

**An-Najah National University**

**Faculty of Graduate Studies**

**Effect of Grape Juice on Characteristics of  
*Nigella Sativa* Protein-Based Film Used for  
Wrapping Sweet Cherries**

**By**

**Dana Yaseen**

**Supervisor**

**Dr. Mohammed Sabbah**

**This Thesis is Submitted in Partial Fulfillment of the Requirements for  
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**This Thesis was defended successfully on 3/11/2021, and approved by:**

**Defense Committee Members**

**Signatures**

**- Dr. Mohammed Sabbah**

  
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**- Dr. Fuad Al-Rimawi**

  
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**- Dr. Mohammad Altamimi**

  
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## Dedication

إلى قدوتي الأولى الذي أعطاني ولم يزل يعطيني بلا حدود، إلى من حصد الأشواك عن دربي ليمهد لي طريق العلم . . أبي الغالي.

إلى نبع المحبة والإيثار صاحبة القلب الحنون، التي علمتني الصمود مهما اشتدت الظروف . . أُمِّي الغالية.

إلى أقرب الناس إلى نفسي، أخوتي، أخواتي، وأصدقائي.

إلى نفسي التي تعبت، وسهرت، واجتهدت لتحقيق هذا النجاح.

إلى جميع من تلقيت منهم النصح والدعم.

أهديكم خلاصة جهدي العلمي.

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**الإقرار**

أنا الموقعة أدناه مقدمة الرسالة التي تحمل العنوان:

**Effect of Grape Juice on Characteristics of *Nigella*  
*Sativa* Protein-Based Film Used for Wrapping Sweet  
Cherries**

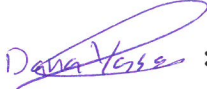
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**Declaration**

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

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الإسم الطالبة: دانا ياسين

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**Abstract**

Edible coatings are environment-friendly materials for extension of the shelf-life and preservation of the nutritional value of fruits. *Nigella sativa* protein concentrate (*NSPC*) powder was dissolved with different concentrations of grape juice (*GJ*) (1, 2, 4, 6, 8,10, 20 and 30% v/v) at pH value 12.0 to obtain a protein based edible films. The obtained results showed for the first time that *GJ* at concentration from 2-10% (v/v) are able to act as plasticizer in the protein based films with promising film properties. The thickness and mechanical results indicated there is no significant difference in films thickness by increasing the *GJ* concentrations. The different *GJ* concentrations have affected significantly ( $p \leq 0.05$ ) the *NSPC* film mechanical properties. The results showed that the tensile strength and Young's modulus of *NSPC* film was reduced significantly when the *GJ* increased. However, the *NSPC* films prepared with 6% *GJ* observed a higher elongation at break compared with other films. Moreover, the *NSPC* /*GJ* films showed a very interesting and promising results for their antioxidant and antimicrobial properties compared to the control films.

In the present research, the sweet cherry (*Prunus avium L.*) which has a very short shelf life, was wrapped with *Nigella sativa* concentrated protein film plasticized with 6% grape juice, as an edible coating before storage. The physicochemical qualities of control (unwrapped) sweet cherries and wrapped samples either in LDPE or NSPC/GJ films were evaluated about the (pH, titratable acidity, total soluble solids, and external color) during 40 days at -20 °C storage, to compare the effects of the applied films.

The results showed that the TSS (Brix) was significantly lower compared to the control (unwrapped) after 10 days of storage at -20 °C, and no significant effect was observed between the TSS of cherries wrapped with LDPE or NSPC films in all storage times, which clearly indicated that the TSS concentration was stable until the end of storage period for cherries wrapped with LDPE or NSPC/GJ films. However, the titratable acidity and pH value of all cherries either wrapped or not was not significantly different in all storage times. And the optical result ( $L^*$  and hue angle) showed that the  $L^*$  value was not significant in all the treatments. Whereas, Hue angle results indicated that the sweet cherries wrapped with LDPE were reduced significantly compared to the ones wrapped with NSPC plasticized with GJ or unwrapped cherries at 20 days of storage. Moreover, the hue angle value for the sweet cherries that wrapped with NSPC was higher than the value at 20 days comparing the control and the value decreased at 40 days of storage at -20 °C, and there were no significant differences between the cherries wrapped with LDPE or NSPC films in 40 days of storage.

It has been determined that *N*SPC/GJ film can be used effectively in protecting the physicochemical properties and increasing the shelf life of sweet cherries at distinct storage temperatures. So it suggests that *N*SCP/GJ film could be considered as a new preservation method for improving postharvest quality and nutritional properties of different sweet cherry cultivars.

## **Chapter 1**

### **Introduction**

#### **1.1 Background**

Today we are living in an era called it “plastic age” plastic pollution has become one of the most urgent environmental issues, with the increase in plastic production exponentially, from 2.3 million tons in 1950 to 448 million tons by 2015. Production is expected to double by 2050. According to National Geographic, the oceans are filled with 18 billion pounds of plastic annually, and people buy about 1 million plastic bottles every minute worldwide [1,2]. The ubiquitous presence of plastic has some strong and valid reasons such as its durability, flexibility, and cheapness due to some additives that make it stronger, more flexible, and durable, so plastic products may take hundreds of years to degrade. However, it is now not hidden that the overuse and disposal of plastic are becoming a major threat to the Earth's environment. Unnecessary use of plastic, improper dumping, excessive use of single-use plastics, and lack of awareness are some of the factors responsible for today's world condition of ecosystems.

Plastic pollution not only affects the land and terrestrials' animals but also harms marine creatures including animals and pollutes our water sources. For example, when they decomposed over a long period of time, many chemicals can leach into the soil and groundwater, or it may produce a lot of harmful toxins that can also be released when burning, which affects air

quality and causes respiratory problems. A large number of animals are killed by plastic consistently, from birds to fish to other marine organic entities. Approximately 700 species, including endangered species, are known to be affected by plastic. Plastic can also negatively affect plants, disturbing the food chain and ecosystem [3]. Single-use plastics generate an enormous amount of waste in many countries. Many of these products, such as plastic bags and food wrappers, have a life span of minutes to hours, yet may remain in the environment for hundreds of years. Although it seems that it is easy to clean up plastic garbage just by implementing the recycling principle of collecting and reusing it in one form or another, the real truth is that plastic pollution ranges from large plastic trash to microplastic and plastic microfibers, which have been reported even in drinking water and the air we breathe. In the current situation, only 9% of the plastic produced is recycled, 12% is incinerated and the remaining 79% either goes to landfill or remains as a pollutant in the environment. So when you think about the amount of plastic that wraps or holds food, it's easy to understand why researchers and companies want to create biodegradable alternatives that are better for the environment.

In recent decades, concerns surrounding conventional plastics have stimulated a focus of attention on environmentally friendly, non-toxic, and biodegradable materials derived from natural ingredients such as polysaccharides, lipids, and proteins due to their sustainable supply and biodegradable potential [4,5]. As viewed as one of the arrangements that



might help solve the problem of plastic, edible food coverings offer a straightforward method to diminish worldwide reliance on plastic and disintegrate effectively in the environment., biodegradable coatings may be one of the most important ways that play an important role in not only reducing plastic problems but also obtaining good quality food wrapped by preserving its properties and providing certain benefits such as edibility, biodegradability, bio-compatibility, environmentally friendly, waste reduction, barrier, preservative, and appearance properties [6]. Biodegradation refers to the ability of materials to degrade and return to nature within a short period of time after they are disposed of - typically a year or less [7]. Natural polymers derived from agricultural products (such as starch, proteins, cellulose, and plant oils) are the main resource for the development of renewable and biodegradable polymer materials.

The edible covering is generally used to protect food items from physiological disturbances and microbial contamination. During the 12<sup>th</sup> and 13<sup>th</sup> centuries, the Chinese developed a waxy coating to apply to lemons and oranges to protect them from microbial spoilage. In the last decade, consumer awareness related to edible, biodegradable, and eco-friendly packaging materials has increased, edible films and coatings are widely used in the food processing sector to maintain the integrity of a wide range of food products (Fruits: Apples, Bananas, Kiwis, Mangoes, Pears, Pineapples, etc. or vegetables: carrots, potatoes, etc. meat, and dairy products). Commercially speaking, the first coating material was invented in 1992 as a

waxing agent for fruit and vegetable applications. Edible packaging is known as eco-friendly packaging material, it can replace synthetic or plastic packaging material and reduces post-harvest damages of fruits and vegetables, can be applied directly to food surfaces by spraying, dipping, panning, or wrapping or others, which can provide many benefits to the product that applied to it; this is because it can be used to extend food stability by reducing moisture exchange, O<sub>2</sub>, CO<sub>2</sub>, lipids and flavor compounds between the food and the surrounding environment, which minimizes a load of pathogenic and spoilage on the surface of the coated food. Increasing quality and extending the shelf life of meat and aquatic products by incorporating natural plant extracts, especially essential oils that possess antioxidant and antimicrobial activity. Hence increased the food stability and quality [8-11]. Prior to applying biopolymers on food products, there are some factors that must be considered such as solubility, transparency, cohesion, mechanical properties, microbiological stability, wettability, oil and grease resistance, sensory and water vapor, and gases permeability [12].

Edible films and coatings are natural polymers obtained from renewable materials such as proteins (animal or vegetable), polysaccharides, lipids, or combinations of these components. It can intended to be an integral part of foods which can consumed with products, so there is no package, no disposal of [13,14]. Natural polymers may also be incorporated with different bioactive compounds such as oregano or thyme essential oil [15-17], cloves

[18], and other that act as an antioxidant or antimicrobial agent in edible coatings or films to keep up food safety and quality and extend the shelf life of food products [19]. Mechanical and barrier properties of edible films and coatings mainly rely on the nature and concentration of the biopolymers, drainage time and viscosity [20], For example, films based on proteins or polysaccharides acted as highly effective barriers to oxygen and carbon dioxide, while their resistance to water vapor transmission is restricted. Multi-component films can also be manufactured to try to mix the benefits of individual materials with film-forming.

Oil seeds are frequently embraced as the essential source of protein for human health and well-being. Their utilization isn't restricted to nutrition but reaches out to the field of traditional medicine or herbal medicine that uses the pharmacological properties of the bioactive compounds within them. These are bioactive components that are natural or artificially derived precursors to numerous pharmacological mixtures or drugs [21]. Physicians consistently try to discover drugs with fewer side effects. Among the most encouraging therapeutic plants, *Nigella sativa* (*N. sativa*) or black seed, of the Ranunculaceae family, is an amazing herb with a historical and religious background. It is developed in numerous nations, however, is native to Southern Europe, Southeast, and Southwest Asia, and is grown as a spice or for its therapeutic worth [22,23]. It is a bushy, self-branching plant with either white or pale to dark blue flowers [24]. Its seeds are mainly used as a spice and relieving agent for various ailments [25-27]. Nutritionally, the

components of *N. Sativa* seeds include, on average, 29% carbohydrates, 35% saturated and unsaturated fatty acids, vitamins [28], 6% crude fibers, 4% ash, 5% moisture, and protein account for 21%, they are rich in lysine and methionine and was considered significant for the dietary application [29-31]. Black seed can be used as directed, active ingredients in herbal medicines or as herbal tea. *N. sativa* seed oil is extracted and may be utilized in traditional medicine to treat a wide range of ailments such as diabetes, hypertension, oxidative stress, epilepsies, ulcers, asthma, inflammatory disorders, and cancers in model organisms as well as in human beings [32-39]. Few of the bioactive compounds extracted from *Nigella sativa* seeds have been specified, studies have shown that the biological activity of *N. Sativa* seeds are mainly attributed to its essential oil component, thymoquinone [40-42], which is a major phytochemical in *N. sativa*, and widely considered to be most important for the broad-spectrum medicinal properties of this valuable plant. Other phytochemicals from different varieties of *N. sativa* include phenolic compounds, various alkaloids, sterols, saponins as well as volatile oils of different compositions [43,44].

There are huge quantities of defatted cake that are certainly created as by-products and a greater part of it's served as animal feed on account of high protein content [45]. In addition, there aren't any within the literature on the exploitation of proteins extracted from *Nigella sativa* defatted seed cake (NSDSC) to get novel bio-materials. Proteins within the hydro-methanolic part of *N. sativa* seeds represent around 35-40% of the total dry weight and

were found to separate into ranges from 10 to 94 kDa on SDS PAGE [46]. This fraction has been found to have pharmacological impacts independent of those exerted by volatile oils and is getting expanding consideration with relation to effects on the health of people. Studies utilizing this concentrate have shown that it goes about as an effective sedative with central nervous system depressant impacts, to incite analgesia, and inhibit progression pathological changes in the lungs, and lowering the blood cytokine levels [47,48].

Several studies have shown that fruits and vegetables have a protective effect against the development of human diseases such as cardiovascular disease, diabetes and cancer [49,50]. It has been hypothesized that the protective role of fruits and vegetables could be because of the different supplements they contain like fiber, nutrients, and phytochemicals. Phenolics are secondary plant metabolites characterized by the presence of at least one aromatic ring with one or more attached hydroxyl groups. A few studies in humans and animal models have shown that phenols are bioavailable and play a defensive part against oxidative stress and free radical damage [51]. Phenolic range from single-aromatic ring compounds with a modest molecular weight to large complex tannins. The quantity and configuration of carbon atoms can be used to classify them. Oxidative damage, caused by free radicals, causes structural and functional changes in the macromolecules of cells and is involved in the molecular mechanisms of chronic human disease. Free radicals can be neutralized or scavenged by antioxidants which are also

necessary to enable other molecules to perform such a function [52,53]. Many studies have shown that cherries contain numerous nutrients like phytonutrients and antioxidants, this causes them to play a defensive part against oxidative stress and free radical damage.

