An-Najah National University

Faculty of Graduate Studies

EFFECT OF FOOD NANOPARTICLES ON EDIBLE FILMS DERIVED FROM INDUSTRIAL BY-PRODUCTS FOR FOOD COATINGS AND WRAPPING

By

Nisreen Wageah Saead Mansour

Supervisor

Dr. Mohammed Sabbah

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This Thesis was Defended Successfully on 17/02/2022 and approved by

Dr. Mohammed Sabbah	
Supervisor	Signature
Dr. Ziad Ayyad	
External Examiner	Signature
Dr. Mohammad Altamimi	
Internal Examiner	Signature

Dedication

(قُلْ إِنَّ صَلاتِي وَنُسُكِي وَمَحْيَايَ وَمَمَاتِي لِلَّهِ رَبِّ الْعَالَمِينَ)

I would like to dedicate my work to humanities' teacher, Prophet Mohammad peace be upon him. I would also like to dedicate my work to all those who seek success in life. My work is a dedication to my beloved country Palestine which is worth everything.

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I would like to express my utmost thanks for each and every person that supported me and took my hand to success in my studies- my university, An-Najah University, especially the faculty of Agriculture and Veterinary Medicine; for the scientific centers who opened their doors for me and gave me all the time and help I needed.

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Finally, my thanks is continued to my dear friend **Dana** for all that we have shared together throughout our educational journey.

Declaration

I, the undersigned, declare that I submitted the thesis entitled:

EFFECT OF FOOD NANOPARTICLES ON EDIBLE FILMS DERIVED FROM INDUSTRIAL BY-PRODUCTS FOR FOOD COATINGS AND WRAPPING

I declare that 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.

Student's Name: Nisrean Wageal Sagal Mansur

Signature: _____

Date: 17/2/2022

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Nisreen Wageah Saeed Mansour
Supervisor
DR. Mohammad Al-sabbah

Abstract

Background: The need for the production of bio-friendly plastic is rising, corresponding to the daily evidence of the harmful effects of petroleum plastics on environment. Plastic wastes accumulate in forest and waterways, which are not stopped even in the environment, but also shares in atmosphere disturbances through the greenhouse effect. The edible or bio-based packaging can be derived from another natural resource as protein, polysaccharides, lipids.

Aim: This research aims to identify the influence of cellulose nanoparticles' impact on the properties of *Nigella sativa* (*NS*) edible films. Furthermore, to evaluate the effect of enhancement of *NS* film with Moringa oil on their antimicrobial and antioxidant activity. And then incorporate the *NS* films with Moringa edible oil (MEO) to investigate the influence of such incorporation on the film activity as antimicrobial and antioxidant for packaging utilization.

Materials and methods: The alkaline-acid treatment was used of *Nigella sativa* defatted seed cake (*NS*DSC) to get the *NS* concentrated protein extract (*NS*CPE). The protein percent of the *NS*PCE determined, which is raw materials for *NS* film preparation at pH (12). Coconut defatted fibers consider as raw materials used for cellulose nanoparticles (C-NPs) production through the acid hydrolysis method.

The *NS* film produced by casting method, in which *NS* film reinforced with C-NPs at different concentrations ranged between (0.1, 0.3, 0.5, 1, 2, up to 3% (w\w)), (C-NPs\ protein) with 30% glycerol (GLY) (w\w) (GLY\ protein). The produced films were compared with control *NS* films (without C-NPs), in mechanical parameters and water uptake, and water content to investigate the influence of C-NPs addition on mentioned

properties. Three different C-NPs (0.1, 0.5, 2%) concentration chosen to be incorporated with (2% MEO nanoemulsion, 10% GLY), in *NS*PC based film, thin compared later with the *NS* films without (2% MEO and 30% GLY) with C-NPs (0.1, 0.5, 2%) addition. After new film production (MEO nanoemulsion, *NS*CP film), we investigated it in mechanical characteristics, water content, water uptake, and the activity of different films as antimicrobial and antioxidant. *NS* film incorporated with MEO also was applied for food coating or wrapping purposes.

Results and Discussion: The data showed C-NPs incorporations into the *NS* films increase the tensile strength (TS) values by raising the concentration of C-NPs. And the increase in Young's modulus (YM) was reported at low C-NPs concentration contrasted with the control films. The elongation at break (EB) showed a percentage increase at low concentrations of C-NPs but decreased as the concentration increased from 1 to 3% C-NPs. The results can be related to the inter-intramolecular bonds between C-NPs and protein. We also explain such results to the C-NPs dispersions in the film matrix. While the percent of water content initially increases a little at low concentrations up to 0.5% C-NPs but then decreases at high concentrations. Though the water absorption percent decreases with decreasing C-NPs concentration, because of C-NPs addition can redux the emptiness between the polymer molecules and plasticizers, which reduces the ability to absorb water molecules.

The results of MEO incorporations showed significant differences in film thickness, which increased more for (MEO with C-NPs) than *NS* films (without MEO with C-NPs). This effect can be attributed to the increase in the surface area according to oil and C-NPs interactions. The water content between the incorporated films with MEO was reduced, as the effect of adding C-NPs reduced the water uptake of *NS* films at all concentrations. However, the *NS* film with 0.5% C-NPs incorporated with 2% MEO showed more reduction than the film with the same C-NPs but without MEO addition. We relate this result to the hydrophobic nature of MEO. The results of EB showed a significant reduction when the film was incorporated into MEO. While TS and YM appeared, no significant effects of adding MEO to the *NS* films and *NS* without MEO, except that the 0.5% C-NPs with MEO showed a considerable reduction in TS. This effect refers to the plasticizing capacity of the emulsions (MEO and Tween 80). The emulsions may have a

poor plasticizing effect. However, it showed significant differences in antioxidant activity, there is a positive effect of MEO compared to the *NS* control films.

The effect of MEO addition to the *NS* film's presence at the increases in antimicrobial activity, particularly *staphylococcus arouse* bacteria. The produced films also showed the ability to be used in food packaging applications, such as products susceptible to oxidation, such as oil or butter packaging.

Conclusion: The addition of C-NPs improves the mechanical properties of *NS* films, and it has an optimal C-NPs concentration for these developments. Instead, the MEO addition improves the antioxidant and antimicrobial activity of the film, and these properties could enable *NS* film to be employed for food packaging applications. Because of its low-cost bio-friendly nature and its active packaging.

Keywords. (Edible film, plastic waste, coating, essential oil, nanotechnology)

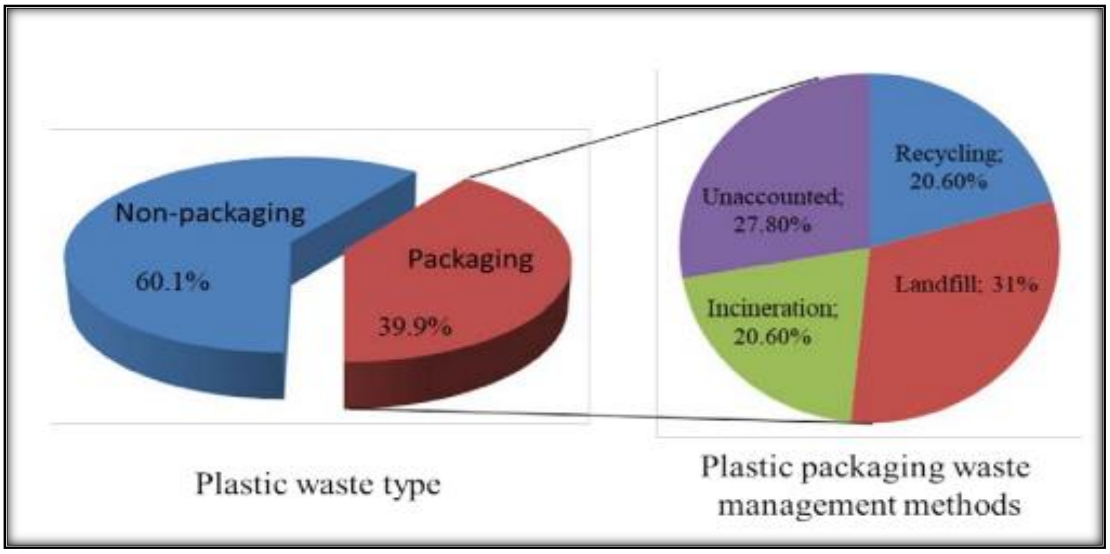
Chapter One

Introduction

Mismanagements of plastic waste have resulted from widely petroleum plastics manufacturing, which presents in a considerable increment in using disposable single-use plastics. Plastic is a non-degradable substance. As a result, it accumulates in seas, oceans, waterways, lands and forests. Plastic pollutants are nowadays a worldwide problems that damages ecosystems [1]. Food packaging materials, in addition to plastic, include glass, metals, paper, and wood. Producers have used plastics for packaging along with the combinations of other materials as composites. For example, concerning packaging waste, in the EU, over 67 million tons of packaging waste were produced in which year, including plastic used for packaging products (Fig. 1) [2]. The plastic petroleum polymers have characteristics such as (easy production, stability, and resilience), which led to a worldwide manufacturing increment. In 2015, the international plastic production reached around 322 million tons, with an increasing percentage of about 35% compared to 2014. Mangaraj et al, reviews mention that's India is an example of big plastic producer, it produces about 83 million tons of plastics from petroleum-based non-renewable resources. The packaging industry sector in India used about 43 of annually produced fabricated polymers [3].

Figure 1

European plastic waste management



The excessive use of plastic products in packaging, driven by the common commercial use of disposable containers. Barnes papers about understanding plastics pollution,

showed that's in the years between 2002 and 2014 in plastic production-consumption industry, packaging accounted for around 45% of all polymer resin generation, followed by building development with 19%, and customer and business enterprise materials with 12% of plastic productions [5]. Unfortunately, petroleum is used along with polyethylene, polystyrene, and polypropylene. These are described as "a non-eco-friendly material". It cannot be decomposed or biodegraded [3].

The international manufacturer of plastic waste was expected, since the start of the "plastic-revolution" to be almost around 6 billion tons of plastic waste have been produced worldwide. Nevertheless, the recycled amount of the integrated manufactured waste was much less than 10% of the 300 million lots of plastic wastes, while the remaining emerges in landfills and oceans [6]. Regarding the environmental impact of plastic production and plastic waste processing, it is considered as a resource of energy exhaustive process, which promotes global warming as greenhouse gaseous effect. Plastic burning releases toxic emissions, such as hydrochloric acid, dioxin, furans, carbon monoxide, and chlorine amines, causing detrimental effects on the environment and public health [3].

1.1 Food packaging.

Food packaging main principle "Protection and preservation" is a method that aims at protecting food products from external factors and deterioration to offer the high quality food for consumers with both nutritional information and ingredients. Monitoring, satisfaction, and tamper sign are additional functions for food packaging with more importance [7]. The economical view of food packaging is based on two points 1- to wrap products at a low cost 2- to satisfy customer requirements. in addition to maintaining food safety and minimizing environmental impact [8].

The food industry use plastics on a large scale, in particular the food packaging. The food packaging industry represents 37% of the total market for packaging materials according to its properties' like low cost, mechanical strength, and low weight . The purpose of food packaging is to protect produced meals from the surrounding environment, thus minimizing or delaying exposure to deteriorating components such as oxygen, microorganisms, temperature, and humidity, consequently, maintaining food nutritional value and allowing extension of the shelf life of products [7]. Food packaging can delay product disintegration, help to keep the benefits and effects of processing, and increase

food safety and quality reflected in the shelf life extension. packaging can protect foods from external chemical, biological, or physical factors [8]. Mainly, all materials in contact with packaged food should not filtrate chemicals into the food items at amounts that may be unsafe to consumer. To Control the health effects of food packaging it is necessary to be aware of the chemical composition of food packaging components, and migration capability. Migration is defined as the amount of packaging chemical composition that can seep into foodstuffs. An example of the food packaging materials with different chemical compositions; ceramics, glass, metal, paper, plastics, wax, and wood [9].

The continuous efforts to find green plastic have led to the innovation of bio-based packaging degradable materials. Degradation of polymeric materials means the loss of mechanical properties or fragmentation or modification, such as chemical degradation by microbial activity. Also, degradation is a complex process that causes the decomposition of the polymer ultimately into CO_2, H_2O, inorganic compounds, and CH_4. The environmental conditions that influence package biodegradation include climate, humidity, atmospheric pollutants [10].

The most prominent characteristics of polymeric materials which affect biodegradation are:

1. The polymer chain length (the smaller chain can be degraded more rapidly than a longer chain).
2. Chemical-formula complexities (because of the variation of chemical bonds).
3. Crystallites (the hardness of crystalline phases is much bigger than an amorphous phase) [11].

Bioplastics are considered one of the food packaging materials. Which are described as biodegradable, compostable, and renewable materials. Actually, (bioplastics) are not ideal. However, using technology such as nanotechnology may enhance more improvements for bioplastics [12].

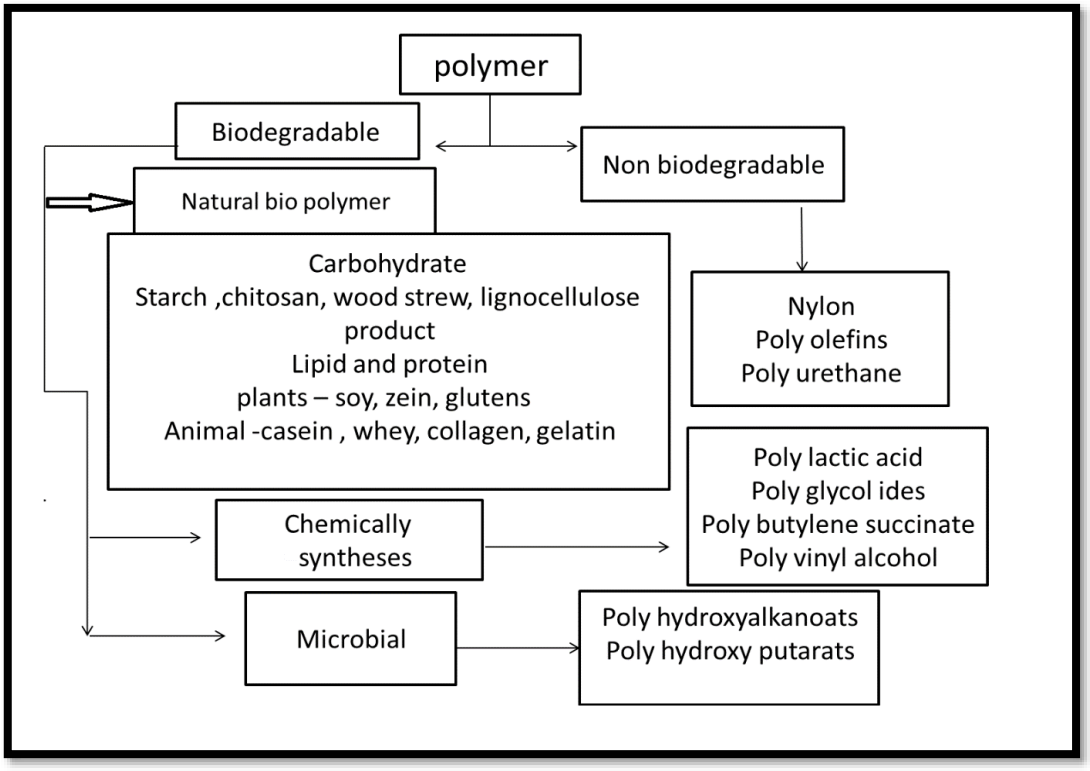
1.2 Edible films.

The continues work in order to minimize the environmental effect of plastic packaging leads food packaging innovators to form biodegradable composites, or edible coating martials "Edible film", based on completely natural polymers, which degrade without producing toxic residuals, are environment-friendly packaging [13].

The term edible film means it has two fundamental properties, though introductory part. Edible means: The film can be consumed with foods. It has a direct contact with food surface as a natural part and will adhere to all properties of safe-food ingredients according to the Food and Drug Administration (FDA), and recognized by the Generally Recognized as Safe (GRAS) status [14]. Second part, edible films means, covering material including packaging properties which is protecting the internal part from external environmental factors, can limit water vapor and gaseous transportation between food material and the external environment. Edible material forms a thin layer that can use for coating food or by wrapping food items in the formed film without changing the original ingredients [15]. Edible films are often made from hydrocolloids protein, polysaccharides, resins, lipids, and composites (Fig. 2). There are several protocols for forming films directly in contact with food surfaces. Such as film-forming monomers spread in liquid solutions, thinly applied directly on food items. In other protocols, solvent removal is required for solid film formation, particularly to achieve the mechanical properties accuracy needed to offer suitable temperature to get adequate plastic film drying rate [16].

Figure 2 (A)

Different sources for polymer bio-nanocomposite films used for food packaging.



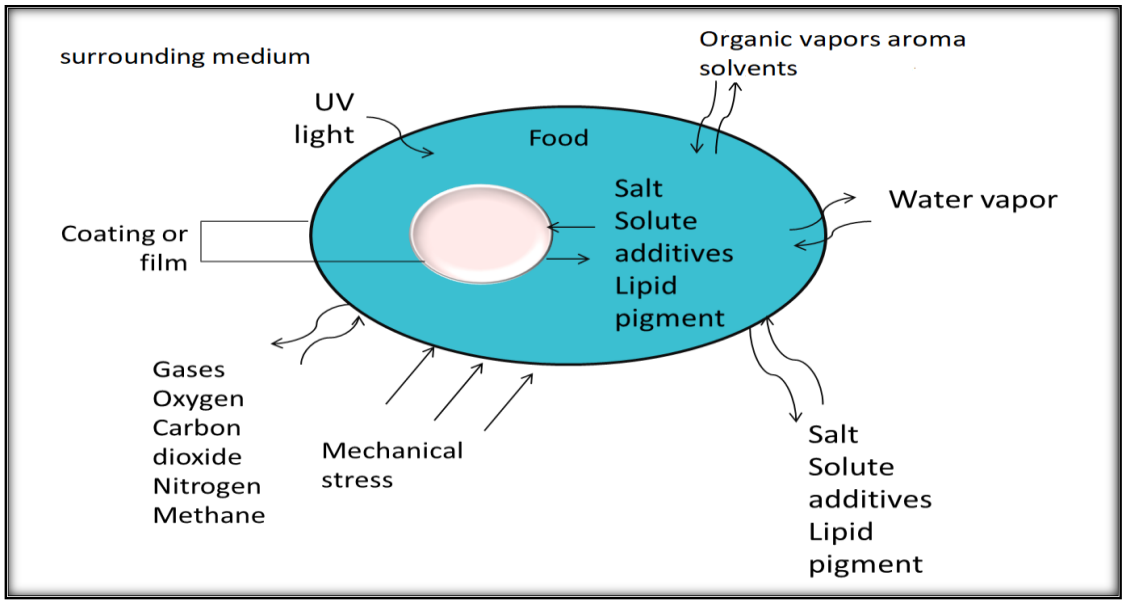
In addition to the general functions of the edible film, it also includes antioxidant activity that preserves the nutritional quality of foods during storage through properties of functional barrier of edible film. Edible films can reduce oxygen permeability, prevents lipids oxidations, colorants, and flavours in food products. Subsequently, it is recommended that fresh food to be packaged with edible film, because of its oxygen barrier properties that delay the food respiration rate [16]. Edible films are used singly or through layers of edible films from the dried thin materials. They define edible coatings as thin material used for covering or wrapping products (food, drugs) to extend the shelf life, which may be consumed together or removed before consumption [15]. This material should not change the desired properties of the packaged product, such as alters the appearance, smell, and taste. To achieve film functionalities mechanical properties are required to have a suitable thickness, which is possible to protect products from food deterioration factors (Fig. 3) [18].

