KONYA FOOD AND AGRICULTURE UNIVERSITY
INSTITUTE OF SCIENCE
GRADUATE SCHOOL OF BIOTECHNOLOGY

DEVELOPMENT OF PROBIOTIC INCORPORATED EDIBLE COATINGS AND EFFECTS ON SHELF-LIFE OF FRESH STRAWBERRIES

MASTER OF SCIENCE THESIS

Naime Nur TEMİZ

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Meram-KONYA
JULY, 2020

KONYA GIDA VE TARIM ÜNİVERSİTESİ
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PROBİYOTİK İLE ZENGİNLEŞTİRİLMİŞ YENİLEBİLİR KAPLAMALARIN GELİŞTİRİLMESİ VE TAZE ÇİLEKLERİN RAF ÖMRÜ ÜZERİNE ETKİSİ

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TEMMUZ, 2020

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This study titled “Development of Probiotic Incorporated Edible Coatings and Effects on Shelf-Life of Fresh Strawberries” and presented as Master Thesis by Naime Nur TEMİZ has been evaluated in compliance with the relevant provisions of Konya Food and Agriculture University (KFAU) Graduate Education and Training Regulation and KFAU Institute of Science Education and Training Direction and jury members written below have decided for the defense of this thesis and it has been declared by consensus / ~~majority~~ of votes that the candidate has succeeded in thesis defense examination dated 01.07.2020.

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ABSTRACT

DEVELOPMENT OF PROBIOTIC INCORPORATED EDIBLE COATINGS AND EFFECTS ON SHELF-LIFE OF FRESH STRAWBERRIES

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MSc in Biotechnology

Supervisor: Asst. Prof. Dr. Kübra Sultan ÖZDEMİR BİLİCİ

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In this thesis work, strawberries (*Fragaria x ananassa*) were used as an alternative product to deliver probiotics, and the study aimed to investigate the effect of *Lactobacillus rhamnosus* HN001 incorporated gelatin films on microbial and physicochemical quality of strawberries during refrigerated storage. For this purpose, probiotic *L. rhamnosus* and inulin were added to gelatin-based coatings and applied onto fresh strawberries and stored at 4^{o}C. Probiotic survivability, microbial, and physicochemical quality parameters of strawberries were monitored during 16 days of refrigerated storage on certain intervals.

According to the results, gelatin-probiotic and gelatin-probiotic-inulin coatings improved the shelf-life of strawberries compared to the control group. These coatings significantly decreased the weight loss in strawberries. Weight loss of strawberries were found as 38%, 24.7%, 17.6%, and 21.3% in control, gelatin, gelatin-probiotic, gelatin-inulin-probiotic coated samples, respectively. Also, the coatings did not alter the pH, titratable acidity, and total soluble solids content during storage. Gelatin-probiotic

coatings slowed down the fungal growth and decay rate of strawberries. Probiotic counts were found as 8.9 log CFU/g up to 10 days and 7 log CFU/g on the 15th day of storage in inoculated strawberries. These results suggest that probiotic incorporated gelatin films can be an alternative to deliver probiotics and improve the shelf-life of perishable fruits and vegetables.

Keywords: *Lactobacillus rhamnosus*, probiotics, strawberry, ready to eat, functional foods, shelf life

ÖZET

PROBİYOTİK İLE ZENGİNLEŞTİRİLMİŞ YENİLEBİLİR KAPLAMALARIN GELİŞTİRİLMESİ VE TAZE ÇİLEKLERİN RAF ÖMRÜ ÜZERİNE ETKİSİ

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Bu tez çalışmasında, çilek meyvesi (*Fragaria x ananassa*) probiyotik bakteriler için alternatif bir taşıyıcı olarak kullanılmış ve *Lactobacillus rhamnosus* HN001 içeren jelatin kaynaklı yenilebilir kaplamaların çileklerin buzdolabı koşullarında 16 gün depolama süresince mikrobiyal ve fizikokimyasal kalitesi üzerine etkisi incelenmiştir. Bu amaçla, taze çilekler inulin ve *Lactobacillus rhamnosus* HN001 içeren jelatin film çözeltilerine daldırılarak kaplanmış ve 4^{o}C'de depolanmıştır. Bu koşullarda 16 gün boyunca depolanan çileklerde probiyotik canlılığı, mikrobiyal ve fizikokimyasal kalite parametreleri belirlenen aralıklarla takip edilmiştir.

Elde edilen sonuçlara göre, jelatin-probiyotik ve jelatin-probiyotik-inülin içeren yenilebilir kaplamaların kontrol grubuna kıyasla çileklerin raf ömrünü artırdığı tespit edilmiştir. Bu kaplamaların, çileklerdeki ağırlık kaybını önemli ölçüde azalttığı görülmüştür. Çileklerdeki ağırlık kaybı kontrol, jelatin, jelatin-probiyotik, jelatin-inülin-probiyotik ile kaplanan örneklerde sırasıyla %38; %24.7 ; %17.6 ve %21.3 olarak tespit edilmiştir. Ayrıca depolama süresince kaplanmış çileklerin pH, titre edilebilir asitlik ve

toplam çözünür katı madde miktarında önemli bir değişiklik gözlenmemiştir. Jelatin-probiyotik içeren kaplamalar, çileklerdeki maya-küf gelişimini ve mikrobiyolojik bozulmayı yavaşlatmıştır. Probiyotik canlı sayısı, çileklerde depolamanın 10. gününe kadar 8,9 log CFU/g ve 15. Gününde ise 7 log CFU/g olarak tespit edilmiştir. Sonuç olarak, yenilebilir jelatin kaplamaları probiyotikler için taşıyıcı olabileceği ve bu kaplamaların hızlı bozulabilen meyve ve sebzelerin raf ömrünü uzatmak için kullanılabileceği tespit edilmiştir.

Anahtar sözcükler: *Lactobacillus rhamnosus*, probiyotik, çilek, tüketime hazır gıdalar, fonksiyonel gıdalar, raf ömrü

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Dedicated to my beloved family...

TEXT OF OATH

I declared and honestly confirm that my study titled “Development of Probiotic Incorporated Edible Coatings and Effects on Shelf-Life of Fresh Strawberries” and presented as Master’s Thesis has been written without applying to any assistance inconsistent with scientific ethics and traditions and all sources I have benefited from are listed in bibliography and I have benefited from these sources by means of making references.

01.07.2020

Naime Nur TEMİZ

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LIST OF ABBREVIATIONS & SYMBOLS

Abbreviations	**Explanation**
GRAS	Generally Recognized As Safe
LAB	Lactic Acid Bacteria
Lb	*Lactobacillus*
MRS Agar/Broth	Man, Ragosa and Sharpe Agar/Broth
PCA	Plate Count Agar
PDA	Potato Dextrose Agar
C	Control
G	Gelatin
G-P	Gelatin and Probiotic
G-I-P	Gelatin, Inulin and Probiotic
TSS	Total Soluble Solid
TA	Titratable Acidity
TPC	Total Phenol Content
MPO	*Mentha Pulegium* Oil
NASS	National Agricultural Statistics Service
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
SD	Standard Deviation

Symbols

kg	Kilogram
g	Gram
mL	Milliliter
L	Liter
mg	Milligram
µg/ml	Microgram per Milliliter
CFU/g	Colony Forming Unit per Gram
nm	Nanometer
Mg GAE/kg fw	Milligram Gallic Acid Equivalents per Kilogram Fresh Weight
DPPH	Diphenyl picrylhydrazyl
mMol	Millimole

1. INTRODUCTION

Strawberry (*Fragaria×ananassa*) is one of the widely consumed fruits due to its micronutrient content such as vitamins, minerals, flavonoids, anthocyanins, proteins, and phenolic compounds. Strawberry has a very large production quantity and is one of the most produced fruit in the greenhouses besides conventional strawberry fields. Turkey is one of the largest strawberry producers in the world with 0.44 million tons per year while the whole market worldwide is reported as 8.337 million tons in 2018 (FAOSTAT, 2020). Strawberries are generally consumed as fresh. However, they are highly perishable fruits and show a tendency to physical injuries and fungal spoilage easily (NASS, 2018). For this reason, the strawberries' shelf-life is quite limited and preservation of their quality is important until consumption.

Currently, due to the consumer choices and rapid living conditions, the demand for fast eating, high-quality, fresh, low-calorie and healthy foods has increased. The need to reply to this trend has affected also the fruit and vegetables market. So, there is an increased interest in ready-to-eat foods and functional foods. The main goal of the ready-to-eat fruits and vegetables is to keep their quality with slight differences during storage. Functional foods also help increasing shelf life of the food and decreasing harmful microorganisms and intoxications.

Edible coating or active packaging is one of the efficient and promising methods with multiple functions, including respiratory control, injury prevention, carrier for antimicrobial and antioxidants to prolong the shelf life of food products (Zhao and McDaniel, 2005). They could increase the shelf life of the product while protecting its nutritional and sensorial characteristics. They build a barrier around the fruit and this

barrier reduces respiration, protect the food against dehydration and decreases oxidation (Olivas and Barbosa-Cánovas, 2009). In addition, using edible films and coatings, it is possible to improve the features and functions of foods such as the inclusion of nutrients, antioxidants, vitamins, minerals, prebiotics and probiotics or antimicrobials that may slow down spoilage (Guimarães et al., 2018). Gelatin which is an animal - sourced collagen is one of the protein based coating materials. Beside it is biodegradable, it has also excellent mechanical characteristics as a film coating material (Liao et al., 2009).

Consumption of foods enriched with probiotics has increased because they are described as "live microorganisms that, when ingested in sufficient quantities, confer a health benefit on the host" (Bambace et al., 2019). Its viability and benefits for the host are key to refer bacteria as a probiotic. *Lactobacillus* and *Bifidobacterium* are widely used genera as probiotics, commercially. *Lactobacilli* are an essential part of human microflora; in addition, they have some significant impacts such as antimicrobial activity, immunity improvement, antitumorigenic behavior, can regulate inducing host immunomodulation and relieve symptoms of a wide range of digestive problems. *Lactobacillus rhamnosus* is one of the well-known and investigated facultative LABs between the probiotics and it is a rod-shaped, non-spore forming and gram-positive bacteria (Gorbach et al., 2017; Kailasapathy, 2014). It has many beneficial effects on human microflora. Probiotics have been incorporated in a number of dairy products till now. However, the use of probiotics has not been commonly used especially in non-dairy products in industrial scale such as milk, yogurt, cheese, dairy desserts, ice-cream and fermented milks (Espitia et al., 2016). This situation creates a deficit in a probiotic foods market especially for those who cannot consume milk and milk based products

due to health problems, vegan food preferences, lactose intolerance or allergic reactions (Panghal et al., 2018).

In this thesis, we designed an alternative product that probiotics (*Lactobacillus rhamnosus* HN001) was incorporated in edible coatings and applied on the surface of fresh strawberries for the purpose of extending their shelf life and to enhance their probiotic value. The effect of gelatin, gelatin-probiotic and gelatin-probiotic-inulin coatings on the storage quality of fresh strawberries during 16 days in refrigerated storage was investigated. Physiochemical quality (weight loss, fungal decay, pH, titratable acidity, total soluble solid content, total phenolic content, antioxidant activity) and microbial quality (total yeast and mold, total aerobic mesophilic bacteria, *Lb. rhamnosus* HN001 survival) were investigated throughout storage time.

2. BACKGROUND INFORMATION

2.1. Strawberry (*Fragaria* × *ananassa*)

Strawberry (*Fragaria* × *ananassa*) is worldwide known and cultivated fruit which belongs to Rosaceae family and Fragaria genus. It is a red and sweet fruit with its own smell, texture and aroma. It is also called as 'garden strawberry'. According to Mangelsdorf from Harvard University, strawberry fruit (*Fragaria* × *ananassa*) is a hybrid of *F. chiloensis* and *F. virginiana* and its size is larger than other Fragaria species (Mangelsdorf, 1927). The spots appearing outside the fruit are called 'achenes' and they are the combination of the seed and ovary tissue of the strawberry (Perkins, 2010).

In recent years, a noticeable increase was seen in healthy diet choices and has become a substantial trend. Consumers prefer the products which are high source of probiotics, prebiotics, vitamins, minerals and antioxidants. Strawberry (*Fragaria*×*ananassa*) is a highly consumed fruit due to its sensorial properties (taste, color) and nutritional value (Chu et al., 2020). It is a rich source of vitamins, minerals, flavonoids, anthocyanins, proteins, and phenolic compounds and antioxidants (Van De Velde et al., 2013).

Plants are notable to possess wide range of naturally occurring antioxidants. Strawberry is one of the main fruits known for containing antioxidants and consumed by consumers. Strawberries have natural antioxidants and antioxidant enzymes in high content, which protect toward free radicals and play a significant part for human health defense mechanisms. People who eat enough fruits and vegetables like strawberries was

linked to lower levels of cancer risk and mortality, cardiovascular disease and a variety of other illnesses (Wang, 2014).

Besides having many nutritional values, strawberries are among the fruits that are rich in phenolic compounds. Phenolic compounds are such molecules distinguished by their composition containing minimum one phenol unit. These are secondary metabolites and phytochemicals observed in certain plant tissues, including fruit and veggies. As long as their chemical compositions change, subgroups of phenolic compounds can vary from one to another, such as phenolic acids, flavonoids, tannins, lignans, and curcuminoids (de la Rosa et al., 2019; Gan et al., 2019).

