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Functionality of Films from *Nigella sativa* Defatted Seed Cake Proteins Plasticized with Grape Juice: Use in Wrapping Sweet Cherries

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Abstract: The main aim of this work is to improve the functionality of *Nigella sativa* protein concentrate (*NS*PC) films by using grape juice (GJ). The film's mechanical, antioxidant, and antimicrobial activities were evaluated. The obtained results showed, for the first time, that GJ at concentrations of 2%–10% (*v*/*v*) are able to act as plasticizer for the *NS*PC films with promising film properties. The results showed that the tensile strength and Young's modulus of *NS*PC films were reduced significantly when the GJ increased. However, the *NS*PC films prepared with 6% GJ observed a higher elongation at break compared with other films. Moreover, the obtained films showed very interesting and promising results for their antioxidant and antimicrobial properties compared with the control films. The sweet cherries wrapped with *NS*PC film showed that the TSS (Brix) was significantly lower compared to the control, after 10 days of storage. However, the titratable acidity, pH value, and L^* of all cherries, either wrapped or not, was not significantly different in all storage times. On the other hand, hue angle was significantly lower after 10 days of storage at −18 °C compared with control films. GJ has a multi-functional effect for protein-based films as plasticizer, antioxidant, and antimicrobial function.

Keywords: edible film; *Nigella sativa*; sweet cherry; grape juice; functional edible films

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1. Introduction

Today we are living in an era called a "plastic age", in which plastic pollution has become one of the most urgent environmental issues, with plastic production increasing exponentially, from 2.3 million tons in 1950 to 448 million tons in 2015. Production is expected to double by 2050. Approximately 1 million plastic bottles are sold every minute worldwide, according to National Geographic, and the oceans contain 18 billion pounds of plastic every year [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 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 the current condition of the world's ecosystems.

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 [3,4].

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 [5]. Natural polymers derived from agricultural products (such as polysaccharides, proteins, and plant oils) are the main resource for the development of renewable and biodegradable or edible films polymer materials. The edible films can be integral part of foods and can be consumed with products, so there is no packaging and no disposed material [6,7]. It may also be incorporated with some different bioactive compounds such as virgin coconut oil [8], oregano essential oil [9,10], thyme essential oil [10], cloves [11], lacto peroxidase enzyme [12], pomegranate peel extract [13], and others, which act as an antimicrobial and antioxidant agent in edible coatings or films to maintain food safety and quality and enhance the shelf life of food products. The mechanical and barrier properties of edible films depend on many factors, such as the biopolymer sources, concentration, and viscosity [14]. For example, films based on proteins or polysaccharides have very efficient oxygen and carbon dioxide barriers; whereas, their resistance to water vapor transmission is limited. Multicomponent films have also been manufactured in an attempt to combine the advantages of individual materials with film forming.

Seeds are classified as the main source of protein and other important nutrients for supporting health and well-being. Seeds are not only used for nutrition but can also be used in traditional medicine or herbal medicine for their pharmacological properties. Many pharmacological applications derive from these bioactive components [15]. Physicians always endeavor to find drugs with fewer side effects. Nigella sativa (*N. sativa*) belongs to the Ranunculaceae family and has a spiritual and historical background that makes it one of the most promising therapeutic plants. It is cultivated in many countries but is native to Southern Europe, Southeast Asia, and Southwest Asia, and is grown as a spice or for its medicinal value [16,17]. The flowers may be white, pale blue, or dark blue, and the plant is self-branching [18]. Its seeds are mainly used as a condiment and as a relieving agent for different ailments [19–21].

Black seed can be used as directed or active ingredients in herbal medicines or as herbal tea. *N. sativa* seed is extracted, and its extracted oil may be exploited 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 [22–29]. Some of the bioactive compounds obtained from *N. sativa* have been identified and studies have shown that the biological activity of *N. sativa* seeds is mainly attributed to its essential oil component, thymoquinone [30,31], 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 sterols, saponins, phenolic compounds, various alkaloids, as well as volatile oils of different compositions [32,33].

Huge quantities of *N. sativa* defatted cake are produced as by-products and most of it is served as animal feed due to the high bioactive materials and protein content [34]. The *N. sativa* proteins' hydro-methanolic fraction account for about 35%–40% of the total dry weight and it separates into ranges from 10 to 94 kDa on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) [35]. Despite its high pharmacological activity, this fraction has been found to exert effects independent of those exerted by volatile oils, and its effects on human health are receiving increasing attention. Based on research, this extract functions as an effective sedative and has depressant effects on the central nervous system, induces analgesia, prevents progression of pathological changes in the lungs, and decreases blood cytokines in the body [36,37]. In a recent paper by Sabbah et al. [38] the use of proteins from *N. sativa* oilcakes to prepare ecofriendly materials was also investigated [38]. The authors investigated the possibility of preparing hydrocolloid films from proteins obtained from *N. sativa* defatted seed cake (*NS*DSC), when

the latter were modified by means of the enzyme microbial transglutaminase, and they characterized the produced materials from a biological and technological point of view.