Sweet cherry (*Prunus avium L.*) is one of all the foremost appreciated fruits by consumers because of its high content of essential nutrients and is principally consumed unprocessed. Several epidemiological investigations have lately shown the health-promoting impacts identified with its content in phytochemicals like antioxidant, anthocyanin, and phenolic compounds [54]. The fundamental attributes identified with the quality of cherry fruits are color, sweetness (due to glucose and fructose), sourness (due to the presence of organic acid; malic acid ), and firmness [55]. Consumer acceptance appears to depend on sugar to acid concentrations ratio [56]. Nutritionally, sweet cherries have a high substance of simple sugar (13g/100g), contain water-soluble vitamins (C, B) and fat-soluble vitamins (A, E, K) and a few carotenoids, specifically beta-carotene. Cherries additionally contain minerals like calcium (14 mg/100 g), magnesium (10 mg/100 g), phosphorous (20 mg/100 g) and potassium (200 mg/100 g). After harvest, high respiration rate and metabolic activities result in a lessening in acidity and phytochemical content, weight reduction, change in color, and total soluble content cause the rapid deterioration of sweet cherries [57]. To maintain the good quality of the fruits, it is necessary to extend the shelf life of sweet cherries and to provide the necessary packaging and storage

conditions. There are many examinations on expanding the shelf life of sweet cherries that are acquiring exceptional importance, different procedures like cold storage, controlled atmosphere storage, modified atmosphere packaging, and edible film coating have been utilized to keep up with the quality and broaden the shelf life of sweet cherries after harvest [58-60].

In recent years, customers have gone to environmentally friendly and recycled materials. Consequently, there is a developing interest in edible film coatings as alternative packaging materials. As of late, natural products quality is kept up and shelf life of realistic usability expanded using edible coatings that can defer some physiological processes like respiration and transpiration [61], It can also function as a barrier, reducing quality loss, inhibiting gas exchanges, controlling respiration rates, and preventing the growth of deteriorating microorganisms. In general, a film of alginate, chitosan with, proteins, and/or lipids is utilized commercially and can be utilized to ensure protection for the whole fruit [62,63].

Grapes (*Vitis vinifera*) is a widely cultivated crop within the world, native to the Mediterranean region and Central Asia. Grapes are non-climacteric fruits for fresh consumption and are botanical groups of true berries. Since grapes have an extremely short shelf life of usability, a lot of grape loss happens because of degradation. Thus, grapes should be handled in a structure that can be stored for a protracted time without loss within the nutritional value. Grapes are one of the broadest organic product crops around the world, their structure and properties have been widely studied, with some reports of the

presence of a lot of phenolic compounds. The majority of the phenolic compounds can go about as antioxidants. Likewise, wine creation deposits are additionally described by the high substances of phenolic compounds because of their fragmented extraction during wine production [64,65]. By-products obtained after the production of wine and juice, (seeds and pomace) constitute a cheap source for extracting antioxidant compounds, providing important economic advantages [66]. The composition of grapes predominantly comprises (w/w) 40% fiber, 11% protein, 16% essential oil, sugars, minerals, 7% complex phenolic compounds such as tannins, and other substances [67]. Grape skin is a wellspring of anthocyanidins and anthocyanins, which are natural pigments with antioxidant properties agent that demonstrate by inhibiting lipid oxidation and furthermore have anti-mutagenic activities [68]. Moreover, they are good sources of vitamins A, C, K, flavonoids, carotenes, and B-complex vitamins such as pyridoxine, riboflavin, and thiamine.

Nutritionally, fruit juices are an important source of energy in the form of sugars such as glucose, fructose, and sucrose are most abundant in fruit and fruit products [69]. In grapes, an enormous part of the dissolvable solids is sugars. Glucose and fructose are the base sugars in the juice. The sugar content of the juice of ripe grapes ranges from 150 to 250 g. In unripe berries, glucose is the predominant sugar. In the ripening stage, glucose and fructose are usually present in equal quantities (1:1 ratio). In overripe grapes, the concentration of fructose surpasses that of glucose. In ripe grapes, there is



some difference in the ratio of glucose to fructose between grape varieties. During the maturing of the grapes, other metabolic changes happen, like the amassing of sugars as glucose and fructose in the vacuoles (flesh and skin), after the exchange of sucrose from the leaves. The accumulation of sugar as glucose and fructose inside the vacuole is one of the principal provisions of the ripening process in grape berries. Sucrose, which was not distinguishable in grape cells in the vitro, is additionally found at extremely low levels just in the flesh and skin of grapes. Grape juice is made from whole grapes, including skin, flesh, and seeds. It contains most of the vitamins and minerals found in table grapes, as well as the health benefits of nutrient-rich seeds. Grape juice is rich in antioxidants, which can help the body protect itself from free radicals. Grape juice is defined as an energy drink unfermented, non-alcoholic, with characteristic color, aroma, and flavor, and its consumption is associated with many health benefits such as increased antioxidant capacity, improvement of endothelial function, inhibition of platelet aggregation, reduction of oxidation of plasma proteins, and reduction of low-density lipoproteins (LDL) and improve cardiovascular oxidation and neurocognitive function [70]. It is known that grape juices contain a very high content of phenolic compounds and depending on their chemical composition, they are divided into several classes that are directly responsible for the special properties of grapes and their derivatives [71-73]. The phenolic compounds included in grape juice may potentially have a role in defining the sensory qualities of this product, in addition to its functional performance.

## **1.2 Objective and Research hypothesis**

Biodegradable film production and potential applications for nutritional applications are gaining increasing interest as alternatives to conventional food packaging polymers. Consumers often demand higher quality products, more safety, and longer shelf life in foods while reducing disposable packaging materials and increasing recyclability. To reduce environmental problems and pollution, we can get environmentally friendly products by using edible film, as this film can extend the shelf life of food and protect it from microorganisms, in addition, some natural additives can be added to this film to give food new functional properties. Fresh cherries are highly perishable fruits, with a short shelf life and high respiration rates, and they undergo a series of physiological and biochemical changes that lead to a loss of their quality, which may persist after harvest, and may degrade before reaching consumers. Therefore, it was necessary to develop new alternative practices necessary to maintain the quality of fresh cherries after harvest.

There are many objectives that were pursued during the research; The main objective of this experiment, is to produce an edible film from renewable and biodegradable resources.

The specific objectives;

- Produce an edible film using the protein extracted from *Nigella sativa* and fortifying it with grape juice, which is rich in different functional

foods such as polyphenols, antioxidants, and antimicrobials, in order to obtain a functional edible film.

- Characterizing *N*SPC film using mechanical, physical, and biological tests.
- Using the protein-based edible film to wrap the cherries in order to maintain their in high quality and increase their shelf life.
- Characterizing physicochemical analysis of the sweet cherries wrapped with *N*SPC/GJ film, to evaluate the effect of film on their properties.

### **1.3 Literature review**

Many experiments and studies have succeeded in forming and applying edible films for packaging food products, such as fresh or processed fruits and vegetables, some fresh meat, and many other products, in various areas and times, and few experiences are described in comparison to many others. Browning and ripening times are important factors in determining the product shelf life of fresh-cut products. Protein-based edible coatings with moderate oxygen, carbon dioxide, and water vapor permeability can be applied to fresh-cut products surfaces to extend product shelf life by delaying ripening, inhibiting enzymatic browning, reducing water loss, and minimizing aroma loss [74]. The two coatings effectively prevented browning by acting as oxygen barriers, according to the color analysis of apple and potato slices coated with calcium caseinate or whey protein solutions. The films' antioxidant characteristics were achieved using a model that allowed oxidative species to be released via electrolysis of saline buffer.

Calcium caseinate had a lower antioxidant capacity than whey proteins. Moreover, adding carboxymethyl cellulose to the formulations increased their antioxidative power dramatically. Films based on whey proteins and carboxymethyl cellulose were found to have the best scavenging of oxygen free radicals and reactive oxygen species, inhibiting the synthesis of colorful compounds formed by the interaction of the oxidative species with N, N-diethyl-p-phenylenediamine by 75% [75].

#### **1.4 Shelf-life and postharvest quality of fruits**

The ability of gelatin (GEL) coatings containing cellulose nanocrystals (CNC) to extend the shelf-life of strawberry fruit (*Fragaria × ananassa*) for 8 days was assessed in one of the studies. Strawberries were immersed within the film-forming solution for 1 minute until being coated and dried at 15°C for 24h, then kept under refrigeration and characterized in terms of their properties (weight loss, ascorbic acid content, titratable acidity, and water content). The results showed that samples covered with GEL/CNC had a major increase in shelf life, as an example, after 8 days of storage, the weight loss for the control sample (without coating) was around 65%, while the loss for the covered samples was within the range of 31-36%. The edible coating was also useful in retaining ascorbic acid in strawberries, whereas the control sample showed a rapid decay in ascorbic acid content, covered samples showed a slow decay in ascorbic acid concentration. Furthermore, the edible GEL/CNC coating had an antimicrobial effect on the fruits [76].

One review was evaluated the physicochemical properties (thickness, solubility in water and acid, vapor permeability, opacity, and mechanical properties) of complex films that contained corn starch (native, modified waxy, or waxy) and gelatin, plasticized with glycerol or sorbitol, these films were then applied as an edible composite coating to Red Crimson grapes so as to increase their shelf life of usability. The addition of gelatin maximized the mechanical strength, water-solubility, permeability to vapor, and thickness of the films while limited their opacity. Composite films arranged with sorbitol had fundamentally lower vapor permeability and better tensile strength than glycerol-plasticized films. Coated grapes had a preferred appearance after 21 days of refrigerated storage, and that they had a lower weight loss than the control group. The sensory assessment revealed that none of the coatings influenced acceptability scores [77].

The effects of Zein and gelatin coatings on the physicochemical properties, softening, and antioxidative enzyme activities of mango fruits stored at ( $32 \pm 1$ ) °C were evaluated and compared with control at regular intervals during the storage period. Coatings of Zein and gelatin appeared to have a favorable effect on postponing changes in weight loss, titratable acidity, sugar content, pH, soluble solids, and total carotenoids. In comparison to the control, Zein and gelatin coatings resulted in the highest retention of phenolic content and ascorbic acid, delayed mango fruit ripening by inhibiting the activity of softening enzymes like polygalacturonase, cellulose, pectin, methylesterase, and -galactosidase, and maintained the best induction of defense-related

peroxidase enzymes, that the application of Zein 5% and gelatin 10% coatings may well be wont to delay ripening, maintain quality attributes, and extend the shelf life of mango fruit during storage [78].

The effect of a Chitosan-coating treatment on the physical and chemical, nutraceutical, and sensorial traits of three sweet cherry crops (*Prunus avium* L., cvs. Ferrovia, Lapins, and Della Recca) harvested when it ripened was studied. Cherries were coated with 0.5 % Chitosan, stored at 2 °C for 14 days, weekly sampled, and stored at 24 °C for 3 days to work out the fruit's shelf life. Physical-chemical (weight loss, Brix, and acidity), nutraceutical (total polyphenol, antioxidant capacity, and ascorbic acid content), and sensory assessments were distributed. In a cultivar-dependent manner, water loss significantly decreased and changes in color, acidity, ascorbic acid content, and respiration rate were delayed by chitosan coating. By using the Chitosan coating the total changes in polyphenol, anthocyanin, and flavonoid content, in sweet cherry fruits furthermore, as antioxidant capacity, were delayed. This treatment increased the postharvest lifetime of sweet cherry, improved its storability, and increased its nutraceutical value [79].

To preserve the quality and safety of crimson seedless table grapes during cold storage and subsequent shelf life, an edible covering based on Aloe-Vera gel was applied. Uncoated groups deteriorated rapidly, with an expected shelf life of 7 days at 1 °C in addition to 4 days at 20 °C, in light of the quick weight reduction, color changes, accelerated softening, and ripening, rachis browning. Those clusters coated with Aloe-Vera gel edible

covering, on the other hand, greatly delayed the above parameters linked to postharvest quality losses, and storability may be extended up to 35 days at 1 °C. And this edible covering was able to minimize the initial microbial counts for both mesophilic aerobic and yeast and molds, which grew dramatically in uncoated samples after storage. Besides, sensible investigations exhibited helpful impacts as far as postponing delaying rachis browning and dehydration, just as keeping up with the visual part of it without influencing taste, aroma, or tastes [80].

An edible coating was formed from defatted walnut flour and applied to walnut kernels, which were then put away at 40 °C close by uncoated pieces for 84 days. On day 84, the coated walnuts showed the least oxidation (13.33%), the maximum carotenoid (2.01 mg/kg), and  $\gamma$ -tocopherol contents (306.78 mg/kg), as well as the least decrease of oleic/linoleic fatty acids ratio. Furthermore, the kernels wrapped in this coating received greater general customer acceptability. The walnut flour coatings also inhibited the breakdown of polyunsaturated fatty acids, which prevent deterioration processes in walnuts. The coating based on walnut flour could be used as a natural option to broaden the shelf life of walnut kernels without adding synthetic substances to the food [81].

Overall, the use of coating was found to delay the ripening process and increased anthocyanins, particularly those composed of a Chitosan with OLE. At the end of 20 days of storage, Chitosan and alginate coated samples supplemented with OLE reduced the loss of total ascorbic acid and phenolic

contents. Higher levels of antioxidant activity were shown to be associated with higher levels of phytochemical content. So Chitosan and alginate coating enhanced with OLE was effective in extending the shelf life of sweet cherries [82].

