag phase and thus reducing their growth rates (Shima. et al., 2018). The decrease in total bacterial numbers was attributed to the effectiveness of the green tea extract against Staphylococcus aureus, which was confirmed when examining the lower limit of inhibition (Sajed. et al., 2019). Also, oxygen has an important role in controlling the growth of aerobic organisms through the great role it plays in the water activity (a<sub>w</sub>) necessary for the activity of these microorganisms, Arantzazu. et al., (2017) indicated that some pathogenic organisms such as Staphylococcus aureus, whose minimum requirements of water activity  $(a_w)$  depend on oxygen concentration. Aerobic bacteria by reducing oxygen entry.

# **4-11-3-6:** Detection of *Salmonella bacteria* in rapped mozzarella cheese with (G.T.E) casings during storage period

The results shown in Table (4-14) show that the enveloped sample content was free from Salmonella bacteria in which the whey protein wrappers were used, which were supported by green tea extract throughout the storage period, in contrast to the control sample, which showed an apparent growth at the end of storage period after it was free in the first period, where It was at the end of the storage period CFU/gm 10 x 3.1. These results were in agreement with what was found by Al-Badrani (2011), which showed that the casein-coated mouterine cheese was free of Salmonella bacteria throughout the storage period of the cheese. These results indicated the effectiveness of the casings of whey protein isolates supported with tea extract. green tea prevents the growth and proliferation of microorganisms due to its anti-microbial activity because it contains active phenolic compounds such as catechins, and determination of the ECGC compound found in green tea extract that supported the casings against bacterial species (gram-positive and gram-negative), (Lorenzo JM et al., 2016).

# **4-11-3-7:** Yeasts and molds detected in mozzarella cheese wrapped with (G.T.E) casings during storage period

It was possible that yeasts and molds were present in dairy products through contamination, especially after the pasteurization process, because pasteurization in itself was a determining factor for the presence of this type of microorganisms. This group of organisms can lead to protein and fat decomposition, which was usually accompanied by the production of substances that affect the taste, the flavor of cheese was evidents in table (4-14) where no growth of molds and yeasts appeared at the first day in control and treated enveloped sample. However, with the progression of the cheese storage period, growth began to appear in the unwrapped control sample till the end of the storage period,  $10^2 \times 8.1$  CFU/ gm unlike the enveloped sample treatment of coated cheese, growth did not appear in it throughout the storage period. These results agreed with Al-Jaruri (2014) where it was found that the number of molds in triumphant cheese coated with whey protein casings was less than in non-wrapped cheese. In preventing the proliferation of molds and yeasts by preventing the entry of

oxygen, as well as the effectiveness of the natural antifungal agents found in whey proteins and anti-microbial additives. It also agreed with Henriques *et al.* (2013), as it was found that the number of molds and yeasts decreased during storage of cheese with whey proteins with natamycin for 45 days at 11 °C and humidity of 85% compared to unwrapped cheese.

# 4-11-4: Sensory evaluation of mozzarella cheese wrapped with (G.T.E) casings during storage period

Sensory evaluation was an expression of the extent to which the consumer accepts the product and also indicates to what extent to achieve the desired goal of the manufacturing process (Al-Dalali and Al-Hakim, 1978). Sensory evaluation when making some modifications in the manufacturing process, such as product packaging, such as effective packaging with protein films supported by active extracts. On this basis, a sensory evaluation was conducted for mozzarella cheese samples and for samples control and enveloped one, where the control sample was the unwrapped cheese, and the enveloped sample represented the cheese coated with an isolated casing whey proteins fortified with green tea extract at a concentration of 3%. The evaluation process group postgraduate studies from the Department of Food Sciences .

Table (4-16) shows the results of the sensory evaluation of mozzarella cheese for control and treated enveloped samples after a day of manufacturing and during storage at a temperature of  $(5 \pm 1)$  C for 90 days. The table indicated that the characteristics of taste, flavour and colour were given higher degrees for enveloped sample coated cheese compared with control sample unwrapped cheese was at the end period of preservation. The reason for this was because whey proteins possess a palatable and acceptable flavor and taste that made it a good choice in the packaging processes of food products, especially those whose flavor was affected during preservation and the role of protein films and their ability to seize fat and flavor compounds because of their properties A compact structure and interconnected protein chains increased the attachment process, which qualified them for use in preservation processes, especially foods that were perishable due to oxidative processes and others (Frederico. et al., 2019). The active antimicrobial compounds in green tea also play a role in reducing decomposition that causes the appearance of strange, undesirable flavours (Lorenzo. et al., 2016), which made the assessors gave higher degrees to the cheese samples wrapped than the unwrapped because of the unpalatable flavours that appeared at the end of the storage period.