Among the most popular fruits by consumers, sweet cherry (*Prunus avium* L.) is known for its high nutritional content and is mostly eaten raw. Several epidemiological studies have recently demonstrated the health-promoting effects related to its content of phytochemicals such as ascorbic acid, anthocyanin, and phenolic compounds [39]. The main characteristics related to the quality of cherry fruits are color, sweetness, sourness, and firmness [40]. Consumer acceptance of sweet cherry depend mainly on the sugar and acid concentrations [41]. Nutritionally, sweet cherries have a higher content of simple sugars (13 g/100 g). Cherries contain water-soluble vitamins (C, B), fat-soluble vitamins (A, E, K), and some carotenoids, in particular beta-carotene. Cherries also contain minerals such as magnesium, calcium, phosphorous, and potassium (10, 14, 20, 200 mg/100 g, respectively). As a result of the high respiration rate and metabolic activity of sweet cherries, they deteriorate rapidly after harvest, causing diminished acidity and phytochemical content, weight loss, and color change [42]. To maintain the good quality of the fruits, it is necessary to work to extend the shelf life of sweet cherries and to maintain the necessary packaging and storage. There are many studies on extending the shelf life of sweet cherries that are gaining great importance. Various techniques such as cold storage, controlled atmosphere storage, modified atmosphere packaging, and edible film coating have been used to maintain the quality and extend the shelf life of sweet cherries after harvest [43–45]. Nowadays, frozen sweet cherries become very popular around the world, and one of the common food quality degradations of frozen food is the freezer burn that occur due to moisture migration in frozen foods. The product appearance becomes glassy due to the evaporating of the ice crystals from the surface area of a products and brownish spots occurring on the food surface that cause the tissue to become dry and tough. The way to help to prevent the freezer burn is by using plastic packaging during the freezing process or to separate the food surfaces from the freezer environment [46].

Grapes (*Vitis vinifera*) is a widely cultivated crop in 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. Turkey is an important grape-producing country and is the fifth largest producer of grapes in the world. Since grapes have a very short shelf life, large amounts of grape loss occur due to deterioration. For this reason, grapes must be processed in a form that can be stored for a long time without loss of the nutritional value. Grapes are one of the most widespread fruit crops worldwide and their composition and properties have been extensively studied, with several reports of the presence of large amounts of phenolic compounds [47]. Most of the phenolic compounds in grapes can act as antioxidants [48]. Similarly, wine production residues are also characterized by high contents of phenolic compounds due to their incomplete extraction during wine production [49,50]. By-products obtained after the production of wine, (seeds and pomace) constitute a cheap source for extracting antioxidant compounds, providing important economic advantages [51]. The composition of grapes mainly consists of (*w/w*) 40% fiber, 16% essential oil, 11% protein, and 7% complex phenolic compounds such as tannins, sugars, minerals, and other substances [52]. Grape skin is a source of anthocyanidins and anthocyanins, which are natural pigments with antioxidant properties that act by inhibiting lipid oxidation and also have anti-mutagenic activities [53]. In addition, they are excellent sources of vitamins A, C, K, carotenes, flavonoids, and B-complex vitamins such as pyridoxine, riboflavin, and thiamine.

The main objective of this study was to produce edible films using the protein extracted from *Nigella sativa* defatted seed cakes, functionalized with different concentrations of natural grape juice. The obtained films were evaluated for their mechanical properties as well as for the water content, water uptake, antioxidant activity, and antimicrobial activity. Despite the high biological value of these proteins [54], there are no studies about the shelf-life of foods when protected by *NS*DSC proteins-based materials. Therefore, in this work, the films with the best properties were selected to wrap the sweet cherries and the

quality of unwrapped and wrapped frozen cherries (−18 °C), stored at different times, was evaluated.

2. Materials and Methods

2.1. Materials

The grapes (*Vitis vinifera*) were harvested, washed, had their seeds removed, and their juice extracted with a fruit juicer. The juice was filtrated with cheesecloth to separate the skins and stored at −20 °C until usage. Sweet cherries (*Prunus avium* L., cv Sweetheart) were obtained from a local market in Nablus, Palestine transported, and stored at room temperature until treatment. *Nigella sativa* defatted seed cake (*NS*DSC) 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 (St. Louis, MO, USA). Mueller Hinton Broth Himedia M391 500 g was obtained from HiMedia Leading BioSciences Company (Mumbai, India). Bacterial strains from American Type Culture Collection were *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 13883), and *Enterococcus faecium* (ATCC 700221), while *Salmonella typhi* were obtained from the microbiology laboratory of An-Najah National University (Nablus, Palestine).

2.2. Methods

2.2.1. Protein Extraction

The proteins were extracted from *NS*DSC by the acid-base extraction method, as previously described [55], with some modifications. After dispersing the dry powder, NSDSC, in distilled water at a ratio of 1:10, w/v, the pH was adjusted to 12.0 by adding 1 N NaOH and stirring at medium speed at room temperature for 2 h. Supernatant was obtained from centrifugation at 4000 rpm for 20 min, and the pH was adjusted at 5.4 with 1 N HCl to precipitate the protein, which was then picked up and dried at 30 °C and 20% relative humidity (RH). The obtained protein concentrate was finely ground by using an electrical miller and stored inside an airtight container at room temperature.