The effects of Chitosan 1% and 1.5%, calcium chloride (CaCl<sub>2</sub>) 1% and 1.5%, Chitosan 1% + gibberellic acid 100 ppm, Chitosan 1.5% + gibberellic acid 100 ppm, jojoba wax, and glycerol (98%) coatings were tested on the shelf life and postharvest quality attributes of banana fruits stored at 34 ± 1°C and 70–75% relative humidity, while uncoated fruits served as a control. When contrasted with uncoated examples, Chitosan, Chitosan + gibberellic acid, and jojoba wax coatings slowed back changes in weight loss rate, deterioration percentage, Brix, pH, acidity, accumulation of sugar, pigments retreating, and ascorbic acid. Furthermore, the fruits treated with Chitosan and Chitosan + gibberellic acid had the lowest pathogen incidence. As a result, coating with Chitosan and Chitosan + gibberellic acid has the ability to control deterioration percentage, broaden shelf life, and maintain essential banana characteristics [83].

Chitosan-based edible coatings were employed in a study on strawberries (*Fragaria × ananassa*) and red raspberries (*Rubus idaeus*) to increase the shelf-life and improve their nutritional content at the point when they were stored at either 2 °C and 88% relative moistness (RH) for 3 weeks or 23 °C for up to 6 months. Chitosan-based coatings were investigated (Chitosan and Chitosan with 0.2% DL- $\alpha$ -tocopheryl acetate). The outcomes showed that



high calcium or Vitamin E concentrations in Chitosan coatings had no impact on their anti-fungal and moisture barrier properties. The coatings reduced decay incidence and weight loss while also delaying changes in color, pH, and titratable acidity of strawberries and red raspberries during cold storage. Coatings also helped to decrease drip loss and preserve the textural integrity of frozen strawberries after thawing. Furthermore, calcium or Vitamin E-containing Chitosan-based coatings greatly enhanced the amount of these nutrients in both fresh and frozen fruits [84].

### **1.5 Antimicrobial activity of films**

During storage at 10°C, the goodness of fresh-cut 'Josapine' pineapple covered with a hydrocolloid-based edible coating based with respect to gelatin was assessed. . Gelatin was applied to cut pineapple in concentrations of 0.5, 1.0, and 1.5 %. Samples without coating were used as controls, and all samples were stored at 10°C for 8 days. The physicochemical changes (color, texture, pH, TA, and TSS) and microbiological changes (total plate count, total coliform, and total yeast and mold) of the samples were assessed at 2-day intervals. Generally, fresh-cut pineapple stayed good for just 6 days when stored at 10°C because of the fungal infestation that was seen in all treatments with prolonged storage. There was no significant difference in any of the physicochemical parameters between the treated and control samples. However, the overall microbiological examination showed a slight decrease in total bacteria counts, total yeast, and mold in 0.5% gelatin, just as a decrease in total coliform in 1.0% gelatin [85].

The impacts of whey protein isolate (WPI) coatings containing a lactoperoxidase system (LPOS) on the repression of *Salmonella enterica* and *Escherichia coli* O157:H7 on roasted turkey were examined by testing both the underlying initial inhibition and the inhibition over time. With varying inoculation levels and LPOS concentrations, the initial antimicrobial effects of WPI coatings incorporating LPOS (LPOS-WPI coatings) were investigated. Initial 3- and 2-log CFU/g reductions of *S. enterica* and *E. coli* O157:H7 were observed in LPOS-WPI coatings containing 7 and 4% LPOS, respectively. *S. enterica* (6.0 log CFU/g) and *E. coli* O157:H7 (5.6 log CFU/g) inoculated sliced turkey was stored for 42 days at 4 and 10°C. LPOS concentrations in the coating solution and an LPOS solution for spreading were 5 and 3 % (wt/wt) for storage studies of *S. enterica* and *E. coli* O157:H7, respectively. After 42 days at both 4 and 10°C. LPOS-WPI coatings inhibited the growth of *S. enterica* and *E. coli* O157:H7 in the turkey. The LPOS-WPI coatings inhibited *S. enterica* and *E. coli* O157:H7 more effectively than the LPOS solution-spreading treatment. LPOS-WPI coatings also slowed total aerobic growth during storage [86].

The antimicrobial activity of gelatin–chitosan-based edible films infused with clove essential oil was tested against six selected microorganisms: *Pseudomonas fluorescens*, *Shewanella putrefaciens*, *Photobacterium phosphoreum*, *Listeria innocua*, *Escherichia coli* and *Lactobacillus acidophilus*. All of these microorganisms were inhibited by the clove-containing films, regardless of the film matrix or microorganism type. In

another experiment, when complex gelatin–chitosan film containing clove essential oil was applied to fish during chilled storage, Microorganism growth was drastically reduced in gram-negative bacteria, particularly enterobacteria, while lactic acid bacteria remained nearly constant for the majority of the storage period. The impact on microorganisms during this time term was steady with biochemical quality indexes, showing the reasonability of these films for fish protection [87].

The impact of various concentrations of lemongrass oil (0.1 %, 0.3 %, and 0.5% w/v) incorporation into an alginate-based [ glycerol 1.16 % (w/v), sodium alginate 1.29 % (w/v), and sunflower oil 0.025 % (w/v)] edible coating on the physicochemical properties, respiration rate, and microbiological and sensory quality of fresh-cut pineapple during 16 days of storage ( $10 \pm 1$  °C,  $65 \pm 10\%$  RH) were evaluated. The covered fresh-cut pineapple without lemongrass and uncoated fresh-cut pineapple which were controls, have been kept under similar conditions. The results appear that total plate counts, yeast, and mold counts, significantly ( $p < 0.05$ ) lower in coated samples containing 0.3 and 0.5 % w/v lemongrass than in other samples. However, the addition of 0.5% w/v lemongrass to the coating reduced the firmness and sensory scores (taste, texture, and overall acceptability) of fresh-cut pineapples significantly ( $p < 0.05$ ). As a result of the findings, an alginate-based edible coating containing 0.3 % (w/v) lemongrass can possibly expand the shelf-life and keep up the quality of cut pineapple [88].

Sweet cherries were coated with 1% Chitosan derived from shrimp waste and stored at 4°C for 25 days or 20°C for 15 days, then the impact on the physicochemical and microbiological properties of sweet cherries (*Prunus avium L.*) was evaluated. Weight loss, total soluble solids, pH, water activity, titratable acidity, total carbohydrate content, respiration rate, and other physicochemical and microbiological properties were weighted. The least weight loss after storage was specified to be 8.85 % in Chitosan-coated sweet cherries kept at 20 °C and 16.18 % in the control group stored at 4 °C. The control group and the Chitosan-coated sweet cherries had the lowest titratable acidity value (0.657 %) at 4 °C, and (0.6 %) at 20°C, respectively. At the end of each period, the water activity value was determined to be (0.969-0.974) for all sample groups and storage conditions. At 4 °C, the general count of mesophilic aerobic bacteria in sweet cherry covered with Chitosan was observed to be less than detectable (2 log CFU/g) whereas the control group had 2.74 log CFU/g. At 4 °C for 25 days, yeast and mold growth were suppressed in sweet cherries coated with Chitosan, while the highest yeast and mold count was determined to be 4.75 log CFU/g in control sweet cherries at the end of storage. It was discovered that different Chitosan coatings have varying effects on several quality attributes at different storage temperatures. When microbiological investigations are taken into account, it is possible to infer that Chitosan, particularly those derived from shrimp wastes, has strong antimicrobial properties and can be utilized effectively to extend the shelf life of sweet cherries [89].

To preserve the quality and safety of crimson seedless table grapes during cold storage and subsequent shelf life, an edible covering based on Aloe-Vera gel was applied. Uncoated groups deteriorated rapidly, with an expected shelf life of 7 days at 1 °C in addition to 4 days at 20 °C, in light of the quick weight reduction, color changes, accelerated softening, and ripening, rachis browning. Those clusters coated with Aloe-Vera gel edible covering, on the other hand, greatly delayed the above parameters linked to postharvest quality losses, and storability may be extended up to 35 days at 1 °C. And this edible covering was able to minimize the initial microbial counts for both mesophilic aerobic and yeast and molds, which grew dramatically in uncoated samples after storage. Besides, sensible investigations exhibited helpful impacts as far as postponing delaying rachis browning and dehydration, just as keeping up with the visual part of it without influencing taste, aroma, or tastes [85].

The study examines the impact of Chitosan-based edible coverings containing Aloe Vera extract on postharvest blueberry product quality during storage at 5 °C. Coatings containing 0.5 % (w/v) Chitosan + 0.5 % (w/v) glycerol + 0.1 % (w/v) Tween 80. Physicochemical (titratable acidity, pH, weight loss) and microbiological studies of coated blueberries were carried out over a period of 25 days. Microbiological growth and water loss levels were reduced by roughly 50% and 42%, respectively, in coated blueberries after 25 days when compared to uncoated blueberries. Weight reduction after 15 days was 6.2% for uncoated blueberries and 3.7% for Chitosan–A.Vera

coated blueberries. Mold contamination was found in uncoated fruits after 2 days of storage ( $2.0 \pm 0.32 \log \text{CFU g}^{-1}$ ), whereas it was ( $1.3 \pm 0.35 \log \text{CFU g}^{-1}$ ) in fruits with Chitosan-based coverings with A.Vera after 9 days of storage. Generally, the coatings formed in this work increase the shelf-life of blueberries by roughly 5 days, which reveals that for the first time that the combination of Chitosan and A.Vera liquid fraction as edible coating materials has massive potential in increasing the shelf-life of fruits [90].

In one study, Chitosan-based films sachets with inserted chestnut extract (CE) were used to pack fresh pasta, a comparative analysis was performed to assess the sachet's effects on microbial development, moisture mobility (with respect to phenolic content), and microstructural modifications of packed material. To improve the antioxidant and antibacterial characteristics of Chitosan-based film materials, a tannin-rich chestnut extract was utilized. After nine days of retention in a Chitosan-chestnut extract (CH-CE) sachet, the results indicate that a retrogradation process of pasta occurred, resulting in a hard-like texture. Changes in water activity ( $a_w$ ) indicate a total phenolic content concentration or dilution when pasta and CH-CE sachet comes into touch. Regardless, the pasta in the CH -CE sachet was free of microbiological deterioration for the whole 60-day shelf life [91].

## **1.6 Film and Coating Preparation**

Arrangement of biodegradable and/or edible films incorporated the usage of at least one film-framing agent (macromolecule); polysaccharides, proteins,

and/or lipids, a solvent, and a plasticizer. Proteins and polysaccharides are the foremost widely investigated biopolymers within the field of edible coatings and films. Films and coatings are additionally made of proteins of both animal and plant origin. The properties of the final film are affected by the intrinsic properties of proteins include amino acid composition, crystallinity (of the protein and/or plasticizer), hydrophobicity/hydrophilicity, surface charge, molecular size, and three-dimensional shape. For example, the presence of cysteine allows for potential disulfide bridge formation, as noted for beta-lactoglobulin, High concentrations of leucine, alanine, and other nonpolar amino acids can create hydrophobic proteins, as seen during a Zein and extrinsic processing factors that include processing temperature, pH, salt type, drying conditions, ionic strength, ratio during processing and storage, shear, and pressure [92,93]. In general, protein films are effective barriers to lipid, oxygen, and aroma at low relative humidity (RH) conditions, therefore, proteins are widely used to form edible films. Proteins such as gelatin, whey protein, wheat or corn proteins, and soy protein have been widely studied [94,95]. Protein-based edible films get significant consideration as of late in view of their benefits, including their utilization as edible packaging materials, over-synthetic films. Additionally, protein-based edible films can work as transporters for antimicrobial and antioxidant agents. Through a similar application, they also can be used at the surface of the food to control the diffusion rate of preservative substances from the surface to the interior of the food.

Distilled water and ethanol are the two solvents that choose to make edible films and coatings because they are safe to consume. However, if the application of film or coating from an agricultural protein is not intended for the application of food, other organic solvents such as acetone or other organic materials may be used. In addition, the properties of a formed film may be affected by the solvent used, Such as in a study by Yoshino et al. (2002), Zein films were made utilizing either fluid ethanol or aqueous acetone as the dissolvable system. At the point when ethanol was used as the dissolvable, coming about films at first had higher rigidity when contrasted with those made with acetone; however, they were likewise more exposed to moisture and high humidity environments [96]. But instead of these solvents, it may be used soaked water/ juices for several types of fruits or vegetables, because they are considered rich sources of flavonoids, polyphenols, and antioxidants in order to obtain them to give special properties to the film and obtain a functional edible film. Edible films based on these plant materials indicate an alternative way to consume nutrients including pigments and polyphenols with an antioxidant capacity [97].

Like most synthetic polymers, edible film materials require property modifiers to work on the physical and mechanical properties of the film. As with synthetic plastics, plasticizers are incorporated into edible coating film materials that overcome the brittleness caused by the extensive forces of intermolecular. Plasticizers; low volatility molecule, are added to polymeric materials to modify their three-dimensional coordination, reduce the forces



of attractive particles, rise free volume and increase chain transport [98,99]. Ordinarily utilized plasticizers in film frameworks are monosaccharide, disaccharides, or oligosaccharides (e.g. glucose, fructose-glucose syrups, sucrose, and honey), polyols (e.g. glycerol, sorbitol, glycerol derivatives, and polyethylene glycols), and lipids and their derivatives (e.g. phospholipids, fatty acids, and surfactants). Plasticizers are generally required at around 10-60% on a dry premise, contingent upon the firmness of the polymer they are essential for making films more flexible and smoother and stay away from pores and cracks in the polymeric matrix [100].