Phenolic group contain various bioactivities and dietary consumption of these phenolics has a health-protective activity, so post-harvest treatment options have been used to improve or retain phenolics in fruit and vegetables (de la Rosa et al., 2019).

Phenolic acids are profitable elements naturally found in fruit and vegetables but are also mainly recognized for their antioxidant capacity. Strawberry has a high nutritious and non-nutritious content including minerals, vitamin C and folic acids, or phenolic compounds, which are important for health (Giampieri et al., 2014).

Phenolic acids are directly correlated to the flavor, color, sensorial and textural qualities of strawberry. Strawberry phenols are widely regarded for their antioxidant and anti-inflammatory functions. The famous polyphenolic strawberry substances are flavonoids, especially anthocyanins, which are the largest group of such phenolics (Giampieri et al., 2014).

Strawberry is categorized as non-climacteric fruit which is not ripening after harvested (Perkins, 2010). Fruit ripening can be explained as physical and biochemical changes of fruit during development. Biochemical changes include pigment & volatiles biosynthesis, degradation of macromolecules such as starch, production of sugars and organic acids & physical changes include formation of cell wall and texture of fruit (Giovannoni, 2001). Fruit softening, one of the ripening stages is a marker in determining shelf life after harvesting. Especially, enzymes cause cell wall degradation is responsible from softening (Moya-León et al., 2019). Strawberry is a fruit prone to tissue softening, mechanical damage, rapid increase in yeast and mold ratio, physiological disorders throughout storage (Vu et al., 2011).

Therefore, it has a very short shelf life and senescence time (Gol et al., 2013). When the strawberry is stored in refrigerator conditions, its shelf life is less than a week (Wills, 1998). This situation affects the shelf-life of fresh strawberry and result in significant quality loss during storage and transport.

Total production of strawberry in the world is 8.337 million tons in 2018. China and United states are the largest producers and Turkey ranks fourth with 0.44 million tons in the world (FAOSTAT, 2020). Nearly 81 percent of total strawberry production is in fresh market (NASS, 2018). However, besides being consumed fresh, strawberry is also widely used fruit in industry such as food, confectionery and cosmetic industries.

2.1.1. One of the most prevalent reason of strawberry degradation: Gray mold

Strawberries are highly perishable fruits and prone to physical injuries, decay and fungal spoilage (Dhital et al., 2018; Park et al., 2005). The main reason of decay of

strawberries that have susceptibility to rot-causing fungi and these fungi decrease the strawberries' shelf life rapidly. Although many pathogens can cause postharvest spoilage including *Rhizopus stolonifer, Mucor spp., Colletotrichum spp.,* and *Penicillium spp,* one of the biggest reason of rot-causing fungi and the main strawberry pathogen is gray mold caused especially by *Botrytis cinerea* (Feliziani and Romanazzi, 2016). *B. cinerea*, which is hosted by about 200 plant species (Williamson et al., 2007), is among the 10 most important pathogens in the world causing enormous economic damage on important crops (Dean et al., 2012).

2.1.2. Methods to prevent gray mold

The conventional strategy is to use some fungicides while controlling of fungal decay, especially controlling of *B. cinerea.* However, there is an international apprehension about using synthetic fungicides in agriculture due to their harmful impacts on human health (Norman, 1988). This leads to a search for options other than synthetic fungicides such as biological control agents for controlling post-harvest degradation of fruits, including gray mold on strawberries. Nevertheless, biocontrol agents have been shown to be less efficient than chemical agents. (Zhang et al., 2010).

Contaminations by *B. cinerea* could happen before harvesting, and it can stay dormant until storage. Once the relative humidity rises and temperature decreases about 0-5°C, the fruit resistance is declined and the microorganisms start growing and infection spread. Contamination begins from mechanical injuries on the fruit (Feliziani and Romanazzi, 2016).

Biocontrol agents, natural compounds, decontaminating agents, and physically treatments may be chosen as new alternatives to the use of chemical fungicides but

these alternatives are still on trial (Mari et al., 2009). Likewise, modified atmosphere storage has been demonstrated to be sufficient in suppressing microorganism development and diminish the rot frequency of strawberries. However, this application can reduce the satisfaction of consumers due to the changes on the color and taste of the strawberry (Fan et al., 2009).

2.2. Probiotics

Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) in 2001 defined probiotics as “living microorganisms which when administered in adequate amounts confer a health benefit on the host” (Bubnov et al., 2018). In order to be evaluated a microorganism as a probiotic; it must satisfy certain conditions given in follow:

- Firstly, it should be alive even on an industrial process.
- It should be durable during shelf life.
- It should be durable in the intestine after consumption.
- It should confer health benefits to the host person (Fuller, 1992).

The human intestine carries more than 1000 species of bacteria including various probiotic strains which compose the microbiota. Some of probiotic strains are listed in Table 2.1. The probiotics in the intestinal microflora has conducted toward many beneficial influences. Probiotics can struggle many diseases and harmful pathogens (Singh et al., 2018). Thus, probiotics show hostile effect against pathogens due to the synthesis of various organic acids, which leads to a reduction in pH, lysozyme (Hill et al., 2014). In addition to preventing pathogen microorganisms, probiotics also have a

role in boosting the immune system, nutrients metabolization and process toxic metabolites, and energy balance control (Bubnov et al., 2018). Moreover, probiotic strains are capable of synthesizing digestive enzymes (amylase, lipase, proteases, pectinases, and endoglucanases), vitamins A, B, E, C, K (Lilly and Stillwell, 1965) and generate metabolites such as short-chain fatty acids. In this way, probiotics are able to regulate microbiota and can demonstrate immunomodulatory and antitoxic properties. Although regarded as valuable food additives, probiotics are easily affected from the harsh conditions of the gastrointestinal (GI) tract such as low pH, bile juices or from storage conditions affecting the probiotic viability (Singh et al., 2018).

Table 2. 1 The List of Some Probiotic Strains (Holzapfel et al., 2001).

Lactobacillus species	*Bifidobacterium* species
Lb. acidophilus	*B. adolescentis*
Lb. casei	*B. animalis*
Lb. crispatus	*B. bifidum*
Lb. gallinarum	*B. breve*
Lb. gasseri	*B. infantis*
Lb. johnsonii	*B. lactis*
Lb. paracasei	*B. longum*
Lb. plantarum	
Lb. reuteri	
Lb. rhamnosus	
Other lactic acid bacteria	Non-lactic acid bacteria
Enterococcus faecalis	*Bacillus cereus var. toyoi*
E. faecium	*Escherichia coli* strain nissle
Lactococcus lactis	*Propionibacterium freudenreichii*
Leuconosioc mesenteroides	*Saccharomyces cerevisiae*
Pediococcus acidilactici	*S. boulardii*
Sporolactobacillus inulinus	
Streptococcus thermophilus	

2.2.1. Health benefits of probiotics

Since each microorganisms cannot be accepted as ‘probiotics’, there are some compulsory and determinative criteria to identify them. Some of these criteria have given below. They have to:

- exhibit a beneficial influence on the human and they should not have any pathogenic effect;
- be endurable in foods and remain alive during the shelf life of food,
- endure throughout the gastrointestinal tract,
- hold on to intestinal epithelium cell and colonize in it ,
- resist to harsh conditions such as bile salt and gastric acid on stomach and intestine,
- generate antimicrobial substances,
- stand up some tough technological process and useful in industry ,
- provide the health benefits and regulate the intestinal microflora (Shewale et al., 2014).

Probiotics are proven microorganisms that have many health benefits if taken in sufficient amounts. Some of these benefits are shown in the Figure 2.1. Although many scientific sources specify that the probiotics concentration on foods should be at least 10^6 cfu/g or cfu/ml to be beneficial. In order to utilize therapeutic effects of probiotics,

the probiotics' daily consumption level should be 10^8-10^9 cfu/g or cfu/ml (Kechagia et al., 2013).

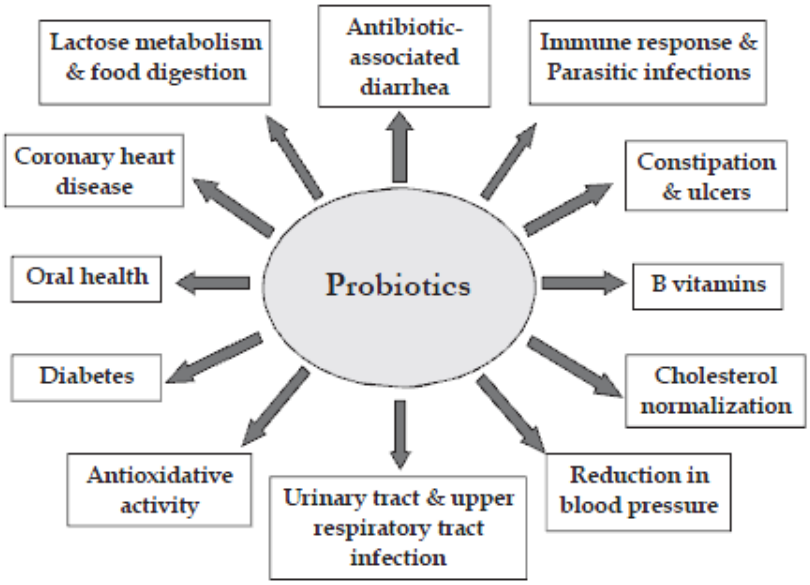


Figure 2. 1 Some health attributes of probiotic microorganisms (Nagpal et al., 2012).

Probiotics have various constructive outcomes on human health, and new researches are included day by day. Some of these health benefits and related mechanisms in human body are demonstrated in Table 2.2.

Table 2. 2 Medical advantages of probiotic microorganisms to the host, and related mechanism (Nagpal et al., 2012).

Health Benefits	**Related mechanisms**
Effective against allergic reactions	Controlling level of immunological responses by adjusting antigen amount and inhibiting translocation of it.

Table 2. 3 (Continued) Medical advantages of probiotic microorganisms to the host, and related mechanism (Nagpal et al., 2012)

Strengthening the immune system	Reinforcing and supporting of antigen-explicit defense against contamination and tumors. Adjusting Th1/Th2 level. Diminishing discharge of harmful metabolites
Decreasing high blood pressure	Peptidase activity brings about antihypertensive tripeptides and cell wall constituents pretend ACE inhibitors.
Decreasing cholesterol level	Change in the action of BSH enzymes, deconjugation of the bile acids & antioxidative impact
Anti-carcinogenicity, anti-tumor	Assimilation of the mutagen, trigger of the immunomodulatory, hindrance of cancer-causing agent creation by the intestinal microflora.
Helicobacter pylori contamination	Preventing the growth and reproduction of *H. pylori* by allowing it to adhere to mucous cells.

Table 2. 4 (Continued) Medical advantages of probiotic microorganisms to the host, and related mechanism (Nagpal et al., 2012)

Regulate digestive system & indications influencing the gastrointestinal tract.	Regulating intestinal microflora, especially by the help of *Lactobacilli* activity, reducing harmful metabolite.
Intensify nutritional value	Production of vitamin and cofactor required for the activity of an enzyme as a catalyst
Beneficial effect against colon cancer	Destroying harmful metabolites shown carcinogenic properties, improving immune system, adjustment in harmful enzymatic movement of colonic microorganisms.
Urogenital health	Helping adhesion to urogenital site, inhibitor formation such as biosurfactants, restraining pathogenic activity

2.2.2. Probiotic strains

Although there are certain criteria to be classified as probiotic, all the probiotics do not have same effect and the identical therapeutic properties. In order to achieve the desired therapeutic effects, the correct strain selection and amount are quite important. Therefore the best possible choice of probiotic is significant. The essential goal of choosing probiotics is that they ought to have safe and desirable characteristics and created several advantages. Furthermore, they should hold their ability till reach to consumer (Shewale et al., 2014).

2.2.3. Selection of strain

The selection of the correct probiotic strain is a crucial factor in formulating products with viable probiotic cultures and to exert health benefits (Terpou et al., 2019). While selecting the probiotics strain, its durability to processing, viability and growth in intestines and therapeutic indications on human have great importance. In vitro and vivo investigations can be utilized to ensure above standards and also these investigations qualify the probiotics and get information about the strain and its activity.

It is prescribed to utilize a blend of phenotypic and hereditary methods to achieve the recognizable proof and characterization of strains. First of all, in vitro experiments are applied on animals. When animal trials are in success, clinical examinations, patient investigations and major human investigations are applied to characterize the strain (Robinson, 2005).

Some of the features that food producers should consider during production of probiotic foods are jointly determined by the FDA and WHO and given in follows:

1. Appropriate description to the degree of strain of all probiotics in the item, with store of all strains in a worldwide culture assortment

2. Honest and not deceiving marking of effectiveness claims and substance through the finish of time span of usability (shelf life).

3. Portrayal of each strain for characteristics essential to its security and capacity

4. Approval of medical advantages in human investigations, including recognizable proof of the amount of the microorganism required to give the advantage (Sanders, 2008).