Moreover, coatings should have the following desired requirements:

- Palatable sensory attributes favorable or neutral to the food product to be coated.
- Good barrier (biochemical, microbial, physicochemical, stability).
- GRAS
- Simple handleable technology .
- Environmentally friendly .
- Low cost for both raw material and processing [19].

Figure 2 (B)

Edible packeging barriers controlling the transfer of gaseous and vapors besides the external factors present in the surrounding medium



1.3 Different methods for edible film preparation.

Edible films usually range between 50 to 250 µm in thickness and can be used for wrapping products or making pouches and bags. Also, films can combine with several films to form laminated sheets [15]. Preparation of the edible film achieved by two methods; the first casting wet methods (Fig. 3, a) and secondly extrusion dry processes. Some techniques can use edible films enclosed with product surfaces, such as spraying, dipping, fluidized-bed, panning (Fig. 3, b). Suhag, and his coauthors, characterized the application of the casting method of film preparation and coating deposition as: Being easy to handle and a suitable method for laboratory work. But for commercial production of edible film, the preferred methods were extrusion and spraying [17].

Figure 3 (A)

Casting method for edible film preparetion.

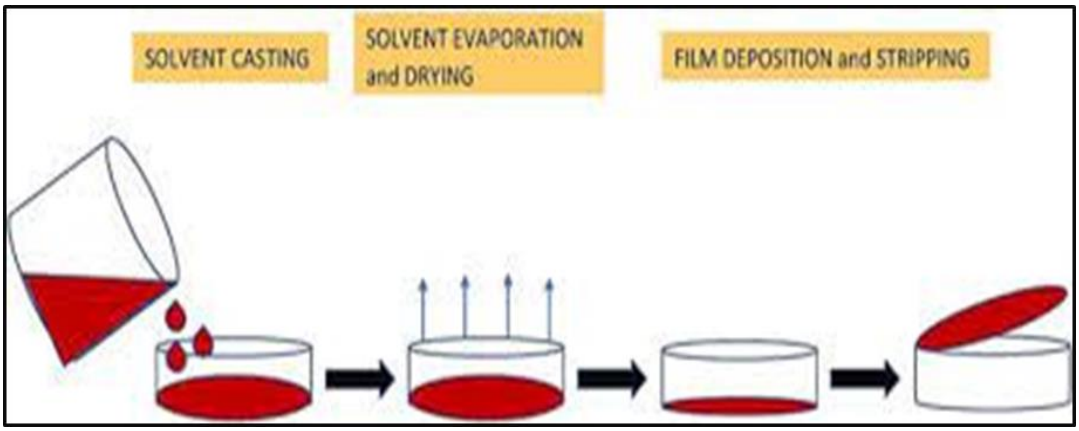
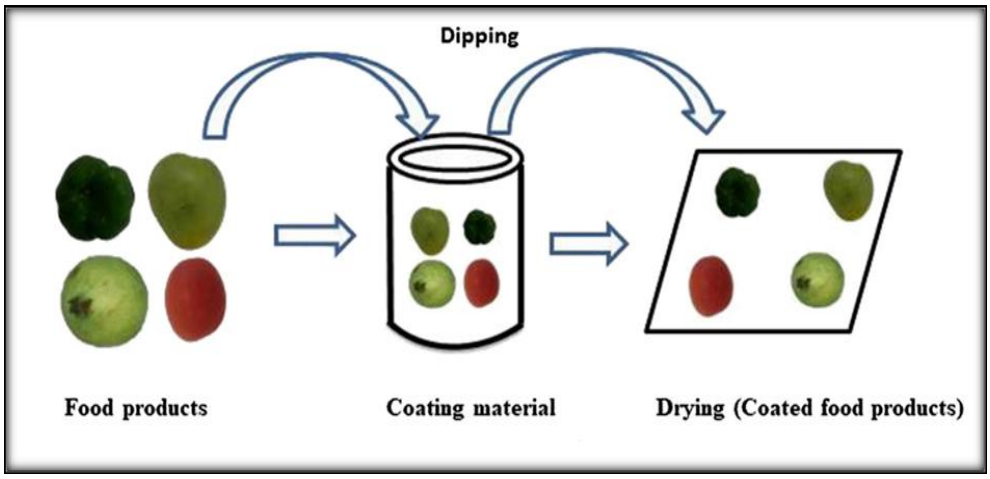


Figure 3 (B).

Dipping method for edible film product coating [17].



1.4 Polysaccharide edible films.

Edible films from polysaccharides have proper gas barriers properties while protein maintain mechanical properties and lipid decrease the water permeability [20] . This characteristic may result from their hydrogen bonds in large numbers, which enable adjacent chains to bind tightly with each other [19]. Such as chitosan (CS), which is linear chitin that belongs to the polysaccharide group, employed to make edible films. CS contains N-acetyl D-glucosamine and D-glucosamine units. Chitosan can be obtained by partial deacetylation of chitin. As known chitin was founded in marine invertebrates, insects' exoskeleton, and cell walls of certain fungi [21]. The biopolymers produced from CS have been applied for different food coating, and they have been proved to extend the shelf life of foods like fruit and vegetables as described in the strawberry coating in the

Petriccione et al. Published study, the edible coating by 1% and 2% chitosan on frozen strawberries, causes significant declines in water loss, color loss, and ascorbic acid content, besides reducing changes in the anthocyanin, phenolic, and flavonoid contents [22]. Another application of CS films with nanoparticles reinforcement proved to extend the shelf life of meat products and freshly produced fish. Because of their antimicrobial properties and mechanical barrier properties. The fabricated films had positive results in improving food quality and shelf-life extension [7].

The production of an edible film based on polysaccharides has not stopped with chitosan as a polymeric material. Also, it includes other types of polysaccharides, such as starch and pectin. in alginate pectin-based edible coatings, Sahraee and his coauthors apply alginate film on fresh-cut melon, the positive effect of melon edible coating, through good water vapour resistance, this presented from decreased dehydration of coated melon. As well, alginate pectin coating prevents ethylene formation. Also, coated melons showed a decline in CO_2 and O_2 permeability, where antioxidants such as vitamin C and total phenolic compounds increased [20]. In a review article on different starch-based edible film coatings. Sapper, and Chiralt, found that the use of edible coatings incorporated with active compounds, such as compounds with antimicrobial activity, represents an advanced preservation technology. In addition to the mentioned advanced technology, edible coating also can offer a change in the gaseous composition of the coated food by creating a changed atmosphere, through regulating gas exchange like oxygen, carbon dioxide, and volatiles compounds. Starch-based films have advantages like low cost (because of natural availability), excellent film thermogenic capacity, tasteless, colorless, and high oxygen barrier capacity. However, starch films are sensitive to water and show limited water vapor barrier properties, and weak mechanical resistance [23].

Emamifar et al. study on polysaccharides edible film by salep (*Orchis mascula*) as a polymer for film formations (Salep is derived from dried and milled tuberous wild orchids). The new addition in this study is the grape seed (GSE) extract, in order to improve film functionality as, antimicrobial and preserve the food quality. The effect of salep coating solution (SS) enriched with GSE was evaluated at the quality of coated fresh strawberries. They store strawberries at different storage times, 0, 4, 8, 12, 16, and 20 days at 1C^{o}and 95% relative humidity conditions. The coated strawberries with (1.5% SS + 3% GSE) had the results of the lowest growth of microorganisms with low ascorbic

acid, a decline in weight loss, and reduced anthocyanin degradation. Also, coated strawberries had the minimum peroxidase activity while ultimate superoxide dismutase activity. Also it had the optimum classifying sensory attributes. In conclusion, the blending of GSE into the SS coating formula could improve antimicrobial properties and extend the shelf life of coated strawberries compared with the uncoated ones by up to 20 days [24].

1.5 Lipid-based edible films

This hydrophobic compound has chemical and physical especial properties. Lipid low water affinity is a causative factor for lipid-based films to reduce moisture permeability, which describes the lipid-based edible coating. Lipid-molecule polarity has happened because each molecule has its electrostatic potentials, which cause variations in charge distribution. This character depends on the aliphatic chain length and chemical group, in addition to the presence of unsaturation [25].

Unlike other macromolecules, lipid and resin compounds have a few variations of monomers. Instead, other macromolecules can form polymers or large molecules by forming covalent bonds. For this reason, lipids lonely are ambitious to be biopolymers base. Lipids cannot form self-supporting cohesive film structures, so it create a fragile film with poor mechanical properties. As mentioned above, the properties of resin and lipid enable them to be blended with film-forming substances for improving moisture barriers properties to the produced films. However, this addition has disadvantages in edible packaging materials, for example, their texture, waxy taste, greasy surface, and oxidation potential rancidity [26].

Edible film types as polymers depend on their monomer hydrophilic natures. So the improvement of moisture barriers is fundamental for the produced edible film, which has hydrophobic compounds such as edible fatty acids or waxes. Film formation approaches comprise two primary formation systems designed for lipid-based edible films: The first emulsion films and secondly bi-layer films. Film properties such as barriers for water and gasses and film structure, thermal, mechanical, and optical properties usually depend on the film preparation and formulation techniques [27]. Also, in a study on films containing paraffin wax and methylcellulose, Debeaufort and his coauthors have characterized the prepared film with a bilayer system with equal values for barrier efficiency, which seems

like synthetic plastic. Opposing the emulsion prepared films, which have a higher value than synthetic plastic. An internal and external factors affected film permeability as desperation, the study result have proved homogeneity also affects permeability in the film structure [28], and external factors, such as temperature, relative humidity, and the application methods of edible packaging. The lipid-based edible films with specified functional characters enable producers to use them for different demands, such as (food, pharmaceutical, and agricultural industries) [27].

In food applications, vegetables and fruit are preserved for a few days or weeks of coating with variant formulations, including natural waxes and paraffin, to minimize water loss. Respiration occurrence is another problem besides water loss for preserved fruit and vegetables. Respiration products, CO_2, H_2O, and heat, because of its happening, a considerable loss in carbohydrate amounts, along with the change in organoleptic properties, there will be undesirable changes in taste, color, and odor in fruit or vegetable . A study by Kahve and Ardic, have proved that bee wax incorporation into chitosan-based edible films causes a significant decrease in the respiration rate of the coated strawberries. So it is an efficient choice for edible packaging, but it has the disadvantage of affecting taste palatability with waxy taste [29].

1.6 Protein-based edible films.

Proteins from different origins, animal or plant proteins, are employed to form edible films. Animal-originated proteins include whey protein, collagen, casein, gelatin, fish myofibril protein, keratin, and egg-white protein. Plant-origin proteins: Wheat gluten, soy protein, Zein, peanut protein, and cottonseed protein [30].

The twenty amino acids are the building block or monomers of proteins. Amino acids contact together in different methods, at many kinds of interactions and chemical reactions, which could give a variety of heteropolymers. When polysaccharides are compared to protein, polysaccharides contain a fewer monomers. For example, glucose is the only monomer for starch and cellulose. As a result, a few heteropolymers are composed. Also, the polysaccharides have hydroxyl groups in the reactive part, but proteins have much more available interactions and reaction parts. They may react through covalent and non-covalent bonds [16]. Protein-based films have better mechanical properties than lipid and polysaccharide-based films because protein

structures may enable many functional properties [30]. The hydrophobic interactions occur between nonpolar groups of amino acid chains because of their ability to form multiple bonds or different bonding positions, which is considered another property of proteins [16,31]. Therefore, the protein films in water have high hydrophilicity. The low affinity for nonpolar and polar substances permeability depends on plasticizer concentration and relative humidity [30]. The best plasticizer model based on the changes in the observed tensile properties and oxygen permeability for films was the one with up to 30% plasticizer [32]. A multi-study designed for protein-based edible films and future applications, as well in Lee et al, study which based on whey protein edible film, whey protein derived from dairy products is used to form edible film and applied to study lipid oxidation in peanuts, as lipid oxidations are the dominant cause for minimizing the shelf life and the causative factor for deterioration in peanuts. The peanut samples coated in different formulations of whey-protein-based coatings were observed in duplicate at various temperatures 40 ºC, 50 °C, and 60 ºC for 45 days. The result was showed a considerable reduction in rancidity in whey-coated peanuts than uncoated peanuts once performed [33] .

Edible films are also made by blending polysaccharides, proteins, and lipids, as done in this study. Researchers Saha et al, use chitosan with coconut oil and whey protein combined in various combinations. To determine the effects of edible coatings on the coated potatoes by measuring the nutritional quality and shelf life during (60) days of storage at 20 ºC. They stored the tested potatoes coated and controlled under the same conditions. The product quality characteristics evaluated during this study are respiration rate, visual appearance, and weight loss, in addition to the pH, soluble solids, ascorbic acid, and firmness. Chitosan study results showed that edible coating of potatoes considerably reduced rates of decay, soluble solids, weight loss, wrinkle development, and respiration shrinking compared to uncoated ones. Also, the shelf life of uncoated potatoes lasted up to 45 days, compared to coated potatoes, with a shelf life extended to 60 days [34]. Andrade Pizarro et al, study on carboxy methylcellulose and soy protein as coating solutions (Each solution alone) were used for potato pellet chips coating. The results showed a reduction in the fat uptake in the coated products with soy protein compared with carboxy methylcellulose [35].

Nowadays, researchers Sabbah and Al tamimi et al, are working on waste from the food industry [39]. It was known the waste from the oil industry as oilseed cake, which is rich in proteins and polysaccharides. Nigella Sativa (*NS*) seed cake is environmentally unfriendly industrial waste. So in this research the protein concentrated extract would be used for edible films formations.

1.7 Nigella sativa

*Nigella sativ*a are common as annual flowering plants used in traditional medicines. Many health benefits attributed to *NS* seeds, such as analgesic, anti-inflammatory, immune-stimulant, antiallergic, antihistaminic, antiasthmatic, hypoglycemic, antihypertensive, and antimicrobial activities. In addition, they traditionally used it to treat nasal congestion, headaches, toothaches, and intestinal worms [36]. Apart from the medicinal herb oil and *NS* seeds benefits. The oil extraction byproduct " seed cakes" contain almost protein, phenol and carbohydrate, seed cakes has high nutritional value and improves the immune system [37]. In addition, Saleh et al, report the *NS* seed cake has high phenolic content, notably antioxidant fractions. *NS* extracts and their essential oil contain a variety of phytochemicals, such as flavonoids, phenols, and tannins that are rich in natural antioxidants that may enhance protection against oxidation. Many studies have shown a high correlation between the antimicrobial activity of such plants and the content of phytochemicals [38]. Recently, researchers Sabbah, Altamimi et al. have used the defatted NS seed cake to make edible films. Their experiment was about making the *NS* films and investigating their properties. In the presence of glycerol as a plasticizer, with enzymatically cross-linked microbial transglutaminase, the results showed that films have good antimicrobial and mechanical barrier properties. So the *NS* films can be used in various food packaging applications depending on 1-film functionality affecting food shelf life extension, 2-protecting vegetables and fruits by controlling ripening or soil mulching [39].

1.8 Plasticizer

Plasticization applies to produce a change in the mechanical criteria and thermal properties of a manufactured polymer, comprising the effect as : (a) reducing stiffness at room

temperature, (b) improving elongation at break at room temperature, (c) increasing gas solubility and water vapor permeability that results from a reduced film cohesion [40]. To achieve considered effects of plasticizers, the edible film could be incorporated with low molecular weight compounds, or by blending with other polymer co-monomers to increase composite chain elasticity and decrease such chain crystallites [41].

The main plasticizer types used for protein-based films include glycerin (GLY), sorbitol, and polyethylene glycol. An example, Corn Zein films are blended with various plasticizers, Zein films are blended first type with organic compounds in the liquid phase such as polyols. In addition to the second type of incorporated plasticizers are in solid-phase compounds such as mono-oligo-saccharides, lipids, and lipid derivatives [30]. Dangaran et al, determined the usefulness of a plasticizer was affected by the microstructure and chemical nature of the plasticizer. For example, plasticization with glycerol is more effective than other plasticizers in the whey protein film matrix. The plasticizers empirical model fits the changes in the measured tensile properties and oxygen permeability of films with up to 30% plasticizer. Scientists consider the association between film property and plasticizer percentage as an exponential correlation. Plasticizer-protein interaction is affected by protein crystallinity, hydrophobicity, and hydrophilicity [32].

Most protein edible films alone are weak, so there is a need for modification and improvement for protein films to optimize their mechanical characteristics by plasticizers' additions. Previous research has proved that, for example the researchers Tanaka et al, in a study that examined the effects of plasticizers on protein-based films, mainly on fish water-soluble protein (FWSP). Films prepared primarily without plasticizer addition are very weak, and the formed films break easily, and peeling them off is difficult because of being brittle. Tanaka, and his team use a variety of plasticizers such as polyethylene glycol (PEG), ethylene glycol, sucrose, GLY, and sorbitol with 50% of fish water-soluble protein, to assess the mechanical film improvements as a suitable plasticizer type for film preparation. The effect of using ethylene glycol on the properties of an FWSP film is clear in preventing film formation with ethylene glycol addition. While sucrose and sorbitol addition to FWSP films resulted in poor mechanical character, the formed film was brittle and fragile to be used. Whereas glycerol or PEG have good plasticizing action on such formed films, it described them as flexible films. The significant results of GLY proved

to be the most effective plasticizer compared to other tested plasticizers. This related to its natural characteristics:

1- High solubility in water at boiling temperature .
2- Large protein miscibility .
3- Non-volatility [42].

In research Al-Hassan and Norziah conducted to study the properties of edible films formed from sago starch and sago starch blended with fish gelatin, and using GLY plus sorbitol as plasticizers, they evaluated the physical and mechanical characteristics to examine the effect of protein and plasticizers. The outcomes illustrated that starch/gelatin solutions at ratios of (3:1), (4:1), and (5:1) were shown to produce good flexible films with GLY combinations, while starch/gelatin content (2:1) have the higher value of tested characters. When different ratios of sago starch and fish gelatin blends were plasticized with GLY or sorbitol, it gave results that prove its effectiveness in the mechanical, physical, and water vapor permeability of the produced films. The two polymers at different ratios can change the extensibility and strength of the formed films. Also, scanning electron microscope (SEM) micrographs of GLY films show they contain uneven surfaces, as opposed to sorbitol film surfaces, while less protein in tested samples gave smoother films whether they have sorbitol or GLY plasticizers [43].

1.9 Use of nanotechnology in edible films

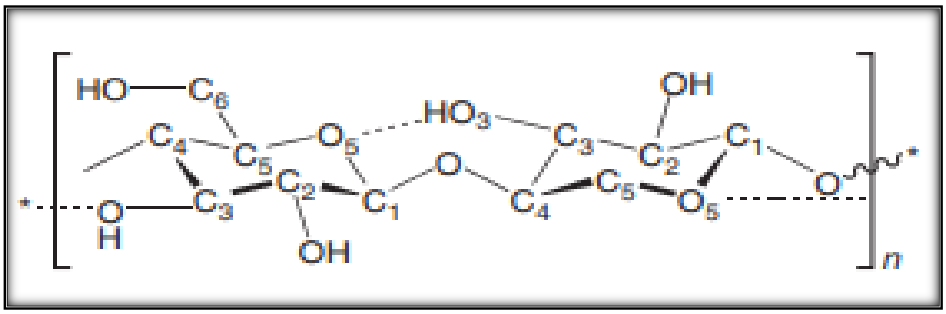
Most edible films and coatings made of single polymer are brittle, thus affecting their mechanical properties. To overcome this problem, plasticizers are employed. Plasticizers were successful in enhancing mechanical properties. However, this also had disadvantages, such as increased oxygen barrier- permeability, and water-barrier properties, so more research is needed to select an efficient plasticizer [30]. This led food innovators to use nanoparticles technology more accurately with fewer side effects. The application of nanotechnology is present in different aspects of food industries, including active packaging, as it is in contact with the food product or the headspace inside food items, to inhibit the risk of microbial growth if it is present on food surfaces. Food packaging innovators do not limit it to the application of nanoparticles to get antimicrobial properties; the nanotechnology have actively used for food packaging as nanocomposites. Researchers (Shankar et al.), (Tajeddin et al.) have applied nanolaminates to strengthen a barrier properties from extreme thermal and mechanical

fragility, thus extending food shelf life. Integrating nanoparticles into packaging industries has led to improvements in food quality with long shelf life [11,13]. The purpose of creating polymer composites is to gain more mechanical strength. Many inorganic or organic nano-fillers are used to achieve improved polymer composites. Incorporating nanoparticles into polymers has allowed the development of more resistant to temperature and humidity, packaging material with cost-effectiveness [44]. Using inert nanoscale fillers such as clay, silicate nanoplatelets, silica (SiO_2), nanoparticles, chitin or chitosan into the polymer matrix means obtaining its lightweight, strength, fire resistance, and thermal properties [45].