2.2.2. Proximate Analysis

*NS*DSC and *N. sativa* protein concentrate (*NS*PC) was analyzed for its proximate. Moisture content was measured by using infrared moisture analyzer (Sartorius™ MA100C, Sartorius, Goettingen, Germany), the temperature was 105 °C, where applied during analysis, and the measure was stopped automatically when the constant weight was reached. Protein content was determined by Kjeldahl's method [56] using a nitrogen conversion factor of 6.25.

Crude fat is the term used to refer to the crude mixture of fat-soluble material present in a sample. The ANKOM^{XT15} extraction system (ANKOM Technology, Macedon, NY, USA) 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 or 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:

$$\text{Crude fat } (\%) = \frac{W2 - W3}{W1} \times 100 \quad (1)$$

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 when 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:

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

where M(ASH) and M(DRY) refer to the mass of the ashed sample, and the original masses of the dried samples, respectively.

Carbohydrate was calculated based on the following formula:

$$\text{Total carbohydrat} = 100 - (\text{moisture} + \text{crude fat} + \text{ash} + \text{crude proein}) \quad (3)$$

2.2.3. Film Preparation

The GJ at different concentrations (1%, 2%, 4%, 6%, 8%, 10%, 20%, and 30% v/v) were firstly prepared, then used for dissolving the *NS*PC powder (4 g/100 mL for each GJ concentration) under continuous stirring. The pH value was adjusted to pH 12.0 by using 1 N NaOH. The film-forming solutions were casted onto 15 cm diameter polystyrene Petri dishes and allowed to dry inside the oven dryer at 30 °C for 24 h. Finally, the dried films were peeled off and stored inside desiccator (50%–54% RH $Mg(NO_3)_2$ $6H_2O$) at room temperature for further analysis and use.

2.2.4. Film Characteristics

In order to compare the effects of various treatments to protein films, their mechanical and physical properties have to be determined. For mechanical properties, dry films were peeled off 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 $Mg(NO_3)_2$ $6H_2O$. Afterwards, their thickness was measured with a micrometer screw gauge (0–25 mm, 0.1 μm), at different positions for each film sample. The mechanical characteristics were measured according to ASTM D882 method [57], using a universal testing instrument (Brookfield CT3 Texture Analyzer, model CT3 50K, Brookfield, Chandler, USA), were described in the relevant literature 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 the break for the degree to which the film can stretch before breaking; (c) Young's modulus, which provides information about a film's resistance to deformation [58]. Film strips (1 cm wide) were 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) is calculated by dividing the load at break by the original minimum cross-sectional area. The result is expressed in megapascals (MPa).

$$\text{Tensile strength } = \frac{(\text{Load at break})}{(\text{original width})(\text{original thickness})} \quad (4)$$

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

$$\text{Percent elongation } = \frac{(\text{elongation at rupture}) \times 100}{(\text{initial gage length})} \quad (5)$$

Young's modulus (YM) 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 the purposes of this calculation, the tensile stress was

calculated by dividing the load by the average original cross-section of the test specimen. The result is expressed in megapascals (MPa).

$$\text{Young's modulus} = \frac{\frac{(\text{load at point on tangent})}{(\text{original width})\ (\text{original thickness})}}{\frac{(\text{elongation at point on tangent})}{(\text{initial gage length})}} \quad (6)$$

The moisture content was measured according to Galus and Lenart [59], determined by the mass loss of 1 g of the film after 24 h of oven drying at 105 °C and was 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 24 h. Three repetitive analyses of each film were made, and the results were expressed as mean value. A total of 3 squares (2 cm × 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, the squares were conditioned at 25 °C and 50% RH for 24 h by placing the film samples into a desiccator over a saturated solution of $Mg(NO_3)_2$ $6H_2O$, after which they were weighed (W3).

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

$$\text{Water content } (\%) = \frac{(W1 - W2)}{(W1)} \times 100 \quad (7)$$

$$\text{Water uptake } (\%) = \frac{(W3 - W2)}{(W3)} \times 100 \quad (8)$$

The antioxidant activity of the films and 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 [60], 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:

$$\text{DPPH scavenging effect } (\%) = 100 - \left[\frac{(\text{Abs DPPH} - \text{Abs sample})}{(\text{Abs DPPH})} \times 100\%\right] \quad (9)$$

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 at least 3 times.

Film antimicrobial activity, the bacterial strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia*, *Enterococcus faecium*, and *Salmonella typhi* were activated twice in a Mueller Hinton broth to reach a cell concentration corresponding to 0.5 turbidities at OD 600. Antimicrobial activity testing of the edible films was carried out using the agar diffusion method, according to Pranoto et al. [61]. 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 inoculum containing approximately 10^5–10^6 CFU/mL of tested bacteria. The plates were then incubated at 37 °C for 24 h. Finally, the inhibition zones were observed and evaluated. Experiments were carried out in triplicate.