2.2.4. Lactic acid bacteria

Lactic acid bacteria are major part of gram positive bacteria and they can be classified as non-spore forming, acid durable, non-respiring rod or cocci microorganisms. Their names come from the lactic acid which they produce after fermentation of carbohydrates (Axelsson, 2004). *Lactobacillus* and *Bifidobacterium* are widely used genera commercially as probiotic bacteria and, among them; *Lactobacilli* represent an important part of human microbiota. *Lactobacilli* genus commonly exists in foods, especially dairy products, fish, meat and fermented food products. They can cope with stomach pH, which can be deadly for microorganisms. They are among the bacteria that are very dense in the small intestine and the human vaginal microbiota (Heeney et al., 2018). Moreover, besides playing an important role in food fermentation, they have some important medical effects such as antimicrobial activity, enhancement of immunity, anti-tumorigenic activities and are able to stabilize inducing host immunomodulation and reducing the symptoms of a wide range of gastrointestinal

disorders. The most abundant lactobacilli consist of *Lb. casei, Lb. delbruckeii, Lb. murinus, Lb. plantarum, Lb. rhamnosus,* and *Lb. ruminus,* and *Lb. rhamnosus* are hardly isolated from conditions outside the digestive system and are considered as gut autochthonous microorganisms since they colonize the skin and mucosal surfaces (Heeney et al., 2018).

2.2.5. *Lactobacillus rhamnosus*

Lactobacillus rhamnosus GG (LGG) is one of the most broadly utilized probiotic strains. It is one of the well-studied LAB that is a rod shaped, non-spore forming and facultative gram positive (Gorbach et al., 2017; Kailasapathy, 2014). It can grow between the range of 5 to 6.4 pH and its ideal growth temperature is around 37°C.

Lactobacillus rhamnosus has many health benefits including the prevention or treatment plan of gastrointestinal illnesses and diarrhea, triggering the immune responses (Goyal and Shukla, 2013) and preventing certain allergic symptoms (Spacova et al., 2018). According to a clinical research, *Lactobacillus rhamnosus* GG is efficient in preventing anti-microbial related diarrhea in kids and grown-ups treated with antibiotics (Szajewska and Kołodziej, 2015). In another study, *Lactobacillus rhamnosus* GG is helpful for different kinds of loose bowels, for example, passenger's diarrhea, intense watery looseness of the bowels, and intense gastroenteritis-associated diarrhea (Basu et al., 2008). In addition to this, *Lactobacillus rhamnosus* GG is helpful for irritable bowel syndrome and urinary tract infections in women (Grin et al., 2013; Han et al., 2018; Saha, 2014). *Lactobacillus rhamnosus* GG can also act as an antimicrobial onto some destructive bacteria and helps increment of short-chain fatty acids and B group vitamins (LeBlanc et al., 2017; Verdenelli et al., 2009).

In addition, many in vitro studies have been found that the HN001 strain of *Lb. rhamnosus* has several beneficial effects on human health. Dietary intake of *Lb. rhamnosus* HN001, originally obtained from milk, appears to improve systemic cellular immune responses, which may be beneficial as a nutritional supplement to boost immune system (Sheih et al., 2001; Gill and Rutherfurd, 2001).

Furthermore, another in vitro work proved that *Lb. rhamnosus* HN001 has strongly inhibited the adhesion of *Gardnerella vaginalis*, which causes bacterial vaginosis, to the line of cancer cells and in vitro development. This research found that vaginosis was decreased by the *Lb. rhamnosus* HN001 and by probiotic combination of *Lb. rhamnosus* HN001 and *Lb. acidophilus* (Jang et al., 2017).

2.3. Ready to Eat Food

Nowadays, the change in consumer preferences and fast living conditions has created a market for quickly eaten, high-quality, fresh, minimum-calorie and nutritionally healthy foods. In the fruit and vegetable market, the need to respond to this demand has arisen. Recently, ready-to-eat foods are winding up exceptionally well known in worldwide due to their convenience and ready for directly consumption without prior preparation or cooking. Some ready-to-eat foods may be preserved for at least one year without using refrigeration. This fact may decrease the shelf life concerns, considerably. This situation is accepted as highly significant development on food industry (Dudeja et al., 2016). However, ready-to-eat foods may still contain living organisms or hazardous substances due to the addition of some substances. Furthermore, the shelf-life of ready-to-eat fruits is restricted as a result of physical damage, dehydration, microbial degradation, and the development of unpleasant taste

and odor ingredients (Olivas and Barbosa-Cánovas, 2005; Perera et al., 2010; Rojas-Graü et al., 2008). The main aim of the ready-to-eat fruits and vegetables is to keep them fresh.

Many ways have been tried in the food industry to increase the shelf life of perishable fruits and vegetables. Various techniques such as modified atmosphere and controlled temperature applications, edible film coating, enzyme inhibitors, reactive oxygen traps, acidity regulators, competing substrates and the incorporation of interacting substances with browning reaction intermediates are performed in order to increase the shelf life of these fruits and vegetables (Garcia and Barrett, 2002; Olivas and Barbosa-Cánovas, 2005).

One of these techniques is modified atmosphere storage, which is an effective technique in inhibiting microbial growth and decrease the decay rate of fruits however it can diminish strawberry color and this can prompt dissatisfaction in buyer (Caleb et al., 2013; Harker et al., 2000; Smith and Skog, 1992).

Another effective technique to raise the shelf life of perishable fruits and vegetables is edible film coating. Edible film coating is a thin layer of natural polymer for coating on food products as a barrier. Using natural polymer on edible films is a significant vantage instead of using synthetic polymers (Ebrahimi et al., 2018). Edible coatings meet the consumer's expectation of preserving the fresh properties of the product for a long time without any chemical preservatives. The use of natural edible film coatings is therefore a process which can fulfill consumer standards and is extensively utilized nowadays (Garcia and Barrett, 2002; Olivas and Barbosa-Cánovas, 2005).

2.4. Edible Film Coating

Over the last decade, interest in the production and use of bio-based packaging materials has been increasing significantly to extend shelf life and improve the standards of fresh, frozen and processed food products (Diab et al., 2001). It is born of the demands of consumers for high quality, safety and healthy food and for more environmentally friendly preservation technique. Edible films and coatings can be useful in increasing product's shelf life while preventing spoilage and protecting the sensory and nutritional qualities of food.

Edible films and coatings have been used for centuries to improve food quality and shelf-life. In fact wax-coated lemon and orange was practiced in China between the 12th and 13th centuries. Whereas the Chinese could not precisely decide how the storage time increased by decreasing the respiratory rate of fruits by edible films, they concluded that the storage time of coated fruits were higher than uncoated ones. Moreover, waxes were commercially used in the 1930's to cover apples and pears (Park, 1999). Many other different film ingredients have been used in coating of fruits and vegetables in previous decades, and their effects on shelf life have been investigated.

Edible coating is one of the efficient and popular methods with several functions, such as controlling respiration, carrier for antimicrobial agents, antioxidants to extend shelf life of food products. Edible films can be used to preserve perishable food products from degradation by retarding dehydration, creating a selective barrier to moisture , oxygen and carbon dioxide, restricting respiration, increasing the quality of texture, helping to preserve volatile flavor compounds and reducing microbial production (Lee et al., 2003).

Edible films and coatings, which supply a protective layer, could increase product's shelf life while protecting their sensory and nutritional qualities by preserving quality and controlling the inside atmosphere of the product. They create a barrier around the fruit and this barrier slows down respiration, retards dehydration and reduces oxidation and rancidity.

The water vapor and gases barriers between food and surrounding provide preservation of food (Guimarães et al., 2018). Water vapor barrier retards dehydration and good gas barrier reduces oxidation and rancidity.

Although edible coating is not popular in usage as much as traditional forms of packaging, it has several advantages. By the help of edible films and coatings, it is feasible to enhance functionalities of food products such as adding nutrients, antioxidants, vitamins, minerals, prebiotics, and probiotics or antimicrobial agents that can delay spoilage microorganisms (Guimarães et al., 2018). In addition, many positive aspects of edible coatings are as listed below (Zuhal et al., 2018):

- Edible & biodegradable
- Enhancing the view by giving brightness to the outside of product
- Decreasing weight loss
- Applicable to various innovations and technologies
- Ensuring product's texture preservation
- Lowering respiration rate and ethylene creation and in this way postponing the ripening
- To protect fruits and vegetables from cold-caused injuries,
- Lowering microbiological-caused damage

- To protect nutritional value of product by preserving aromatic constituents, nutrients and antioxidant agents, anthocyanins, colors and reducing their browning reactions.

Although there are many benefits of edible coatings, increased cost, allergic symptoms due to the film materials, applicability of materials, need for a second packaging material could be disadvantages (Dhall, 2013). Also, when the edible coating material is thick, this situation causes some negative effects on the coated material due to low internal oxygen level and high carbon dioxide level (Dhumal and Sarkar, 2018). The following ways can be followed to prevent negative effects;

- building up a few edible film coatings,
- controlling the moisture gradient of edible coatings,
- estimating the gas pervasion properties of chosen coatings,
- estimating the diffusion properties of fruits & vegetables,
- anticipating inner gas balance for fruits & vegetables covered with edible films,
- analyzing coating impacts on the quality changes of fruits & vegetables (Park et al., 2014).

Edible coating technique have been effectively applied in processed foods like meat, cereals, confectionaries, dried fruits, nuts and fresh and fresh-cut fruits and vegetables (McHugh and Avena-Bustillos, 2011).

2.4.1. Edible coating techniques

Edible coatings can be applied to fruits and vegetables through various methods. The following techniques are, dipping, brushing, extrusion, spraying and solvent casting.

In fruits and vegetables, the most common application of edible coating technique is dipping. Application is simple and fruits or vegetables are dipped in about 1-5 minutes into the coating solution and then left to drying. Brushing technique also generates good outcomes when applied to beans and perishable vegetables and fruits. In food industry, additional three methodologies which are spraying, extrusion, and solvent casting are also preferred (Sharma et al., 2019).

2.4.2. Coating materials

The physical and chemical properties of the biopolymers used in the films and the active substances added to the mixture have significant effect on the coated material. For this purpose, the film material must be chosen according to the product. Some properties should be considered when choosing a film material. A film material should be simple to apply, non-toxic, recognized as safe (GRAS) and have strong mechanical properties, reasonable sensory characteristics, low oxygen permeability. Moreover, the material's water vapor permeability must be taken into consideration to prevent product from drying out (Park, 1999; Park et al., 2014; Rodrigues and Fernandes, 2012).

Edible coatings are typically categorized according to their structures. They can be protein, lipid or polysaccharide based or combination of them (Zuhal et al., 2018). Some examples of these film materials are given:

- Protein based biopolymer film materials: Collagen, gelatin, casein, whey protein, wheat gluten, soy protein, egg albumin etc.
- Polysaccharide based biopolymer film materials: Starch, modified starch, modified cellulose, alginate, carrageenan, agar, pullulan, pectin, chitosan, gellan, gum and xanthan gum.
- Lipids based biopolymer film materials: Animal and vegetable oils, waxes (beeswax, paraffin, carnauba wax, candelilla wax, jojoba), resins (guarana, shellac, terpene) and emulsifiers, surface agents (fatty acids, lecithin).

2.4.2.1. Gelatin

Gelatin is a kind of protein, produced from collagen by thermal degradation. While gelatin has biodegradable property, it has also good mechanical properties for film forming as a coating material (Liao et al., 2009).

The major industrial sources of gelatin are cattle bones, hides, pig skins, fish and, lately, insects (Mariod and Fadul, 2013). It is typically produced from collagen sources, which are accessible for the manufacturer both in supply and at a fair price.

Gelatin is an essential hydrocolloid that has common use in food industry due to its gelling and thickening abilities. Gelatin differs from other hydrocolloids, which many of them are polysaccharides, since it is a digestible protein which includes all essential amino acids besides tryptophan (Fakhouri et al., 2015). Gelation, stabilization, emulsification, adhesivity and sedimentation are particular functional uses of gelatin in food industry (Baziwane and He, 2003).

Gelatin is one of the most water-soluble polymers in the food industry which can be used as gelling, thickening, or stabilizing agent (Baziwane and He, 2003) and it is considered as generally regarded as safe (GRAS). It is able to form a sustainable and three-dimensional gel (Mariod and Fadul, 2013). It makes thermally reversible gels with water, and the melting temperature of gel is below body heat, by this means it gives special organoleptic properties to gelatin and flavor discharge (Glicksman, 1969). The effectiveness of the gel varies on the amount of gelatin and its type. Two main forms of gelatin exist obtained from porcine (type A) and bovine (type B) (Mariod and Fadul, 2013). Nevertheless, gelatin is marketed with a wide variety of special properties to suit specific applications, such as gel strength and viscosity. However, gelatin is an ideal environment of proliferation for most bacteria and therefore strict hygiene is required during processing.

2.4.2.2. Prebiotic

Prebiotics are described as "a non-digestible food component that helps the host by selectively promoting the growth and/or function of one or a limited number of bacteria in the colon" (Gibson and Roberfroid, 1995).

In order to identify a food product as a prebiotic:

1) It must not be hydrolyzed or absorbed in the upper part of the GI tract;

2) It must be a suitable substrate for one or a small number of beneficial symbiotic organisms to the colon and triggered to develop and/or active metabolically;

(3) It must also be able to modify the flora of the colonies in favor of a healthier formation;

(4) It must cause luminous or systematic outcomes which are beneficial for host health (Gibson and Roberfroid, 1995).

Non-digestible carbohydrates (oligo- and polysaccharides), several peptides and proteins, and some lipids (ethers and esters) are the potential food sources for prebiotics due to they cannot be hydrolyzed by human digestive enzymes and cannot be absorbed by GI tract's upper part. But as soon as they reach the large bowel, at least some of the colonic bacteria ferment the prebiotics (Roberfroid, 2003). This fermentation generates excreted gases, and short-chain fatty acids, which the host can absorb. Short-chain fatty acids play a role in cell differentiation, cell reproduction and regulatory metabolic processes (Roberfroid, 2003). Today, it has been known that the prebiotics most commonly used in the food industry are fructo-oligosaccharide, galacto-oligosaccharide and inulin (Sezen, 2013).