As for the characteristics of texture, and external appearance, the two control and treated enveloped samples were given relatively close degrees with a less significant difference (P>0.05).from the previous characteristics between the enveloped sample cheese and the control sample cheese. control sample packaged cheese was somewhat similar to that of the packaged cheese samples, as the appearance of the packaged and unwrapped cheeses seemed to some extent similar, especially in the early days of storage.

As for the flavor characteristic, enveloped sample gave higher scores compared to cheese samples with control sample, especially at the end of storage period. It limited the decomposition processes that cause the appearance of strange and undesirable tastes, which made the assessors gave higher degrees to the cheese samples than it was to the unwrapped cheeses, which were given lower degrees for the unpalatable flavors that appeared at the end of the storage period. As for the bitterness, it was a qualitative phenomenon in cheese treated enveloped sample gave higher scores compared to samples of unwrapped cheese control sample from the beginning of the storage period to its end due to the bitter taste of green tea due to the catechins it contains (Lixue Dou *et al*, 2018)

# Table (4-16) Sensory evaluation results of mozzarella cheese packaged with green tea extract casings during storage period

mozzar ella cheese sample s	storag e perio d of cheese (days)	Color 5 dgree	Taste and flavor 30 degrees	Texture 15 degrees	Appeara nce 10 degrees	Bittern ess 20	Exotic flavors 20 degrees	The final grade
control sample	1	5 ±0.43	28 ±2.54 ª	15 ±0.75 a	10 ±0.36 a	20 ±1.09 <sup>a</sup>	19 ±0.92 ª	97 ±5.10 a
	30	5 ±0.41 ª	28 ±2.40 ª	15 ±0.68	10 ±0.42	20 ±1.25 <sup>a</sup>	18 ±1.14 <sup>a</sup>	96 ±4.87 a
	60	4 ±0.37 <sup>a</sup>	21±1.63 b	12 ±0.49	8 ±0.35 <sup>b</sup>	20 ±1.04 <sup>a</sup>	15 ±0.67 <sup>b</sup>	80 ±4.29 c
	90	2 ±0.18 <sup>b</sup>	13 ±0.78 °	11 ±0.55	5 ±0.27 °	16 ±0.94 <sup>bc</sup>	10 ±0.52 °	57 ±3.16 e
envelop ed model	1	5 ±0.47 ª	28 ±2.37 ª	15 ±0.69	10 ±0.37 a	19 ±1.13 <sup>a</sup>	19 ±1.39 <sup>a</sup>	96 ±5.03 a
	30	5 ±0.38 <sup>a</sup>	28 ±2.50 ª	15 ±0.54	10 ±0.42	18 ±1.08 <sup>ab</sup>	19 ±1.06 <sup>a</sup>	95 ±4.79 a
	60	4 ±0.40 <sup>a</sup>	26 ±2.07 <sup>a</sup>	15 ±0.61	8 ±0.54 <sup>b</sup>	15 ±0.63 °	18 ±0.87 <sup>a</sup>	86 ±4.28 b
	90	4 ±0.35 <sup>a</sup>	20 ±1.37 <sup>b</sup>	13 ±.38 <sup>ab</sup>	7 ±0.49 <sup>b</sup>	14 ±0.57 c	16 ±0.72	74 ±3.92 d

Means in the same column not bearing similar superscript letters are significantly difference (P>0.05).