2.2.5. Sweet Cherry Wrapping

Sweet cherries of uniform size, color, without physical damage, and without fungal infections, were selected, washed with tap water, and dried at room temperature. They were randomly divided into three groups, one was wrapped (W) by sealed *NS*PC bags (10 cm × 10 cm), one 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 −18 °C, the

quality of both wrapped and control samples was evaluated during storage every week for 40 days.

2.2.6. Physicochemical Analyses of Wrapped and Unwrapped Cherries

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 [62].

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, KRÜSS, Hamburg, Germany).

3. Results and Discussion

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 the meal and 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% ± 0.6% and after the extraction of protein, based on the acid base extraction method, it was 43.1% ± 2.5%. Moreover, the fat concentration of raw seeds was 45.4% ± 0.5% while in the *NS*PC was 3.1% ± 0.3% 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 (*NS*DSC), and *Nigella sativa* protein concentrate (*NS*PC), obtained from defatted seeds cake.

Compositions (%)	*NS* Seed *	*NS*DSC	*NS*PC
Protein	20.3 ± 0.6	34.0 ± 2.7	45.1 ± 2.5
Moisture	7.1 ± 0.2	7.5 ± 0. 1	5.0 ± 0.3
Ash	7.4 ± 0.3	5.5 ± 0.1	3.7 ± 0.7
Fats	45.4 ± 0.5	18.2 ± 0.5	3.1 ± 0.3
Carbohydrate	19.7 ± 0.4	34.8 ± 2.3	43.1 ± 1.4

* Results was according to [63].

3.2. Nigella Sativa Edible Films Obtained in the Presence of Different Concentrations of Grape Juice

Nigella sativa protein concentrate (*NS*PC) 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 was critical to obtain very good film appearance and properties, which the *NS*PC films could not obtain at a pH less than 8.0 by using GJ. The *NS*PC–GJ film-forming solutions were casted in a Petri dish and dried for at least 48 h. Figure 1, showed that adding 2%, 4%, 6%, 8%, and 10% was sufficient to obtain handleable films that were homogenized, flexible, and easily to peel off from the Petri dish, without any observed defects cracks or pores. When GJ concentrations were 1%, 20%, and 30%, the film with 1% GJ was rigid, brittle, and easily broken with many cracks—which occurred because the GJ concentration was not enough to plasticize the protein polymers—while, at 20% and 30%, the obtained films were sticky and difficult to separate from Petri dish due to the high concentration of GJ. Therefore, films containing 1%, 20%, and 30% of GJ were excluded. Almost all protein-based films required plasticizing agent to reduce film fragility and obtain certain plastic properties [64,65].

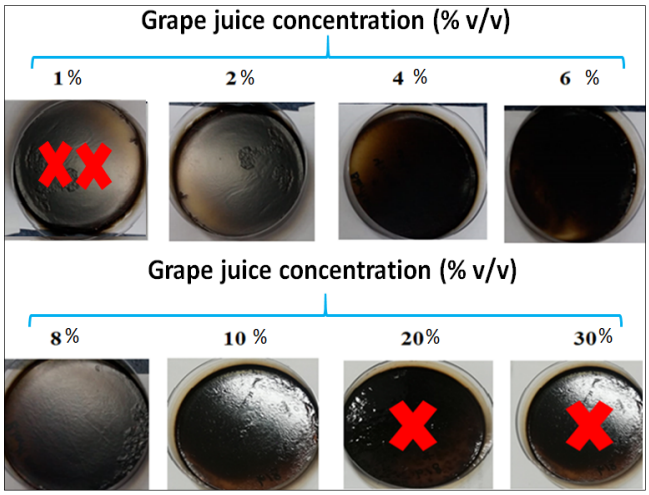

Figure 1. Images of NSDSC protein film containing different concentrations of grape juice (GJ), obtained at pH 12.0. Not-handleable—either brittle (XX) or sticky (X). Further experimental details are given in the text.

Plasticizers are molecules that are able to reduce intermolecular interactions along polymer chains, resulting in increased flexibility, extensibility, and toughness [66]. Plasticizers, on the other hand, decrease films' mechanical resistance and barrier characteristics [67]. The most common use plasticizers are polysaccharides, monosaccharides, disaccharides, 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 [68,69]; therefore, the main reason for the formation of a film based on *NS*DSC protein, with good physicochemical characteristics, without adding glycerol as a plasticizer, is the sugars that presented in GJ that act as plasticizers as in the case of sucrose. Veiga-Santos et al. [70] successfully obtained cassava starch films by using sucrose or invert sugar. Moreover, the pea starch-guar gum also plasticizes with different sugars [71].

The film color was black due to the seed pigmentation represented by phytomelanins, which are high-molecular weight polymers, which formed by the oxidation of phenols [72–74]. The black color of the obtained film will help to protect food products, medicines, or other products from the oxidation that may affect these products.