There are many functional effects of prebiotics and some are stated in below;

- Increase absorption of calcium/other minerals
- Increase production of short-chain fatty acids.
- Stimulate probiotic bacteria,
- Increase the faeces volume,
- Increase healthy microbiota in colon
- Reduce caloric value
- Raise folate and B vitamins
- Drop cholesterol and triglycerides

- Improve immune reaction
- Decrease chance of colon cancer risk
- To have an energy value less than 9 kJ/g,
- Not being digested in metabolism (Anadón et al., 2016; Sağdıç et al., 2004).

2.4.2.2.1. Inulin

Inulin is a water-soluble polysaccharide and derived from plant sources only. Inulin was described as a polydisperse carbohydrate material composed mainly, if not exclusively, of β (2→1) fructosyl-fructose links (Flamm et al., 2001).

It is mainly present in vegetables like onion, garlic, leek, artichoke, and chicory. However, chicory roots are seen as the main source of inulin (Yabancı, 2010). Inulin is an indigestible carbohydrate and it is widely used in many sectors, especially in the food industry due to its positive effects on human health and product.

Inulin gained the status of GRAS worldwide. It is widely used as a prebiotic, fat substitute, sugar substitute, texture modification and also for the production of nutritious foods to promote health due to its advantages on gastrointestinal health (Shoaib et al., 2016).

In the studies conducted, researchers stated that fructo-oligosaccharides could not be metabolized by *Escherichia coli* and *Clostridium difficile*, which also supports the reproduction of *Lactobacillus* and *Bifidobacterium* in the digestive system (Glibowski and Kowalska, 2012). Another research showed that adding inulin with oral administration to the diet of a group of obese people, decreases reduced total

cholesterol, LDL cholesterol and trygliceride levels in dyslipidemic and obese subjects, without modifications in the insulin sensitivity (Balcázar-Muñoz et al., 2003).

Furthermore, another advantage of inulin for its use in food processing is that the inulin does not raise the caloric content of the food product and does not affect the sensorial properties (Debon et al., 2010). To summarize, inulin; is a low-energy, prebiotic, oligo-fructose that can be widely used in food industry.

2.5. Aim of the Study

The aim of this thesis was to improve shelf life of strawberries by using edible coating technique and to enrich the strawberries with probiotics. The thesis objectives involve:

- To investigate the effect of probiotic incorporated edible films on the physicochemical and microbial quality of strawberry during refrigerated shelf life by using gelatin and inulin as coating materials;
- To investigate the combined advantages of two substantial subjects those are probiotics and edible films;
- To increase shelf life of strawberries;
- To investigate the survival of probiotics on fresh strawberry during storage.
- To investigate the potential usage of probiotics in edible film coatings on a non-dairy product.

3. MATERIALS AND METHODS

3.1. MATERIALS

Fresh strawberries (*Fragaria* × *ananassa*) were obtained from a greenhouse in Antalya, Turkey and were taken to the laboratory keeping cold chain. Then the uniform-sized fruits without decay and visual fungal growth were selected and stored at 4^{o}C until the experiments. Gelatin was supplied from SelJel, Balıkesir (Turkey). Inulin was purchased from Beneo Orafti, (Belgium) and glycerol was purchased from Evyap (Turkey). Folic acid was obtained from Gnosis (Milan, Italy). Sodium hydroxide and sodium bicarbonate were purchased from Sigma Aldrich (St. Louis, MO, USA). *Lactobacillus rhamnosus* HN001™ (HOWARU *Rhamnosus* 450B, HN001™, Danisco US – Madison Plant, Material No: 1245378) was used as the probiotic microorganism. Man, Rogosa and Sharpe (MRS) agar, plate count agar (PCA) and potato dextrose agar (PDA) were obtained from Merck (Darmstadt, Germany). The MRS broth was sold from VWR International.

3.2. METHODS

3.2.1. Preparation of *Lb. rhamnosus* HN001 culture

All culture media, glassware, pipette tips, centrifuge tubes and buffer solutions were sterilized at 121°C for 15 min. Freeze-dried cells of *Lactobacillus rhamnosus* HN001 were rehydrated in 10 ml sterile peptone solution and after vortex, bacterial aliquots were activated into MRS broth in 3 sterile 10 ml tubes. Tubes were incubated at 37^{o}C for 48 hours. Then, 0.9 ml of culture was transported in 32 sterile tubes, each containing 40 ml of MRS broth. These tubes were kept at 37^{o}C for 48 hours in

anaerobic jars. Pellets were collected from the tubes and the initial cell concentration was determined on MRS agar. The tubes were centrifuged at 4000 rpm, 4°C for 20 minutes. The supernatant was discarded and 20 ml of sterile water was added. Tubes were centrifuged and bacteria washed again. In this way, the broth was completely removed. Then 20 ml of sterile water was added to each of the settled bacteria and vortexed. For each probiotic including film solution, 16 pellets were used.

3.2.2. Preparation of film formulations and sample treatments

To prepare the coating solutions, gelatin was dissolved in distilled water 5 g/100mL by gently stirring at 40°C until the solution became clear. Glycerol was used at a concentration of 0.15 g/g. Film concentrations were determined according to the data obtained from the preliminary trials and academic researches. Four different treatments were applied as follows and given in Table 1: (1) distilled water (control), (2) 5% gelatin (G), (3) 5% gelatin + probiotic (G-P), (4) 5% gelatin + 2.5% inulin + probiotic (G-I-P). To formulate gelatin-probiotic (G-P) and gelatin-inulin-probiotic (G-I-P) coatings, gelatin, gelatin-inulin were mixed aseptically with the biomass sediment of *Lb. rhamnosus* HN001 to obtain suspension of about 10^{11} colony forming units (CFU) per mL. Then, fruits were dipped in the coating solutions for 1 minute and then dried at room temperature till there were no drops on the surfaces. Uncoated samples were dipped into distilled water as control. All treated and untreated strawberries were placed in perforated PET packages (clamshell PET containers with a volume of 500 mL and low oxygen and moisture permeability) and stored in darkness at 4°C for 16 days. Due to the fact that the single package is inconvenient commercially, it was found appropriate to place 10 strawberries in each package. At each sampling day (0, 4, 8, 12

and 16 for physiochemical analyses and 0, 5, 10, 15 for microbiological analyses) three containers of each treatment were removed from storage and used for analyses.

3.2.3. Microbial quality and fruit decay

3.2.3.1. Total yeast and mold

Total yeast and mold counts were performed on 0, 5, 10, 15 days of storage for each treatment by using the spread plate method. For this, 10 grams of strawberries were randomly selected and cut into tiny pieces and suspended in 90 mL of peptone water (Maximum Recovery Diluents). The suspension was blended with Bag Mixer in a Stomacher bag. Serial dilutions (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}) were made and plated on to PDA as two parallels and they were incubated at 28°C for 5 days for yeasts and mold counts. After incubation, the results were given as log CFU/g (logarithm colony forming units per gram) of strawberries.

3.2.3.2. Total aerobic mesophilic bacteria

Total aerobic mesophilic bacteria counts were performed during storage (0, 5, 10, 15 days) for each treatment by using the spread plate method. 10 grams of strawberries were selected and blend in 90 mL of peptone water (Maximum Recovery Diluents) with Bag Mixer in a Stomacher bag. Dilution tubes containing 9 ml peptone water were prepared and serial dilutions (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}) were made and plated onto plate count agar (PCA) in duplicate and they were incubated at 25°C for 2 days. Serial dilutions for two treatments without probiotic were determined as 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}. For the other two treatments including probiotic, dilutions were determined as 10^{-6},

10^{-7}, 10^{-8} and 10^{-9}. After incubation, results were expressed as log CFU/g of strawberries.

3.2.3.3. Determination of viable *Lb. rhamnosus* HN001

Viability of *Lb. rhamnosus* HN001 analyzes was performed on 0, 5, 10, 15 days of storage for each treatment by using the spread plate method. The effect of storage on the survival of *Lb. rhamnosus* HN001 in samples was determined. For this purpose, 10 grams of strawberries were chosen and mixed with 90 mL of peptone water in a Stomacher bag. Serial dilutions (peptone water) were prepared (from 10^{-2} to 10^{-9}) and plated into MRS agar (containing vancomycin with a concentration of 20 µg/ml) (Björneholm et al., 2002). Plates were placed in anaerobic jars and incubated 37°C for 48 hours. After incubation, results were expressed as log CFU/g of strawberries.

3.2.4. Physiochemical quality during storage

3.2.4.1. Weight loss

Samples were weighed at the end of each sampling day. 10 strawberries were used for each treatment. Weight loss was calculated as the percentage difference between the initial weight and the final weight of the strawberries. Weight loss was calculated as follows:

$$Weight\ Loss\ \% = \frac{(initial\ weight - final\ weight)}{initial\ weight}\ x\ 100$$

3.2.4.2. Fungal decay

The evaluation of the decay rate was done according to Alvarez et al. (2018). Fungal decay was visually observed in 10 strawberries per treatment on 0, 4, 8, 12 and 16 days. While making the physical observation, softening, spoilage and brown spots of fruits were marked to be infected. The results were given as the percentage of infected fruits (Alvarez et al., 2018).

3.2.4.3. Determination of pH

The juice of strawberries was obtained by using a kitchen blender than filtered from a porous cloth. The pH values of the juices were determined during storage in sampling days. A pH-meter (WTW Inolab Ph-7310P, Germany) was used for the determination of the pH value of the juice.

3.2.4.4. Titratable acidity rate

The juice of strawberries was obtained by using a kitchen blender than filtered from a porous cloth. Titratable acidity (TA) of the juices was determined during storage in sampling days. TA was measured on 5 ml juice by adjusting the pH to 8.2 with 0.1 M NaOH. Phenolphthalein was used as an indicator. The results were expressed as citric acid equivalent by calculated with following formula:

$$\%\, Asit = \frac{(mL\ NaOH\ used)x\ (0{,}1\ N\ NaOH)x\ (milliequivalent\ factor)}{grams\ of\ sample} x100$$

3.2.4.5. Total soluble solid content

The juice of strawberries was obtained by using a kitchen blender than filtered from a porous cloth. The total soluble solids (TSS) of the juices were determined during storage in sampling days. The TSS content of juices was obtained by using a digital refractometer (Rudolph J257, USA) at 25^{o}C.

3.2.4.6. Total phenolic content

Total phenolic content of strawberries was measured on the sampling days for each treatment by using Folin Ciacalteu Method (Panico et al., 2009; Singleton and Rossi, 1965). For each group, 10 grams of strawberries were randomly selected and suspended in 200 mL of 80% methanol with the aid of blender for 10 minutes. The suspensions were shaken in a 40^{o}C water bath for 2 hours. The obtained suspensions were filtered with the aid of filter paper. 0.5 ml sample was mixed with 2.5 ml 10% Folin Ciacalteu reagent and 2 ml 7.5% Na_2CO_3. The samples were vortexed and waited in a water bath at 50^{o}C for 5 minutes. Samples taken from the water bath were kept in the dark until they reach room temperature for 10 minutes. The absorbances of samples were measured at 765 nm with a Shimadzu UV-1800 model spectrophotometer. The calibration curve was constituted by the gallic acid standard as given on the Figure 3.1. Total phenolic content was expressed as mg gallic acid equivalents per kg fresh weight (mg GAE/kg fw).

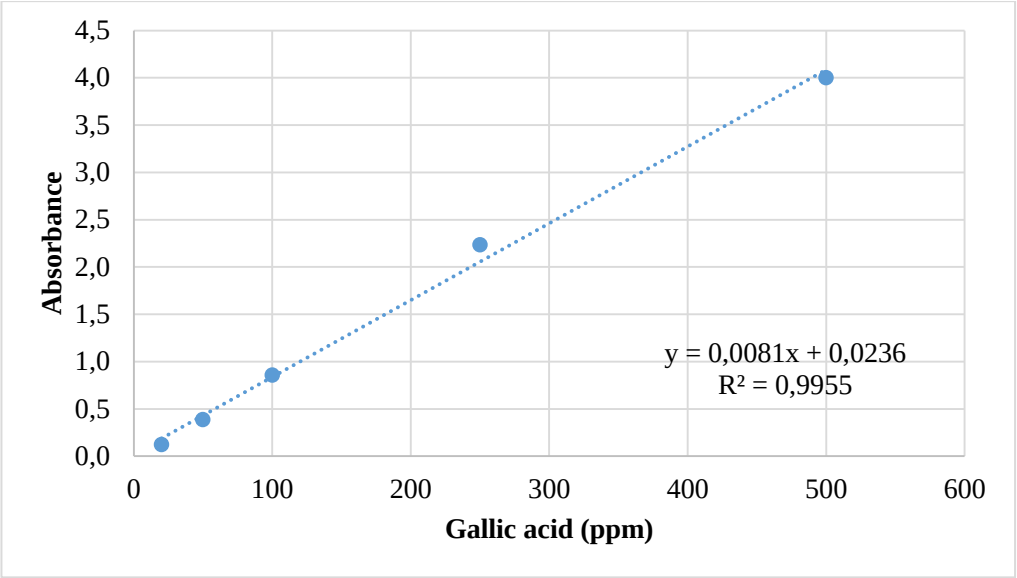


Figure 3. 1 Gallic acid standard curve prepared according to Folin Ciocalteou method.