### Chapter 5

### **Conclusions and Recommendation**

#### **5-1: Conclusions**

- The possibility of natural packing materials for cheese was made from green tea extract by reinforcing whey protein casings with green tea extract being bioactive materials and using them as natural food wrappers, due to their added nutritional value and ease of use.
- Whey protein films enriched with alcoholic extract of green tea have proven their ability to inhibit the growth of a number of microorganisms, in addition to their high antioxidant activity, which made them efficient for preservation purposes.
- The alcoholic extract with a concentration of (50%) was chosen as the most appropriate solution due to its anti-microbial activity compared to the rest of the concentrations and because the colour of the resulting extract was acceptable after adding it to the coating films used in cheese packaging.
- The casings prepared for packaging have good mechanical and retaining properties, and their low permeability to O2, and their use led to prolonging the shelf life of mozzarella cheese while preserving its good sensory properties throughout the storage period.
- The use of casings isolated whey proteins and supplemented with alcoholic extract of green tea had the effect of reducing the microbial content of the coated cheese samples compared to the unwrapped samples, as well as slowing the chemical changes of cheese during storage, which in turn was reflected on the sensory evaluation.
- Ease conducting the wrapping process with isolated whey proteins carrying antimicrobial growth factors compared to wax wrapping.

#### **5-2 Recommendation**

- 1- Developing edible casings by using nanotechnology in their manufacture, in addition to integrating other natural compounds with them to impart new features that were desirable for some manufacturing processes.
- 2- Using it in packaging of perishable food products, especially products with high moisture content, such as pastries, cakes and meat products e.s burgers, and studying their packaging for cooked food.

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### Appendices

### Appendix (1)

Table Apparatus and equipment used in This research

Apparatus	Manufacturer		
Sensitive balance	Sartorius (Germany)		
Water dwastillator	Rosamar (England)		
Whatman filter paper No:1	Sigma Aldrich (China)		
Autoclave	Gallen Kamp (England)		
Refrigerator			
UV Spectrophotometer	Cecil (England)		
Magnetic stirrer with Hot Plate	PhilipHorrwas (England)		
Laminar			
Moisture analyzer	Germany(Precwasa)		
Micro Kjeldahl	Gerhardt (Germany)		
Tensile strength tester	TiniusOken (England)		
Oxygen permeability	Testing Machies		
Brightness and color meter	Testing Machies (Englwash)		
Incubator	Julabo (West Germany)		
Water bath	Julabo (west Germany)		
Electric Mixer	Panasonic (Malaysia)		
Timer watch	Casio (China)		
Electrical mill	Panasonic (Malaysia)		
Muffle furnace	Carbolite (England)		
Air oven	Gallen Kamp (England)		

Vortex	Gallen Kamp(England)
Digital Micrometer	IDM (Estralia)
pH-meter	Sartoruwas (Switzerland)
Centrifuge	Beckman (England)
Rotary evaporator	labtech-grou (China)

### Appendix (2) control casings (without extract)



Appendix (3) casings discoloration by increasing the concentration of green tea extract



Appendix (4) Apparatus for the estimation of casing permeability for oxygen



Appendix (5) Elongation or tensile testing device



Appendix (6) Apparatus for estimating optical and chromatic properties with a scheme for estimating the color of films







#### Appendix (7) values of casing thickness, tensile strength and elongation ratio for gelatinous casing samples

#### Appendix (8) casing oxygen permeability values





#### Appendix (9) The permeability values of the films to water vapour

# Appendix (9) solubility values in water for the control treatment casing and the additive casing for the alcoholic extract of green tea



Appendix (10) Moisture percentages of treatments for unwrapped mozzarella cheese (control sample) and cheese samples coated (enveloped sample) after a day of processing and during storage at a temperature of (5±1) C°. After a period of 90 days



Appendix (11) Protein percentages for treatments of unwrapped mozzarella cheese (control sample) and cheese samples coated (enveloped sample) after a day of processing and during storage at a temperature of  $(5\pm1)$  C°. After 90 days



Appendix (12) Fat ratios of treatments for unwrapped mozzarella cheese (control sample) and cheese samples coated (enveloped sample) after a day of processing and during storage at a temperature of  $(5\pm1)$  C°. 90 days pass


Appendix (13) shows the pH values of parameters of unwrapped mozzarella cheese (control sample) and cheese samples coated ( enveloped sample) after a day of processing and during storage at a temperature of (5±1) C°. After a period of 90 days has passed



Appendix (14) total acidity percentages of treatments for unwrapped mozzarella cheese (control sample) and cheese samples coated ( enveloped sample) after a day of processing and during storage at a temperature  $(5\pm1)C^{\circ}$ . After 90 days has passed