3.3. Film Characterizations

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 [75]. Figure 2 reports the film thickness and mechanical properties of *NS*DSC protein-based films, prepared with different concentrations of grape juice (2%, 4%, 6%, 8%, and 10% v/v), and casted at pH 12.0.

Results clearly indicated that there is no significant difference in film thickness by increasing the GJ concentration. The film thickness was between 76–72 µm. The different GJ concentrations have significantly affected ($p \leq 0.05$) the *NS*PC films TS, EB, and YM. The obtained results showed that the *NS*CP films TS and YM were reduced significantly when the GJ increased; whereas, film EB increased significantly until 6% GJ, and remained at almost same value at 8% and 10% GJ. Those film mechanical properties were observed by using glycerol, which is recognized as the most used plasticizer to obtain films, even in protein- or polysaccharide-based films [38,76]. Therefore, we recognized that GJ has plasticizing effect for NSPC film, based on the main ingredients of GJ, which are mainly sugars, well known for plasticizing effects. Previous work demonstrated this effect in monosaccharide, especially with glucose produced thinner films, due to the similarity of

its chemical structure to the repeating units of polymers [71]. Veiga-Santos [70] concluded that, by increasing the sucrose or invert sugar as plasticizer to cassava starch, the film EB can be significantly increased.

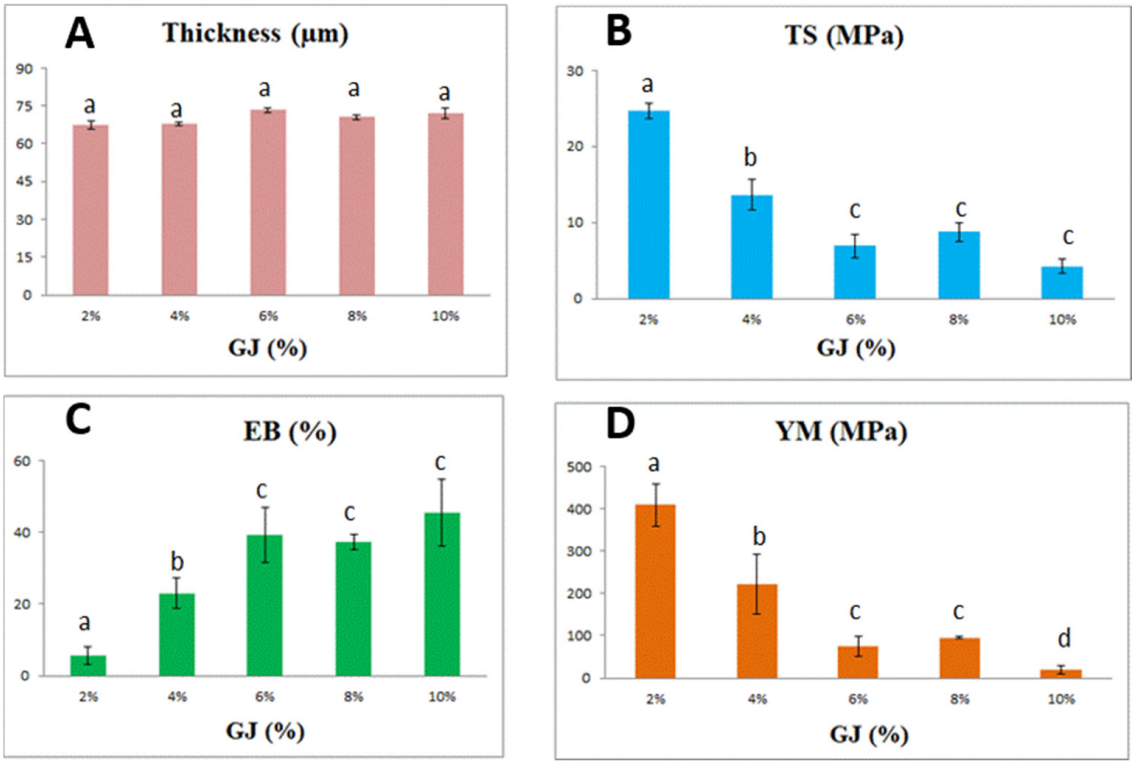

Figure 2. Demonstrates the effect of different concentrations of grape juice (GJ) on the film thickness (**A**) and mechanical properties (TS—tensile strength (**B**); EB—elongation at break (**C**); YM—Young's modulus (**D**) of NSPC edible films obtained at pH 12.0. Different statistical symbols (**a**–**d**) indicate significant difference between treatments ($p < 0.05$).

Table 2 compares the mechanical properties of *NS*DPC films incorporated with 2% and 6% GJ—as well as those of some commercial edible casing called (Viscofan NDX, Tajonar, Spain) that is obtained from gelatin—with plastic high-density polyethylene (HDPE) materials analyzed in previous studies [77]. 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 as Viscofan (NDX), the commercially available material. Film prepared at 6% GJ had similar YM properties as HDPE, a commercial material.