3.2.4.7. Antioxidant activity

Total antioxidant activity of strawberries were analyzed according to the method given by Hangun-Balkir and McKenney with some modifications (Hangun-Balkir and McKenney, 2012). For each treatment, 10 grams of strawberries were randomly selected and suspended in 200 mL of 80% methanol with the aid of blender for 10 minutes. Then the suspensions were shaken in a 40^0C shaken water bath for 2 hours and then filtered. The 200 µl filtered sample was added on 3.80 mL of diphenylpicrylhydrazyl (DPPH) solution. The samples were kept in the dark for 30 minutes. The absorbances were measured at 515 nm by using a spectrophotometer (Shimadzu UV-1800, Japan). The calibration curve was constituted by the Trolox standard as given on the Figure 3.2. and the antioxidant activity results denoted as mMol Trolox eq./kg fresh strawberries.

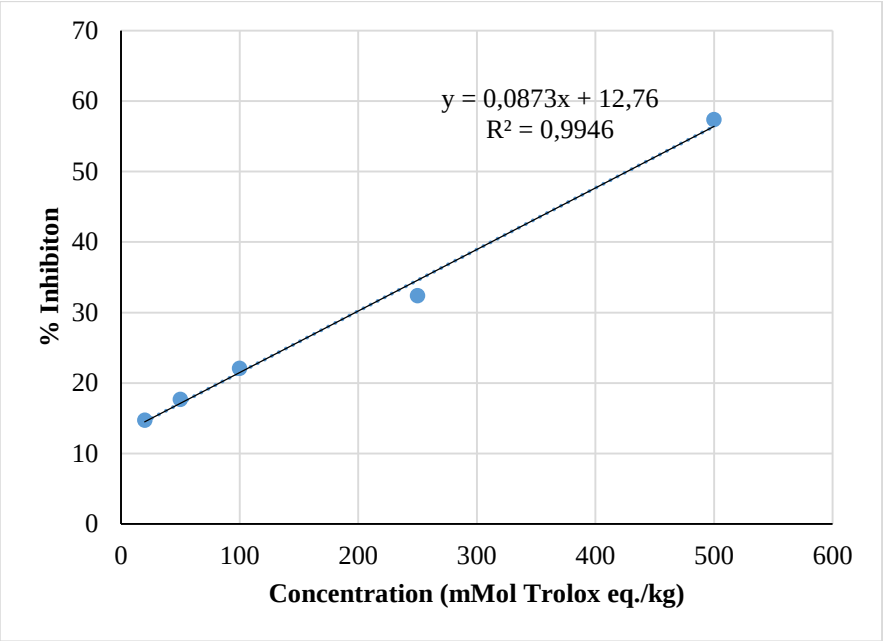


Figure 3. 2 Trolox standard curve prepared according to DPPH method.

3.2.5. Statistical analyses

The results were given as average ± standard deviations. Significant difference ($p<0.05$) were evaluated by Tukey's test via analysis of variance (ANOVA). Independent sample t-test was used to compare gelatin-probiotic and gelatin-inulin-probiotic coated samples for *Lb. rhamnosus* HN001 survival. Statistics of the experiments were done by using Minitab 17.

4. RESULTS & DISCUSSION

4.1. Physiochemical Quality During Storage

4.1.1. Weight loss

Weight loss percentages of control group and three different treatments which are (1) 5% gelatin (G), (2) 5% gelatin + probiotic (G-P), (3) 5% gelatin + 2.5% inulin + probiotic (G-I-P), were determined on 4°C throughout 16 days period. The results are given in Figure 4.1.

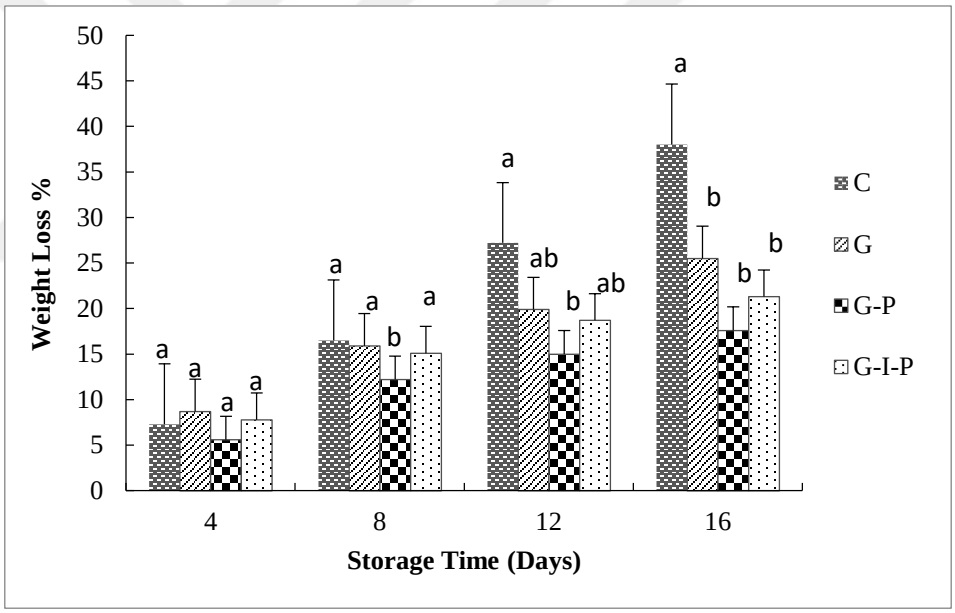


Figure 4. 1 Weight loss percentage of strawberries during 16 days storage at 4°C.

One of the important factors for measuring the shelf-life of strawberry is weight loss and the major cause for weight loss is water vaporization from strawberry skin (Jiang et al., 2020). The epidermis layer and stomata of the fruits provide moisture loss and gas exchange, which is the main cause of decay and perishability of the strawberries

(Luksiene and Buchovec, 2019). This leads to the dehydration of fruits and reduce shelf-life (Dhital et al., 2018; Luksiene and Buchovec, 2019).

Weight loss increased throughout storage for all samples as shown in Figure 4.1. Although an increase in weight loss was observed in all applications during storage including control group, it was found that the weight loss in the coated samples was relatively low compared to the control samples. The most significant feature that separates strawberries from other fruits which have short shelf-lives is the absence of an outer protective layer. Since strawberry fruits have very thin skin, rapid water loss by moisture evaporation occurs. Loss of weight due to moisture evaporation depends on the resistance of the fruit surface to vapor diffusion. It also depends on the difference in vapor pressure between the fruit tissues and the atmospheric ambient air, which is measured by temperature and relative humidity (Kader, 2002).

According to the Figure 4.1., there was no statistically significant differences ($p > 0.05$) observed in weight loss on the first days of storage time. Weight loss in gelatin-probiotic coated strawberries was significantly lower than other samples after 8 days of storage. At the end of the storage time, it was observed that uncoated (control) treatment has the highest weight loss 38% while it was 24.7%, 17.6%, and 21.3% in gelatin, gelatin-probiotic, gelatin-inulin-probiotic coated samples, respectively. Probiotic addition resulted in less weight loss compared to only gelatin coated strawberries. The results showed that gelatin-based coatings decline the respiration rate and the water loss of strawberries hence retarded the perishability of the strawberries. In literature, it was reported that edible films have low oxygen and carbon dioxide permeability in order to decrease respiration and metabolic activity, retarding decay (Sharma et al., 2019).

In another study, strawberries were coated with pectin, beeswax and gelatin. While a group of the coated and control strawberries had been stored at room temperature, the other group had been stored at refrigerated temperature and analyses had been performed. Similar to our results, among all strawberries, 21% weight loss was observed in gelatin coated strawberries after 12 days of storage at refrigeration temperatures as a best option. (Barrazueta-Rojas et al., 2018). Gelatin is a hydrophilic substance and the hydrophilic substances that established the film coating may have assisted with keeping up much more humidity in the surface of the fruit tissue, therefore it may help maintaining from dehydration by not allowing the sample to loose water (Vargas et al., 2008).

Our results also showed less weight loss compared with values reported study about the chitosan, pectin and pullulan based coated strawberries (Treviño-Garza et al., 2015). All coated strawberries in this study showed a weight loss of over 35% after 15 days of storage at refrigerated temperature. Moreover, our results demonstrated less weight loss than another gelatin based edible coating containing cellulose nanocrystals on strawberry fruit (Fakhouri et al., 2014). This difference may be related with the relative humidity of storage condition or the polymer concentration in film formulations (Garcia et al., 2010).

4.1.2. Fungal decay rate

Fungal infection has a quite significant role on strawberries' shelf life. Senescence makes the product more sensitive to pathogenic infection due to tissue loss. However, film coatings can limit deterioration by slowing down senescence. The fungal decay rate

of strawberries for each treatment groups were observed throughout storage for 16 days and shown in the Figure 4.2.

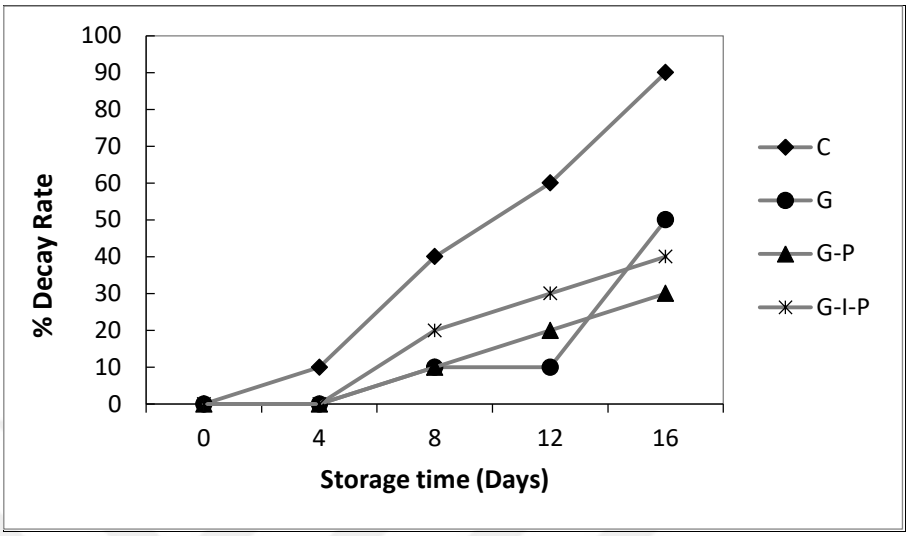


Figure 4. 2 % Fungal decay rate of strawberries during 16 days of storage at 4^{o}C.

The fungal decay rate of strawberries increased substantially for all samples during storage time. In control samples fungal decay was observed almost for all fruits at the end of storage time while in coated samples less rotting was observed. Among the coated samples, G-I-P and G-P coatings resulted in less decay rate compared to solely gelatin coatings. In the final day of storage, fungal decay rate of C, G, G-P and G-I-P fruits were found as 90%, 50%, 30% and 40%, respectively. Antimicrobial activity of *Lb. rhamnosus* could be one of the major reasons of retarding spoilage of strawberries. Our results in a similar pattern that the presence of *Lb. rhamnosus* positively affected the shelf-life due to it has antimicrobial activity and restricts the proliferation of pathogens (Ambalam et al., 2009). *Lb. rhamnosus* HN001 is also known with its antifungal mechanisms. In a scientific research, the effect of *Bifidobacterium longum* BB536 and *Lb. rhamnosus* HN001 against gram-negative, gram-positive bacteria and Candida, a pathogenic yeast-type fungus, was investigated. The research indicated that

Bifidobacterium longum and *Lb. rhamnosus* HN001 displayed inhibition against the genus Candida and slows down their growth. By this means, it had been found that the use of *Bifidobacterium longum* and *Lb. rhamnosus* HN001 can be a promising approach to treatment of gastrointestinal and vaginal pathogens (Inturri et al., 2019).

The results obtained in this thesis show that visual decay rate of strawberries were less than the values obtained in another study which investigated 4% gelatin based edible coatings enriched with different concentrations of *Mentha pulegium* oil (MPO) (Aitboulahsen et al., 2018). The researchers found that even the most efficient treatment preserve 60% of fruits from rotting after 13 days. The variation between the studies may originate in the different gelatin concentration or the addition of MPO. In addition, Blake (1966) stated that there are several scenarios where coatings can potentially rise rotting, including high spore load from the field on the fruit or poor sanitary conditions in the packaging (Tanada-Palmu and Grosso, 2005). Additionally, there are situations where coating can be so repressive to gas exchange that it can cause physical problems on the surface, likely by causing fermentation and toxic metabolite formation. This condition can result in tissue apoptosis and can increase the amount of rotting (Risse et al., 1987).

The samples' images were also given in Figure 4.3. The decay rate can be also confirmed visually from the images given in Figure 4.3.