Cellulose is the structural building component of the primary cell wall of green plants and many forms of algae. A few species of bacteria can secrete it to form biofilm. Cellulose is the most common organic compound on earth [46]. Chemically, it comprises $(C_6H_{10}O_5)$ n. molecules. It is an organic compound from the polysaccharide class forming a linear chain of several hundred to over ten thousand β (1→4) linked D-glucose units (Fig. 4).

Figure 4 (A).

Cellulose structure. Single cellulose chain repeat unit showing the directionality of the β (1–4) linkage and internal hydrogen bonding (dotted line).



Researchers consider the low cost of cellulose as an advantage. Along with its independence from synthetic petroleum sources, the availability of renewable resources, and its capability to design large scales of diameters of size, with a range from nano-size to micro size [48]. Recently, the trend is the blending of cellulose in composite materials to reinforce "engineering polymer systems" because of its good mechanical properties. In addition, cellulose is available in different types or forms, which will support mechanical properties improvements. Khalil and his coauthors relate the variation between cellulose types for the particle size, shape, and crystallinity [49]. The regulatory restrictions are because of the uncertain toxicology of nanocellulose. Since the ecological and organic

toxicity of cellulose nanomaterials is of utmost significance when considering its use in environmental remediation. The risk nanoparticles present when inhaled, the small dimension of nanoparticles ease its uptake into cells or body fluid (blood and lymph) circulation and could probably reach sensitive target areas [50]. Most eco-toxicology research based on nanocellulose composites was not widely studied, although it had a critical and sped-up stage. The nanoparticles' risk on public health increases with decreasing particle size of non-toxic substances besides seldom toxic substances. There have been reports of high concentrations' side effects on living cell viability and proliferation [51].

In this research coconut defatted cake were chosen for cellulose nanoparticles preparations. Because the blending at nanoscale filler could give a larger contact surface area between filler and matrix, that may lead to the improvement of the mechanical properties and the moisture resistance of plasticized starches [48]. Nanosystem functionality remarked above, it can be incorporated the nanoparticles with antioxidants, which will improve the application to extend the shelf life of food packaging. Also, the application of natural nanosystems allows using smaller proportions of such substances and prevents flavor change in food [52].

In the nanofiller mechanism at the nanoscale level, the size of the nanophase leads to good enhancement in the surface area of the fillers. Nanocomposite needs the mentioned mechanism, because bio-nano composites depend on the high surface area of the nanosized fillers, which contributes to a wide interfacial or boundary area in the middle of the matrix of biopolymer and nanofillers .Bio-nanocomposites with a higher interface may enable conversion of relaxation behavior and molecular mobility, besides improving mechanical, barrier, and thermal properties, especially for food packaging applications. Bio-nanocomposite materials are often formed to preserve (mechanical and thermal stresses) during food processing, storage, and transportation [53, 54].

In the experimental area, Jancy, and Shruthy et al have produced films containing cellulose nanoparticles and polyvinyl alcohol with fennel seed essential oil by casting methods. The results showed that the film tensile strength improved seven-fold and six-fold in elongation at break compared to polyvinyl alcohol film because of incorporating cellulose nanofiber. This material is effective compared with the traditional food packaging materials [55]. Using adsorbing materials affected the nano-laminate coating properties, such as gas

permeability, mechanical properties, water- content, and water uptake characteristics, besides the sequence and the preparation factors- pH, temperature, and ionic strength. A nano-systems gas barrier properties are also an advantage. It shows permeability to water, oxygen, and water vapor more than traditional edible coatings [56]. The nanofillers which are used for food packaging industries, were classified into nano types nanoparticles, nanofibrils, nanorods, and tubs [52].

In addition, different resources for producing the nanofillers, from both organic and inorganic resources as the following, 1- Clay (montmorillonite), 2- Natural biopolymers (Chitosan), 3- Antimicrobial active natural compounds (nisin, grape seed extract), 4- Metal (silver) or metal oxides (ZnO, TiO2). (Fig. 4 (B) plotted in appendices (A)) represents a part of the nanofillers that we can employ for improving nanocomposite films [11].

The researchers Shankar, and Teng et al. have used various fillers and biopolymers to create bio-nano composite films, as discussed in the reviewed studies. A blend of zinc oxide- nanoparticles with gelatin composite films was investigated for properties, like antimicrobial activity and mechanical character. This study showed significant effects of the nanoparticle on mechanical properties and antimicrobial activities [57]. Andrade Pizarro et al, research aimed to investigate the effect of gelatin, GLY, and cellulose nanofiber (CNFs) concentrations on film properties, such as (mechanical, water vapor permeability, and color). The results showed the significant effect of gelatin addition at different concentrations on color. While mechanical assessment showed that with increasing concentration of gelatin and CNFs there is an increase in tensile strength, moreover an increase in GLY concentration causes an increase in elongation at break, making the films more flexible. An increased concentration of gelatin and GLY makes the film more permeable to water vapor, while an increase in the concentration of CNFs reduces this property. Finally, the addition of CNFs to gelatin-based films improves their mechanical and barrier properties (water vapor) without affecting the film color appearance [35].

Recently Al asmar et al. study the effect of incorporated mesoporous silica nanoparticles with citrus peel pectin, whether in the presence or absence of GLY, successfully improved the film mechanical parameters. This shows that pectin nanoparticles containing films, a minor improvement in tensile strength. While considerably reduced in Young's modulus compared to pectin films without nanoparticles. Besides the reduction of barrier

properties of pectin films with nanoparticles. Whereas the thermal stability and seal strength positively increased. They produced films used for wrapping strawberries. The results showed the strawberries wrapped with pectin films had more stability, a shelf-life the strawberries lasted up to (8) days contrasted with the control group that was unwrapped [58].

In Tabari et al. study, the casting methods used to produce a sago-starch film were film incorporated with carboxyl methylcellulose nanoparticles. Besides, use of (sorbitol/ glycerol) plasticizing agent. Nano carboxymethyl cellulose with different concentrations was added to the film before casting. The results showed that, by increasing the concentration of nanoparticles, the tensile strength significantly increased, where the prolongation parameter significantly decreased, while the sago film seal strength showed a decrease with a high percentage of nanoparticles. In conclusion, sago film characteristics, such as cost-saving and biodegradability, make it possible to use in industries, particularly food packaging, specifically as film reinforced with nanoparticles [59].

The crystalline nanocelullose (CNC) used for edible pectin films, with three concentrations of CNC (2, 5, and 7% w/w), and tested to understand its effect on thermal, mechanical, and water vapor barrier properties of pectin-biodegradable films. Chaichi, and his team used the solution casting evaporation method for film preparation. The best result obtained, based on the CNC concentration, was happening at (5%) corresponding to water vapor and mechanical properties. The tensile strength improved up to 84%, and a 40% reduction in water vapor permeability. Finally, pectin film reinforced with 5% CNC, it can recommend for food packaging purposes. Because of its renewable, effective improvements, and complete biodegradable [60].

Starches from different plants or vegetal sources (legumes, tuber, and cereal) were used with different GLY concentrations. Besides reinforcement with cellulose nanocrystals, through a protocol of solution casting method. This work by Montero et al, aimed to get bio-based thermoplastic starch films for replacing petroleum-derived ones in packaging industries, especially for short-life product application. Montero et al, study the effect of different starch and cellulose filler percentage. Which are needed to get an improvement of plasticization and crystallinity of produced film. Through (X-ray diffraction and SEM) examination to have thermoplastic-starch morphologies, they also analyzed mechanical properties. The result showed an extension in the plasticization of high amylopectin

starches, it creates matrices with large starch-rich domains. Starches are also acceptable for thermal stability, with low rigidity, and resistance to water absorption [13] .

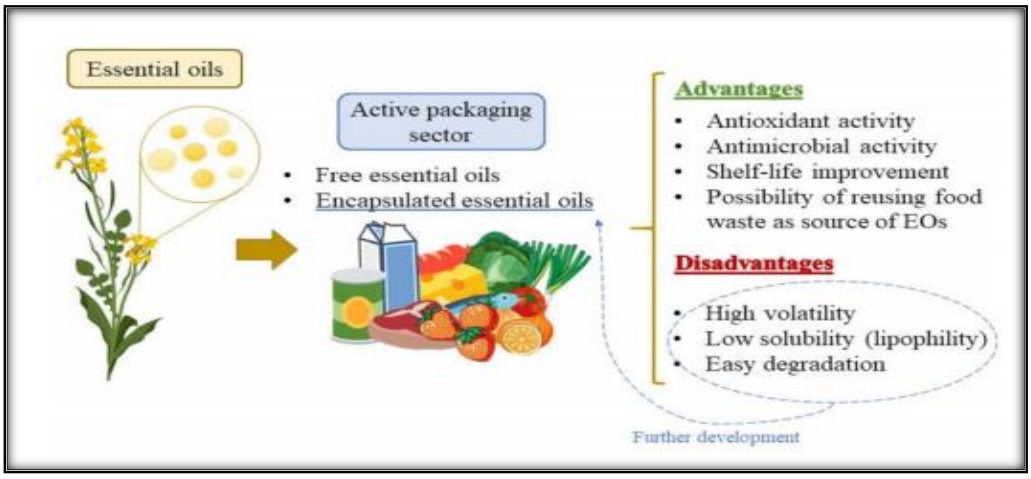
1.10 Use of essential oil for active packaging.

Active packaging is a novel method in which the coating substance can integrate compounds with biological properties applied to being slowly released into food. This function of essential oil could improve the water barrier properties, thus extending the shelf life and ensuring criteria such as quality preservation of food items [61]. Antimicrobial or antioxidant bioactive compounds applied to the films were released and delivered up to the food surface without adding large concentrations of active ingredients, ensuring that it kept an adequate level of preservative at the food's surface in the long term [60].

Essential oils (EOs) are a group of active agents extracted from various parts of plants, such as leaves, stems, flowers, and roots, and have high antioxidant and antimicrobial effects (Fig. 5 (A)). The plant characteristics like variety, geographical origin, age, season, and condition of the plant when harvested, and part of the plant used for EOs extraction affects the EOs' composition besides the EOs' quality. The extraction method also influenced on quality of EOs. Besides conditions of the assessment, and the solvent being used [62].

Figure 5 (A)

essential oils properties for active food packaging



nowadays, EOs have more broad classes of applications in the food industry, pharmaceuticals, cosmetics [63].

1.10.1 Antimicrobial activity of essential oils

Microorganism (M.O) contamination reduces food shelf life. It could occur at any stage of the food supply chain and, anywhere food is unprotected from the environment. M.O are food spoilage factors can begin from simple alterations of sensory features to dangerous health hazards on consumers. According to the developed technologies for the (welfare of the consumer), antimicrobial packaging can be a beneficial technology that improves food safety and extends food shelf life to reduce food deterioration and economic losses [64]. Antimicrobial packaging handling aims to extend food shelf life and preserve foods by further inhibiting microorganism growth This functionality could be achieved by applying a coating layer within the packaging material. Or by incorporating an active agent on packaging materials. Corresponding to the different properties of antimicrobial agents, their effect differences depend on the characteristics of pathogenic microorganisms as follows:

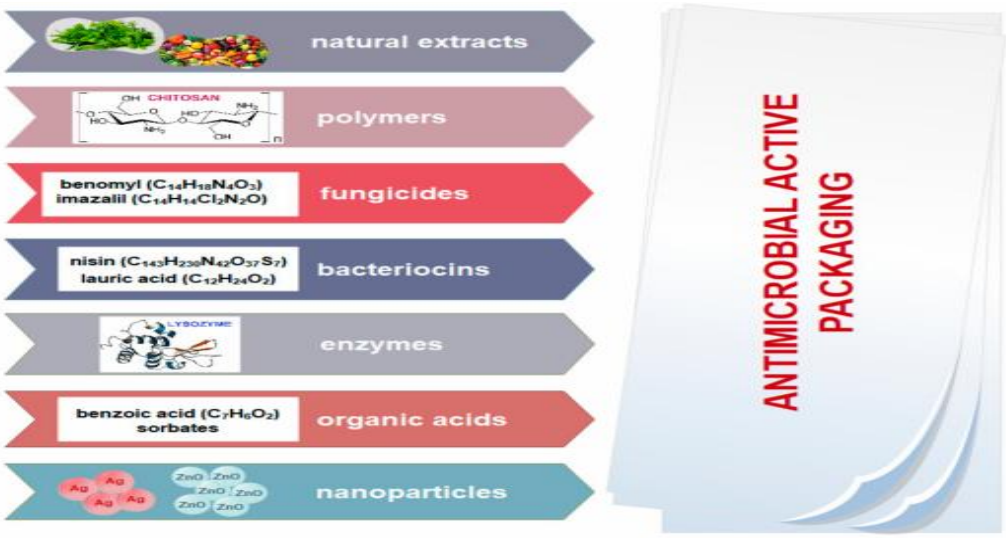
- Cell wall composition (Gram-negative or Gram-positive) .
- Oxygen requirements (aerobes or anaerobes).
- Growth stage (spores or vegetative cells) .
- Acid/osmosis resistance .
- Optimal growth temperatures (mesophilic, thermophilic) [65].

So food packaging innovators should consider all the above when choosing the proper antimicrobial agent. Different microorganisms include *Staphylococcus aureus*, *Bacillus*

cereus, *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Pseudomonas*, *Klebsiella*, and *Lactobacillus* spp (spoilage bacteria). There are two mechanisms of antimicrobial activities. The first mechanism is the inhibition of the essential metabolic pathways of microorganisms. The second mechanism is impairing the cell wall/membrane structure. EOs contain various antimicrobial substances with the possibility of incorporation into food packaging systems (Fig.5 (B)) [44].

Figure 5 (B)

Different antimicrobial substances [44].



1.10.2 Mechanism of antimicrobial action

Determining the exact mechanism of essentials as antimicrobial agents is not fully clear, but some researches have discussed some of EOs mechanisms or hypotheses which were related to EOs. The permeability of bacterial cell membrane increases by the action of EOs according to the dissolution of the integral lipid structure found in the bacterial (or microbial) cell membrane [66], which is an action related to the EO's hydrophobicity. The molecular composition of essential oils can give them the ability to penetrate and disrupt the lipid content of the microbial cell membrane; as a result, this will lead to the destruction of the cell structure and then an increase in the permeability [67].

The increment of permeability causes the leakage of the organelles of a microbial cell, consequently leading to microbial cell death. Moreover, EOs can play a role in the degradation of the microbial cell membrane proteins. The effects of EOs have more activity against the gram-positive bacteria. Because of a 'peptidoglycan layer on the outer surface of the external membrane, while the gram-negative bacterial species have

lipopolysaccharides, which could prevent EOs diffusion or internalization of bacterial cell walls [66]. Other researchers have illustrated that a few compounds can inhibit the action of cellular metabolic pathways or may affect proteins of the cytoplasm membrane. Researchers linked the effectiveness of the EOs antimicrobial activity to specific compounds at high concentrations, that doesn't mean using the isolated bioactive compounds from EOs alone. They found the whole EOs more beneficial than mixing isolated bioactive compounds as an antimicrobial agent [67].

1.10.3 Moringa oleifera (Moringaceae)

In this research, Moringa unrefined seed oil is used, which is extracted by the cold extraction method, as a source of lipid for performing active packaging. *Moringa oleifera* (*Moringaceae*) is a fast-growing softwood tree found in the Middle East, African and Asian countries. Because of its adaptability, it is spreading to other areas, especially tropical and subtropical lands. All the different parts of Moringa tree, such as leaves, seeds, roots, and flowers, can be used for human and animal consumption (Fig. 8). The leaves are rich in minerals, protein, β-carotene, and bioactive compounds like antioxidants [68]. The seeds have attracted scientific interest as *M. oleifera* seed kernels contain a significant amount of oil (up to 40%) with a fatty acid (recognized as a high-quality acid) (Table 1), with a composition of oleic acid > 70%. Recently, the characteristics of Moringa species mature seeds include having edible oil contents of about 38–54%.

The Moringa oil composition has unsaturated fatty acids at high levels. It contains palmitic, stearic, lauric acid, linolenic, and linoleic acid. Also the oil after seed refining had remarkable resistance to oxidative degradation [68, 69]. (Fig. 6) plotted in appendices shows . moringa plant leaves, seeds and oil.

Table 1

Chemical composition of Moringa oleifera seeds (g/100g of dry weight) [68].

Nutrients	Mean
Fat	36.7 ± 2.8
Proteins	31.4 ± 1.3
Carbohydrates	18.4 ± 1.4
Fiber	7.3 ± 0.5
Ash	6.2 ± 0.9
Moisture	7.0 ± 1.2

The Moringa seeds oil sterols profile are β-sitosterol, avenasterol, campesterol, lambda 5 sterol, and stigmasterol, where the (δ-tocopherols and α-, Ɣ-), are considered the main sterol components of the Moringa seed oil [69]. Moringa seed oils have similarities with vegetable oil at physicochemical parameters and present a part of nutritional benefits for human health and the ability to be used in food formulation. These benefits relate to the oil fatty acid constituents of mono and unsaturated fatty acid [70]. The nutritional benefit besides the value of the fatty acid composition, also, has antimicrobial benefits when the extract of Moringa was examined, and this benefit may be used in different application [69].

1.10.4 EOs effect on edible film properties

Researchers have investigated the effects of different essential oils on edible films at many properties. For example, Shojaee-Aliabadi and his coauthors study on kappa-carrageenan films were incorporated with summer savory oil *Satureja hortensis* (SEO). The results showed that films' water barrier properties significantly improved with the combination with SEO and SEO-incorporated film had good antioxidant properties, which were maximum action at a 3% SEO concentration. When films combined with SEO effectively restricted the growth of examined microorganisms [71].

Strawberry fruit has a short shelf life because of its susceptibility to fungal and bacterial growth, so Maringgal and his team applied bioactive coatings using essential oils with chitosan. Essential oils' activity, as antimicrobials were evaluated on molds and total flora, which were isolated from strawberries. Oregano extract (OR), limonene (LIM), and red thyme (RT) essential oils proved to be effective bioactive agents against total flora and molds isolated from strawberries. Even though RT and LIM had lesser antimicrobial properties. Maringgal et al. also used peppermint PM and LIM essential oils because they contain bioactive compounds. EOs cover strawberries via a spraying method. The team also found essential oils to be efficient preservative agents for strawberries within 14 days

of storage. The coating based on chitosan containing LIM emulsified with Tween 80 showed more antimicrobial activity than PM essential oils [72].(Table 2) summarizes the applications of the different EOs functionalities on edible films.

Table 2

EOs antimicrobial activity incorporated with different edible films.