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

Film	Thickness (µm)	TS (MPa)	EB (%)	YM (MPa)
*NS*PC + GJ 2%	67.5 ± 1.5	24.6 ± 1.1	5.6 ± 2.3	409.5 ± 50.0
*NS*PC + GJ 6%	73.3 ± 0.81	6.90 ± 1.5	39.3 ± 7.6	74.5 ± 23.10
Viscofan (NDX) *	30.0 ± 0.4	36.6 ± 8.1	13.1 ± 2.9	356.0 ± 29.0
HDPE *	36.2 ± 1.7	13.1 ± 1.4	501.9 ± 43.3	75.2 ± 2.70

* The results were obtained from previous study [77].

3.3.2. Moisture Content and Uptake

Among the properties of the obtained films that have been evaluated are their water moisture content and uptake, which play important roles in determining the texture and mechanical properties of edible films as coating materials, and are highly essential for potential food packaging applications [78]. 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 no significant change in water content from increasing the GJ concentrations; whereas, the water uptake of the *NS*CP film prepared with 4% GJ has the highest water uptake (11.1% ± 0.3%), which significantly differs from the films prepared with 10% GJ (6.4% ± 1.8%) (Table 3). Plasticizing the NSCP with GJ at 4% showed the maximum water uptake that, due to the higher water-holding capacity inside the film matrixes, then gradually decreased with an increase in GJ concentration. Previous work showed that fructose plasticized cassava starch films absorb less water compared with other films obtained with urea, tri-ethylene glycol, or triethanolamine [79].

Table 3. Water content and uptake of *NS*PC prepared with different concentrations of grape juice (GJ).

Film	Water Content (%)	Water Uptake (%)
*NS*PC + GJ (2%)	12.1 ± 1.4 [a]	7.6 ± 1.1 [a,b]
*NS*PC + GJ (4%)	17.4 ± 1.4 [a]	11.1 ± 0.3 [a]
*NS*PC + GJ (6%)	18.0 ± 0.9 [a]	8.9 ± 0.8 [a,b]
*NS*PC + GJ (8%)	15.5 ± 3.7 [a]	9.8 ± 0.7 [a,b]
*NS*PC + GJ (10%)	16.7 ± 3.4 [a]	6.4 ± 1.8 [b]

[a,b] The values with different letters are significantly different.

There are many studies reporting the moisture content of protein-based edible films under different conditions. Bamdad et al. [80] studied the moisture content of films made from lentil protein concentrate with 50% GLY, which was 23.15% ± 1.6%. Mahmoud and Savello [81] reported that the moisture content in whey protein films ranges from 26.3% to 26.5% when their glycerol content was 1.5% and increased when the concentration of glycerol increased. In a study on peanut protein concentrate film, 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%) [82].

3.3.3. Antioxidant Activity of the Film

DPPH scavenging assay was used to indicate the antioxidant activity of the film, when the DPPH solution was mixed with the sample mixture (as a reagent), 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 [83]. The results showed that the DPPH scavenging activity of the films significantly increased with an increase in GJ concentration, as shown in comparison with the control films that obtained this with 30% glycerol as plasticizer (Figure 3). The film's scavenging action is connected to the fact that free radicals can react with remaining free amino (NH_2) groups to generate stable macromolecule radicals, and NH_2 groups can form ammonium (NH_3) groups by absorbing a hydrogen ion from the solution [84].

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

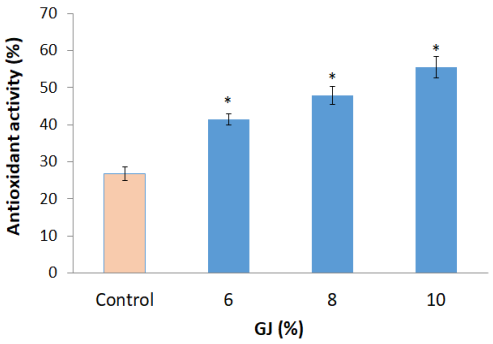

Figure 3. Effects of different concentrations of grape juice (GJ) on the DPPH scavenging activity of obtained *NS*PC edible films, control was the *NS*PC film obtained with 30% glycerol at the same pH value 12. Values with (*) were significantly different compared with the control.

3.3.4. NSDSC Protein Film Antimicrobial Activity

The microbial strains used in this study were of medical importance. They may cause sepsis, pneumonia, and diarrhea infectious diseases, and, more importantly, they may resist conventional antibiotics. The results have shown that there was antimicrobial activity for the films as observed around the film. The inhibition zones, although irregular, increased as the GJ concentration was increased (Figure 4). However, bacterial strains showed different responses with *K. pneumonia*, which was the most affected strain. *K. pneumonia* is a biofilm-forming bacterium that uses extracellular materials to support cell growth. GJ contains active ingredients such as polyphenols that may inhibit or interfere with biofilm formation [86]. Such active ingredients in GJ were also expected to disrupt the cell wall of gram-negative bacteria. Gram-negative bacteria have complex cell walls and are more resistant to antimicrobial agents than gram-positive bacteria [87,88]. Similar work has found that gram-positive bacteria were more susceptible to polyphenols than gram-negative bacteria. This is an advantageous result to promote edible films and increase their functionality. With more attention given to polyphenols as antimicrobial agents, edible films enriched with polyphenols can serve functions for both preserving food and decreasing foodborne diseases. On the other hand, it was documented that polyphenol were not active against probiotics or beneficial microbiota in the gastrointestinal tract [86]. The difference between the results of current studies and other studies, in terms of antimicrobial effects, is mainly due to variations in types of polyphenols, concentrations, method of application, mode of action, and strains of target microorganisms.