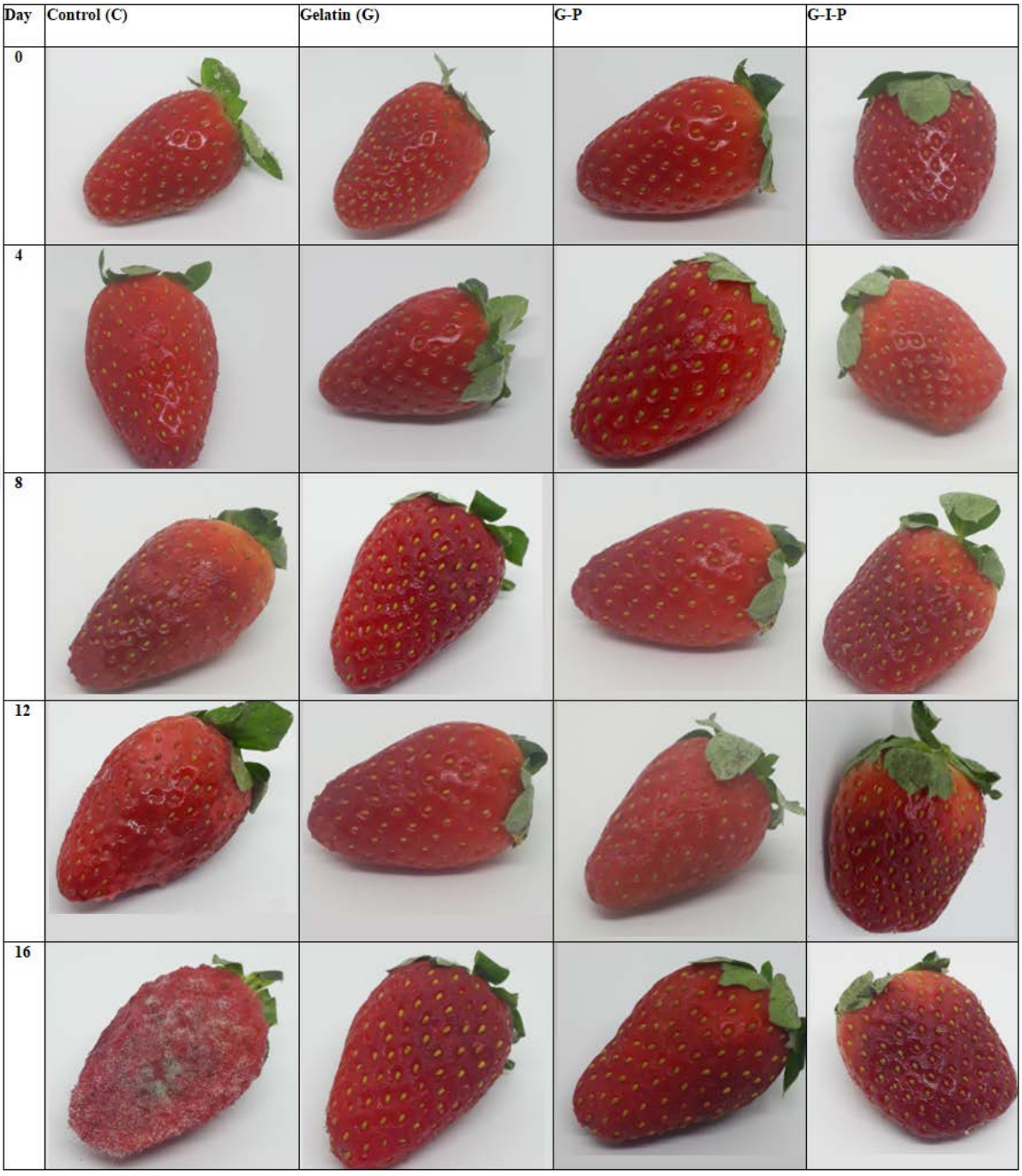


Figure 4. 3 Appearances of coated and uncoated strawberries during 16 days of storage at 4^{o}C.

4.2. Microbial Quality During Storage

4.2.1. Yeast & mold

The effects of different coatings on yeast and mold growth on strawberries are given in Table 4.1.

Table 4.1 Effect of different treatments on total yeast and molds (log CFU/g) on strawberries during storage at 4°C for 15 days (n=3)[*]

Time	Treatment			
Day	**C**	**G**	**G-P**	**G-I-P**
0	$3.70^{Aa} \pm 0.44^{*}$	$4.00^{Aa} \pm 0.05$	$3.79^{Aa} \pm 0.01$	$3.90^{Aa} \pm 0.14$
5	$4.74^{Bb} \pm 0.10$	$4.35^{ABb} \pm 0.12$	$4.28^{Ab} \pm 0.05$	$4.69^{ABb} \pm 0.27$
10	$6.00^{Bc} \pm 0.07$	$5.48^{Ac} \pm 0.19$	$5.32^{Ac} \pm 0.18$	$5.34^{Ac} \pm 0.13$
15	$6.44^{Bc} \pm 0.08$	$5.93^{Ad} \pm 0.10$	$5.80^{Ad} \pm 0.20$	$5.77^{Ac} \pm 0.17$

[*]Different superscript lowercase letters (within each column) show differences between the storage times within the same analysis group ($p < 0.05$). Different superscript uppercase letters (within each row) show differences between treatment groups within the same analysis day ($p < 0.05$).

The number of yeasts and molds increased in all samples during 15 days of storage. It was observed that in the control strawberries as 3.70 CFU/g in the first day of storage and increased to 6.44 CFU/g in the end of storage time. The growth of yeasts and molds in fresh produce is undesirable due to their ability to generate different enzymes which can deteriorate the food and affect the sensory quality of the product (Bierhals et al., 2011).

The maximum level of yeast and mold in fresh fruits was reported as 10^6 CFU/g by Institute of Food Science & Technology (Azarakhsh et al., 2014). Total yeast and mold counts in the all coated strawberries were found to be lower than control. Although there were no statistically difference between coated strawberries, G-P and G-I-P coated groups had numerically lower yeast and mold than G group. Moreover, it had been found that all coated groups were statistically different than control fruits. While the count was 3.90 CFU/g at the first day of storage, was increased to 5.77 CFU/g at the end of storage. This could be associated with *Lb. rhamnosus* since it produces antimicrobial agents. Because LAB produce bacteriocins, organic acids, hydrogen peroxide, and diacetyl, they can decrease pathogens and microorganisms that cause decay in fresh fruit (Herreros et al., 2005). Based on these results, the counts in control group were found to be quite high as 6 log CFU/g on the 10th and 15th days, the yeast and mold load of all other coated strawberries was lower than 6 log CFU/g even in the final day. In the control samples, wet (watery) appearances were observed on the last day. However, although the coated strawberries lose their shine, no wet (juicy) appearance is observed. This may be related with dehydration rate which was slower in coated strawberries than control ones. Since edible coatings create a barrier around the fruit, this barrier slows down respiration rate and retards dehydration. In addition, probiotic creates a competitive environment for other microorganisms due to resource consumption. For this reason, it may have caused a decrease in the number of yeast and mold.

The similar pattern was observed in another study (Shahbazi, 2018), which reported that strawberries coated with carboxymethyl cellulose and chitosan enriched with *Mentha spicata* were strongly repressed the growth of yeasts and molds during 12

days of storage. Our findings indicate that gelatin-based coatings have a beneficial effect inhibiting yeast and mold growth on strawberry samples.

In several different experiments, similar findings were obtained. In a study performed with fresh melon slices, it was realized that lactic acid bacteria helped to prevent survival and production of microbial pathogens (de Oliveira et al., 2014). In another study, Russo et al. (2015) demonstrated that although probiotic enriched fresh-cut cantaloupes had less yeast and molds regard to uncoated fruits, the presence of probiotics was not statistically affected. Similarly, the effect of *Lb. rhamnosus* GG on the quality of fresh-cut pear treated with $CaCl_2$ was investigated in a different study. Bio-preservative effects of *Lb. rhamnosus* towards harmful microorganisms were proved in this experiment (Iglesias et al., 2018).

4.2.2. Total aerobic mesophilic bacteria

Total aerobic mesophilic bacteria count of each treatment was determined and given in the Table 4.2 below.

Table 4. 2 Effect of different treatments on total aerobic mesophilic bacteria count (log CFU/g) on strawberries during storage at 4°C for 15 days (n=3)*

Time	**Treatment**			
Day	**C**	**G**	**G-P**	**G-I-P**
0	$4.87^{Aa} \pm 0.22^{*}$	$5.90^{Ba} \pm 0.10$	< 2	< 2
5	$5.18^{Aa} \pm 0.25$	$6.03^{Ba} \pm 0.16$	< 2	< 2
10	$7.94^{Cb} \pm 0.14$	$6.78^{Bb} \pm 0.30$	$4.78^{Aa} \pm 0.12$	$4.41^{Aa} \pm 0.11$
15	$8.33^{Cb} \pm 0.20$	$7.62^{Bc} \pm 0.16$	$6.49^{Ab} \pm 0.18$	$6.13^{Ab} \pm 0.06$

*Different superscript lowercase letters (within each column) show differences between the storage times within the same analysis group ($p < 0.05$). Different superscript uppercase letters (within each row) show differences between treatment groups within the same analysis day ($p < 0.05$).

In the presence of *Lb. rhamnosus* HN001, coated strawberry groups' total aerobic mesophilic bacteria were counted as 6.13 log CFU/g and 6.49 log CFU/g in G-I-P coated fruits and G-P coated fruits at the final day of storage respectively. As it can be seen on the Table, there is no significant difference between these two treatments, mostly due to the presence of *Lb. rhamnosus* HN001. In case of *Lb. rhamnosus* HN001 absence, gelatin coated strawberries was found as 7.62 log CFU/g at the end of the storage. Control samples' total aerobic mesophilic bacteria count were determined as 4.87 log CFU/g at the initial day and 8.33 log CFU/g at the final day. As a result of the calculations, there is a substantially difference between these two treatments and control samples has quite higher aerobic mesophilic count than gelatin based coated group. In the lights of these findings, it can be easily said that coating strawberries with gelatin restricted the aerobic microorganisms' growth. Sogvar et al. (2019) found similar conclusions in their study related with aloe vera and ascorbic acid coated strawberries. They found that coated fruits have less microbial growth than uncoated fruits during 18 days of cold storage.

4.2.3. Survival of *Lb. rhamnos*us HN001

The results of survival of *Lb. rhamnos*us HN001 during storage are given in Table 4.3. Initially, the counts of *Lb. rhamnosus* HN001 in the film coatings were 11.98 log CFU/g and 11.87 log CFU/g in the G-I-P coated samples and G-P coated samples, respectively. On the first day of storage, *Lb. rhamnosus* HN001 was determined as 10.9 log CFU/g and 11.05 log CFU/g, in G-P and G-I-P coated strawberries, respectively.

Table 4. 3 Stability of *Lb. rhamnosus* HN001 (log CFU/g) in different treatments during storage at 4°C for 15 day (n=3)[*]

Time	Treatment	
Day	**G-I-P**	**G-P**
0	$10.90^{Aa} \pm 0.425^{*}$	$11.05^{Aa} \pm 0.37$
5	$10.44^{Aa} \pm 0.93$	$10.43^{Aa} \pm 0.72$
10	$8.86^{Ba} \pm 0.12$	$8.48^{Bb} \pm 0.10$
15	$7.43^{Ca} \pm 0.06$	$7.00^{Cb} \pm 0.20$

[*]Different superscript uppercase letters (within each row) show differences between the storage times within the same analysis group ($p < 0.05$). Different superscript lowercase letters (within each column) show differences between treatment groups within the same analysis day ($p < 0.05$).

The viable cell number of *Lb. rhamnosus* HN001 decreased during storage time. However, at the end of the 15th day 7 log CFU/g and above *Lb. rhamnosus* HN001 survival was found in both coated samples which is the sufficient amounts (10^{6} - 10^{7} CFU/g) to improve consumer's health (Food and Agriculture Organization of the United Nations and World Health Organization., 2006). While the value of the G-I-P coated group on the last day of storage was 7.43 log CFU/g, this value was determined as 7 log CFU/g in the G-P coated group. It was observed that there was a significant difference between these groups on day 10 and day 15. It is assumed that this is due to the presence of inulin. Inulin is considered as a highly efficient prebiotic compound and a significant ingredient in food industry (Kolida et al., 2002). Inulin can support the reproduction of lactic acid bacteria, improves systemic metabolism and has benefits for human health related with these beneficial effects on gut microbiota (Hoffman et al., 2019). The survival of *Lb. rhamnosus* HN001 was relatively high due to the presence of inulin in the G-I-P coated group.

Similar to our research, *Lb. plantarum* was added into carboxymethylcellulose coatings and these coatings were applied on strawberries and the shelf-life of strawberries during storage period was investigated. According to the results, viable *Lb. plantarum* did not fall below 6 log CFU/g in treatments and it helped to reduce the growth of yeast and molds on the surface of strawberries compared to control samples which it is mainly due to competitive and antimicrobial properties of probiotic bacteria (Khodaei and Hamidi-Esfahani, 2019). Our results also confirm the research which reported inulin was to be the most successful prebiotic among wheat dextrin, glucose oligosaccharides and polydextrose in regulating sublethal effects on *Lb. rhamnosus* GG during storage at 4^{o}C and 25^{o}C (Soukoulis et al., 2014).

Likewise, García-Argueta et al. (2013) designed several edible film formulations with different ratio of whey, gelatin, inulin and probiotic (*Lb. casei*) and observed how the formulations affect the survival of lactic acid bacteria and reported the physicochemical and textural properties of film dispersions. They found that the best formulation was the combination of 2% inulin, 1% *Lb. casei* and 3.5% gelatin and the coating ensured highest viability environment for LAB. Also, the researchers remarked that these coatings could be performed on non-dairy foods, to increase probiotic dietary intake.

4.3. Chemical Quality during Storage

4.3.1. pH, titratable acidity and total soluble solid content

Titratable acidity of coated and uncoated strawberries is given in Table 4.4.