Essential oil	Food Application	Antimicrobial activity	Reference
Lemongrass essential oil	Coating cut Fuji apples	Inhibited the natural micro flora completely at both concentrations 0.5 and 1% to the cut Fuji apples during 14 day storage, at 4ºC	(Salvia-Trujillo. et al) [73]
Thymol nano emulsion	Coating Strawberries	Low fungal and yeast load than uncoated at 5ºC with 90% RH during storage. The coated strawberries were able to resist fungi at least 10 days under the similar commercial storage environment	(Robledo. et al) [74]
Rosemary essential oil	Fresh cheese	Retained minimal numbers of Coliform improving the microbiological cheese quality at 30-day storage, 35ºC for alginate coated with rosemary essential oil.	(Pieretti. et al) [75]
Garlic essential oil	Roasted peanuts	The banana flour nanocomposite film, with garlic essential oil was able to inhibit the growth of *Aspergillus flavus*, and good antimicrobial activity.	(Orsuwan. et al) [76]
Lemon essential oil	Coated the rucola leaves	Prolongs the shelf life by a minimum of 3 days to a maximum of 7 days compared to the untreated samples.	(Sessa. et al) [77]
Lemongrass essential oil	Coated papaya fruit	Starch based solution with lemongrass essential oil was significant inhibiting the microbial growth compared to controls	(Praseptiangga .et al) [78]

The essential oils migrations into the surface of edible coated product are considered as significant benefit of reinforcing the edible film. So, the essential oil mechanism is described by slowing down the releasing rate of antimicrobial bioactive compounds as proved in Ju j et al study on peppermint essential oil which was incorporated with chitosan coated solution applied on grapes fruit it was notable inhibition of the fungal infection

during storage time [79]. Therefore, preserving high concentrations of the antimicrobial agent on the product surface, as the contamination is commonly available. So, the use of such a process on food items directly can reduce the growth of microorganisms [80].

1.10.5 EOs antioxidant and reactive oxygen species

Antioxidant are natural compounds consider as common practice. This is known by molecules' ability to react with free radicals. Also, it may provide a reducing power to counteract the oxidative stress caused by free radicals [81]. Food spoilage occurs as a result of reactions occur when food comes in connection with O_2. Or through the action of oxidative damage resulting from the interactions of "reactive oxygen species" (ROS) with any compound capable of being oxidized, that's, which leads to structural variations in the food product [83]. The different free radicals, such as superoxide (O_3), hydroxyl (OH), and hydrogen peroxide (H_2O_2) are classified as ROS. When ROS production is elevated, it can damage varied biomolecules, such as protein, lipids, DNA, and RNA [82,83]. As a result, ROS will exert several undesirable defects in foods, such as shortening their shelf life, delaying nutritional value, besides loss of color, flavor, or odor at least. All these changes result in food rancidity and lipid peroxidation [62].

Lipid peroxidation is defined as a damaging oxidative process in the presence of free radicals, which react with lipid (lipid play as electron donors). Meanwhile, ROS is a damage indicator under oxidation conditions, though it is measured by the concentration of lipid peroxidase. The structure of phospholipid molecules encourages the ROS to be reacted to its molecules. It contains two reaction sites, the first is the unsaturated double bond between two carbon atoms, and the second is the ester bond between glycerol and fatty acids. In addition, the unsaturated fatty acids which were presented in membrane phospholipids are also sensitive to ROS attacks. The presence of one molecule of hydroxyl radicals can cause many unsaturated fatty acids to be oxidized [81].

The innovators in food packaging technology founded that incorporating EOs into edible films can help in two issues: The first reduction of plastic packages, secondly lowering the use of artificial food additives. EOs extracted are used from different plant species and various plant parts for active food packaging, which will be mentioned below. Savory spices and herbs, which have high amounts of phenolic compounds, may be used in functional food packaging, while they also give desirable flavor and aromas. Instead, the

most EOs used are rosemary, oregano, thyme, cinnamon, and basil. In addition, they could include the isolated compounds from different origins in the packaging film, such as (α-tocopherol or β-carotene) [62]. Recent findings in a Tongnuanchan et al. study showed the antioxidant capacity generated by a combination of different composites and EOs, such as a study on fish skin gelatin films formed with various citrus EOs. The EOs addition lowered water vapor permeability, while antioxidant capacity, tested by 2,2-diphenyl-1picrylhydrazyl (DPPH), fluorescence recovery after photobleaching (FRAP), and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, improved [84].

EOs properties as antioxidant and antimicrobial activity, in direct or indirect use of food is still a trend in food research. As Dashipoor, A.et al. published study, which focused on the physical, chemical, antioxidant, and antimicrobial properties of the edible coating, based on carboxymethyl cellulose (CMC) integrated with clove essential oils (CEO). In this experiment, the casting method was used to form CMC edible films with CEO or without CEO. Many characteristics, like water vapor permeability (VWP), thickness, elongation at break, tensile strength, antimicrobial, antioxidant, and microstructure properties of the films, were tested. Results showed that tensile strength values and elongation at break were higher when compared with control films (pure CMC film). Antioxidant and total phenolic compounds also increased with the presence of EOs. Antimicrobial activity of the films showed that films with EOs are effective against microorganisms (pathogenic bacteria) [85].

While Alizadeh et al used Rosemary essential oils in nanocomposite films which were based on whey protein isolation (WPI) and incorporated with nanoparticles titanium dioxide (TiO_2) and natural-based nanoparticles from cellulose nanofibers (CNFs). The film formation approach was the casting/evaporation method. TiO_2 with various concentrations was tested (0.5, 1, and 1.5%), cellulose nanofibers (2.5, 5, 7.5, and 10%), and REO with two concentrations 1.5% and 2%. Specific characteristics were studied, such as the activity against microbial growth, and antioxidant functionality, in addition to barrier analysis of the whey protein-based films. The addition of different nanoparticles in WPI films and REO resulted in a significant effect on films. According to the obtained results, the easy combination of TiO_2 and phytochemical of REO, with (WPI/CNFs). CNF films had remarkable effects on the film qualities, in properties like water-resistance of

the formed films. Also, the addition of TiO_2 and REO caused an increase in such properties. The mechanical investigations of the TiO_2 (1%) and CNFs (7.5%) led to a significant improvement in Young's modulus and tensile strength. Instead, elongation at break was negatively affected and decreased. Conversely, the action of REO, despite TiO_2 nanoparticles, lowered YM and TS, whereas EB considerably increased. Meanwhile, 7.5% (WPI/CNFs) composite films that combined with both (TiO_2 and REO) demonstrated a notable reduction in EB, while TS and YM were improved. So the combination of TiO_2 and REO into WPI/CNFs films had a considerable action against pathogenic microbes or spoilage microorganisms, in particular, gram-positive bacteria. Finally, the findings support the use of CNF as a natural reinforcement, as a replacement for mineral reinforcement, also encouraging the usage of these eco-friendly biodegradable films in packaging industries [86].

In an experimental study, Drago and his coauthors blend cinnamon essential oil with edible sodium caseinate films (SC), at a practical part, they add 2.5 and 5% w/w of SC/cellulose nanofiber (CNF) as a reinforcing additive and (5% w/w of SC/CEO-NE) prepared nanocomposite active films. Based on the result of investigated films, composed from CEO-NE and 2.5% CNF proved the highest antioxidant activity which reached about (66.04%), besides low antimicrobial activity against (*E.coli*, *Staph.aureus P.aeroginosa, and S.enteritidis*) species. This experiment showed that the sodium caseinate films reinforced with CNF and activated by CEO-NE could be a suitable candidate for different food packaging issues, such as shelf-life extending agent[64].

The researchers Orsuwan, and Sothornvit, R., study, which investigates the effects of garlic essential oils incorporations to edible films based on Banana flour raw material, active banana nanocomposite film (ABNCF) preparation. which was then assessed to determine the activity and efficiency of the combinations ABNCF was prepared by the casting method to package roasted peanuts. To evaluate the properties of the composite first, then assess its activities to protect the quality of roasted peanuts. The results show the positive antioxidant activity of garlic essential oil 1 mg/ml (garlic essential oil/APBNF) which, enabled the preservation of the roasted peanuts from further oxidation. The evaluation of filled peanut in two packaging materials (APBNF and polyethylene terephthalate PET) corresponded to its deterioration rats, through measurements peroxide value (PV), which was relative to the storage temperature of

about (45 °C). As will APBNF has a variety of functional properties as utilization for food packaging, to preserve the nutritional value and achieve consumers' needs. Notably, roasted peanuts or foods, which are sensitive to oxidization. Such as oily foods and their derivatives [76].

In Hafsa and Smach et al. published study, a combination of chitosan and *Eucalyptus globulus* (EG) essential oil was used for new film preparation, to investigate the film application ability for food coating. Through, evaluating the film's functionality as an antioxidant, antimicrobial, besides assessments the film quality according to physical parameters. In detail, EG essential oils in this study were added at different percentages, initially from 0, 1, 2, 3 to 4% (v/v). And the dry films were formed by the casting and solvent-evaporation method. The evaluation of antioxidant activity was assessed with DPPH, NO, and H_2O_2 assays. Simultaneously, the determination of the antimicrobial activity of the developed films was tested, by the agar disc diffusion method against the following pathogens, *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli*, and *Candida Albicans*, *Candida_Parapsilosis*). The results indicated that the developed chitosan films with EG essential oil caused a significant reduction in both parameters, water solubility and moisture content. Film analysis in scanning electron microscopy SEM can enable a full explanation of such results when viewing the microstructure of the films. The produced film characteristics as antimicrobial and antioxidant properties positively improved. Particularly, the significant antimicrobial activity against the desired pathogens occurred due to the EG essential oil addition [87].

In Ghamari et al. study, on an edible film based on milk protein incorporated with *Nigella sativa* essential oil, they investigated the different types of plasticizers with different concentrations on film properties with added essential oil. The research team added different sorbitol concentrations (5, 7, and 9% W/V), glycerol concentration (5, 7, and 9% W/V), and *Nigella sativa* essential oil (*NS*EO) amount (0, 1, and 2% W/V) to be evaluated using a Box–Behnken design. The film assessed its different properties to choose the best model of film constituents according to its desired functionality as the minimum values of water vapor permeability and moisture content, along with the optimum whiteness index. Fourier-transform infrared spectroscopy, Scanning electron microscopy (SEM), differential scanning calorimetry, in addition to mechanical analyses were used to test the film functional groups, microscopic structure, and thermal

characteristics, like melting point, in addition to mechanical properties. The assessments indicate that is the optimal preparation conditions to create edible films, were achieved at 5% of plasticizers (glycerol, sorbitol) and 1.27% *NS*EO. The SEM analysis presented regular morphological characteristics of edible protein-based film incorporated with *NS*EO. While the mechanical properties TS, YM showed a reduction in its value, where the EB increases with an increasing concentration of plasticizer and *NS*EO amount. The film melting temperature of the best film model was about 130 °C[88].

In this study, Abdel Aziz et al. researchers were aimed to produce novel active edible films, based on sodium alginate as polymers and castor oil (CO) as bio-active addition. The active sodium-alginate film was investigated for its crystallinity and chemical structures using XRD and FTIR respectively. Also, the mechanical properties were assessed too. The incorporation of CO with sodium alginate caused improvements in mechanical properties when compared with neat sodium alginate film. Also, film water vapor permeability was decreased while the total color difference was affected after CO addition. Meanwhile, the antibacterial investigation showed a notable inhibitory effect of the examined films against Gram-positive bacteria. Whereas no effect was observed for Gram-negative bacteria [89].

The presented study also aimed to investigate the effect of oil addition on edible film. The blends of Mung bean starch (MBS) as filmogenic biopolymers and guar gum (GG) as thickeners, whereas sunflower seed oil (SSO) as a hydrophobicity-imparting substance, were used to produce the edible film in this study. The effect of SSO content was assessed on the film's mechanical, optical, and physicochemical properties. Lee, J. S and his team were added different SSO concentrations (0, 0.5, 1, and 2%, w/w). Consequently, the results showed increasing SSO% causes a decrease in the following properties: elongation at break, tensile strength, water-solubility, water vapor permeability, and crystallinity. In contrast, an SSO increment could increase the oxygen transmission rate. In conclusion, the addition of SSO to the MBS-based films reduced their mechanical strength while effectively improving the film's water-resistance properties. As a result, the new MBS-based film produced here can be used as a coating film in products that need high water resistance properties but do not depend on high mechanical strength [90].

2. Objectives

Using industrial food wastes as a source to get edible films is considered a good choice for two reasons. First is the low cost of such materials, and second is due to the fact that these films are eco-friendly films because the food industry discard its waste in the environment.

The main aims of this research is to develop edible\biodegradable films from the plant-based protein derived from the industrial food waste- defatted *NS* seed cakes .

In addition to the following aims:

- Produce cellulose nanoparticles (C-NPs) derived from the industrial food waste coconut defatted cake as a reinforcing material.
- Evaluate the effect of C-NPs on the film properties such as mechanical, water content water uptake.
- Incorporats *Moringa* edible oil (MEO) to improve the film's functionality as an antioxidant and antimicrobial agent.
- Evaluate the effect of addition of C-NPs and MEO on film properties (mechanical, water content, water uptake, antioxidant and antimicrobial).
- Evaluate the suitability of the formed films for food packaging, in terms of oil holders and putters wrapping.

Chapter Two
Materials and Methods

2.1 Materials

The used materials in this research included *Nigella sativa* defatted seed cake, coconut defatted cake, Moringa oil purchased from Al-Hethnawy General Trade Co. (Jenin, Palestine). All chemicals (NaOH, HCl, NaClO, magnesium nitrate, and Tween 80 and

glycerol) purchased from Sigma-Aldrich, (Denmark), and Mueller Hinton broth was from Hi-Media leading biosciences company (Mumbai-India). Bacterial strains from American Type Culture Collection were*Escherichia coli* (ATCC700221), *Staphylococcus aureus* (ATCC 25923) 2,2-diphenyl-1-picrylhydrazyl (DPPH) from the laboratory of An-Najah National University (Nablus, Palestine).

2.2 Protein extraction from *NS* defatted seed cake

Concentrated protein extracts were obtained from *Nigella sativa* defatted seed cake (*NS*DSC) by acid base extraction method as described by Sabbah et al. [39]. Dry *NS*DSC was grounded using a rotary mill until the suitable smooth powder was formed. Then the fine powder was dispersed in distilled water (1:10, w/v), and the solution's pH was adjusted to a value of 12.0 with 1 N NaOH and the mixture then was stirred at medium speed for 2h at room temperature. The supernatant was collected by centrifugation at 4000 rpm for 20 min. The pH of the collected supernatant was then adjusted to a value of 5.4 using 1 N HCl. The supernatant then was centrifuged at 4000 rpm for 20 min, and the precipitate that contained the protein was collected. The collected protein pellet was then dried at 30°C, after which and the dried pellets were grounded to obtain fine protein concentrated powder that contained about 45% protein. This was determined by Kjeldahl's method using a nitrogen conversion factor of 6.25.

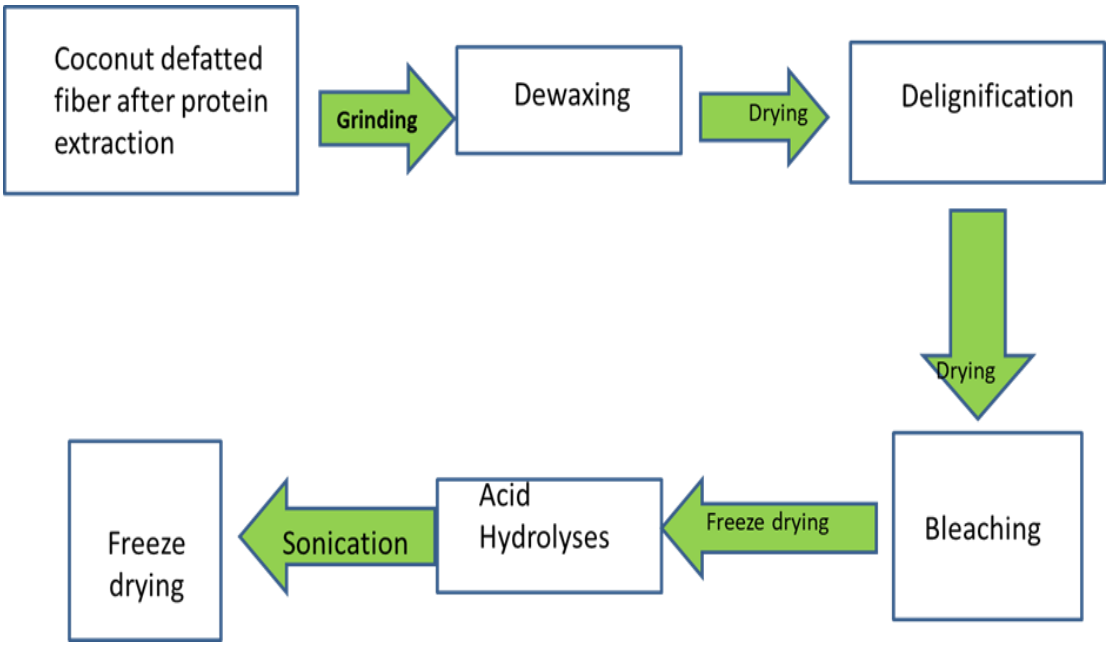
2.3 Cellulose extraction and nanoparticle formation

The cellulose extract was obtained from coconut defatted cake after protein extraction through 6 stages (Fig. 6) as described by Abu-Thabit et al [91]. The dewaxing step is to dissolve any lipid residuals in the coconut defatted cake. The ground defatted fibers about 40 g were soaked overnight in a chloroform and ethanol mixture with a ratio of (2:1), then the solution was filtered to be dried at room temperature. After that, in the delignification step, the dewaxed coconut defatted cake was boiled in an alkaline medium (2.3 M NaOH) with a ratio of 1/10 for three hours at 80-90°C with countenance stirring, the solution was lifted to cool down, then was washed to remove the alkaline solution, (which contains dissolved lignin). The washing step after discharge the alkaline solution and with addition DW to the alkaline fibers to be then centrifuged the at 3000 rpm for 15 minutes 7 times until the pH value reached pH 7.0. Then the resulting coconut cakes were dried overnight at 45^{o}C. In the bleaching step: The resulting coconut cakes were ground to be smooth enough for optimum dissolving into 10% sodium hypochlorites (NaClO) with a ratio of

(1\20) powder\solute. The bleaching solution was placed in a water bath at a temperature 50^{o}C, to avoid foaming for 2 hours or until the white color of the mixture strongly appeared. The bleaching solution to cool down and then centrifuged at 3200 rpm for 10 minutes first, then 5 minutes until the pH value reached 7.0. The centrifuged pellets were dried in the freeze dryer for 24 hours. Finally, the acid hydrolyses step was done by mixing the freeze deride powder with 3 N HCl at a ratio of (1\20), in a water bath for 2.5 h and then washed with DW, by centrifuge at 3000 rpm for 5 min several times until the pH reached 3.0. Then the cellulose hydrolyzed particles were sonicated for one hour, then dried at freeze-dryer, also to get the final fine powder of cellulose nanoparticles C-NPs. (Fig 6) plotted in appendices summaries the cellulose nanoparicls preparation procedures

Figure 6

Coconut cellulose nanoparticles preparation diagram



2.4 Preparation of film forming solution

The film forming solution method was described by sabbah et al. [39]. Different film forming solutions (FFSs), prepared as following: *Nigella sativa* concentrated protein extract *NS*CPE was dissolved in distilled water (4 g/100 mL), pH value of the solution was adjusted to pH 12.0 by using 1 N NaOH with constant stirring until the powder was completely solubilized. In preliminary experiments, FFSs was prepared with different glycerol (GLY) concentrations (10%– 50% v/w protein) to find out the minimal GLY amount needed to obtain handle able films. The C-NPs solution was prepared at concentration 0.4g for 100mL DW stirred at room temperature for 48 hours and then sonicated until completely dissolved. The C-NPs added to FFSs (w\w) at different concentrations (0.1, 0.5, 1, 2 and 3%). Finally, the pH value of the FSSs was adjusted to pH values 12.0 by 1N NaOH and then casted at petri dish for 48 hours for drying after which, the dried films were peeled.