However, plasticizers can add a significant change in film properties including; diminishing tensile strength (TS), expanding film permeability to water, and increasing the adsorb water capacity of the films, In this regard, the sort and amount of plasticizers utilized and their functions in films contribute significant provisions to the final performance of films [101-103]. Most protein-based films and coatings are exceptionally strong, however extremely brittle when not plasticized [104]; so a plasticizer is important to further develop the application capability of protein-based films. In polymer science, plasticizers can be either described as internal or external. External plasticizers are low volatile substances that are added to polymers, for this situation, plasticizer particles interface with polymer chains, yet are not synthetically joined to them by essential bonds and can, hence, be lost by evaporation, migration, or extraction. Otherwise, internal plasticizers are intrinsic parts of the polymer atoms and become part of the item, which can

be either co-polymerized into the polymer structure or reacted with the original polymer [105]. Internal plasticizers for the most part have massive constructions that provide polymers with more space to move around and keep polymers from approaching together, they soften polymers by reducing glass transition temperature ( $T_g$ ) and accordingly decreasing the elastic modulus, it can synthetically change a protein chain through the addition of substituent groups joined by means of covalent bonds and makes a steric boundary between the protein chains, leading to expanded free volume and improved flexibility. For the two types, it was observed that the properties of the materials are strongly dependent on temperature, although this dependence is more pronounced for the internal plasticizers. The advantage of utilizing external plasticizers, contrasted with internal ones, is the opportunity to choose the right substance relying upon the ideal item properties [100,106].

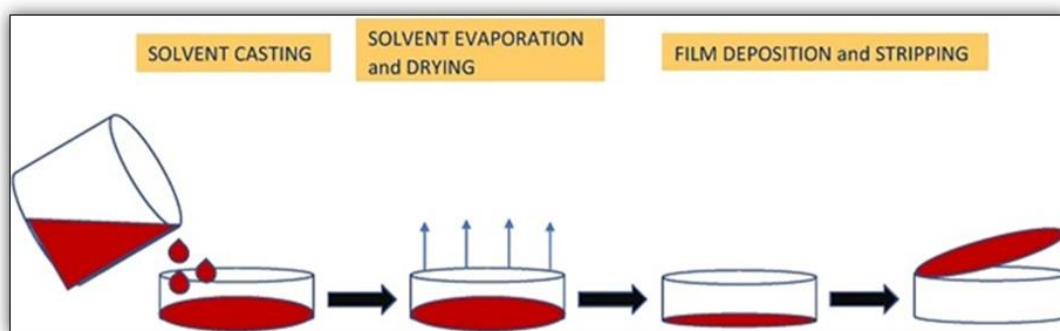
The edible film is generally wrapped over the outer layer of a food item as a solid matrix or food was dipped in the film-forming solution, film can serve as primary packaging without any sensory or nutritional appeal. In these cases, it is assumed to be flavorless, colorless, and does not interfere with the sensory features of the food item [107]. The chemical and structural properties of film-forming biopolymers and additives should be surely known and adjusted to develop films for specific applications. Edible films can be obtained from edible materials in two different ways: wet (solvent casting) and dry (extrusion) processes. The solubility of film-forming

biopolymers and additives is vital to the casting method, and thermoplasticity of biopolymers alongside the phase transition, glass transition, and gelatinization properties should be perceived in the extrusion process to form the film [108-110].

The film-forming procedures can be determined by the structural chemistry of biopolymers, whereas the film characteristics such as mechanical strength, moisture, and color, etc. are controlled by the physical chemistry of biopolymers. For example, the cohesiveness of film-forming materials can influence the mechanical strength and the analogous structure of films; weak mechanical strength film will form from film-forming materials with poor cohesion, which can be reduced by using plasticizers [111,112]. There are different protocols to use to prepare edible film and coatings from agricultural proteins such as extrusion, casting, spinning, and others, which the properties of the final film or coatings are affected by it. Depending on the starting material several processes can be used to form films. The biopolymers present in a solution can form films depending on several factors including changing the conditions of the solution, application of heat, addition of salt, or a change in pH [113].

The preferred technique used to frame edible protein films for research, includes three stages to set up a film from biopolymers: a) Solubilization of biopolymer in an appropriate solvent, b) Casting solution in the plates, c) Drying off the casted film [114]. It is a cost-efficient and productive strategy, and various types of equipment are available for film casting from simple

casting plates to more advanced batch and continuous lab coaters, which is the most ordinarily utilized technique for forming protein film samples for research by manually spreading dilute film solutions (usually 5–10% solids) of protein and plasticizer into Petri dishes or plates, then drying them under fitting conditions or controlled relative humidity. Taking into consideration; the method of drying can fundamentally influence the actual properties of the final film, including film morphology, appearance, and barrier or mechanical properties [115].



**Fig 1. Lab casting method of film formation.**

Wet-spinning is a preparation procedure usually utilized in the textile industry to form fibers. During formation, a solution of polymer is gone through a pin hold spinneret under tension. In spite of the fact that they were effective in forming films, there no expansion in the orientation of the protein chains was noticed. Since the modified film-forming process uses fewer tension factors than conventionally used material preparation, the authors proposed that shear could assume a part in orientation [116].

Extrusion is an alternative option in contrast to solvent casting, which uses raised temperature and shearing to soften and melt the polymer, permitting a

cohesive film matrix to shape. Extrusion of proteins into films has specific benefits over solvent casting. Relatively, extrusion is speedier and requires less energy, on account of the way that more concentrated film solutions can be dealt with into the extruder. While solvent casting is energy-intensive and time-consuming, due to vaporizing ethanol, or water, specifically, expands the costs of manufacturing edible films. The utilization of extrusion diminishes time and energy contributions to bring the value of biopolymer film development to a competitive scale with manufactured film creation. Results from recent studies on the preparing parameters to extrude transparent, adaptable whey protein sheets utilizing a twin-screw extruder explored that feed composition, temperature, and screw speed differed. These extruder measurements and working conditions considered sufficient heat denaturing and cross-linking of the whey protein to supply sheets that had altered tensile properties when contrasted with solvent-cast heat-denatured whey protein films [117-119].

### **1.7 Edible coating applications**

Fresh produce quality factors are important to ensure marketability. Post-harvest losses in fresh produce are an important issue due to their rapid decomposition during handling, transportation, and storage. It is well known that most foods are susceptible to mechanical spoilage, physiological deterioration, water loss, and decay during storage. Reduced turgidity due to water loss leads to faster wilting and depletion of nutrients and sensory properties which is a major cause of deterioration. Cold storage of some

foods is common, but other foods such as fruits are usually stored at room temperature in markets between harvest and consumption. For this reason, these product losses are very high. By using edible coatings/film and cold storage, spoilage can be reduced. Edible Films can be applied to fruits, vegetables, meats, poultry, and grains that can be frozen, processed, or freshly processed. Essentially, the purpose of edible coatings is to increase the natural barrier of food products. Likewise, a very important fact of edible coatings is that they can be eaten safely as part of the products and are environment-friendly at the same time extending the shelf life of fresh produce. To maintain the quality of food products, the application of edible coatings and films on these products can be considered an effective method. The edible coating/film provides steady nature of food items with market integrity, nutritional value, and economic cost of creation in the food treatment sector [120], as it keeps up of boundaries of significant value and appearance upgrades the shelf life of food products (vegetables, fruits, meat, fish, and dairy products, bakery, etc) on account of the promising properties of the barrier against moisture, gas transmission, lipid oxidation and control of enzymatic activities and microbial deterioration [121]. Regular antioxidants and antimicrobial agents have likewise been joined into the edible film to delay autoxidation of high-fat-rich items just as increment the oil resistance of fried food items [122].

Edible materials give obstruction properties against moisture and gases of fresh items during the ability to upset the enzymatic oxidation, which shields

food from browning discoloration and softening of texture. It likewise can prevent the deficiency of natural volatile flavor and color components [123]. The nature of food items relies upon sensory, and microbiological properties in addition to nutritional, which are dynamically changed during handling, storage, or marketing. These progressions are the consequences of food items and environmental interactions and may prompt changes that debase food quality, for example, water and gas reduction [124]. Edible coatings and films have been represented to adequately protect the nutritional, sensory, and microbiological properties of various food products. According to Sharma et al. (2019) Edible coatings and films assist with preserve phytochemicals (antioxidants, phenols, pigments), and phytochemical properties (pH, soluble solids content, respiration rate, weight loss) throughout an extensive period of time in fresh and minimally processed fruits and vegetables [125]. Edible coatings and films have generally been made unflavored, transparent, which don't upset the organoleptic nature of food items. However, the new outcomes show that organoleptic properties, such as color, flavor, appearance, so on, are fundamental in some applications such as sushi rolls and pizza toppings [126].

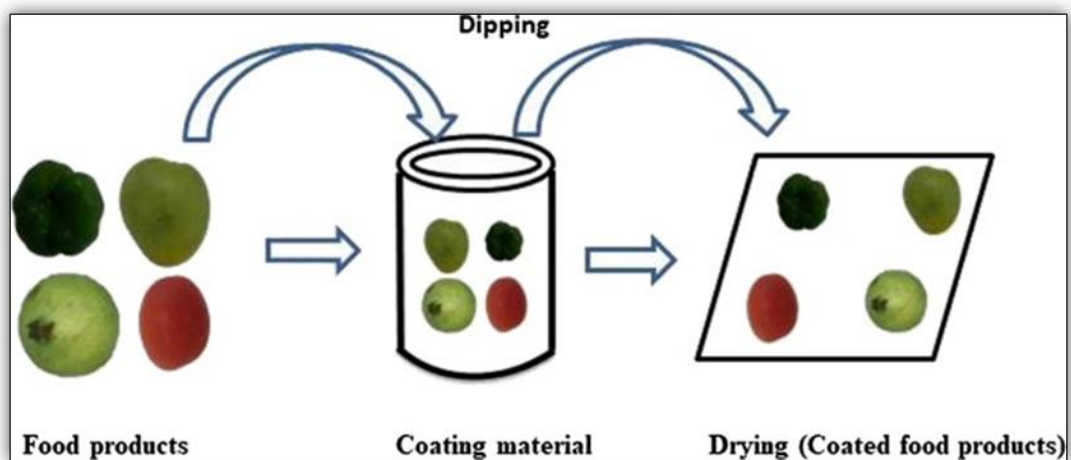


**Fig 2. Scheme illustrating the main characteristics of edible films and coatings**

Dipping is the most commonly recognized technique for covering food items, it is the immersion of a food sample in the film-forming solution (dipping process) [127]. The dipping technique for applying edible covering on food products consists of three steps: i) immersion & dwelling, ii) deposition, and iii) solvents evaporation [128,129]. The substrate is immersed in the coating emulsion/solution at a continuous speed, ensuring an adequate amount of solution for wet the substrate and complete connection between both substrate and coating matrix [130], thin layers of

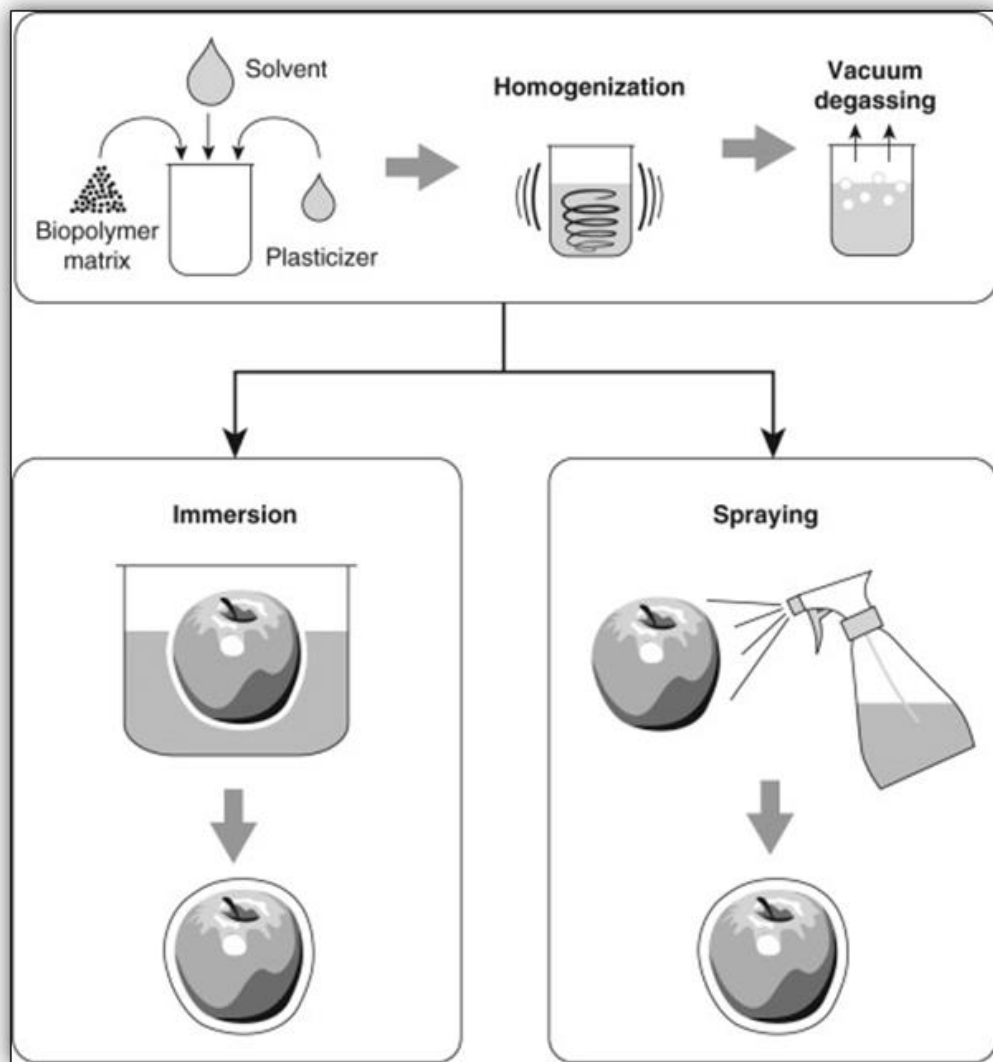


precursor emulsion are shaped on the outer layer of food items. The overabundance of surface liquid is drained and taken out by deposition [129]. The solvent and excess liquid are then permitted to evaporate from the outer layer of the food items utilizing heating and drying methods. The item is dried either at room temperature or with the assistance of a dryer when the excess coating is drained away [128].