Table 4. 4 Effect of different treatments on titratable acidity (TA) of strawberries during storage at 4°C for 16 d. (n=3)*

Time	Treatment			
Day	**C**	**G**	**G-P**	**G-I-P**
0	$0.90^{Da} \pm 0.01$*	$0.83^{Ca} \pm 0.00$	$0.72^{Aa} \pm 0.00$	$0.80^{Ba} \pm 0.01$
4	$0.92^{Cab} \pm 0.01$	$0.86^{Bab} \pm 0.02$	$0.75^{Ab} \pm 0.01$	$0.83^{Bb} \pm 0.01$
8	$0.92^{Cab} \pm 0.01$	$0.87^{Babc} \pm 0.02$	$0.76^{Abc} \pm 0.01$	$0.85^{Bbc} \pm 0.01$
12	$0.94^{Dbc} \pm 0.01$	$0.90^{Cbc} \pm 0.01$	$0.78^{Ac} \pm 0.00$	$0.86^{Bcd} \pm 0.02$
16	$0.97^{Dc} \pm 0.01$	$0.92^{Cc} \pm 0.01$	$0.79^{Ac} \pm 0.01$	$0.88^{Bd} \pm 0.03$

*Different superscript lowercase letters (within each column) show differences between the storage times within the same analysis group ($p < 0.05$). Different superscript uppercase letters (within each row) show differences between treatment groups within the same analysis day ($p < 0.05$).

It was observed that, in all treatment groups, TA of strawberries showed a slight increase and relatively higher in control groups. Samples with gelatin based coating demonstrated lower numbers than control ones on day 0 due to the intervention from the pH of gelatin dispersion. At the end of storage, the TA values of whole groups were found statistically different from each other. Among whole groups, *Lb. rhamnosus* HN001 incorporated edible coated strawberries have less titratable acidity than the control and gelatin coated groups during cold storage. The gelatin coated fruits had also lower acidity than the control fruits, but G-P coated fruits have the least acidity.

Similar pattern was observed in the study on sodium alginate coated carrots enriched with *Lb. acidophilus*. Control group, sodium alginate coated carrots and probiotic and sodium alginate coated carrots were compared throughout storage time. Although there were no big differences, probiotic enriched ones has lower acidity in regard to others (Shigematsu et al., 2018).

The effects of different coating groups on the pH value of strawberries were investigated and presented in the Table 4.5. The pH values of strawberries increased slightly in all groups during storage.

Table 4. 5 Effect of different treatments on pH of strawberries during storage at 4°C for 16 d (n=3)*

Time	Treatment			
Day	**C**	**G**	**G-P**	**G-I-P**
0	$3.159^{Ca} \pm 0.001^{*}$	$3.208^{Ba} \pm 0.001$	$3.206^{Ba} \pm 0.002$	$3.254^{Aa} \pm 0.001$
4	$3.275^{Ab} \pm 0.002$	$3.264^{Bb} \pm 0.001$	$3.185^{Db} \pm 0.001$	$3.234^{Cb} \pm 0.001$
8	$3.298^{Bc} \pm 0.001$	$3.292^{Cc} \pm 0.002$	$3.302^{Ac} \pm 0.002$	$3.230^{Dc} \pm 0.001$
12	$3.313^{Ad} \pm 0.001$	$3.314^{Ad} \pm 0.001$	$3.298^{Bd} \pm 0.001$	$3.275^{Cd} \pm 0.001$
16	$3.359^{Ae} \pm 0.003$	$3.321^{Be} \pm 0.001$	$3.306^{Dc} \pm 0.001$	$3.309^{Ce} \pm 0.001$

*Different superscript lowercase letters (within each column) show differences between the storage times within the same analysis group ($p < 0.05$). Different superscript uppercase letters (within each row) show differences between treatment groups within the same analysis day ($p < 0.05$).

Respiration is one of the factors affecting pH. Because of the fruit 's excessive exposure to oxygen, concentrations of carbondioxide and ethylene may affect respiration differently (Kader and Ben-Yehoshua, 2000). It explains increasing of pH value for all treatments during storage. However, until day 8, pH value declined slightly in G-P & G-I-P coated strawberries. This is due to the fact that *Lb. rhamnosus*, as a lactic acid bacteria, produces lactic acid and effect the pH of the strawberries (López de Lacey et al., 2012).

At the end of storage, the pH values of the G-P and G-I-P coated strawberries were lower than control samples and gelatin coated samples. Khodaei and Hamidi-

Esfahani (2019) investigated the effect of addition *Lb. plantarum* to carboxymethyl cellulose coatings on the shelf life of strawberries. Similarly to our results, they found that *Lb. plantarum* loaded strawberries were observed to have substantially lower pH values as compared to control and CMC coated samples.

Total soluble solid content (TSS) of treated strawberries were calculated throughout storage time and as it can be seen in the Table 4.6. The TSS content of control samples was 7.90% and increased to 8.25% at the end of storage. There were no significant differences in TSS content of strawberries between the groups during storage but on day 16, there was a significant difference observed on G-P coated samples (Table 4.6).

Table 4. 6 Effect of different treatments on total soluble solid (TSS) of strawberries during storage at 4°C for 16 d (n=3)*

Time	**Treatment**			
Day	**C**	**G**	**G-P**	**G-I-P**
0	$7.90^{Aa} \pm 0.00$	$7.65^{Bb} \pm 0.07$	$7.60^{Bab} \pm 0.00$	$7.90^{Aab} \pm 0.00$
4	$7.95^{Aa} \pm 0.07$	$7.70^{ABb} \pm 0.14$	$7.45^{Bb} \pm 0.07$	$7.80^{ABb} \pm 0.00$
8	$8.20^{Aa} \pm 0.28$	$7.95^{Aab} \pm 0.07$	$7.70^{Aa} \pm 0.00$	$8.05^{Aa} \pm 0.07$
12	$8.20^{Aa} \pm 0.00$	$8.10^{Aa} \pm 0.00$	$7.60^{Cab} \pm 0.00$	$7.95^{Bab} \pm 0.07$
16	$8.25^{Aa} \pm 0.07$	$8.05^{Aa} \pm 0.07$	$7.75^{Ba} \pm 0.07$	$8.05^{Aa} \pm 0.07$

*Different superscript uppercase letters (within each row) show differences between treatment groups within the same analysis day ($p < 0.05$). Different superscript lowercase letters (within each column) show differences between the storage times within the same analysis group ($p < 0.05$).

TSS increase was higher in control and gelatin coated samples than probiotic coated samples. The raise of the TSS content could be clarified by maturation. Another

explanation is that increased TSS is associated with significant loss of water and disruption of cell wall during storage (Hernández-Muñoz et al., 2008). The presence of probiotics has been shown to have beneficial effects on TSS content.

Similarly, in another search on effect of chitosan coating of three strawberry cultivars, it has been seen that TSS of all coated and uncoated fruits rose gradually during 9 days storage. However the lowest increase was observed on coated ones. This outcome was be related with the internal atmosphere of strawberries in this search. With the transformation of sugar to CO_2 and water molecules, CO_2 rate increases and O_2 rate declines. This causes to respiration ratio diminished (Petriccione et al., 2015).

4.3.2. Total phenolic content

The total phenolic content of strawberries in all treatments was examined on determined intervals (0, 4, 8, 12, 16 days) and results are given in the Table 4.7.

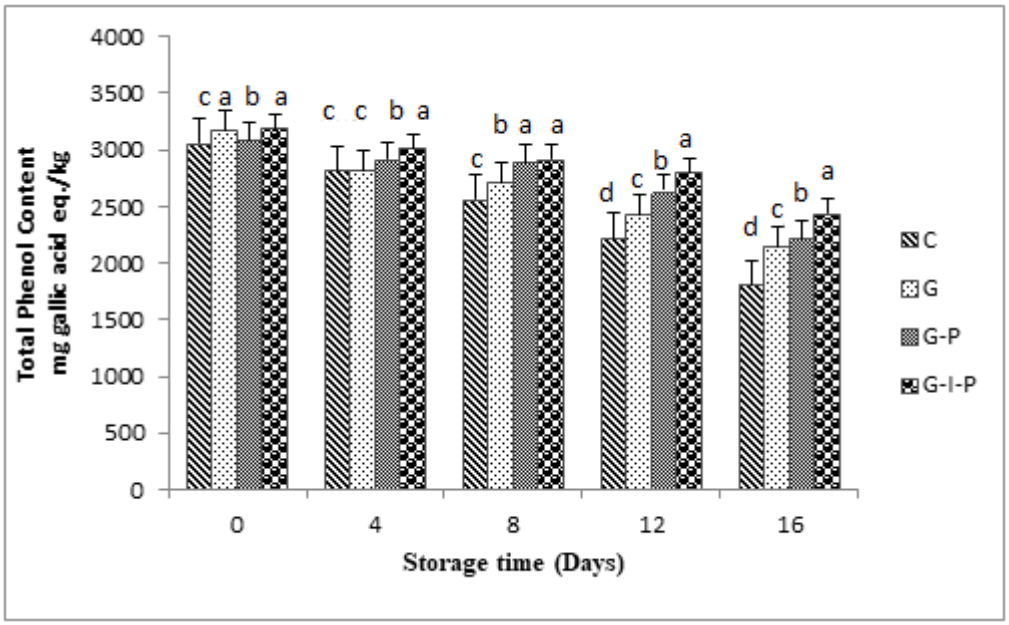
Table 4. 7 Effect of different treatments on total phenolic content (mg GAE/kg) of strawberries during 16 days of storage at 4^{o}C.

Time	**Treatment (mg Gallic Acid Equivalent /kg)**			
Day	**C**	**G**	**G-P**	**G-I-P**
0	$3058.4^{Ca} \pm 10,40$	$3172.3^{Aa} \pm 1,73$	$3085.4^{Ba} \pm 3,47$	$3185.9^{Aa} \pm 6,93$
4	$2813.4^{Cb} \pm 3,47$	$2823.2^{Cb} \pm 0,00$	$2918.8^{Bb} \pm 6,93$	$3015.6^{Ab} \pm 1,73$
8	$2563.5^{Cc} \pm 3,47$	$2719.1^{Bc} \pm 1,73$	$2890.6^{Ab} \pm 12,13$	$2916.3^{Ac} \pm 6,93$
12	$2216.7^{Dd} \pm 1,73$	$2423.8^{Cd} \pm 3,47$	$2634.5^{Bc} \pm 6,93$	$2803.6^{Ad} \pm 6,93$
16	$1805.1^{De} \pm 1,73$	$2140.8^{Ce} \pm 5,20$	$2214.3^{Bd} \pm 5,20$	$2438.5^{Ae} \pm 3,47$

*Different superscript uppercase letters (within each row) show differences between treatment groups within the same analysis day ($p < 0.05$). Different superscript lowercase letters (within each column) show differences between treatment groups within the same analysis group ($p < 0.05$).

The total phenol content (TPC) of strawberries in all groups decreased during storage as shown in the Figure 4.4. In the control group, total phenolic content was 3058.43 mg GAE / kg on the first day of storage while it was decreased to 1805.05 mg GAE / kg on the day 16.

Figure 4. 4 Total phenolic content (mg gallic acid eq./kg) of strawberries during 16 days of storage at 4^{o}C.



* Different lowercase letters of each bar show differences between treatment groups within the same analysis day ($p < 0.05$).

Among the coated groups, the highest phenol content after 16 days was seen in G-I-P coated strawberries. TPC was determined as 2438.48 mg GAE/ kg on the last day. G-P and G-I-P coatings protected phenolic content of strawberry samples better than other treatments. The reduction in TPC may be related to breakdown of anthocyanins during the senescence period (Ventura-Aguilar et al., 2018).

In addition, the reduction in TPC in all groups can be originated due to cell permeability. Some phenolic acids can affect the cell permeability and this cause exudation of macromolecules from the pores. Likewise, phenolic acids may affect each other with proteins and cause interruption of membrane structure (Taylor and Kahan, 2007). According to a research, this situation cause to decrease phenolic compounds throughout storage (Khodaei and Hamidi-Esfahani, 2019). Moreover, total phenolic content may affect from numerous variables. Agricultural factors, cultivation modifications, mulch color and fertilization level were found effective on the total phenolic content of strawberries (Anttonen et al., 2006).

In a similar trend, in a research about gelatin based edible coated strawberries enriched with *Mentha pulegium*, TPC was found above 2000 mg GAE/kg in the beginning of storage for all groups, decreased in all coated and uncoated samples at the end of 10 days. The highest TPC was found in gelatin and *Mentha pulegium* coated strawberries with a value of almost 1500 mg GAE/kg at the end of storage (Aitboulahsen et al., 2018). Likewise, the strawberries coated with chitosan, *aloe vera* gel and sodium alginate separately and observed for 12 days in another research. The results shows similar decline model with our outcomes though the initial number of strawberries were lower than normal. When TPC of non-coated strawberries on day 12 had the lowest number with 315 µg GE/mL, *Aloe vera* and Sodium alginate coated fruits were higher value than non-coated fruits and the results were found as 365 and 357 µg GE/mL, respectively (Qamar et al., 2018).

4.3.3. Total antioxidant content

The total antioxidant contents of the strawberries during storage were investigated and the results were given in the Table 4.8. The antioxidant contents of all groups decreased during storage (Figure 4.5). Among these groups, the greatest decrease in antioxidant content was the control group, as expected. While the antioxidant content of the control group was 602 mM Trolox/kg on the 1st day, this value decreased to 323 mM Trolox/kg on the 16th day of storage.