2.5 Nanoemulsion preparation

The nano emulsion was prepared following the method of Ranjbaryan et al. with some modifications [63], For preparing *Moringh*a edible oil (MEO) nanoemulsion (MEO-NE). The amount of MEO was 2% v/v (MEO/water), while Tween 80 was added at the concentration of 60% w/w (Tween80/MEO). Then MEO mixtures were added to the C-

NPs solution at different NPs concentrations (0.1, 0.5, and 2%) with continuous stirring for 15 minutes. After that the ultra-sonication machine used for the produced solutions which were sonicated for 30 min, at $25C^0$.

2.6 Film preparation with nanoemulsion

The nanoemulsion was added gradually to the dissolved *NSPC* solution with continuous stirring for 10 min, then sonicated for 10 min, after that the solution pH was adjusted to 12.0. GLY was then added and mixed well, then casted on a petri dish.

2.7 *NS* film assessment for antimicrobial activity

The antibacterial effects of *NS*CP nanoemulsion films were tested by the disc diffusion method, as described by Alizadeh-Sani et al. [85]. *Escherichia coli* O157:H7, *Staphylococcus aureus* suspensions were prepared for 18h in Mueller Hinton broth, then adjusted to 0.5 McFarland standard turbidity and diluted (1:10) to from the bacterial density (1.5×10^6 CFU/mL). Then *NS*PC films were aseptically cut into discs with around 5 mm inner diameter and then fixed on the surface of (Mueller Hinton) agar plates, which were inoculated with the bacterial suspensions. The agar plates were then incubated at 37°C (*E. coli* O157:H7, *and S. aureus*) for 24h. The inhibition zone around the discs were examined under the microscope.

2.8 *NS* film assessment for antioxidant activity

DPPH radical scavenging assay was used to evaluate the antioxidant activity of the films, according to the method of Sukhtezari, Almasi, Pisa, Zandi, and Pirouzifard [92], with a few modifications. Approximately 20mg of the *NS*PC film (0, 0,1, 0.5, 2% C-NP+ 2% MEO) were placed in Eppendorf tubes containing (0.8) ml of DW pulse (0.2) ml methanol, to be liquefied by vortex until the film was ultimately dissolved at room temperature. The DPPH methanolic solution was prepared, with the addition of 1 mg DPPH to 20 ml of methanol, and it should be kept in a dark condition. Next step, 0.1 ml of the prepared *NS*PC solution was mixed with 0.9 ml of DPPH methanolic solution. Then the mixture was vortex vigorously and incubated for 30 minutes in the dark at room temperature. The absorbance was measured at 517 nm compared to the corresponding blank solution by using UV Schimadzo [86] Equation (1). $Antioxedant\ activity = 100 - \frac{asorbance\ control - absorbance\ sample}{absorbance\ control} \times 100$

2.9 Approximate analyses for *NS*DSC and protein concentrated extract

2.9.1 Protein and *NS*DSC characterization.

The raw material and protein-concentrated extract material was characterized according to several parameters as their composition, fat content, ash, moisture, and protein.

2.9.1.1 Ash content.

Ash content is defined as the inorganic residue, which results after the burning process or by complete oxidation of food components. The ash content for *NS*DSC and protein concentrated extract were calculated [93] based on Equation (2):

$$Ash\ (\%dry\ basis) = (weight\ after\ ashing \div weight\ of\ original\ sample) \times 100$$

2.9.1.2 Moisture content.

The *NS*PC film may use of research for food packaging. So, the moisture content of *NS* and protein-concentrated extract are critical factors of food product preservation, quality and deterioration resistance for packaged food and *NS*PC itself. Besides that, moisture content determination is, also needed for calculating the other food, dry matter bases, or total solids, for example, dry weight basis, which is formed after moisture evaluation is carried out for further calculations [93]. The moisture content analysis was carried out using the electronic moisture analyzer, Sartorius Moisture Analyzer Models MA100 | MA50.

2.9.1.3 Quantitative protein analyses

Protein quantity is fundamental to be calculated in food analysis, and for edible film-based materials are principal issues. Because protein concentration is a critical point to reach the desired films with the best quality. The Kjeldahl method for quantitative protein analyses was used to calculate the protein percentage of *NS*DSC and *NS*CP extract [93].

Nitrogen % was calculated according to equations (3) and (4).

Equation (3). $$N\% = \frac{volume\ of\ HCl \times N\ HCl \times 1.4007}{sample\ weight\ (g)}$$

Equation (4). $$Protein\ \% = N\% \times 6.25$$

Where N means nitrogen percent in the analyzed materials

2.9.1.4 Crude fat content

The fat content was assessed by the ANKOM extractor, XT analysis. and was calculated according to [94], as in the following. Equation (5).

$$Fat\% = W\ dry(g) - W\ original(g) \div W\ original\ (g) \times 100$$

2.10 Film thickness and mechanical analyses

The thickness of *NSPC* films was determined using stainless steel micrometers. Film thickness was measured in different areas of the film surface, and then reading the average of measure samples was calculated. The ASTMD882-95 method was applied for testing tensile strength and elongation at break measurement of the *NSPC* films. The *NSPC* films were cut into specimens with 1 cm width, each with scissors, and placed in the specific sit in the texture analyzer machine. As the test was by using a TA-XT2i Texture Analyzer (Stable Microsystems, Goldaming, UK).

2.10.1 Tensile Strength (TS)

We carried out tensile strengths analyses using self-tightening roller grips.
The tensile strengths, the elongations at the break, and Young's Modulus of the *NS* specimens were conditioned in a 50% relative humidity atmosphere of salt solution from magnesium nitrate in a closed champer for at least 2 hours at room temperature [95].

Tensile strength is defined as the polymer's maximum stress number, which can handle stretching, i.e. its distance between plasticity and rupture [96].

The result is expressed in mega-pascals and calculations were done according to equations (6), (7), and (8) [97].

Equation (6). *Tensile strength (g/mm)*= $\frac{(load\ at\ break)(g)}{(original\ width)(mm) \times (original\ thickness)(mm)}$

2.10.2 Elongation at break (EB)

Elongation at break is defined as the maximum elongation of the polymer at which it can hold before breaking. It has no unit [96].

Equation (7). *Percent elongation* = $\frac{(elongation\ at\ rupture)(mm)}{(initial\ gage\ length)(mm)} \times 100$

2.10.3 Young's Modulus (YM)

The initial elastic behavior of the material before the first yield point is known as Young's Modulus, which means that material will return to the initial position if it is releasing the applied strength [96]. Young's modulus is evaluated by a draft of a tangent to the initial linear portion of the stress-strain curve, choosing one point on the linear tangent to divide the tensile stress by the corresponding strain [97]. The result is converted into MPa.

Equation (8). Young's modulus=

$$\frac{load\ at\ point\ on\ tangent\ \div (original\ width)(mm)\ \times (original\ thickness)\ (mm)}{(elongation\ at\ point\ on\ tangent)(mm) \div initial\ gage\ length\ (mm)}$$

2.11 Water content and uptake

2.11.1 *NS* film water content

The moisture content was measured of each film gravimetrically based on Al-Asmar et al. [98] with some modifications. The film specimen was cut into squares (2 cm × 2 cm) from different areas of each film type, which weighed, and then placed on aluminum dishes for water vaporization in an oven at 105°C for 24h. Water content evaluated film water content from each *NSPC*. Film water content was calculated based on equation (9):*NS* film water content was calculated based on equation (9):

Equation (9). *Film water content (%)* = $\frac{(W1-W2)}{W1} \times 100$

Where W_1 is the film original weight and W_2 film dry weight.

2.11.2 *NS* film water uptake

Water uptake for each *NS*PC film was assessed in triplicates as described by Giosafatto, C. V. L Al-Asmar et al. [98]. In detail, *NS* films were cut into 2-cm 2-cm squares; We dried the film specimens at 105°C overnight then put them into a desiccator previously adjusted at 50% RH with a salty saturated solution Mg (NO3)2 for 24 hours. The water uptake was calculated based on equation (10):

Equation (10). $Film\ water\ uptake(Dry\ basis\%) = \frac{Ws-Wd}{Wd} \times 100$

Where Ws film weight after 50% RH solution and Wd are the film dry weight. Each measure was carried out in triplicates.

2.12 Food packaging potential applications

*NS*PC film combined with MEO was used for making oil holder, the film with 4cm×4cm squares fixed from a backward corner, alongside sealer machine at practical fixing temperature, to make oil package bag can contain (2-3) ml oil, it shielded at room temperature.

A large sheet of *NS*PC film used for warping butter slices about (25)g of butter was wrapped from all sides in two-layer wrapping and sealed from the outsides thin persevered on the refrigerators.

2.13 Statistical analyses

JMP software 8 (SAS Institute, Cary, USA) was used for data analysis based on the two-way ANOVA test and Tukey post hoc test comparison, $p<0.05$ considered significant differences for different comparisons values.

Chapter Three
Results and Discussion

3.1. *NS*DSC extract and *NS*DSC characterization

Defatted *NS* and concentrated protein extract material were characterized for several parameter including, fat content, ash, moisture and protein percentage the results were plotted in Table 3 addition to the raw *NS* such composition [99].

Table 3

Approximate analyses of NSDSC, and NSDSC extract.

	Raw *NS**	***NS*DSC**	***NS*DSC extract**
Moisture content	7.1 ± 0.2	7.5 ± 0.1	5.0 ± 0.3
Protein	20.3 ± 0.6	34.0 ± 2.7	43.0 ± 2.5
Carbohydrates	19.7 ± 0.4	44.0 ± 2.3	45.1 ± 1.4
Fat	45.4 ± 0.5	10.2 ± 0.5	3.1 ± 0.3
Ash	7.4 ± 0.3	5.5 ± 0.1	3.7 ± 0. 6

* Results based on Nergiz, and Ötleş. study [99].

The results show that the moisture content of all materials was between 7-5% which is essential in reducing microorganism ability to grow since high moisture leads to spoilage or undesired properties' [93]. Meanwhile, the *NS* extract's protein content is considered a rich natural source of protein to form edible packaging materials. The fat content of *NS*DSC extracts was less than the *NS*DSC, and this is may be related to the extraction by centrifugation at low temperatures it was clearly clustered in the upper parts of supernatant, which leads to the additional removal of fats. Instead, the lower ash content in the *NS*DSC extract may be related to the discharge of fibers and other content by an alkaline separation step.

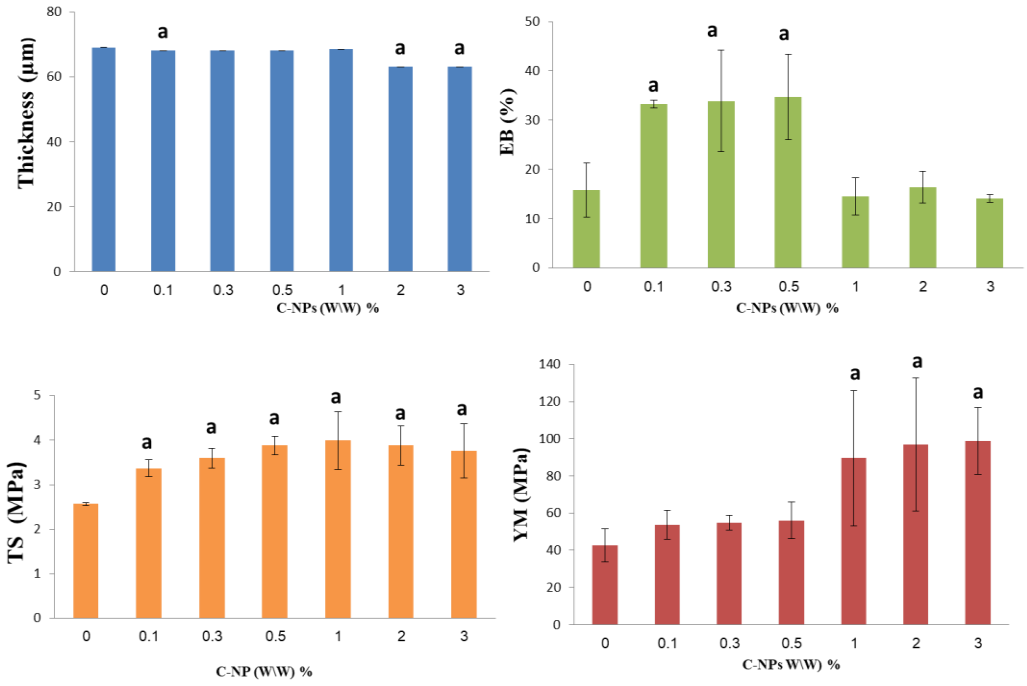
3.2 The effect of cellulose nanoparticles on film thickness and mechanical properties

The edible film was exposed to various types of stress during use, because determination of the mechanical properties involves scientific aspects and technological, practical aspects. The film thickness is a remarkable property of the produced films. Because it has a direct impact on the film's properties, such as mechanical, biodegradability. It also has adverse environmental effects on food and packaging. The obtained results (Fig. 7 (A)) have shown that the thickness of the films obtained with 0.1 up to 1.0% (w/w) C-NPs has no differences compared to the control films (*NS*PC without C-NPs). Whereas, by increasing the C-NPs up to 1%, the film thickness significantly decreased compared to the control films. The films with high C-NPs concentration as shown in (Fig. 7 (A))

reduced the thickness value, which may be because of the aggregation occurrence of C-NPs at a high concentration between polymers matrix. On the other hand, because of the reduction of electronic repulsion, as described in Ngo et al. study of nano-chitosan-pectin-based films [100], Another explanation may be related to the drying process as confirmed by Romani et al. in starch /protein edible film study [101].

Figure 7 (A)

The effect of different concentrations of C-NPs on thickness and mechanical properties of NSCP films plasticized with 30% GLY. "a" Significantly different values as compared to the obtained film under the same experimental conditions in the absence of C-NPs.



Tensile strength (TS) is the maximum tensile stress sustained by the sample during the tension test. TS significantly increased shown in (Fig.7 (A)) as C-NPs concentration increased compared to control films were it reached the maximum TS value after 0.5% C-NPs was added to the *NS*CP.

The research findings (Fig.7 (A)) agree with Bilbao-Sainz et al. study that use cellulose nanoparticles as reinforcement materials. The study results showed improvements in mechanical properties for (hydroxy propyl methylcellulose) HPMC films, especially in TS plus YM mechanical properties. However, the EB did not change with C-NPs addition [102]. Similar results agree with this research findings that a high concentration of C-NPs (Fig.7 (A)), could cause loss of TS at higher concentrations, as resulted in Chaichi and

his team study on pectin film incorporated with C-NPs, the improvement of TS was when the C-NPs were 5.0% (w/w) [60]. While in results (Fig. 7 (A)), C-NPs 1% (w\w) was the highest TS value. It may relate this to polymeric martial, which employs as a base for the C-NPs filling effect.

As defined, elongation at break shows film flexibility and stretch-ability (extensibility) [103]. The EB increased as the concentration of C-NPs increased compared to control films (Fig. 7 (A)); it increased from 22% in control films to 34% in the reinforced films with 0.5% C-NPs. This EB increase may be explained as Zhang et al, suggested it, because of C-NPs at low percent could be enabled interaction between polymers as C-NPs can shorten the distance between bonds of polymeric molecules, thin implements glycerol to form bonds within the matrix of polymers. Also, the C-NPs may form ionic bonds with the polymers matrix, which then changes the original film force [104]. Meanwhile, high concentrations of C-NPs from 1 to 3% have not been shown in differences in EB from control films but reduced the EB of films take place at low C-NPs concentration (Fig.7 (A)).The EB research findings agree with Ljungberg et al, as they evaluated the effect of C-NPs reinforced polypropylene, and concluded that C-NPs good dispersion could give higher EB than nanocomposites with aggregated C-NPs, which achieved by a high concentration of C-NPs[105]. Also Chaichi, M., study on the effect of C-NPs addition on pectin-based films, agree with our findings, where the improvement of EB of pectin films occurred at 5% of C-NPs [59]. In addition to Y. Bao, H. Zhang. [106], research on xylan–chaitosan composite with cellulose nanocrystal supports the research finding in (Fig.7 (A)) .

Young's modulus (YM) designates to *NS*PC film rigidity or may relate to the film's flexibility properties, as it referred to the chemical structure of polymers' materials [103,107]. Consequently, (Fig. 7 (A)) showed that YM increased at a minor difference from control films at low concentrations of C-NPs. While higher concentration ranged 1.0-3.0% (w/w) C-NPs showed higher YM average values contrasted to the average value of control films.

Several studies showed that, by increasing C-NPs, the YM values increased. This increase in YM values may relate to multi hydrogen bonds between C-NPs and *NS* protein according to increasing concentration, so the film rigidity increases. Fathi, and Almasi, study on sesame edible films incorporated with TiO_2 NP agrees with our results(Fig. 7

(A)), which show that the YM improvements increase at TiO_2 NP concentration of 3%, but further addition of TiO2 NP reduced all mechanical parameters [108]. The rigidity of structure nanocomposite materials reinforced with C-NPs may be related to nano cellulose particle relocation in the polymers' matrix. Mechanical improvements may occur because of strong inter–intramolecular hydrogen bonding with matrix polymers, as concluded by Mondal [109].

The low concentration of C-NPs can bond to protein through functional hydroxyl groups and C-NP_S simultaneously through hydrogen bonds. But at high concentration, it may reduce the interaction between the C-NPs and the polymer matrix, according to the non-uniform distribution and agglomeration of the C-NPs. That means there is an optimum limit for C-NPs concentration to get the improvement in desired mechanical characteristics of edible films [60], [110]. These findings agree with another study as Bilbao-Sainz et al. study on films based on alginate and reinforced with C-NPs [102]. In addition to, Fathi et al, study on sesame protein based film supported with TiO_2 NP proved the same theory of nanofiller reinforcement. Revealing that TS and other mechanical characteristics increased to an optimum concentration value, while the increased concentration of TiO_2 NP causes a decline in such properties [108]. Conflicting with our results, the study on starch–chitosan-film reinforced with C-NPs, which done by Al sammarraie et al. proved such reinforcement doesn't change the tensile strength [111].

3.3 The effect of cellulose nanoparticles on water content and uptake of *NS*PC films

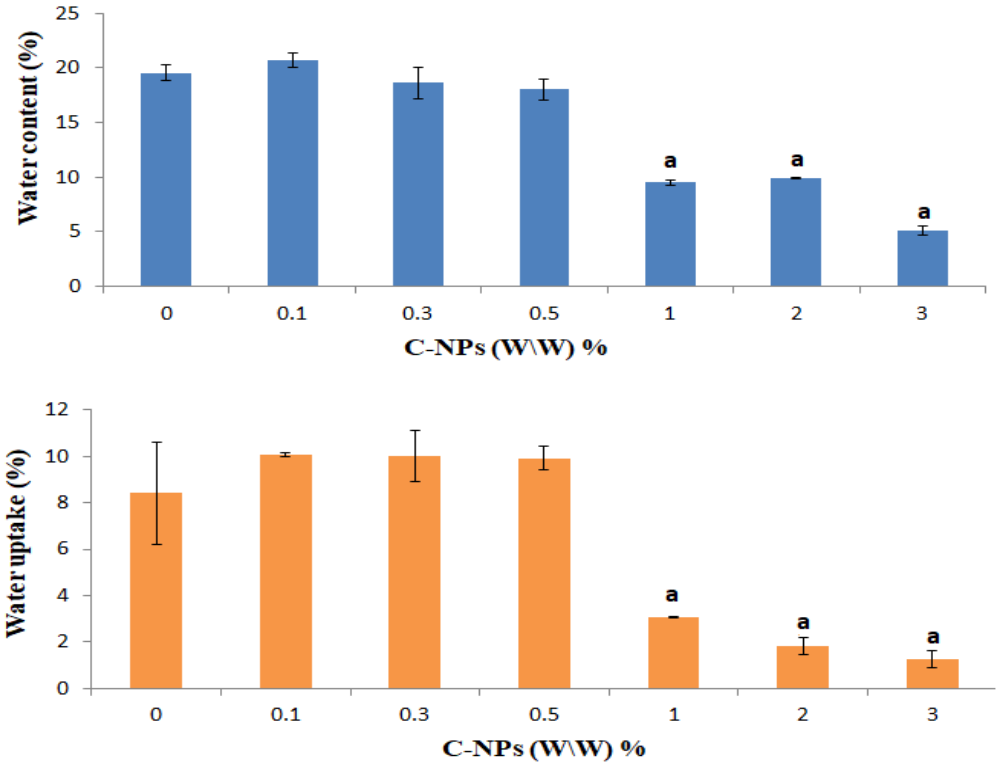
The prepared *NS* films was also characterized for their water content and water uptake. Such characteristics are critical to be determined, because of the possibility of employing film in food packaging sector applications. Especially, with packaging materials used in contact with water or high water activity, or when the film must be in closing with water and acts as a (food protective) barrier [112]. If the edible film contains elevated percentages of water content, this could limit its usage as a packaging material.