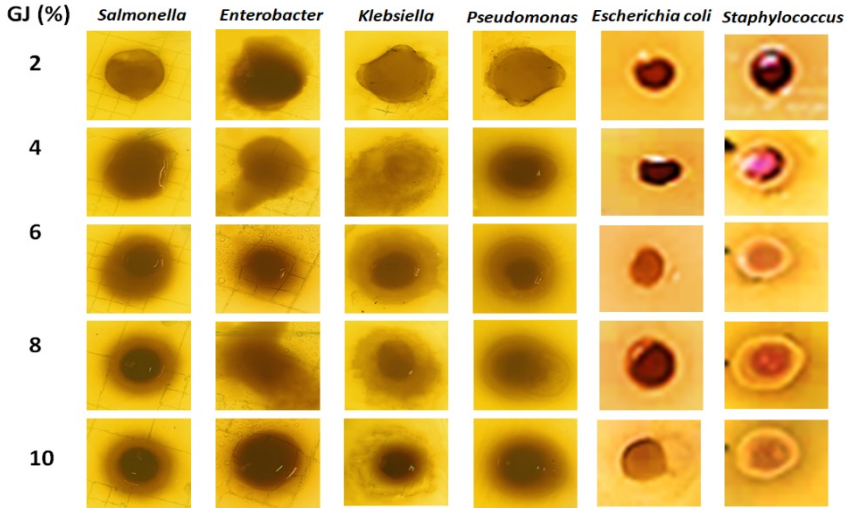

Figure 4. Effects of different concentrations of grape juice (GJ) on the antimicrobial activity of obtained *NS*PC edible films.

3.4. Effect of Wrapping with or without NSPC/GJ Film on Sweet Cherries Quality

Effects of *NS*DPC with 6% GJ films and LDPE on the quality of sweet cherries was shown in (Figures 5–7). The unwrapped sweet cherries and those sweet cherries wrapped with LDPE were the controls in this experiment. The *NS*PC with 6% GJ was selected based on mechanical properties. Figure 5 showed the sweet cherries after removal from freezer where the unwrapped cherries were covered with small ice crystal; whereas, this was not observed in both wrapped cherries when it closed. However, the *NS*PC with GJ films are able to be heat-sealed by a house sealer machine.

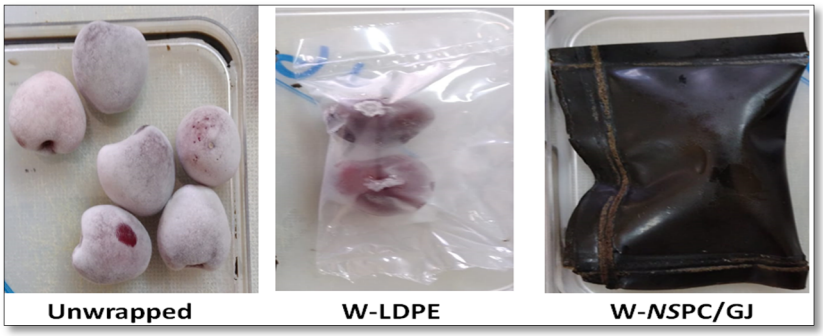

Figure 5. Sweet cherries image after removing from freezer: unwrapped (control) and wrapped (W) with LDPE or *NS*PC with 6% GJ.

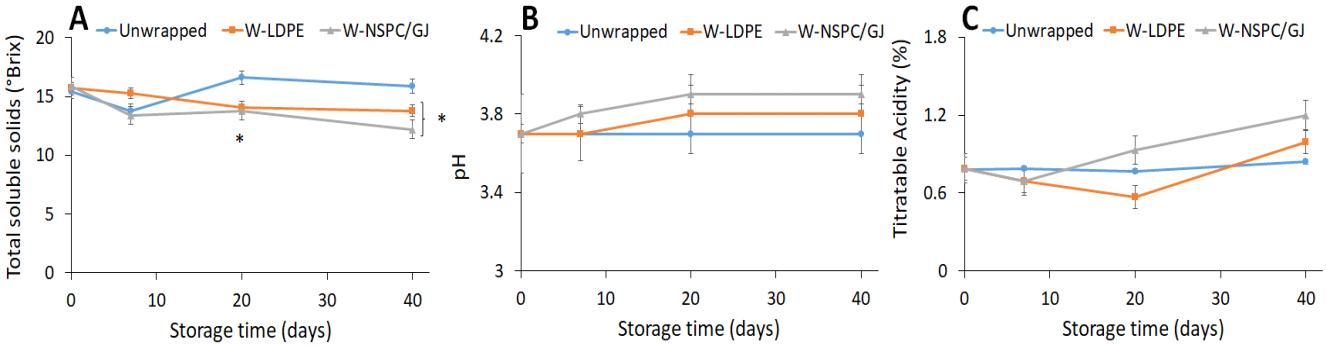

Figure 6. Effect of unwrapped (control) and wrapped (W) with LDPE or *NS*PC plasticized with 6% GJ films on soluble solids content (Brix) (**A**), pH (**B**) and titratable acidity (**C**), of sweet cherries stored at different storage time at −18 °C. Value with (*) is significantly different, compared with the unwrapped sweet cherries.