**Fig 3. Dipping application methods of edible coating for fruits**

Spraying is the most common method used to apply a coating to food products [124,131], used to apply a uniform coating to the surface of food, and is potentially a more controllable coating application method than other methods. It increases the surface of the liquid by creating drops and dispersing them across the food surface with a set of nozzles. There are three kinds of spraying procedures that have been utilized in industries to apply an edible coating to food products; (i) Air spray atomization, (ii) Air-assisted airless atomization, (iii) Pressure atomization.



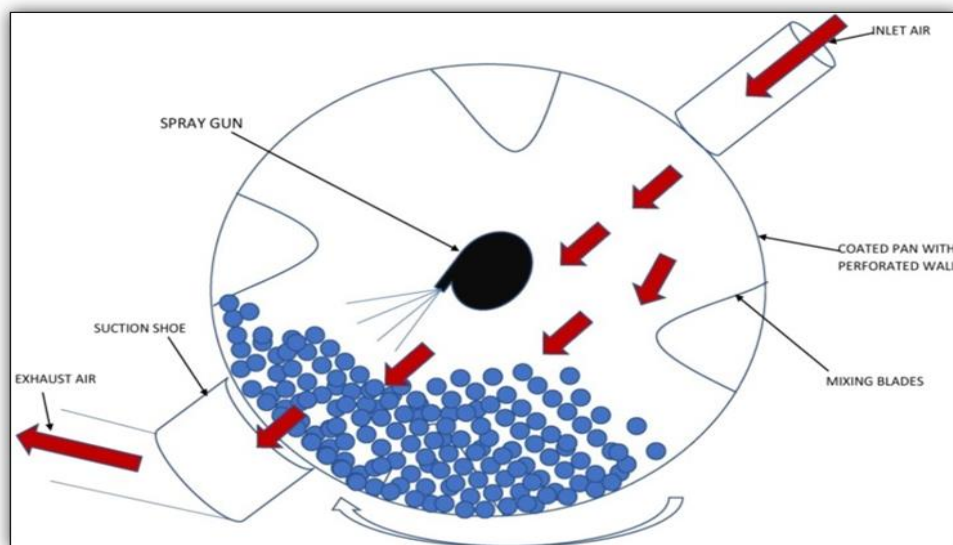
**Fig 4. Spraying application methods of edible coating for fruits**

The fluidized-bed technique of edible coating is generally utilized in food processing and exploration applications. It is utilized to apply slight layers of covering material to dry particles of uncommonly low density as well as a small size [132], and it is generally utilized by the pharmaceutical industry for tablet coating. The coating solution and suspension are showered onto the fluidized powder surface through various nozzles to shape a shell-like structure. The fluidization interaction happens when the liquid stream moves

up through a bed of particles accomplishing sufficient speed to help the particles without redirecting them into the liquid stream. The fluidized-bed process technique is ordered into three classes: top spray, bottom spray, and rotating fluidized bed. Although, in the food industry, the conventional top spraying strategy is more functional than different strategies [133]. The molecule size in the fluidized bed must be more than 100  $\mu\text{m}$  in light of the fact that the powders in the conventional bed don't tend to steadily or shape unreasonable agglomerations at more smaller sizes [134,135]. Powder agglomeration amplifies the scattering and solubility of the coating material. Simultaneously, the stability of the material improved by using a liquid binder, the process takes place under heat treatment before being sprinkled on foods. This interaction prompts the attachment, collection, and drying of the particles and is applicable to both continuous and batch operations. Nozzles generally utilized in fluidized-bed coatings are pneumatic or binary nozzles: the liquid is conveyed at low pressure and the air is sheared into the nozzles. The activity of the fluidized bed during drying of the coating appeared to lessen the development of clusters onto the coated items.

The panning covering technique began in Greek Arabian culture and was utilized by pharmaceutical and candy industries and in the uses of medications. The panning technique is putting the food and other different things that needed to be covered in a huge rotating bowl called a pan. The solution is then sprinkled into a rotating compartment and the product is stirred to ensure that the solution reaches all parts of the food and has good

coverage. Coatings are dried using vigorous air either at room or higher temperatures [136]. In this strategy, heat is made by rubbing with cold air. This technique is separated into three unique kinds of utilization measures subject to the attributes of the food items; hard panning, soft panning, and chocolate panning. The use of the hard shell is hard panning by the steady use of sugar syrup to the surface which dries and solidifies. Soft panning incorporates the usage of corn syrup and a blend of sugar as a syrup for covering, which is dried by applying dry sugar to shape thick, soft, but fewer layers. Chocolate panning is using a fat-based layer around the middle. Chocolate and cocoa-based candy or white (favorable) composite coatings can be used [137].



**Fig 5. Panning application method of edible coating.**

## Chapter 2

### Materials and Methods

#### 2.1 Materials

The black grapes (*Vitis vinifera*) were harvested from house-grown trees, washed, seeds removed, and juice extracted with a fruit juicer. The juice was filtrated with cheesecloth to separate the skins and stored at  $-20\text{ }^{\circ}\text{C}$  until usage. Sweet cherries (*Prunus avium* L., cv Sweetheart) were obtained from a local market in Nablus, transported, and stored at room temperature until treatment. *Nigella sativa* defatted seed cake (NSDSC) was purchased from Alhathnawi General Trade Co. (Jenin, Palestine). All chemicals, BBL™ Mannitol Salt Agar and other solvents used in this study were obtained from Sigma-Aldrich Company. Mueller Hinton Broth Himedia M391-500g obtained from HiMedia Leading BioSciences Company. Bacterial strain *Staphylococcus aureus*, from American Type Culture Collection (ATCC 25923) and *Escherichia coli* (E. coli O157:H7) was obtained from microbiology laboratory An-Najah National University.

#### 2.2 Methods

#### 2.3 Protein extraction and determination

The protein was extracted from NSDSC by the acid-base extraction method as previously described [138] with some modifications. Dry NSDSC was ground utilizing an electric mill at a high velocity, for 5 min and the powder

was dissolved in distilled water (1:10, w/v), the pH was adjusted at 12.0 with 1 N NaOH and stirred at medium speed for 2 h at room temperature. After centrifugation at 4000 rpm for 20 min, the supernatant was collected and pH adjusted at 5.4 with 1 N HCl to form a precipitate which was then isolated by centrifugation at 4000 rpm for 20 min, to be collected and dried at 30°C and 20% relative humidity (RH). The got protein concentrate (PC) was finely ground and the protein moisture content, crude fat, ash, and carbohydrate were evaluated.

Protein content was determined by Kjeldahl's method [139] using a nitrogen conversion factor of 6.25. Moisture content is generally determined by weight loss upon drying, it was determined by using the Moisture Analyzer (Sartorius™ MA100 Infrared Moisture Analyzer), which performs moisture analysis by using the loss on drying method, based on the vaporization of water during the material is heated.

Crude fat is the term used to refer to the crude mixture of fat-soluble material present in a sample. The ANKOMXT15 extraction system is a common approach designed to extract crude fat is based on the solubility of lipids in non-polar organic solvents. The analysis is achieved by measuring the loss of mass due to the extraction of fat/oil from the sample encapsulated in a filter bag. Crude Fat contained within a food or feed sample can be calculated using the following formula:

$$\text{Crude fat (\%)} = 100 \times \frac{W_2 - W_3}{W_1} \quad (1)$$

Where: W1 = Original weight of the sample, W2 = Weight of pre-dried sample and filter bag, W3 = Weight of dried sample and filter bag after extraction.

Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in a foodstuff. Analysis of nutritional evaluation is done by determining the ash content of the food. Ashing is the primary step while preparing a sample for elemental analysis. The dry ashing method with a Muffle Furnace determines the ash content of a variety of food products. The ash content is calculated as follows:

$$\text{Ash (\%)} = \frac{M(\text{ASH})}{M(\text{DRY})} \times 100 \quad (2)$$

Where MASH, MDRY refers to the mass of the ashed sample, and original masses of the dried samples, respectively.

## **2.4 Film preparation**

The film-forming solution was prepared by dissolving the PC under continuous stirring in the diluted grape juice (4g / 100ml juice), and the pH value was adjusted to pH 12.0 by using 1 N NaOH until the powder was completely solubilized. After that, the same volumes (100 mL) of all film-forming solutions FFSs (50 mL each containing 400 mg protein), were casted onto 15 cm diameter polystyrene Petri dishes at pH 12.0 and allowed to dry at room temperature for 72 h, and finally, the dried films were analyzed.

## **2.5 Film characteristics**

In order to compare the effects of various treatments to protein films, their mechanical, physical, and biological properties have to be determined.

### **2.5.1 Film mechanical properties**

The dried films were peeled from the casting surface and conditioned at 25°C and 50% relative humidity for 2 h by placing the film samples into a desiccator over a saturated solution of  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ , then their thickness was measured with a micrometer screw gauge (0-25mm, 0.1  $\mu\text{m}$ ), at different positions for each film sample. The mechanical characteristics measured according to ASTM D882 method [140], by using a universal testing instrument (Brookfield CT3 Texture Analyzer, model CT3 50K), were described in the relevant literature are as follows: (a) Tensile strength (TS) which is the pulling force per film cross-sectional area required to break the film; (b) The elongation at break for the degree to which the film can stretch before breaking, and (c) Young's modulus which provides information about a film's resistance to deformation [141], films strips (1 cm wide) was mounted between the grips of the texture analyzer and tested with an initial grip separation of 50 mm and a crosshead speed of 0.5 mm/s. Three samples of each film type were tested.

**Tensile Strength (TS):** This is calculated by dividing the load at break by the original minimum crosssectional area. The result is expressed in megapascals (MPa).



$$\text{Tensile strength} = \frac{(\text{Load at break})}{(\text{original width})(\text{original thickness})} \quad (3)$$

Percent Elongation (EB): This is calculated by dividing the elongation at the moment of rupture by the initial gauge length and multiplying by 100. Which the distance between the grips is used as the initial gauge length. The result is expressed in percent.

$$\text{Percent elongation} = \frac{(\text{elongation at rupture}) \times 100}{(\text{initial gage length})} \quad (4)$$

Young's Modulus (YM): This is calculated by drawing a tangent to the initial linear portion of the stress-strain curve, selecting any point on this tangent, and dividing the tensile stress by the corresponding strain. For purposes of this calculation, the tensile stress shall be calculated by dividing the load by the average original cross-section of the test specimen. The result is expressed in megapascals (MPa).

$$\text{Young's modulus} = \frac{\frac{(\text{load at point on tangent})}{(\text{original width})(\text{original thickness})}}{\frac{(\text{elongation at point on tangent})}{(\text{initial gage length})}} \quad (5)$$

## 2.5.2 Film physical properties

### 2.5.2.1 Moisture Content and Uptake

The moisture content was measured according to Galus and Lenart [142], it was determined by the mass loss of 1 g of the film after 24 h of oven drying at 105 °C and expressed as the percentage of initial film mass loss during drying, the ability of each specimen to absorb moisture was determined by measuring the weight gain of each specimen at 50% RH after 24h. Three repetitive analyses of each film were made and the results were expressed as mean value. The 3 squares (2 × 2 cm) were cut from the films and weighed (W1), then the films were put in the oven (105 °C) for 24 h and then weighed again (W2), Then squares were conditioning at 25 °C and 50% RH for 24 h by placing the film samples into a desiccator over a saturated solution of Mg(NO<sub>3</sub>)<sub>2</sub>•6 H<sub>2</sub>O, after that they were weighed (W3).

Water content and uptake were calculated according to the following formulas:

$$\text{Water content (\%)} = \frac{(W1 - W2)}{(W1)} \times 100 \quad (6)$$

$$\text{Water uptake (\%)} = \frac{(W3 - W2)}{(W3)} \times 100 \quad (7)$$

### 2.5.3 Films biological properties

#### 2.5.3.1 Antioxidant activity of the film

The ability of films and of every single component of the film-casting solution to scavenge DPPH free radicals was assessed using the method described by Siripatrawan and Harte [143] with some modifications. Briefly, the films (20 mg) were dissolved in water (500 mL). Then sample solutions (100 mL each) were mixed with 900 mL of DPPH methanolic solution (0.05 mg/mL). After 30 min in darkness at room temperature (25 °C), the absorbance was recorded at 517 nm. The percentage of DPPH free radical quenching activity was determined using the following equation:

$$\begin{aligned} & \text{DPPH scavenging effect (\%)} \\ & = 100 - \left[ \frac{(\text{Abs DPPH} - \text{Abs sample})}{(\text{Abs DPPH})} \times 100 \right] \end{aligned} \quad (8)$$

where Abs DPPH is the absorbance value at 517 nm of the methanolic solution of DPPH and Abs sample is the absorbance value at 517 nm for the samples. Each sample was assayed three times.

#### 2.5.3.2 Film Antimicrobial Activity

The bacterial strain of *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (E. coli O157:H7) was activated twice in a nutrient broth to reach a cell concentration corresponding to 0.5 turbidities. Antimicrobial activity testing of the edible films was carried out using the agar diffusion method according to Pranoto et al. [144]. The edible films were cut into 5

mm diameter discs and then placed on Mueller Hinton agar plates. These had been previously seeded with 0.2 mL of inoculums containing approximately  $10^5$ - $10^6$  CFU/mL of tested bacteria. The plates were then incubated at 37°C for 24 hours. Finally, the inhibition zones were observed and evaluated. Experiments were done in triplicate.