The effect of C-NPs on the *NS*PC film water content and water uptake is presented in (Fig.7 (B)). The *NS*PC film reinforced by C-NPs ranged from 0.1to 0.5% w/w showed similar results to the control film. However, increasing the C-NPs caused a considerable decrease in water content for *NS*PC edible films (Fig.7 (B)), it declined from 20.7% for the control film to 5.1% for the reinforced film with 3% C-NPs. Based on previous

research by Mihalca et al, proved that's water content of the edible films depends on film original raw materials and the disintegrating of hydrogen bonds of protein monomers and H_2O molecules [113]. Another study their finding seems like our finding, in Giosafatto and Sabbahet al, study of incorporating mesoporous silica nanoparticles with pectin based films. Proved that nanofillers significantly decrease the water content of the films [98]. Also, the same is found with pectin based films when incorporated with cellulose nanocrystals which done by Chaichi et al [60].

Figure 7 (B)

The effect of different concentrations of C-NPs on water content and uptake of NSPs films plasticized with 30% GLY. "a" Significantly different values as compared to the obtained films under the same experimental conditions in the absence of C-NPs.



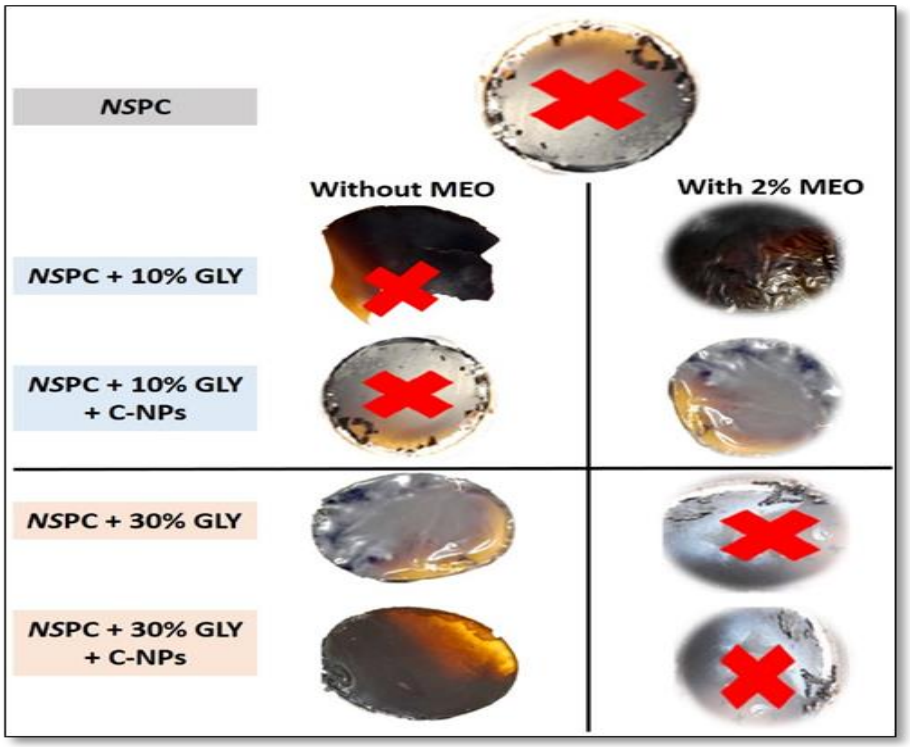
The water uptake of *NS*PC films is shown in (Fig. 7 (B)). The water uptake increased slightly from 8.4% for the control film to 10.06% for films with 0.1% C-NPs. Then, with increasing C-NPs, the water uptake decreased but was still more than or near to the control film. This may be due to C-NPs as hydrophilic and the weak hydrogen bonding between C-NPs due to low concentrations of C-NPs. However, when the concentration of C-NPs increased (Fig. 7 (B)), the water uptake dropped to reach about 1.26% at a concentration of 3.0% for C-NPs. It related this to the filler effect of C-NPs, as mentioned in previous studies [60,98], along with the action of the filling effect of C-NPs, which influences film rigidity. Meanwhile, the addition of plasticizers to edible films could enable the formation of bonds with polymeric materials, so these bonds do not fill all the spaces within the polymers matrix (the concentration and type of plasticizers play a role in its action), so nanoparticles fill in the space instead of water molecule adsorption as explained by Mihalca and Kerezsi et al. study, and Kusumaningtyas et al study too [113,114]. This finding agrees with pectin incorporated with MSNs mentioned above Giosafatto and Sabbah et al [98], and also agrees with Almasi, et al, on starch–CMC–nanoclay biodegradable films' study findings [115], and with pectinbased films reinforced with cellulose nanocrystal study by Chaichi et al [60].

3.4 The effect of C-NPs incorporated with Moringa oil of *NS*PC film

The effect of adding C-NPs% incorporated with 2% MEO,10% GLY, compared with the same C-NPs%, 30% GLY, without MEO, is shown in (Fig.8 (A)). As known, *NS*PC films' incorporation with MEO required Tween 80 (polysorbate 80) to disperse the MEO to the film forming solutions. The investigations indicated that the minimum GLY concentration expected to create films without Tween 80 was 30% (w/w). Though, the obtained results, like the previously proved results by Sabbah et al. [39]. Whereas, when Tween 80 was added to the film forming solution in the presence of MEO plasticized with 30% GLY, the film became sticky and hard to be peeled off from the dish. To overcome this issue, the GLY% minimized to 10% (w/w) in the presence of Tween 80 and MEO.

Figure 8 (A)

NSPC films obtained with different concentrations of GLY in the presence and absence of both C-NPs and 2% MEO.



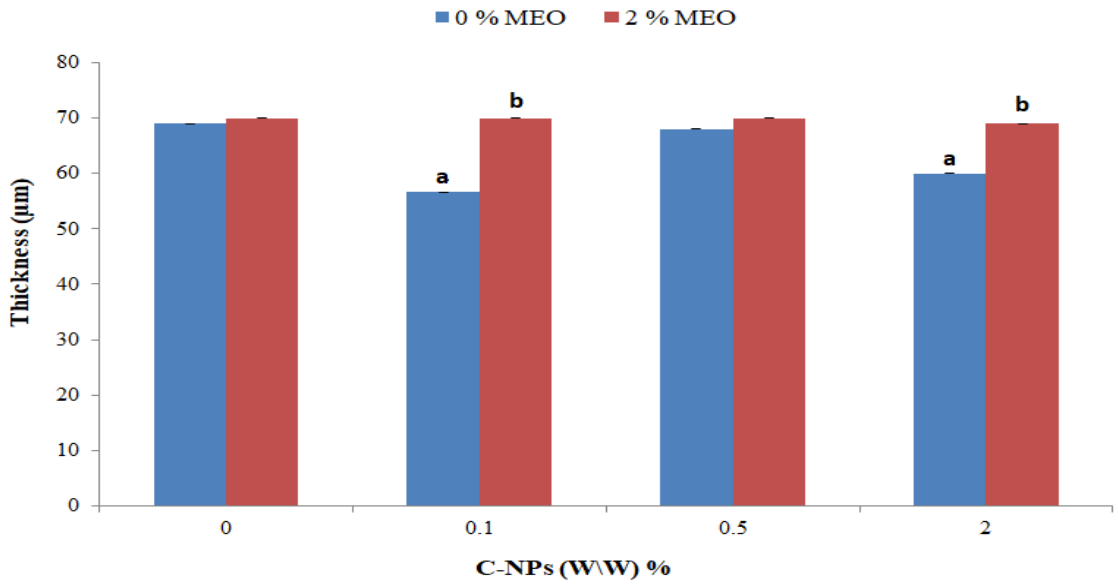
3.5 The effect of C-NPs containing MEO in the presence of glycerol on *NS*PC film thickness and mechanical properties

The thickness measurement of *NS*PC films which made by adding different concentrations of C-NPs containing MEO (Fig. 8 (B)) showed a significant increase in film thickness compared to the films that were prepared without MEO. These results were

like the previous work by Gohargani et al, that showed a significant increase in chitosan/whey protein film thickness when adding *zataria multiflora* essential oil incorporated with TiO_2 NPs [116]. The findings (Fig. 8 (B)) could be explained through the ability of essential oils blended with NPs to create empty spaces between the polymer chains that enable phase separation, which occurred because of the effect of the intermolecular bonding between the polymer's matrix as explained in Gohargani et al study and Asdagh and his team study on whey protein isolated with copper oxide nanoparticles containing incorporated with coconut essential oil and paprika extract [116, 117]. Siracusa and his team mentioned many factors that affect film thickness, starting with film preparation method, dish surface flatness, position in drying place or machine, and film formation during the drying process and kinetics [118].

Figure 8 (B)

The effect of different concentrations of C-NPs with 30% GLY and C-NPs containing 2% MEO in the presence of 10% GLY on NSPC film thickness. "a" Significantly different values as compared to the obtained film under the same experimental conditions in the absence of C-NPs. "b" Significantly different values as



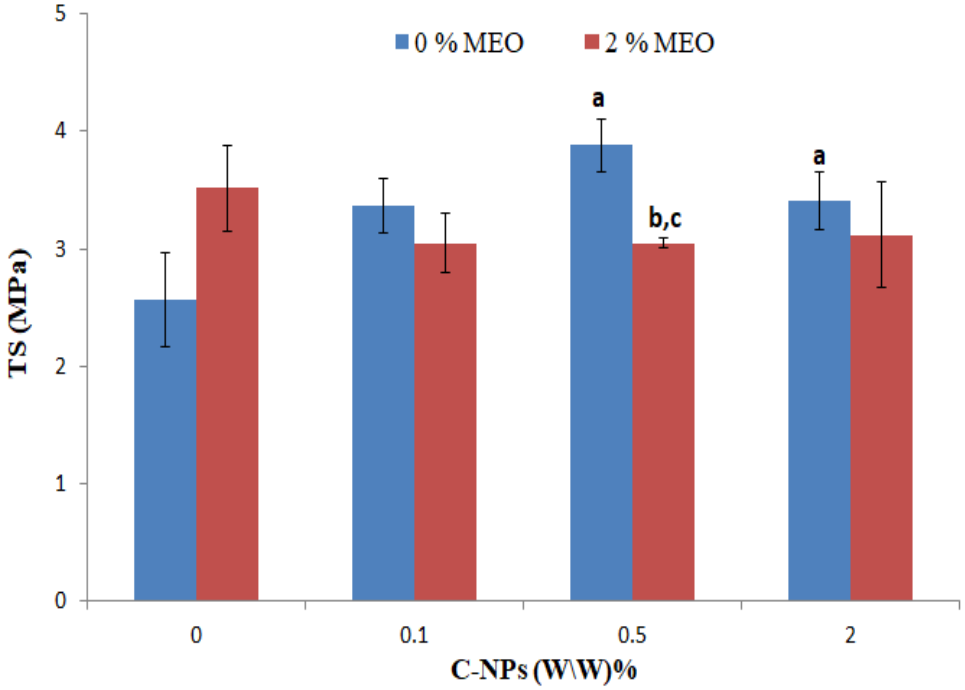
compared to the obtained film under the same experimental conditions at the same C-NPs concentration without MEO.

The statistical analyses showed no significant differences of the addition of different concentrations of C-NPs containing or without MEO on TS to *NS*PC films (Fig 8 (C)). However, the results clearly showed that adding MEO 2% to the *NS*PC without C-NPs

plasticized with 10% GLY had significantly differences higher TS compared to the *NSPC* films that were prepared with 30% GLY only.

Figure 8 (C)

The effect of different concentrations of C-NPs with 30% GLY and C-NPs containing 2% MEO in the presence of 10% GLY on NSPC film tensile strength (TS). *"a"* *Significantly different values as compared to the obtained film under the same experimental conditions in the absence of C-NPs without MEO.* *"b"* *Significantly different values as compared to the obtained film under the same experimental conditions in the absence of C-NPs with MEO.* *"c"* *Significantly different values as compared to the obtained film under the same experimental conditions at the same C-NPs concentration without MEO.*



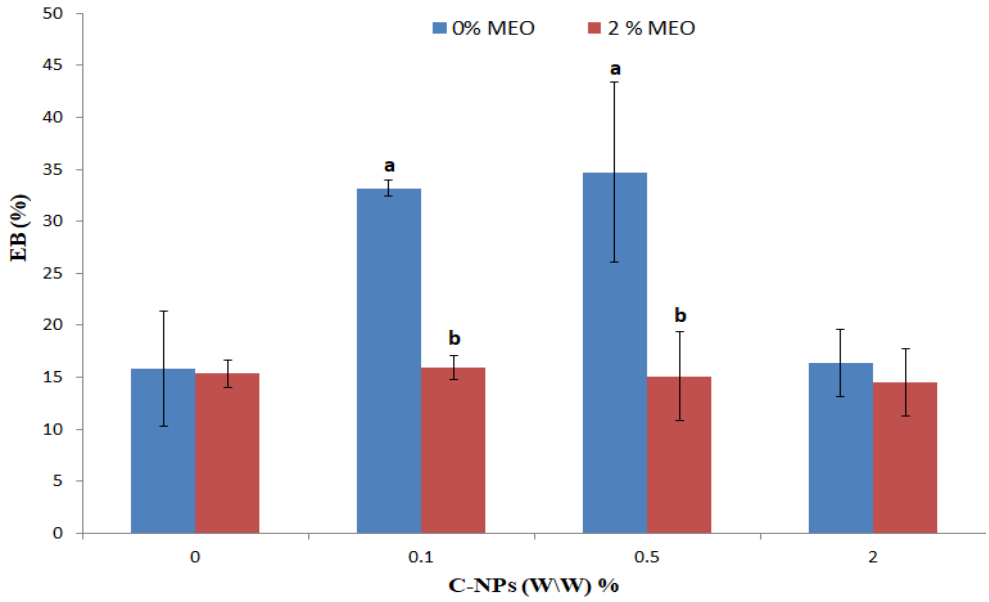
The obtained results (Fig. 8 (C)) are consistent with the previously published study by Frank et al, which concluded that when adding cinnamon essential oil nanoemulsions CEO-NAs to alginate edible films; the TS showed no significant effect compared to the control films [119]. In contrast, some studies had different results. For example, Kavoosi et al, study about the incorporation of *Zataria multiflora* essential oil into gelatin based films, the TS was significantly reduced. Kavoosi et al, related the result to the lowered interaction between gelatins filament, or may impair gelatin (chain-to-chain-interactions); as a result, the decrease of TS occurs [120]. Besides, the studies on thyme essential oil incorporated into chaitosan films by Esmaeili, and Ebrahimzadeh [121]. In

addition to Pelissari. et al, study on oregano essential oil effect on cassava starch film finding in TS is irrelevant for essential oil plasticizing capacity [122].

EB of *NS*PC films showed significant differences (P≤0.05) (Fig. 8 (D)) between films with MEO and films without MEO, all containing C-NPs at different concentrations. In contrast, there were no differences between films with MEO and different C-NPs concentrations together. The little reduction in EB (Fig. 8 (D)) from 15.8% to 15.35 for *NS* film + 0 C-NPs, 30% (w/w) glycerol (control films), to *NS* film 0 C-NPs, 10% glycerol, respectively. While the addition of MEO reduced the EB for *NS*PC films with C-NPs (Fig. 8 (D)). For example, in films with 0.1% C-NPs, the EB significantly reduced from 33.2% to 15.96% when MEO incorporates.

Figure 8 (D)

The effect of different concentrations of C-NPs with 30% GLY and C-NPs containing 2% MEO in the presence of 10% GLY on NSPC film elongation at break (EB). "a" Significantly different values as compared to the obtained film under the same experimental conditions in the absence of C-NPs without MEO. "b" Significantly different values as compared to the obtained films under the same experimental conditions at the same C-NPs concentration without MEO.



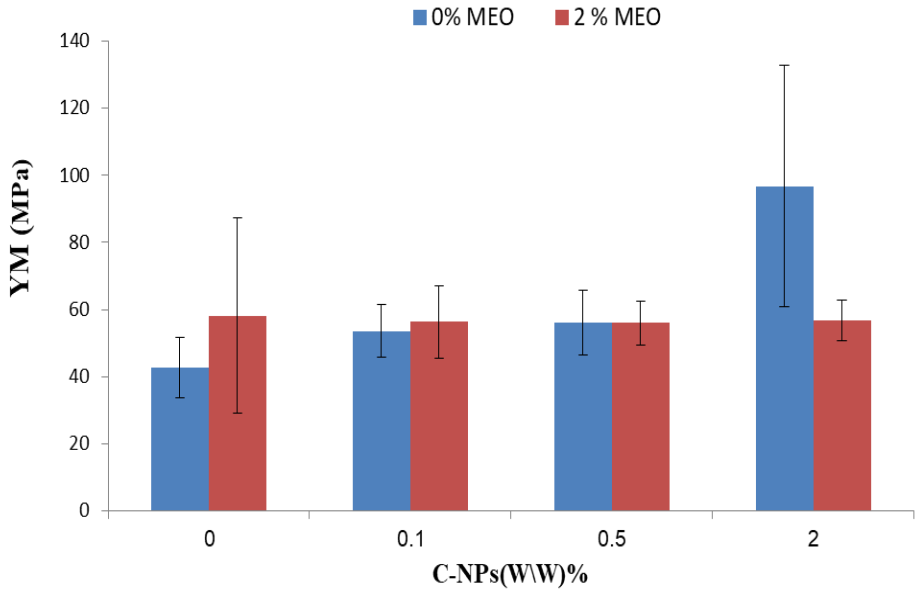
The reported EB results in (Fig. 8 (D)), showed the C-NPs incorporated with MEO do not affect the EB of *NS*PC films compared to the control films with MEO alone. The addition of C-NPs alone showed a significant increase in the *NS*PC films' EB below

0.5%. *NS*CP film, at higher concentrations of 2% C-NPs containing or not MEO there was no difference in EB. The result could be explained by the effect of using Tween 80 (surfactant) to allow the MEO to disperse into the water. Several works concluded that Tween 80 reduces films' EB as (Atef, Rezaei, and Behrooz) study, in addition to (Pranoto et al) study and (Strasakova et al) study, demonstrate tween 80 surfactants cause strong polymeric martial intermolecular bond, due to near distances between them, thou films' flexibility reduces [123, 124, 125]. Brandelero and his coauthors published study on the effects of surfactant effect on cassava starch-based films and poly (butylene adipate-co-terephthalate) (PBAT) blend films. Proved that the addition of Tween 80 could decrease the mechanical properties [126]. The reduction of EB (Fig. 8 (D)), is explained by Vahedikia, and his team to complex biopolymer structures generated by the increase of molecular bonds (intra-inter) between polymer side chains. Even though linear polymer structures have high EB, the rearrangement of protein side chains in polymer matrixes affects EB value [127]. Tween 80 has an notable effect on developing pores in film (cross-sectional area), which makes edible film matrix discontinuous, then reduces (inter-intramolecular) forces as proved in Mei and his team work on carboxy methyl cellulose films incorporated with Chinese fir essential oil [128].