Although there is a rapid decrease in antioxidant content in all groups, it can be seen that there is a distinct difference on the control group compared to coated groups and antioxidant content of G-I-P coated group is higher than other groups even on the last storage day. The antioxidant content of G, G-P, G-I-P coated strawberries were found as 393, 404 and 454 μmol Trolox/kg as shown in the Figure 4.6 and have higher DPPH inhibition with values of 55.7, 56.8 and 62.4% while control has 48% as shown in the Figure 4.5 on the 16th day of storage, respectively. There was no statistical difference between gelatin and gelatin-probiotic coated samples, however inulin addition resulted in significant difference and lower loss of antioxidant activity.

Similar to our results, in the previous study about strawberries' nanocomposite coating based on sodium alginate showed that antioxidant capacity of coated and uncoated strawberries decreased throughout 20 days of storage. While control fruits' antioxidant activity decreased from 70% to 25%, the coated samples' activity decreased to about 50% (Emamifar and Bavaisi, 2020).

Table 4. 8 Effect of different treatments on total antioxidant content (mMol Trolox /kg) of strawberries during 16 days of storage at 4°C.

Time	Treatment (mMol Trolox /kg)			
Day	**C**	**G**	**G-P**	**G-I-P**
0	$602.02^{Ca} \pm 2.86^{*}$	$613.48^{Ba} \pm 5.72$	$618.87^{Ba} \pm 1.91$	$684.23^{Aa} \pm 0.95$
4	$549.47^{Cb} \pm 2.86$	$591.24^{Bb} \pm 2.86$	$560.92^{Cb} \pm 3.81$	$634.37^{Ab} \pm 2.86$
8	$514.43^{Cc} \pm 2.86$	$538.01^{Bc} \pm 1.91$	$510.39^{Cc} \pm 2.86$	$568.33^{Ac} \pm 2.86$
12	$453.79^{Bd} \pm 0.95$	$486.80^{Ad} \pm 1.91$	$449.74^{Bd} \pm 0.95$	$494.89^{Ad} \pm 3.81$
16	$323.07^{Ce} \pm 4.76$	$393.82^{Be} \pm 3.81$	$403.92^{Be} \pm 6.67$	$454.46^{Ae} \pm 3.81$

*Different superscript uppercase letters (within each row) show differences between treatment groups within the same analysis day ($p < 0.05$). Different superscript lowercase letters (within each column) show differences between treatment groups within the same analysis group ($p < 0.05$).

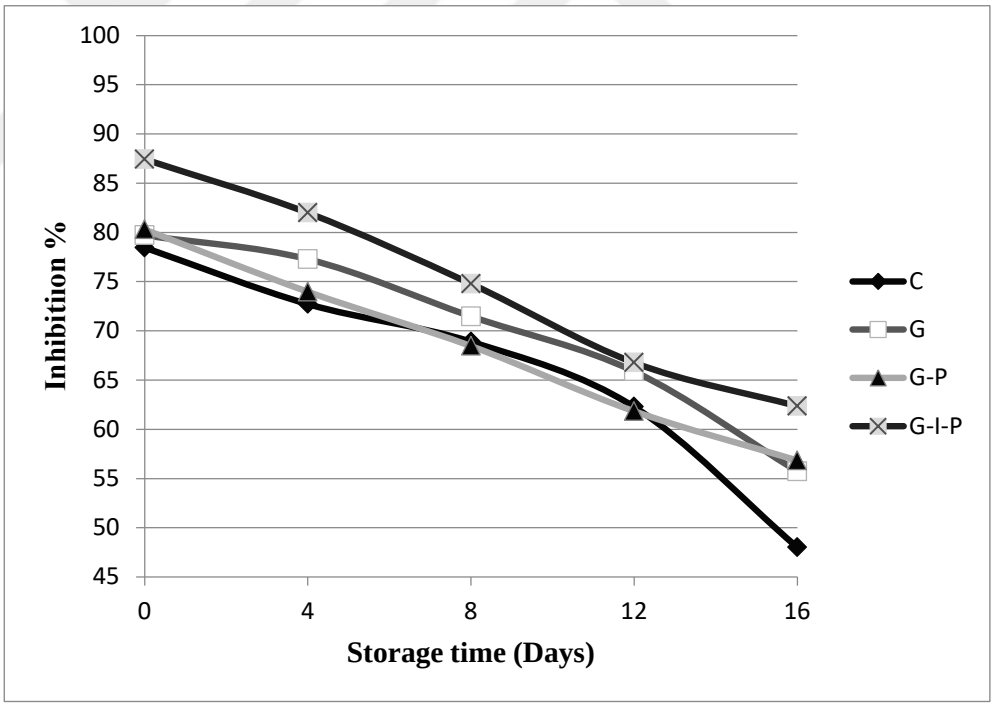


Figure 4. 5 DPPH inhibition rate of strawberries during storage time.

In a similar way, when the antioxidant content of the strawberries coated with methylcellulose and control strawberries compared, antioxidant content of strawberries

decreased during 8 days. However, the decline on control strawberries was higher than the coated ones (Nadim et al., 2015).

5. CONCLUSION

In this thesis, *Lb. rhamnosus* HN001 and inulin were integrated in gelatin coatings and these coatings were applied onto fresh strawberry and stored at 4^{o}C for 16 days. The first part involved the preparation of *Lb. rhamnosus* HN001 and preparation of film formulations. The second part included the strawberries with different treatments where gelatin, gelatin-probiotic and gelatin-inulin-probiotic coating application strawberries and determination of the effect of coatings on the physical, chemical and microbiological properties of strawberries during 16 days at 4^{o}C storage. The following results are obtained at the end of the thesis:

- ✓ Gelatin and gelatin-inulin coatings can be used as a vehicle for probiotics.
- ✓ Inulin supported the viability of probiotic bacteria.
- ✓ G, G-P and G-I-P coatings inhibited the growth of yeasts and mold counts on the strawberries compared to the control.
- ✓ *Lb. rhamnosus* HN001 enriched gelatin coatings beneficially affected on the chemical quality of the strawberries by reducing weight loss, decay, and maintaining the total phenolic content and antioxidant activity while it did not affect significantly the TA, TSS, and pH of fruit.
- ✓ The coatings did not affect the sensorial properties and appearances of coated strawberries.
- ✓ *Lb. rhamnosus* HN001 and inulin enriched gelatin coatings improved the shelf-life quality of strawberries during 16 days of refrigerated storage.

For further studies, these edible film formulations containing pure culture can be designed along with different coating formulations to improve its effectiveness and stability.

Moreover, these coatings can be applied to other foods like meat and fish products, dried fruits, nuts and other fresh/fresh-cut fruits and vegetables. These probiotic edible films integrated in food industry can be a pioneer in several novel foods.

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APPENDIX

Table A. 1 ANOVA Table for the effect of storage and different treatments on weight loss percentage of strawberries.

Variation Source		Parameter values	R-sq.	F value	p value
Storage Time	Day 4	4	71,88 %	1,59	0,229
	Day 8	4	76,36 %	1,11	0,373
	Day 12	4	41,16 %	3,96	0,026*
	Day 16	4	74,39 %	16,46	0,001*

(*)P < 0.05 expresses difference.

Table A. 2 ANOVA Table for the effect of storage and different treatments on pH of strawberries.

Variation Source		Parameter values	R-sq.	F value
Coating Treatments	Control	4	99,96 %	6953,01*
	Gelatin	4	99,94 %	6306,50*
	Gelatin + *Lb. rhamnosus*	4	99,97 %	7421,74*
	Gelatin + Inulin + *Lb. rhamnosus*	4	99,94 %	3974,92*
Storage Time	Day 0	5	99,93 %	3870,17*
	Day 4	5	99,93 %	3663,56*
	Day 8	5	99,88 %	2174,23*
	Day 12	5	99,71 %	933,00*
	Day 16	5	99,72 %	959,92*

(*)P < 0.05 expresses difference.

Table A. 3 ANOVA Table for the effect of storage and different treatments on TA of strawberries.

Variation Source		Parameter values	R-sq.	F value	p value
Coating Treatments	Control	4	94,61 %	21,95	0,002*
	Gelatin	4	92,01 %	14,39	0,006*
	Gelatin + *Lb. rhamnosus*	4	96,16 %	31,33	0,001*
	Gelatin + Inulin + *Lb. rhamnosus*	4	96,87 %	38,69	0,001*
Storage Time	Day 0	5	99,78 %	615,66	0,000*
	Day 4	5	98,36 %	79,93	0,001*
	Day 8	5	98,12 %	69,53	0,001*
	Day 12	5	99,14 %	154,55	0,000*
	Day 16	5	99,02 %	135,24	0,000*

(*)P < 0.05 expresses difference.

Table A. 4 ANOVA Table for the effect of storage and different treatments on TSS of strawberries.

Variation Source		Parameter values	R-sq.	F value	p value
Coating Treatments	Control	4	70,00 %	2,92	0,136
	Gelatin	4	90,51 %	11,93	0,009*
	Gelatin + *Lb. rhamnosus*	4	91,38 %	13,25	0,007*
	Gelatin + Inulin + *Lb. rhamnosus*	4	85,71 %	7,50	0,024
Storage Time	Day 0	5	96,85 %	41,00	0,002*
	Day 4	5	89,83 %	11,78	0,019*
	Day 8	5	74,65 %	3,93	0,110
	Day 12	5	98,81 %	110,33	0,000*
	Day 16	5	92,73 %	17,00	0,010*

(*)P < 0.05 expresses difference.

Table A. 5 ANOVA Table for the effect of storage and different treatments on total yeast and mold in strawberries.

Variation Source		Parameter values	R-sq.	F value	p value
Storage Time	Day 0	4	26,18 %	0,95	0,463
	Day 5	4	70,72 %	6,44	0,016*
	Day 10	4	82,91 %	12,94	0,002*
	Day 15	4	83,43 %	13,43	0,002*
Coating Treatments	Control	4	96,60 %	75,75	0,000*
	Gelatin	4	98,39 %	163,00	0,000*
	Gelatin + *Lb. rhamnosus*	4	98,03 %	132,69	0,000*
	Gelatin + Inulin + *Lb. rhamnosus*	4	95,43 %	55,74	0,000*

(*)P < 0.05 expresses difference.

Table A. 6 ANOVA Table for the effect of storage and different treatments on total mesophilic aerobic bacteria in strawberries.

Variation Source		Parameter values	R-sq.	F value	p value
Storage Time	Day 0	4	93,19 %	54,72	0,002*
	Day 5	4	85,93 %	24,42	0,008*
	Day 10	4	98,91 %	241,58	0,000*
	Day 15	4	97,88 %	123,27	0,000*
Coating Treatments	Control	4	98,86 %	230,59	0,000*
	Gelatin	4	94,82 %	48,82	0,000*
	Gelatin + *Lb. rhamnosus*	4	97,98 %	194,45	0,000*
	Gelatin + Inulin + *Lb. rhamnosus*	4	99,31 %	577,30	0,000*

(*)P < 0.05 expresses difference.

Table A. 7 ANOVA and Independent sample t test table for the effect of storage and different treatments on survival of *Lb. rhamnosus* HN001 in strawberries.

Variation Source		**Parameter values**	**R-sq.**	**F value**	**p value**
Coating Treatments	Gelatin + *Lb. rhamnosus*	2	95,61 %	58,07	0,001*
	Gelatin + Inulin + *Lb. rhamnosus*	2	91,43 %	25,45	0,000*
Variation Source		**Parameter values**	**T-value**	**P-value**	**DF (Degrees of Freedom)**
Storage Time	Day 0	4	-0,48	0,663	3
	Day 5	4	0,01	0,989	3
	Day 10	4	4,23	0,024*	3
	Day 15	4	3,59	0,040*	2

(*)P < 0.05 expresses difference.

Table A. 8 ANOVA Table for the effect of storage and different treatments on total phenolic content of strawberries.

Variation Source		**Parameter values**	**R-sq.**	**F value**	**p value**
Storage Time	Day 0	5	99,29 %	186,17	0,000*
	Day 4	5	99,88 %	1137,48	0,000*
	Day 8	5	99,77 %	1030,10	0,000*
	Day 12	5	99,97 %	4675,77	0,001*
	Day 16	5	99,98 %	7971,87	0,000*
Coating Treatments	Control	4	99,98 %	17684,89	0,000*
	Gelatin	4	99,99 %	34306,44	0,000*
	Gelatin + *Lb. rhamnosus*	4	99,97 %	4084,35	0,001*
	Gelatin + Inulin + *Lb. rhamnosus*	4	99,97 %	4928,60	0,000*

(*)P < 0.05 expresses difference.

Table A. 9 ANOVA Table for the effect of storage and different treatments on antioxidant content of strawberries.

Variation Source		Parameter values	R-sq.	F value	p value
Coating Treatments	Control	4	99,95 %	2381,77	0,000*
	Gelatin	4	99,90 %	1239,07	0,000*
	Gelatin + *Lb. rhamnosus*	4	99,88 %	1021,85	0,001*
	Gelatin + Inulin + *Lb. rhamnosus*	4	99,94 %	1951,40	0,000*
Storage Time	Day 0	5	99,45 %	241,97	0,000*
	Day 4	5	99,55 %	294,66	0,000*
	Day 8	5	99,34 %	201,75	0,000*
	Day 12	5	99,37 %	209,33	0,001*
	Day 16	5	99,46 %	243,40	0,000*

(*)$P < 0.05$ expresses difference.

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