## **2.6 Sweet cherry wrapping**

Sweet cherries were selected, of uniform size, color, without physical damage and fungal infections, washed with tap water, and dried at room temperature. They were randomly divided into three groups was wrapped (W) by sealed NS-PC bags (10 × 10 cm) and in sealed low-density polyethylene (LDPE) bags with the same size of the film, and the control was unwrapped (UW). These samples were placed at -20°C, the quality of both wrapped and control samples was evaluated during storage every week for 40 days.

### **2.6.1 Physicochemical analysis of sweet cherries after storage**

Samples of 2 cherries from each bag were assessed for color, pH, titratable acidity and, soluble solids. Color (L\*a\*b\* mode) was measured with a Konica Minolta CR-400 Chroma Meter. And expressed as hue angle according to the method of McGuire [145].

The pH values of the juice obtained by hand crushing the cherries in the bags were recorded using a pH meter, then it was titrated with 0.1 N NaOH for titratable acidity (TA) which was expressed as the percentage of malic acid (%).

The Total soluble solids concentration (TSS) in the juice was measured with a refractometer (A. KRÜSS Optronic GmbH. DR6100-T).

## Chapter 3

### Results and Discussions

#### 3.1 Proximate analysis of *Nigella sativa* seeds and their derivatives.

Using an Acid-Base extraction technique, protein concentrates were prepared from the defatted seed meals. Consequently, protein, moisture, fat, carbohydrate, and ash contents of concentrate protein were determined immediately after drying and then compared with raw *Nigella sativa* seeds (Table 1). The results showed that the protein concentration of raw seeds was  $20.3 \pm 0.63\%$  and after the extraction of protein based on the acid base extraction method was  $45.1 \pm 2.5\%$ . Moreover, the fat concentration of raw seeds was  $45.4 \pm 0.53\%$  while in the PC was  $3.1 \pm 0.7\%$  due to the defatted process that proceed to extract the *Nigella sativa* oil before the protein extraction.

**Table 1: Proximate analysis of the *Nigella sativa* (NS) seeds, *Nigella sativa* defatted seeds cake (NSDSC) and protein concentrate (PC) obtained from defatted seeds cake.**

Compositions (%)	NS seed*	NSDSC	PC
Protein	$20.3 \pm 0.63$	$34.0 \pm 2.7$	$45.1 \pm 2.5$
Moisture	$7.1 \pm 0.22$	$7.5 \pm 0.1$	$5.0 \pm 0.3$
Ash	$7.4 \pm 0.27$	$5.5 \pm 0.1$	$3.7 \pm 0.7$
Fats	$45.4 \pm 0.53$	$18.2 \pm 0.5$	$3.1 \pm 0.3$
Carbohydrate	$19.7 \pm 0.44$	$34.8 \pm 2.3$	$43.1 \pm 1.4$

\* Results was according to [146].

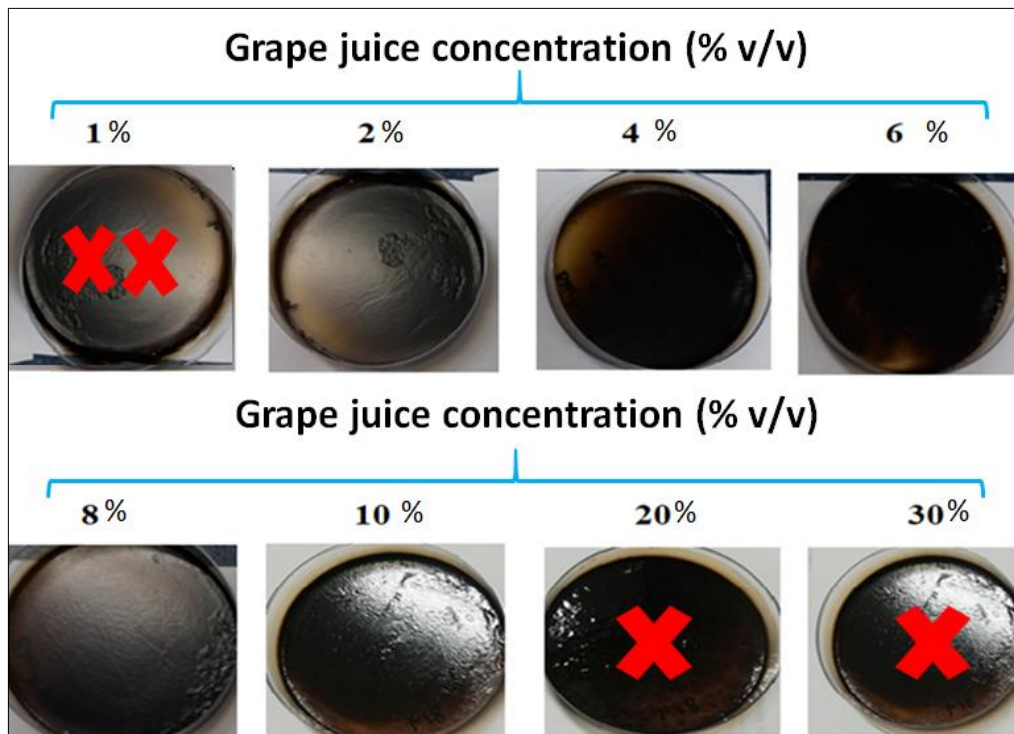
### **3.2 *Nigella sativa* edible films obtained in the presence of different concentrations of grape juice**

*Nigella sativa* protein concentrate (*NSPC*) powder was dissolved with different concentrations of GJ at pH value 12. The initial experiments showed that the pH value of the film forming solution is critical to obtain very good film appearance and properties. The *NSPC* films cannot obtain at pH less than 8.0 by using GJ. The *NSPC*/GJ film forming solution were casted in Petri dish and dried for at least 48h. Fig 6, showed that adding 2, 4, 6, 8, and 10% was sufficient to obtained handleable films was homogenized, flexible, and easily to peel off from the Petri dish, without any observed defects cracks or pores. Except when GJ concentrations was 1, 20, and 30%, where the film with 1% GJ was rigid, brittle, and easily broken with many cracks, that because the GJ concentration was not enough to plasticize protein polymers, while at 20 and 30% the obtained films were sticky and difficult to separate from Petri dish due to high concentration of GJ. Therefore, films containing 1, 20, and 30% of GJ are excluded.

Protein-based films typically contain food materials, which can change over time, resulting in the loss of protective functions and affecting the appearance of the covered food. As a result, protein-based film formulations require the addition of a plasticizing agent above the minimum threshold to reduce film fragility and obtain certain plastic properties [147,148]. Plasticizer molecules reduce intermolecular interactions along polymer chains, resulting in increased flexibility, extensibility, and toughness.

Plasticizers, on the other hand, decrease the films mechanical resistance and barrier characteristics [149]. The most common use plasticizers are polyols, mono-, di- or oligosaccharides. Physicochemical properties of edible films produced from whey proteins and plasticized with sucrose have been investigated by several authors, who found that these films are flexible, strong, and extremely glossy, as well as possessing good oxygen barrier qualities [150,151]. So, The main reason for the formation of a film based on *NSDSC* protein with good physicochemical characteristics without adding glycerol as a plasticizer is the sugars in grape juice that act as plasticizers as in the case of sucrose. Veiga-Santos et al. [152], successfully obtained cassava starch films by using sucrose or invert sugar. Moreover, the pea starch-guar gum was also plastics with different sugars [153]. The film color was black due to the seed pigmentation that are phytomelanins, high-molecular weight polymers which formed by the oxidation of phenols [154-156]. The black color of the obtained film will help to protect food products, medicines or other products from an oxidation that can harm these products.



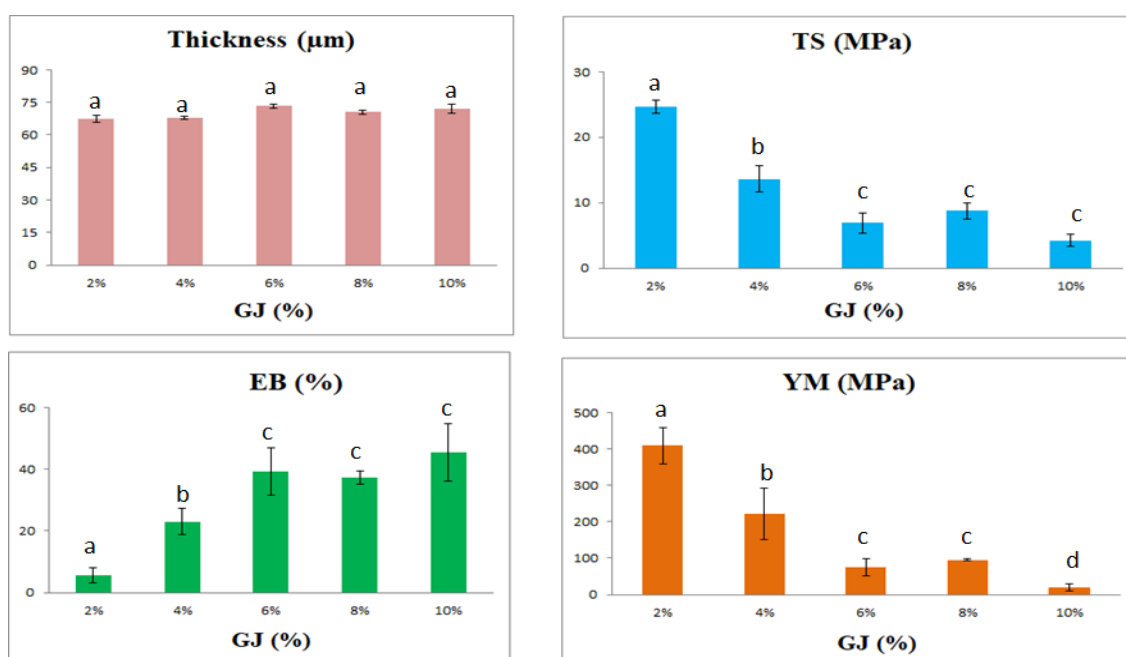


**Fig 6.** Images of NSDSC protein film containing different concentrations of grape juice (GJ), obtained at pH 12.0. Not-handleable—either brittle (XX) or sticky (X). Further experimental details are given in the text.

### 3.3 Film characterization

#### 3.3.1 Film thickness and mechanical properties

Thickness and mechanical properties of edible films are important to ensure that the films have adequate mechanical strength and integrity during transportation, handling and storage of foods [157]. Figure 7, reported that the film thickness and mechanical properties of NSDSC proteins based films prepared with different concentrations of grape juice (2, 4, 6, 8 and 10% V/V) and casted at pH 12.0.



**Fig 7. Effect of different concentrations of grape juice (GJ) on the film thickness and mechanical properties (TS, tensile strength; EB, elongation at break; YM, Young's modulus) of NSPC edible films obtained at pH 12.0. Different statistical symbol (a,b,c,d) indicated significant different between treatments ( $p < 0.05$ ).**

Results clear indicated there is no significant difference in films thickness by increasing the GJ concentrations. The film thickness was between 72-76  $\mu\text{m}$ . The different GJ concentrations have affected significantly ( $p \leq 0.05$ ) the NSPC film TS, EB and YM. The obtained results showed that the NSPC film TS and YM was reduced significantly when the GJ increased. Whereas, film EB increased significantly until 6 % GJ and remain at almost same value at 8 and 10% GJ. Those film mechanical properties were observed by using glycerol that recognized as the most used plasticizer to obtained films even to proteins or polysaccharides based films [158,159].

Wherefore, we recognized GJ has plasticizing effect for NSPC film, based on the main ingredients of GJ that mainly sugars that well known has

plasticizing effects. In the previous work was demonstrated that monosaccharide especially glucose produced thinner films that due to the similarity of its chemical structure to the repeating units of polymers [152,153], concluded that by increasing the sucrose or invert sugar as plasticizer to cassava starch significantly increasing the film EB.

Table 2, comparing the mechanical properties of *NSDPC* films incorporated with 2% and 6% GJ, as well as those of some commercial edible casing called (Viscofan NDX) that obtained from gelatin, and plastic high density polyethylene (HDPE) materials analyzed in the previous studies [160]. As shown in Table 2, film prepared with a concentration of 2% of GJ had higher film thickness. Film prepared with 2% GJ almost has similar TS and YM properties with Viscofan (NDX) commercially available material, as well as film prepared at 6% GJ had similar YM properties with HDPE commercial material.

**Table 2. Thickness and mechanical properties of some commercial materials and of *NSDSC* protein films incorporated with 2 and 6% GJ.**

Film	Thickness ( $\mu\text{m}$ )	TS (MPa)	EB (%)	YM (MPa)
<i>NSPC</i> + GJ 2%	$67.5 \pm 1.5$	$24.6 \pm 1.1$	$5.6 \pm 2.3$	$409.5 \pm 50.0$
<i>NSPC</i> + GJ 6%	$73.3 \pm 0.81$	$6.90 \pm 1.5$	$39.3 \pm 7.6$	$74.5 \pm 23.10$
Viscofan (NDX)*	$30.0 \pm 0.4$	$36.6 \pm 8.1$	$13.1 \pm 2.9$	$356.0 \pm 29.0$
HDPE*	$36.2 \pm 1.7$	$13.1 \pm 1.4$	$501.9 \pm 43.3$	$75.2 \pm 2.70$

\* Results were obtained from previous study [160].

### 3.4 Film moisture Content and Uptake

Among the properties of the obtained films that have been evaluated are their water moisture content and uptake, which play an important role in determining the texture and mechanical properties of edible films as coated materials and are highly essential for potential food packaging applications [161]. Because it is well recognized that high moisture content may allow for increased bacterial and enzymatic activity or mold development under the available conditions, the use of edible films as a food packing material may be severely limited.