The data showed no significant differences in YM between NS films produced without MEO or with MEO incorporated with C-NPs (Fig. 8 (E)).

Figure 8 (E)

The effect of different concentrations of C-NPs with 30% GLY and C-NPs containing 2% MEO in the presence of 10% GLY on NSPC film's Young's modulus (YM).



Several studies proved that the effect of essential oil addition could decrease YM based on its degree of plasticizing, but in our research, it preserved YM at the same value. The oil addition did not affect the rigidity of the film; such incorporation did not affect it. This may relate to the oil concentration and nature, which keep film rigidity. In a study mentioned above by Frank. et al, the addition of CEO-NEs at a low concentration increased the YM, but at a high concentration, it reduced [119]. Some studies as Costa et al and Valencia. et al, studies discussed the theory of a film's mechanical changes with essential oil addition, response to its molecular interaction with heterogeneous emulsion as the action of the film's-inner network [129,130].

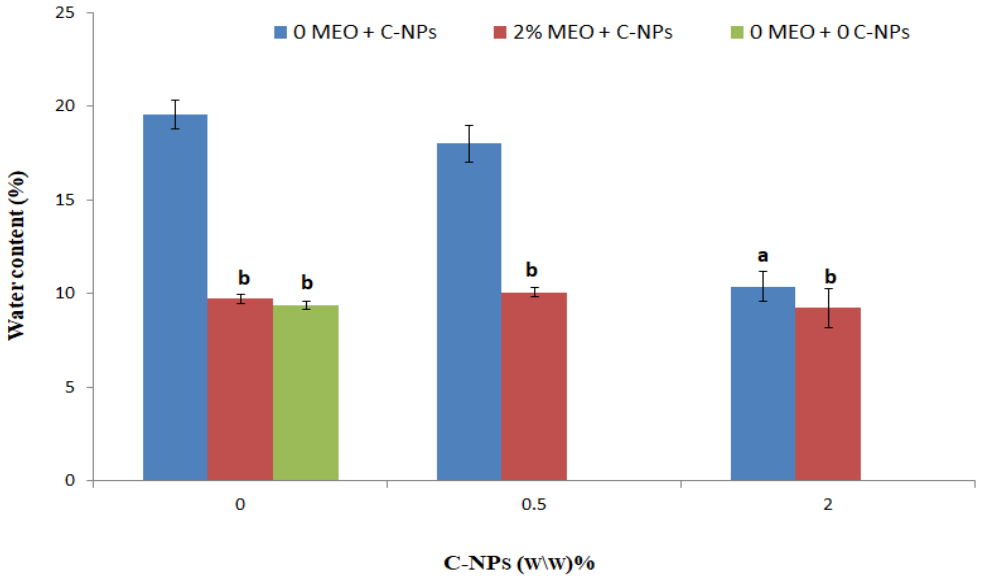
This behavior of oil can be related, or the effect of oil plasticizing, to one theory that explains the principle of plasticization as the researchers Mei, and his team explained. First, gel theory states that the plasticizer obstructs and exchanges polymer-polymer interactions that bond polymer chains in concert. Theory two is the free volume theory, which states that the availability of internal space in polymeric material enables chains of polymer movement. The lubricity theory is the third theory, which considers the plasticizer action as a lubricant to minimize friction and promote mobility of the polymer chains to pass through the matrix, hence reducing deformation [131].

3.6 The effect of C-NPs containing MEO in the presence of glycerol on *NS*PC film water content and uptake.

The results showed that by increasing the C-NPs, a considerable reduction in the water content of the *NS*PC film. Incorporating 2% MEO alone and 0.5% C-NPs containing 2% MEO in the presence of 10% GLY showed a significant reduction in film water content compared to the films without MEO with 30% GLY (Fig 9 (A)). The hydrophobic nature of MEO may explain these results, so minimize the interaction of H_2O molecules within polymers' materials. Our findings (Fig. 9 (A)) agree with Anis, and his coauthors study on tamarind kernel polysaccharide incorporated with geraniol oils [66]. A similar result in carboxy methylcellulose edible films with *Zataria multiflora* essential oils ZEO by Dashipour et al [132]. Contradicting our data, Bharti. et al, research result about, caraway essential oil with starch bio-based composite active film increased water content as CEO increased Bharti et al. [133].

Figure 9 (A)

The effect of different concentrations of C-NPs with 30% GLY and C-NPs containing 2% MEO in the presence of 10% GLY on NSPC film water content. "a" Significantly different values as compared to the obtained film under the same experimental conditions in the absence of C-NPs without MEO. "b" Significantly different values as compared to the obtained film under the same experimental conditions at the same C-NPs concentration without MEO.

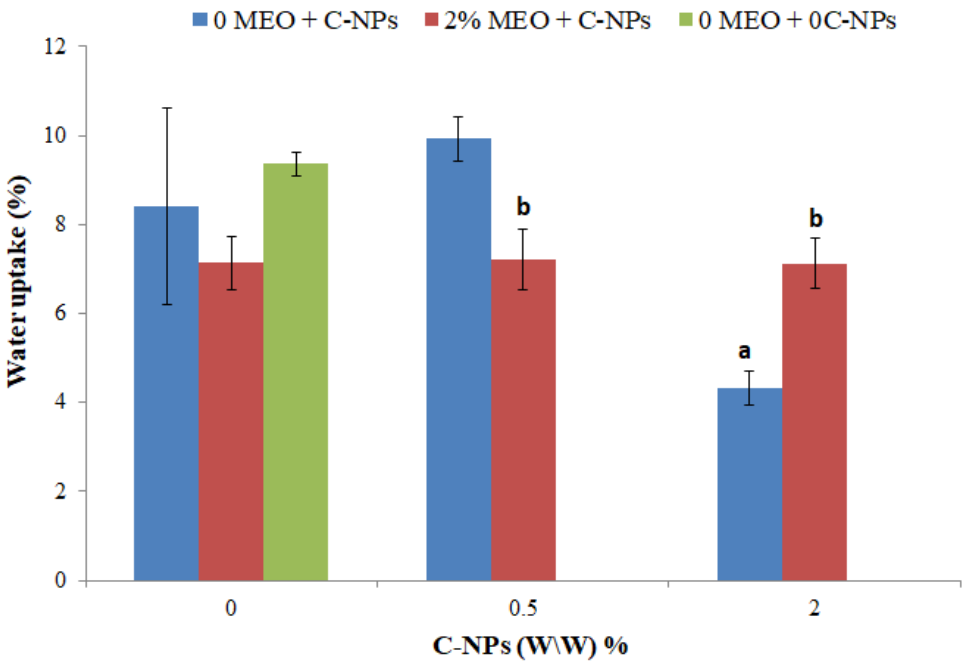


C-NPs showed a significant decrease in film water uptake at higher concentrations in the presence of 30% GLY (Fig.9 (B)). The increase of nanoparticles caused a decline in water uptake, which could be because of the nature and distribution of nanoparticles inside the film, as explained by Giosafatto et al, consequently, the addition of C-NPs could form a

tortuous pathway as MSNs [98]. However, incorporating the 0.5% C-NPs containing 2% MEO (Fig.9 (B)) significantly reduced the film water uptake compared to the film without MEO with the same C-NPs concentration. As Bharti et al, study on starch with the addition of caraway essential oil decreases such properties for edible films [133], but Salarbashi et al, research confirmed that's the addition of ZEO to soluble soybean polysaccharide edible films doesn't affect water uptake for concentrations less than 3%, in other words, the addition of 3% ZEO or more decreases the film water uptake [134].

Figure 9 (B)

The effect of different concentrations of C-NPs with 30% GLY and C-NPs containing 2% MEO in the presence of 10% GLY on NSPC film water uptake. "a" Significantly different values as compared to the obtained film under the same experimental conditions in the absence of C-NPs without MEO. "b" Significantly different values as compared to the obtained film under the same experimental conditions at the same C-NPs concentration without MEO.



3.7 Antioxidant activity of *NS*PC films reinforced with C-NPs containing MEO

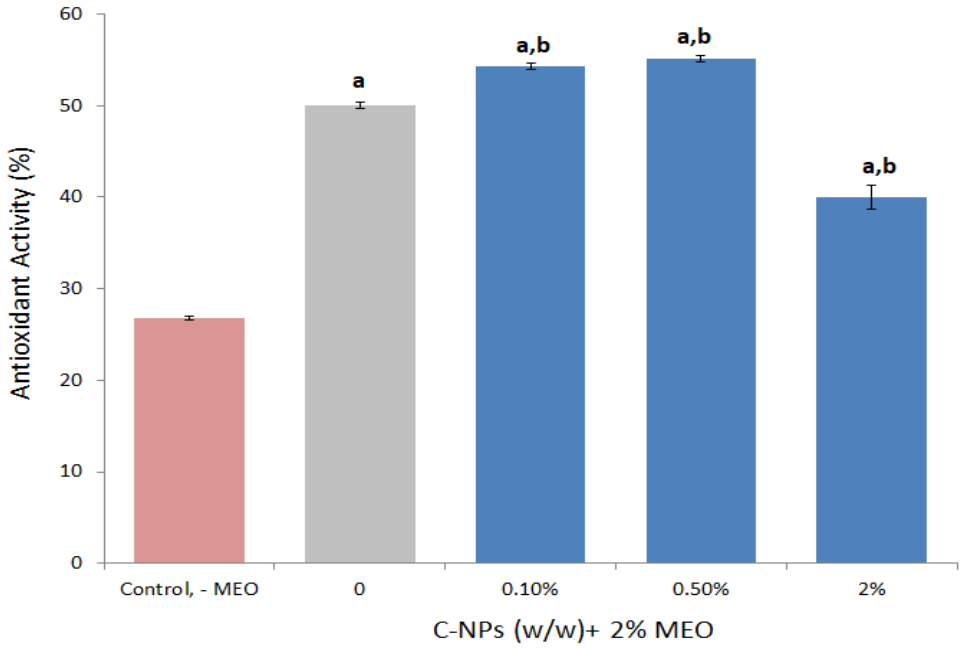
The results showed that the addition of 2% MEO to the *NS*PC films significantly increased the films' antioxidant activity. It even increased more when C-NPs 0.1 and 0.5% contained 2% MEO to the films (Fig 9 (C)). At higher concentrations of C-NPs containing 2% MEO, the antioxidant activity reduced compared to the 0, 0.1, and 0.5% of C-NPs, but was still higher when compared to the *NS*PC alone. The obtained results

agree with the previous study as Dashipour et al, that concluded that blends of carboxymethyl cellulose films containing *Zataria multiflora* essential oil could increase the antioxidant activity [132]. The obtained results (Fig. 9 (C)) may relates the antioxidant activity of MEO to its composition since it is rich in tocopherols and alpha tocopherols [69]. Besides oil activity, *NS* also is naturally have an antioxidant-active compound as proved in Nergiz and Ötleş research [99]. The presented results (Fig. 9 (C)) agree with other studies on the effect of bioactive compounds as essential oil addition on edible film antioxidant activity. The researchers Al-Hashimi et al, study on millets starch film with the clove essential oil addition, increased antioxidant activity. As soon as the concentration of essential oils increased, the antioxidant activity increased too [135]. In addition to agreement studies, Tongnuanchan, Benjakul, and Prodpran, study by addition of lemon and bergamot essential oils to gelatin fish edible films. It also gave positive effects on antioxidant activity, with bergamot being more active than lemon [84].

Figure 9 (C)

The effect of different concentrations of C-NPs with 2% MEO in the presence of 10% GLY on the NSPC films water uptake. Film obtained without C-NPs or MEO in the presence of 30% GLY was the control. "a" Significantly different values as compared to those obtained under the same

experimental conditions in the absence of C-NPC or MEO. Moreover, "b" Significantly different values as compared to those obtained under the same experimental conditions in the absence of C-NPC and in the presence of 2% MEO.



3.8 antimicrobial activity of *N*SPC films reinforced with C-NPs containing MEO

The antimicrobial activity of MEO was evaluated alone for both *Staphylococcus arouse* and *E.coli.* We report the results in (Fig. 10 (A)). The results confirmed that MEO at 10 µL and 20 µL has an effective antimicrobial activity for *Staphylococcus arouse* alone. Moreover, films that were reinforced with different concentrations of C-NPs containing MEO were evaluated, and the obtained results, are presented in (Fig. 10 (B)). The results showed *N*SPC films reinforced with C-NPs having MEO cause inhibition in *Staphylococcus arouse*s more than e *E.coli* bacteria. Considerably *staph* is gram-positive bacteria, instead, *E.coli* gram-negative bacteria, so bioactive compounds found in the oils can easily migrate into the bacterial cell wall of gram-positive species, and can also inhibit the bacterial growth activity mentioned in [136] such as:

- 4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate.
- Methyl N-4-(α-L-rhamnopyranosyloxy) benzyl carbamate.
- 4-(β-D-glucopyranosyl-1→4-α-L-rhamnopyranosyloxy)-benzylthiocarboxamide

Besides the action of bioactive compounds, C-NPs could work as a stabilizer for essential oils, as proved in E. Sogut., study on pomegranate essential oils incorporated into C-NPs in whey protein edible films [137]. Our findings agree with the Strasakova et al, study on alginate-based films incorporated with garlic essential oil, against gram-positive *Staphylococcus aureus* and a study by Al-Hashimi et al, on a millet starch edible film containing clove essential oil showed effect against.*B.* cereus, and a weak effect against *E.coli* [125, 135], and agree with Pelissari et al, study on oregano essential oil with cassava-starch-chitosan films [122].

Figure 10 (A)

The effect of different concentrations of MEO alone on Staphylococcus aureus and Escherichia coli. MEO was in the cotton disc.

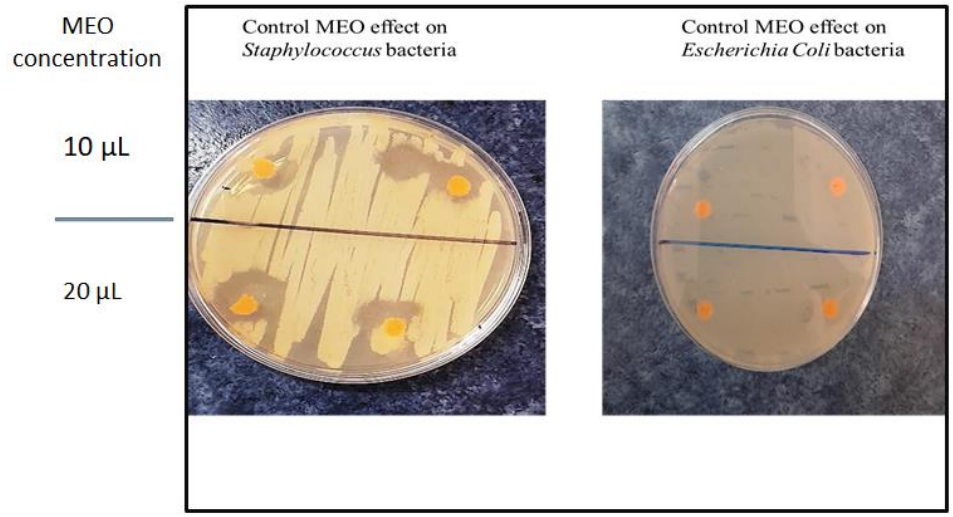
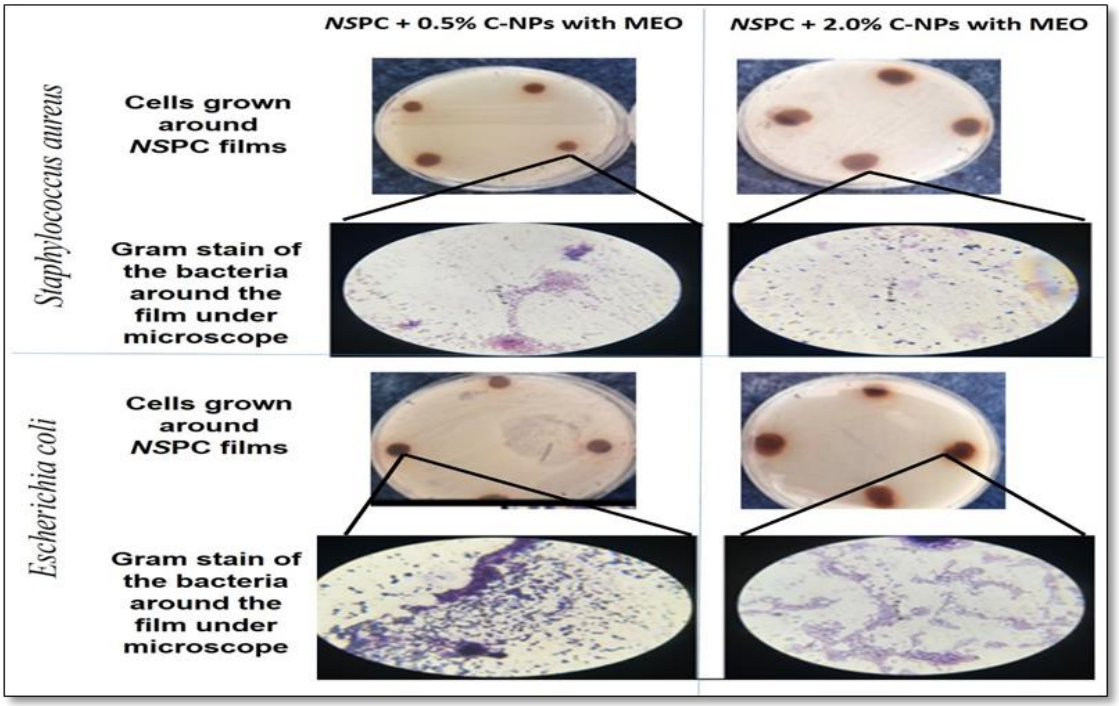


Figure 10 (B)

The effect of different concentrations of C-NPs containing 2% MEO in reinforcing the NSPC films against Staphylococcus aureus and Escherichia coli.



3.9 Film applications

The produced films were assessed for their ability to make small packaging containers to maintain olive oil because of being more opaque and impermeable to light (Fig. 10 (C)). That could decrease the olive oil oxidation and protect the quality of fresh oil with thymoquinone as a constituent of *NS* plants, which works as an antioxidant agent as confirmed by Mariod study [138], in addition to Athikomkulchai, research proved MEO had constituents which improve additional antioxidant action such as α-tocopherol [139].

Another application for the created film is to wrap, vegetable, or butter rather than utilizing plastic materials, which are used these days which used these days. During the primary experiment, after (5) weeks of wrapping, the butter surface color changed to a little brown color because of the migration of some natural black pigment.

Figure 10 (C)

Potential applications of NSPC reinforced with C-NPs containing MEO for olive oil packaging and butter wrapping.

*NS*PC + C-NPs with MEO for olive oil packaging

*NS*PC + C-NPs with MEO for butter wrapping

Chapter Four

Conclusions and Recommendations

4.1 Conclusions

Using food industry waste byproducts as a resource for developing and improving biodegradable food packaging materials for edible films has various benefits. Along with its cheap cost, the production of protein-concentrated materials and cellulose nanoparticles from industrial byproducts, used for developing *NS* formed edible films. The improvement of edible films can be achieved by strengthen of mechanical properties and increase film functionality as antimicrobial and antioxidant activity.