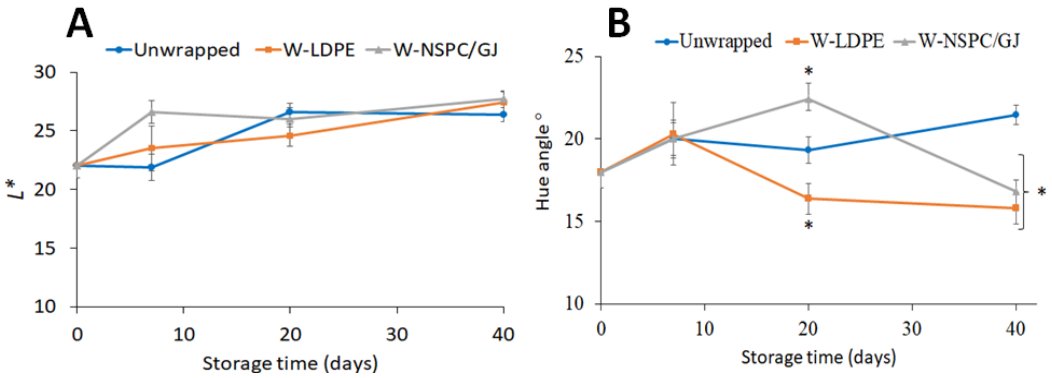

Figure 7. The effects of unwrapped and wrapped (w) with LDPH and *NS*PC plasticized with 6% GJ on optical properties of sweet cherries stored at different storage times at −18 °C. Value with (*) is significantly different compared with the unwrapped sweet cherries. (**A**) *L**; (**B**) Hue angle.

The initial total soluble solids (TSS) were (16.3 ± 1.2 Brix) of fresh cherries, indicated a good maturity as described by Kappel et al. [89]. The results showed that the TSS (Brix)

was significantly lower compared with the control (unwrapped) after 10 days of storage at −18 °C, and no significant effect was observed between the TSS of cherries wrapped with LDPE or *NS*PC films in all storage times (Figure 6). One of the good juice quality indicators is the retention or minimum increase in TSS content of juice during storage [90]. 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 [91]. The results clearly indicated that the TSS concentration was stable until end of the storage period for cherries wrapped with LDPE or *NS*CP–GJ films. However, the titratable acidity and pH value of all cherries, wrapped or not, was not significantly different in any storage times.

The optical result (L^* and hue angle) was analyzed for the sweet cherries, the unwrapped cherries (control), and those wrapped (W) with LDPE or *NS*PC plasticized with 6% GJ, at different storage times, at freezing temperature −18 °C (Figure 7). L^* indicates lightness read from 0 (completely opaque or "black") to 100 (completely transparent or "white") [91]. The results showed that the L^* value was not significant in any of the treatments; whereas, the hue angle results indicated that the sweet cherries wrapped with LDPE were reduced significantly compared with the ones wrapped with *NS*PC plasticized with GJ, or the unwrapped cherries, at 20 days of storage. Moreover, the hue angle value for the sweet cherries wrapped with *NS*PC showed significantly higher values at 20 days compared with the control, and the value decreased at 40 days of storage at −18 °C; additionally, there were no significant differences between the cherries wrapped with LDPE or *NS*PC films after 40 days of storage. Gonçalves et al. [91] and Gutiérrez-Jara et al. [92] concluded that there is a correlation between the hue angle and anthocyanins content, where 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 that the wrapping of sweet cherries is 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 compared to the 0 day hue angle value, due to the loss of the anthocyanins content. Similar results were found by Gutiérrez-Jara et al. [92], where the coated cherries showed the lower hue angle value compared with the uncoated cherries at refrigeration storage control.

4. Conclusions

The use of *NS*CP, plasticized with GJ, represents a stimulating route for creating new food packaging materials. This study indicated that *NS*PC and GJ appear to be interesting raw materials for the formation of functional edible packaging films. The GJ concentrations content was the most important parameter influencing the mechanical properties, as well as the physical properties, due to its plasticizing effects on the biopolymer matrix. The present study revealed, for the first time, that the use of natural GJ in combination with *NS*PC 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. Moreover, the antimicrobial ability of the edible film, together with its antioxidant properties, may act synergistically to preserve food without affecting its properties, in a more environmentally friendly way.

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