The results indicated that no significant change on water content by increasing the GJ concentrations. Whereas, the water uptake of the *N*SPC film prepared with 4% GJ has the highest water uptake ( $11.1 \pm 0.3\%$ ) that significantly different with the films prepared with 10% GJ ( $6.4 \pm 1.8\%$ ). Plasticizing the *N*SPC with GJ at 4% showed the maximum water uptake that due to the higher water holding capacity inside the film matrixes than gradually decreased by increasing the GJ concentration. Previous work showed that fructose plasticized cassava starch films absorb less water compared to other films that obtained with urea, tri-ethylene glycol, or triethanolamine [162].

There are many studies reporting the moisture content of protein-based edible films under different conditions. Bamdad, et al. [163], studied the moisture content of films made from lentil protein concentrate with 50 % GLY it was  $23.15 \pm 1.6\%$ . Mahmoud and Savello [164], reported that the

moisture content in whey protein films ranging from 26.3 to 26.5% when their glycerol content 1.5%, and increased when the concentration of glycerol increased. In a study on peanut protein concentrate film were dried at 70, 80, or 90°C, the moisture content of peanut protein films prepared at 70 °C was 32.57% higher than for those prepared at 80 °C (23.84%) or 90°C (14.79%) [165].

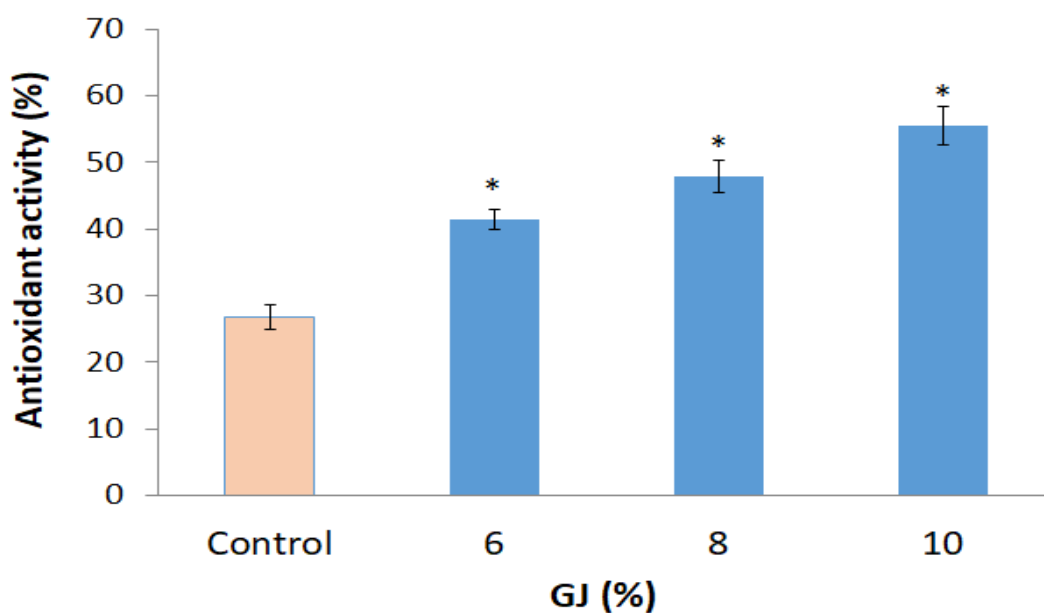
**Table 3. Water content and uptake of NSPC dissolved with different concentrations of grape juice (GJ).**

<b>Film</b>	<b>Water content (%)</b>	<b>Water uptake (%)</b>
NSPC + GJ (2%)	12.1 ± 1.4 <sup>a</sup>	7.6 ± 1.1 <sup>a,b</sup>
NSPC + GJ (4%)	17.4 ± 1.4 <sup>a</sup>	11.1 ± 0.3 <sup>a</sup>
NSPC + GJ (6%)	18.0 ± 0.9 <sup>a</sup>	8.9 ± 0.8 <sup>a,b</sup>
NSPC + GJ (8%)	15.5 ± 3.7 <sup>a</sup>	9.8 ± 0.7 <sup>a,b</sup>
NSPC + GJ (10%)	16.7 ± 3.4 <sup>a</sup>	6.4 ± 1.8 <sup>b</sup>

<sup>a,b</sup> Values in the same column with different letters are significantly different ( $p < 0.05$ )

### 3.5 Antioxidant activity of the NSDSC protein film

DPPH scavenging assay was used to indicate the antioxidant activity of the film, when the DPPH solution (as a reagent) was mixed with the sample mixture acting as a hydrogen atom donor, a stable non-radical form of DPPH is obtained with simultaneous change of the violet color to pale yellow, which was determined by using the spectrophotometry method [166].



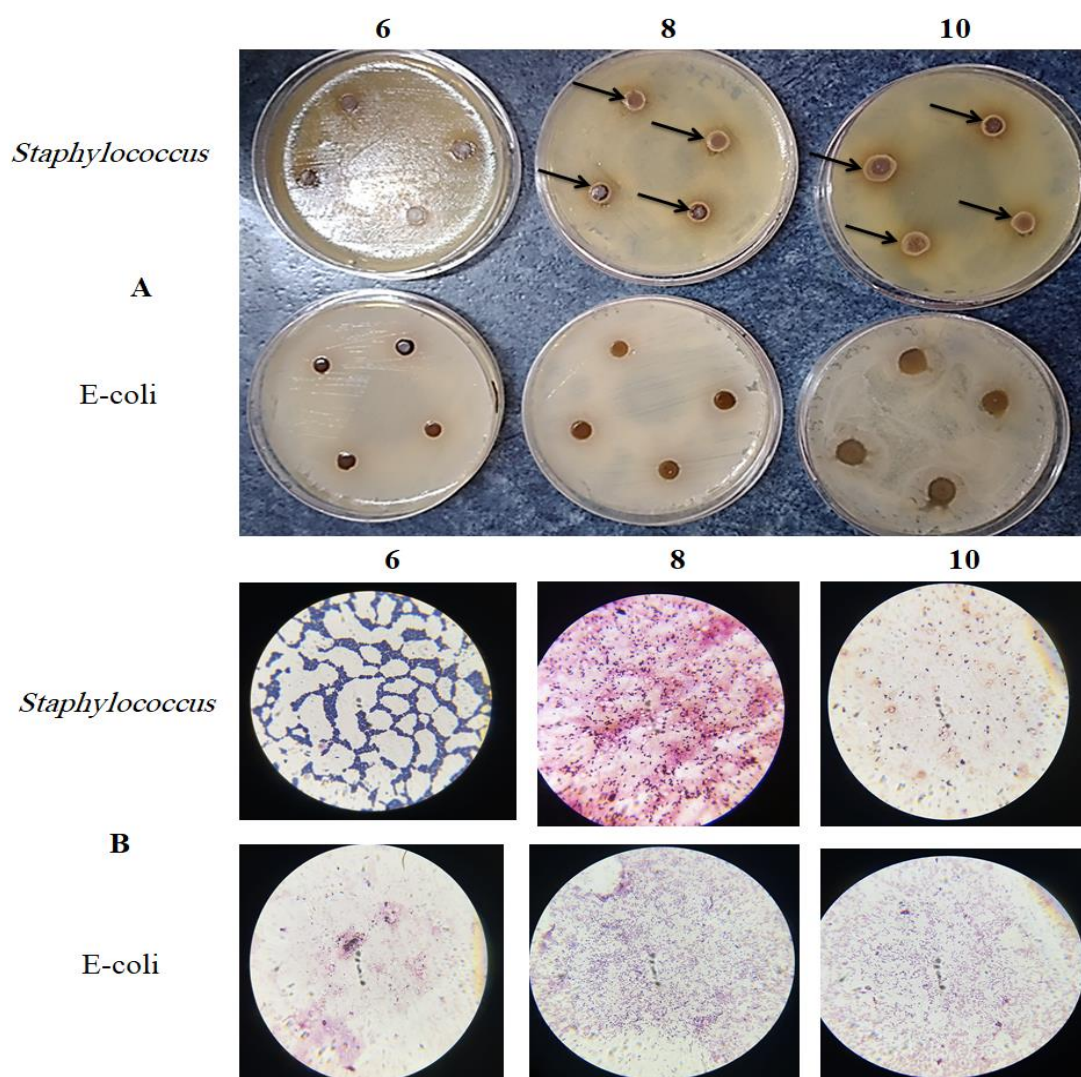
**Fig 8. Effect of different concentrations of grape juice (GJ) on the DPPH scavenging activity of obtained NSPC edible films, control was the NSPC obtained with 30% glycerol at the same pH value 12. Values with (\*) it's significantly different compering to the control.**

The results showed that the DPPH scavenging activity of the films significantly increased with increasing GJ concentration as shown in comparing to the control films that obtained with 30% glycerol as plasticizer (Fig 8). The film's scavenging action is connected to the fact that free radicals can react with remaining free amino ( $\text{NH}_2$ ) groups to generate stable macromolecule radicals, and  $\text{NH}_2$  groups can form ammonium ( $\text{NH}_3$ ) groups by absorbing a hydrogen ion from the solution [167]. In the films containing GJ, the antioxidant activity increased due to bioactive components in grapes which is a good natural source of antioxidants, containing many phytochemicals such as anthocyanin, catechin, epicatechin, resveratrol, and proanthocyanidin, and therefore have strong activity for scavenging free

radicals [168]. The expected antioxidant nature of the active film improved as the GJ concentration in the film formulation raised.

### **3.6 NSDSC protein film Antimicrobial Activity**

Figure 9, presents the antimicrobial activity of protein concentrate films containing different concentrations of GJ, against two pathogenic organisms, *Staphylococcus aureus* (*S. aureus*), and *Escherichia coli* (*E.coli*) (panel A and B). The inhibitory activity was measured based on a clear inhibition zone that surrounds the film disks. If there was no clear zone surrounding the film disks, it was assumed that there was no inhibitory effect. The results indicated that the GJ concentrations were active against *S. aureus*.



**Fig 9. Antimicrobial properties of NSPC edible films at pH 12.0, against two pathogenic organisms, *Staphylococcus aureus*, and *Escherichia coli*. (A) Effect of different GJ concentrations incorporated with NSPC films on microbial activity. (B) Effect of different GJ concentrations incorporated with NS-PC films on *Staphylococcus* and *E-coli* under a microscope.**

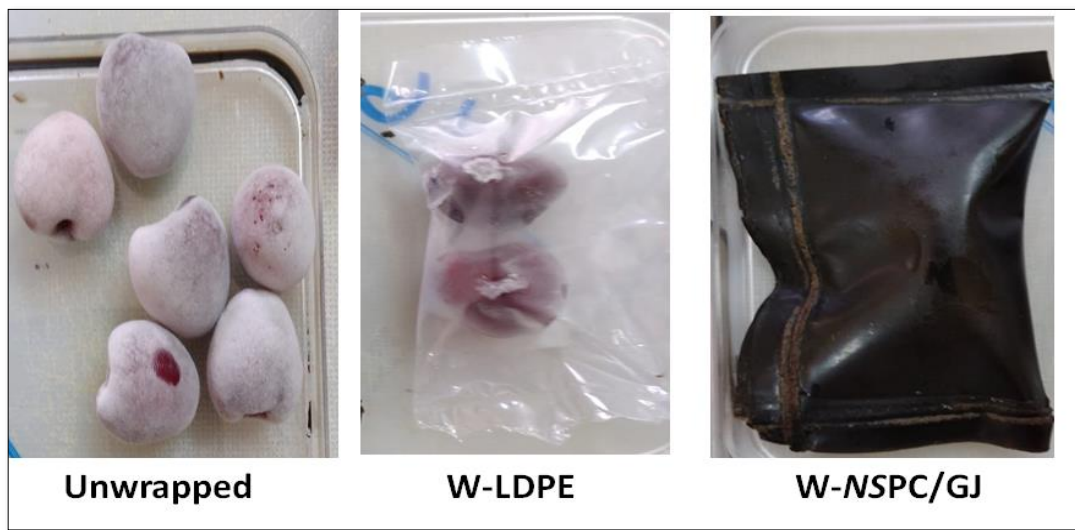
Antimicrobial activity was observed in films prepared with 8% and 10% GJ, particularly against *S. aureus*, with a clear inhibition zone, where the concentration of 10% was more effective against *S. aureus* due to a higher content of grape juice and therefore larger quantities of polyphenols. The



diameters of the inhibition zones were exactly proportional to the total polyphenol content, indicating that these chemicals are responsible for antimicrobial activities [169,170]. Other trials that demonstrated *Staphylococcus* species to be the most sensitive bacterium to polyphenols discovered comparable outcomes [171-173]. Whereas, there was no inhibitory impact of any of the films on *E. coli* at all concentrations tested. In fact, it has regularly been accounted for that polyphenolic extracts are more effective against Gram-positive bacteria. Gram-negative microbes have a low vulnerability to polyphenols when contrasted with Gram-positive microorganisms because of the repugnance between these mixtures and the lipopolysaccharide present in the surfaces of gram-negative microscopic organisms [174,175]. Different investigations have reported the inhibitory effect of polyphenols on gram-negative bacteria, but at higher concentrations of polyphenols than in our review [176,177].

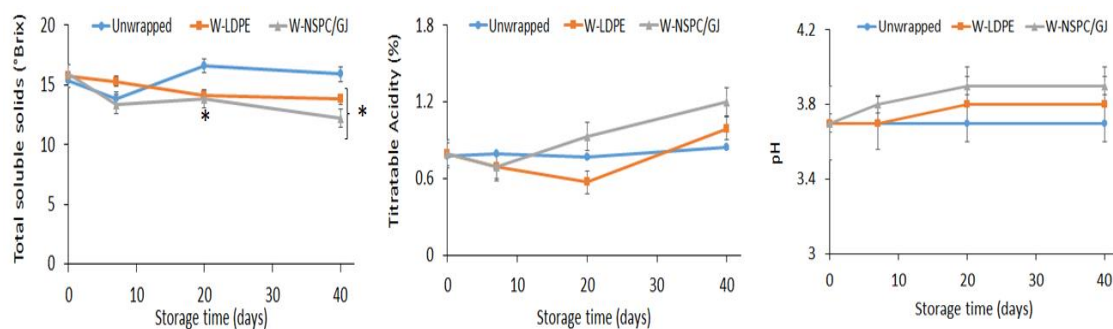
### **3.7 Effect of wrapping with or without NSPC/GJ film on sweet cherries quality.**

Effects of NSDPC with 6% GJ films and LDPE on the quality of sweet cherries was shown in (Fig. 5-7). The unwrapped and sweet cherries wrapped with LDPE was the control in this experiment. The NSPC with 6% GJ was selected based on mechanical properties. Fig 10, showed the sweet cherries after removing from freezer were the unwrapped cherries covered with small ice crystal, whereas not observed in both wrapped cherries when it closed. However, the NSPC with GJ are able to heat sealed by house sealer machine.



**Fig 10. Sweet cherries image after removing from freezer unwrapped (control) and wrapped (W) with LDPE or NSPC with 6% GJ.**

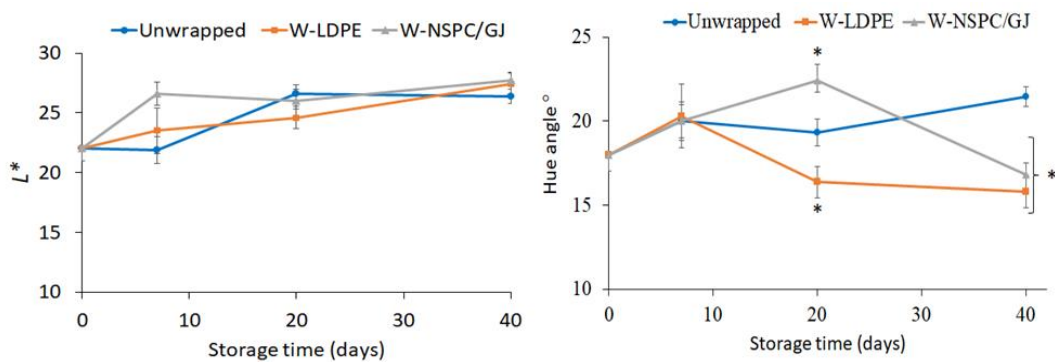
The initial total soluble solids (TSS) were ( $16.3 \pm 1.2$  Brix) of fresh cherries indicated a good maturity as described by Kappel et al [178]. The results showed that the TSS (Brix) was significantly lower comparing to the control (unwrapped) after 10 days of storage at  $-20\text{ }^{\circ}\text{C}$ , and no significant effect was observed between the TSS of cherries wrapped with LDPE or NSPC films in all storage times (Fig 11). One of the good juice quality indicators is the retention or minimum increase in TSS content of juice during storage [179]. The TSS decrease during storage could attribute to the respiration rate or conversion of sugar. While the increase could be explained by the breakdown of starch to sugar [180]. The results clear indicated that the TSS concentration was stable until end of storage period for cherries wrapped with LDPE or NSPC/GJ films. However, the titratable acidity and pH value of all cherries wrapped or not was not significant different in all storage times



**Fig 11. Effect of unwrapped (control) and wrapped (W) with LDPE or NSPC plasticized with 6% GJ films on soluble solids content (Brix), titratable acidity and pH, of sweet cherries stored at different storage time at  $-20^{\circ}\text{C}$ . Value with (\*) it's significantly different comparing to the unwrapped sweet cherries.**

The optical result ( $L^*$  and Hue angle) was analyzed for the sweet cherries, the unwrapped cherries (control) and wrapped (W) with LDPE or NSPC plasticized with 6% GJ at different storage time at freezing temperature  $-20^{\circ}\text{C}$  (Fig 12).  $L^*$  indicates lightness read from 0 (completely opaque or “black”) to 100 (completely transparent or “white”) [180]. The results showed that the  $L^*$  value was not significant in all the treatments. Whereas, Hue angle results indicated that the sweet cherries wrapped with LDPE was reduced significantly comparing to the once wrapped with NSPC plasticized with GJ or unwrapped cherries at 20 days of storage. Moreover, the hue angle value for the sweet cherries that wrapped with NSPC showed significantly higher value at 20 days comparing the control and the value decreased at 40 days of storage at  $-20^{\circ}\text{C}$ , and there were no significant differences between the cherries wrapped with LDPE or NSPC films in 40 days of storage. Gonçalves et al., [180] and Gutiérrez-Jara et al., [181] concluded that there is a correlation between the hue angle and anthocyanins content, were the

lowest value of hue angle is correlated to the cherries with highest anthocyanins content, giving a darker red color. Based on that the obtained results indicated wrapped sweet cherries are very important during freezing to protect the anthocyanins concentration in the products. However, at 40 days of storage the unwrapped cherries hue angle value was higher comparing to the 0 day that due to the loss of the anthocyanins content. Similar results were founded by Gutiérrez-Jara et al., [181], were the coated cherries showed the lower hue angle value comparing to the uncoated cherries at refrigeration storage control.



**Figure 12. Effect of unwrapped and wrapped (W) with LDPE and NSPC plasticized with 6% GJ on optical properties of sweet cherries stored at different storage time at -20°C. Value with (\*) it's significantly different comparing to the unwrapped sweet cherries.**

## Chapter 4

### Conclusion and Recommendations

Edible films have been investigated as potential replacements for traditional plastics in food packaging. Following global trends in environmental preservation, their development gives another option alternative for applying hydrocolloids materials. Many research studies have been conducted to evaluate the overall effect of the addition of different substances such as crosslinkers, strengthening agents, plasticizers, or additives with antimicrobial or antioxidant properties to protein to improve the physical and mechanical properties of edible films and the shelf-life of food products. This study indicated many important points, that;

- The use of *NSCP* plasticized with GJ-based edible films represent a stimulating route for creating new food packaging materials, thus *NSPC* and GJ appear to be interesting raw materials for the formation of functional edible packaging films.
- The GJ concentration content was the most important parameter influencing the mechanical properties, as well as the physical properties due to its plasticizing effects on the polymer matrix.
- An edible coating containing natural extracts has been widely used for extending the shelf life of fruits and vegetables. The present study found for the first time that the use of natural GJ in combination with *NSPC* has a positive influence on the physicochemical traits of sweet cherries.

- The film proved to extend the shelf-life of fresh sweet cherries by delaying changes in color, titratable acidity, total soluble solids, and pH during freezing storage.

In addition, there are several general recommendations that should be taken into account for those with an interest in the same field, some of them;

- Stopped the plastic pollution by reduce use or purchase of plastic, especially single-use plastic, where possible.
- It is necessary to use reusable bags and containers and recyclable materials, or to search for ways that can be used as alternatives to plastic materials.
- Increasing community awareness about edible packaging and highlighting them because of their great importance in terms of the preservation of the environment and protection of products. In addition to Increase marketing of biodegradable packaging.
- Use eco-friendly and low-priced materials to prepare edible films, such as industrial by-products in view of reducing competition on the basic nutritional components.
- Reduce some traditional polymeric packaging materials for specific applications by using biodegradable or edible films as food packaging.
- The edible wraps would not be used alone, they would be used as a primary package to wrap foods inside a secondary package during food distribution and storage.

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جامعة النجاح الوطنية

كلية الدراسات العليا

# تأثير عصير العنب على خصائص غشاء بروتين حبة البركة المستخدمة لتغليف الكرز الحلو

إعداد

دانا ياسين

إشراف

د. محمد الصباح

قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في التغذية وتكنولوجيا الغذاء من كلية الدراسات العليا في جامعة النجاح الوطنية في نابلس-فلسطين.

2021

ب

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### الملخص

يكتسب إنتاج الأفلام القابلة للتحلل والتطبيقات المحتملة للتطبيقات الغذائية اهتمامًا متزايدًا كبديل للبولىميرات المستخدمة في تغليف المواد الغذائية وذلك بسبب طبيعتها المستدامة وأنها تعكس صورة صديقة للبيئة، بالإضافة إلى توافقها مع المواد الغذائية والتطبيقات الغذائية. حيث أنه من الممكن أن يتم تصنيع مواد التعبئة والتغليف الصالحة للأكل من عدد من المواد مثل عديدات التسكر، البروتينات أو الدهون، وإضافة العديد من الإضافات التي تعمل على تحسين خصائص الفيلم المكون وبالتالي الحصول على فيلم بخصائص وظيفية جيدة، التي يمكن أن تكون على شكل أغلفة وأكياس تستخدم لتغليف المواد الغذائية. في هذه الدراسة، تم استخراج بروتين من المنتجات الثانوية لبذور حبة البركة واذابتها بتركيز مختلف من عصير العنب الطبيعي (1,2,4,6,8,10,20,30 حجم/حجم) عند الأس الهيدروجيني 12 للحصول على أفلام صالحة للأكل، واستخدامها لتغليف الكرز الطازج ومتابعة تأثير الفيلم على الخصائص الفيزيائية والكيميائية للكرز (الأس الهيدروجيني، الحموضة القابلة للمعايرة، إجمالي المواد الصلبة القابلة للذوبان، واللون الخارجي) خلال فترة 40 يومًا من تخزينها على -20 درجة مئوية. أظهرت النتائج التي تم الحصول عليها من تحليل خصائص فيلم حبة البركة المدعم بعصير العنب ولأول مرة أن عصير العنب بتركيز من 2-10% (حجم / حجم) قادرًا على العمل كملدن في الأفلام المعتمدة على البروتين وخصائص واعدة. حيث أشارت السماكة والنتائج الميكانيكية إلى عدم وجود فرق في سماكة الأفلام كلما زاد تركيز العصير بينما أثرت التراكيز المختلفة للعصير على الخصائص الميكانيكية لفيلم حبة البركة وبشكل كبير. حيث أظهرت النتائج أن قوة الشد ومعامل يونغ للأفلام انخفض بشكل ملحوظ عند زيادة تركيز العصير. بينما لوحظ قيم أعلى للاستطالة عند الكسر للأفلام المحضرة بتركيز 6% من عصير العنب مقارنة بالأفلام الأخرى.

بالإضافة الى نتائج مثيرة للاهتمام وواعدة للغاية للخصائص المضادة للأكسدة ومضادات الميكروبات للأفلام المحضرة بعصير العنب مقارنة بأفلام التحكم المرجعية.

تم تقسيم الكرز الى ثلاث مجموعات كانت احداها غير مغلفة وأخرى مغلفة بأكياس بلاستيك من البولي إيثيلين قليل الكثافة والتي تم استخدامها كمجموعات تحكم مرجعية وتم مقارنة بمجموعة الكرز المغلفة بالفيلم الذي يعتمد على بروتين حبة البركة المدعم بعصير العنب بتركيز 6 % حيث أظهرت النتائج أن إجمالي المواد الصلبة القابلة للذوبان كانت أقل بشكل ملحوظ مقارنة بالكرز غير المغلف بعد 10 أيام من التخزين على - 20 درجة مئوية ، ولم يلاحظ أي تأثير معنوي للكرز الملفوف بأغشية البولي إيثيلين قليل الكثافة أو أفلام حبة البركة لجميع مراحل التخزين .

حيث كان تركيز إجمالي المواد الصلبة القابلة للذوبان مستقرًا حتى نهاية فترة التخزين للكرز الملفوف بالبولي إيثيلين قليل الكثافة أو أفلام حبة البركة كما ووجد عدم وجود اختلاف في درجة الحموضة القابلة للمعايرة وقيمة الأس الهيدروجيني لجميع أنواع الكرز سواء كانت مغلفة أو غير مغلفة على جميع أوقات التخزين. وأظهرت النتيجة البصرية (اللمعان وزاوية التدرج) أنه لم يكن هناك اختلاف في قيمة اللمعان في كافة المجموعات . في حين أشارت نتائج زاوية التدرج إلى أن الكرز الحلو المغلف بالبولي إيثيلين قليل الكثافة قد انخفض بشكل ملحوظ مقارنة بالكرز الملفوف بأفلام حبة البركة والكرز غير المغلف خلال 20 يومًا من التخزين. علاوة على ذلك ، كانت قيمة زاوية التدرج للكرز الحلو الذي تم لفه بأفلام حبة البركة أعلى قيمة بعد 20 يومًا مقارنة بالمجموعة المرجعية وانخفضت القيمة بعد 40 يومًا من التخزين عند -20 درجة مئوية ، ولم تكن هناك فروق ذات دلالة إحصائية بين الكرز الملفوف باستخدام أغلفة البولي إيثيلين قليل الكثافة أو أفلام حبة البركة خلال 40 يومًا من التخزين.

استنادًا الى هذه النتائج، تم تحديد أنه يمكن استخدام الأفلام المكونة من بروتين حبة البركة المضاف إليها عصير العنب الطبيعي بشكل فعال في حماية الخصائص الفيزيائية والكيميائية وزيادة العمر الافتراضي للكرز الحلو في درجات حرارة التخزين المميزة. لذلك يقترح أن أفلام حبة البركة يمكن

ث

اعتبارها طريقة حفظ جديدة لتحسين جودة ما بعد الحصاد والخصائص الغذائية لأصناف الكرز الحلو المختلفة.