The addition of C-NPs improved the films' mechanical parameters. However, these improvements differ as C-NPs concentration from 1 to 3% (w\w). The reduction in EB occurred at concentrations from 1 to 3% C-NPs, while YM improvement occurred at the same concentrations of NPs. The low concentrations, from 0.1 to 0.5% of C-NPs, had regular rhythms of mechanical behaviors. They proved TS and EB in several studies to be adverse, but this adversity was not notable except at elevated concentrations of C-NPs. Results of water content and water uptake reduced as C-NPs concentration increased. The film incorporation of Moringa oils did not have considerable effects on TS, YM for all concentrations of C-NPs except (0,5%). It preserved the C-NPs fillers effect but decreased EB. The weak plasticizing effect of MEO may explain this result: which does not compensate for the reduction of glycerol from 30 to 10% (w\w). The water content of films with Moringa oil incorporation also reduced the water content, while did not have considerable effects on water uptake. This result was releated to the hydrophobic nature of oils. Regarding antioxidant and antimicrobial activities, the film improvement succeeded as the effect of Moringa oil addition. But it proved its action as an antimicrobial against gram-positive bacteria, which may accord with its cell wall composition.

The film capability for sealing was poor because of lipid components on the surface of *NS* films. So it necessary to adjust to suitable temperatures for sealing; however, wrapping was successful for butter slices, but the leakage of *NS* dark color pigment may affect the palatability of such product.

4.2 Recommendations and future trends

The *NS* concentrated protein may need bleaching steps. To be overcome the film pigment's leakage to the wrapped food items also may reduce the bitter taste of the *NS* films. The oil encapsulation may preserve and increase the oil activity and cause

additional activities. It also protects the oil quality. C-NPs desperation plays a role in its effect as a filler material, choosing optimum concentrations with a fewer agglomeration of nanoparticles. The application methods of such edible films include using them as packaging martial depending on the food items and the way of application. It is critical to protect the film under conditions that preserve its moisture content and structural appearance. The Easily losing water content makes them applicable for being used as secondary packaging martial or in refrigerator containers for freezing food items**.**

List of Abbreviations

Abbreviation	Meaning
ABTS	2,2ˉ-Azino-Bis (3-Ethylbenzo Thiazoline-6-Sulfonic Acid)
APBNF	Active Plasticized Banana Nanocomposite Film

CMC	Carboxy Methyl Cellulose
CS	Chitosan
CNFs	Cellulose Nano fiber
DPPH	2,2-Diphenyl-1-Picrylhydrazy
EB	Elongation At break
EOs	Essential Oils
FDA	Food And Drugs Administration
FFSs	Film Forming Solutions
FRAP	Fluorescence Recovery After Photobleaching
FWSP	Fish Water Soluble Protein
GSE	Grab Seed Extract
GRADS	Generally Recognized As Safe
HPMC	Hydroxy Propyl Methylcellulose
LCA	Life Cycle Assessments
LIM	Limonene
MEO	*Moringa* Edible Oil
MSNs	Mesoporous Silica Nanoparticles
µm	Micro Miters
M.O	Microorganism
NS	*Nigella Sativa*
*NS*DSC	*Nigella Sativa* Defatted Seed Cake
*NS*PC	*Nigella Sativa* Protein Concentrate
*NS*PCE	*Nigella Sativa* Protein Concentrated Extract
OR	Oregano Extract
PE	Polyethylene
PEG	Poly Ethylene Glycol
PLA	Poly Lactic Acid
PM	Peppermints
PV	Peroxide Value
ROS	Reactive Oxygen Species
SC	Sodium Caseinate
SEO	*Saturaja Hotness* Essential Oil
SS	Salep Solution
TS	Tensile Strength
WPI	Whey Protein Isolation
WVP	Water Vapor Permeability
YM	Young's Modulus

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Appendices

Appendix A: Different supporting figures for literature reviews.

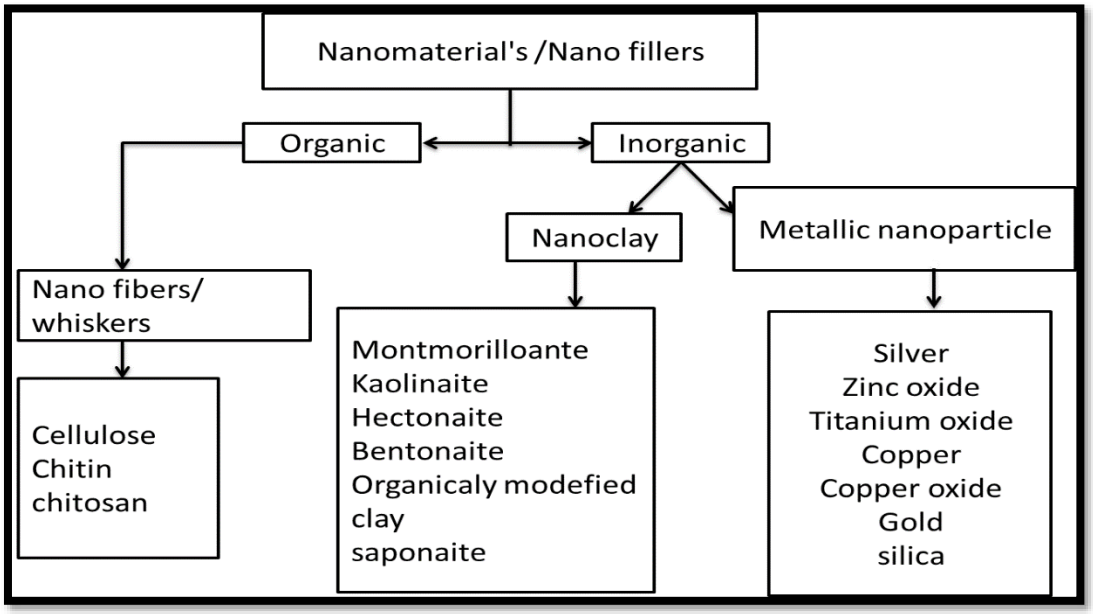


Figure 4 (B). Part of the nanofillers that can be used for the preparation of nanocomposite films [11].

Figure 5 (C). Moringa plant seeds and leaves

Appendix B: Statistical Analyses For Mechanical Properteis, Water Contentm Water Uptake, And Antiocxedant Activity

Least Squares Fit

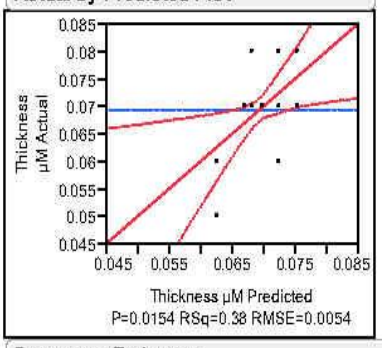
Singularity Details

MOE[0.00] = - GLY[10]
NP %[0.00] = - MOE[0.00]*NP %[0.00]*GLY[10]
NP %[0.10] = - MOE[0.00]*NP %[0.10]*GLY[10]
NP %[0.50] = - MOE[0.00]*NP %[0.50]*GLY[10]

Response Thickness µM

Whole Model

Actual by Predicted Plot



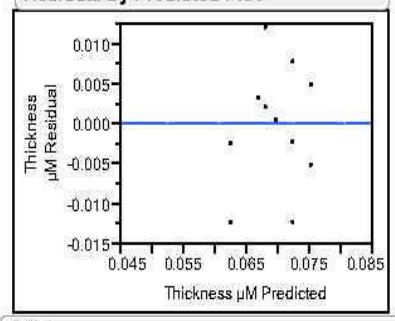
Parameter Estimates

Term		Estimate	Std Error	t Ratio	Prob>|t|
Intercept		0.0692295	0.001008	68.70	<.0001*
MOE[0.00]	Biased	-0.002769	0.001002	-2.76	0.0106*
NP %[0.00]	Biased	0.003232	0.001763	1.83	0.0787
NP %[0.10]	Biased	0.0003748	0.001763	0.21	0.8334
NP %[0.50]	Biased	0.0003748	0.001763	0.21	0.8334
GLY[10]	Zeroed	0	0	.	.
MOE[0.00]*NP %[0.00]*GLY[10]	Zeroed	0	0	.	.
MOE[0.00]*NP %[0.10]*GLY[10]	Zeroed	0	0	.	.
MOE[0.00]*NP %[0.50]*GLY[10]	Zeroed	0	0	.	.

Effect Tests

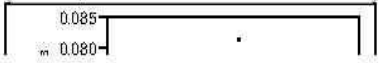
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F	
MOE	1	0	1.0842e-19	.	.	LostDFs
NP %	3	0	-4.235e-22	.	.	LostDFs
GLY	1	0	0	.	.	LostDFs
MOE*NP %*GLY	3	0	0	.	.	LostDFs

Residual by Predicted Plot



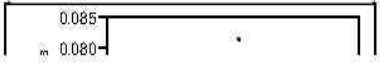
MOE

Leverage Plot



NP %

Leverage Plot

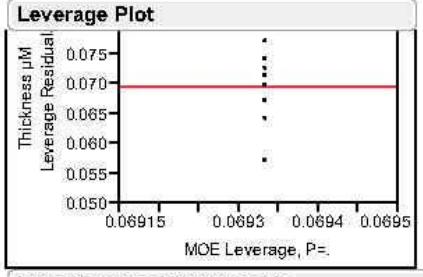


Least Squares Fit

Response Thickness µM

MOE

Leverage Plot



Least Squares Means Table

Level	Least Sq Mean		Std Error	Mean
0.00	.	NonEstimable	.	0.066154
2.00	.	NonEstimable	.	0.071765

LSMeans Differences Student's t

α= 0.050 t= 2.05954

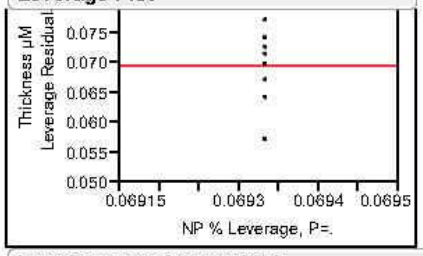
Mean[i]-Mean[j] / Std Err Dif / Lower CL Dif / Upper CL Dif	LSMean[j] 0.00	LSMean[j] 2.00
LSMean[i] 0.00	0 0 0 0	. 0.002 . .
LSMean[i] 2.00	. 0.002 . .	0 0 0 0

Level		Least Sq Mean
0.00	A	.
2.00	A	.

Levels not connected by same letter are significantly different.

NP %

Leverage Plot



Least Squares Means Table

Level	Least Sq Mean		Std Error	Mean
0.00	.	NonEstimable	.	0.072857
0.10	.	NonEstimable	.	0.070000
0.50	.	NonEstimable	.	0.070000
2.00	.	NonEstimable	.	0.065556

LSMeans Differences Student's t

α= 0.050 t= 2.05954

Mean[i]-Mean[j] / Std Err Dif / Lower CL Dif / Upper CL Dif	LSMean[j] 0.00	LSMean[j] 0.10	LSMean[j] 0.50	LSMean[j] 2.00
LSMean[i] 0.00	0 0 0 0	. 0.00291 . .	. 0.00291 . .	. 0.00274 . .
LSMean[i] 0.10	. 0.00291 . .	0 0 0 0	. 0.00291 . .	. 0.00274 . .
LSMean[i] 0.50	. 0.00291 . .	. 0.00291 . .	0 0 0 0	. 0.00274 . .
LSMean[i] 2.00	. 0.00274 . .	. 0.00274 . .	. 0.00274 . .	0 0 0 0

Level		Least Sq Mean
0.00	A	.
0.10	A	.
0.50	A	.
2.00	A	.

Levels not connected by same letter are significantly different.

GLY

Leverage Plot

Least Squares Fit

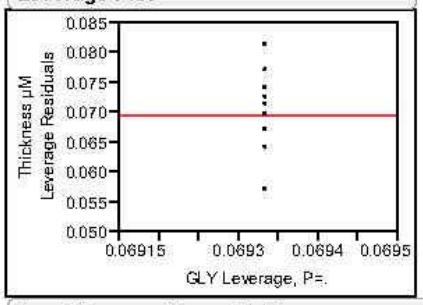
Response Thickness µM

NP %

LSMeans Differences Student's t

GLY

Leverage Plot



Least Squares Means Table

Level	Least Sq Mean		Std Error	Mean
10	.	NonEstimable	.	0.071765
30	.	NonEstimable	.	0.066154

LSMeans Differences Student's t

α= 0.050 t= 2.05954

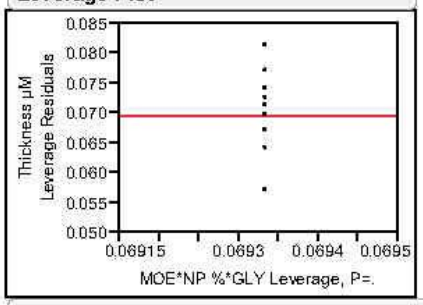
Mean[i]-Mean[j] / Std Err Dif / Lower CL Dif / Upper CL Dif	LSMean[j] 10	LSMean[j] 30
LSMean[i] 10	0 / 0 / 0 / 0	. / . / . / .
LSMean[i] 30	. / . / . / .	0 / 0 / 0 / 0

Level		Least Sq Mean
10	A	.
30	A	.

Levels not connected by same letter are significantly different.

MOE*NP %*GLY

Leverage Plot



LSMeans Differences Student's t

Least Squares Fit

Response Thickness µM

MOE*NP %*GLY

Leverage Plot

MOE*NP %*GLY Leverage, P=.

LSMeans Differences Student's t

α= 0.050 t= 2.05954

LSMean[j]

LSMean[i] Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	0.00,0.00,10	0.00,0.00,30	0.00,0.10,10	0.00,0.10,30	0.00,0.50,10	0.00,0.50,30	0.00,2.00,10	0.00,2.00,30	2.00,0.00,10
0.00,0.00,10	0 0 0 0	. 0 .	. 0.00291 .	. 0.00291 .	. 0.00291 .	. 0.00291 .	. 0.00274 .	. 0.00274 .	. 0.002 .
0.00,0.00,30	. 0 .	0 0 0 0	. 0.00291 .	0.00286 0.00291 -0.0031 0.00884	. 0.00291 .	0.00286 0.00291 -0.0031 0.00884	. 0.00274 .	0.00721 0.00274 0.00157 0.01286	-0.0055 0.002 -0.0097 -0.0014
0.00,0.10,10	. 0.00291 .	. 0.00291 .	0 0 0 0	. 0 .	. 0.00291 .	. 0.00291 .	. 0.00274 .	. 0.00274 .	. 0.00353 .
0.00,0.10,30	. 0.00291 .	-0.0029 0.00291 -0.0088 0.00313	. 0 .	0 0 0 0	. 0.00291 .	0 0.00291 -0.006 0.00599	. 0.00274 .	0.00436 0.00274 -0.0013 0.01	-0.0084 0.00353 -0.0157 -0.0011
0.00,0.50,10	. 0.00291 .	. 0.00291 .	. 0.00291 .	. 0.00291 .	0 0 0 0	. 0 .	. 0.00274 .	. 0.00274 .	. 0.00353 .
0.00,0.50,30	. 0.00291 .	-0.0029 0.00291 -0.0088 0.00313	. 0.00291 .	0 0.00291 -0.006 0.00599	. 0 .	0 0 0 0	. 0.00274 .	0.00436 0.00274 -0.0013 0.01	-0.0084 0.00353 -0.0157 -0.0011
0.00,2.00,10	. 0.00274 .	. 0.00274 .	. 0.00274 .	. 0.00274 .	. 0.00274 .	. 0.00274 .	0 0 0 0	. 0 .	. 0.00338 .
0.00,2.00,30	. 0.00274 .	-0.0072 0.00274 -0.0129 -0.0016	. 0.00274 .	-0.0044 0.00274 -0.01 0.00129	. 0.00274 .	-0.0044 0.00274 -0.01 0.00129	. 0 .	0 0 0 0	-0.0128 0.00338 -0.0197 -0.0058
2.00,0.00,10	. 0.002 .	0.00554 0.002 0.00141 0.00967	. 0.00353 .	0.0084 0.00353 0.00113 0.01567	. 0.00353 .	0.0084 0.00353 0.00113 0.01567	. 0.00338 .	0.01275 0.00338 0.0058 0.01971	0 0 0 0
2.00,0.00,30	. 0.002 .	. 0.002 .	. 0.00353 .	. 0.00353 .	. 0.00353 .	. 0.00353 .	. 0.00338 .	. 0.00338 .	. 0 .
2.00,0.10,10	. 0.00353 .	0.00268 0.00353 -0.0046 0.00995	. 0.002 .	0.00554 0.002 0.00141 0.00967	. 0.00353 .	0.00554 0.00353 -0.0017 0.01281	. 0.00338 .	0.0099 0.00338 0.00294 0.01685	-0.0029 0.00291 -0.0088 0.00313
2.00,0.10,30	. 0.00353 .	. 0.00353 .	. 0.002 .	. 0.002 .	. 0.00353 .	. 0.00353 .	. 0.00338 .	. 0.00338 .	. 0.00291 .
2.00,0.50,10	. 0.00353 .	0.00268 0.00353 -0.0046 0.00995	. 0.00353 .	0.00554 0.00353 -0.0017 0.01281	. 0.002 .	0.00554 0.002 0.00141 0.00967	. 0.00338 .	0.0099 0.00338 0.00294 0.01685	-0.0029 0.00291 -0.0088 0.00313
2.00,0.50,30									

2.00,0.00,30	2.00,0.10,10	2.00,0.10,30	2.00,0.50,10	2.00,0.50,30	2.00,2.00,10	2.00,2.00,30
0.002	0.00353	0.00353	0.00353	0.00353	0.00341	0.00341
0.002	-0.0027 0.00353 -0.01 0.00459	0.00353	-0.0027 0.00353 -0.01 0.00459	0.00353	0.00167 0.00341 -0.0054 0.0087	0.00341
0.00353	0.002	0.002	0.00353	0.00353	0.00341	0.00341
0.00353	-0.0055 0.002 -0.0097 -0.0014	0.002	-0.0055 0.00353 -0.0128 0.00173	0.00353	-0.0012 0.00341 -0.0082 0.00585	0.00341
0.00353	0.00353	0.00353	0.002	0.002	0.00341	0.00341
0.00353	-0.0055 0.00353 -0.0128 0.00173	0.00353	-0.0055 0.002 -0.0097 -0.0014	0.002	-0.0012 0.00341 -0.0082 0.00585	0.00341
0.00338	0.00338	0.00338	0.00338	0.00338	0.002	0.002
0.00338	-0.0099 0.00338 -0.0168 -0.0029	0.00338	-0.0099 0.00338 -0.0168 -0.0029	0.00338	-0.0055 0.002 -0.0097 -0.0014	0.002
0	0.00286 0.00291 -0.0031 0.00884	0.00291	0.00286 0.00291 -0.0031 0.00884	0.00291	0.00721 0.00274 0.00157 0.01286	0.00274
0 0 0 0	0.00291	0.00291	0.00291	0.00291	0.00274	0.00274
0.00291	0 0 0 0	0	0 0.00291 -0.006 0.00599	0.00291	0.00436 0.00274 -0.0013 0.01	0.00274
0.00291	0	0 0 0 0	0.00291	0.00291	0.00274	0.00274
0.00291	0 0.00291 -0.006 0.00599	0.00291	0 0 0 0	0	0.00436 0.00274 -0.0013 0.01	0.00274

Least Squares Fit

Response Thickness µM

MOE*NP %*GLY

LSMeans Differences Student's t

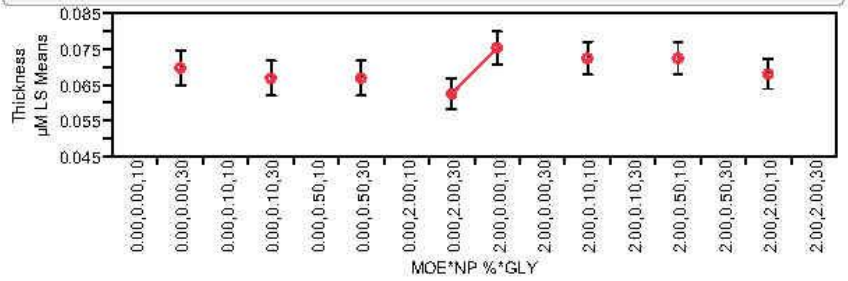
LSMean[j]

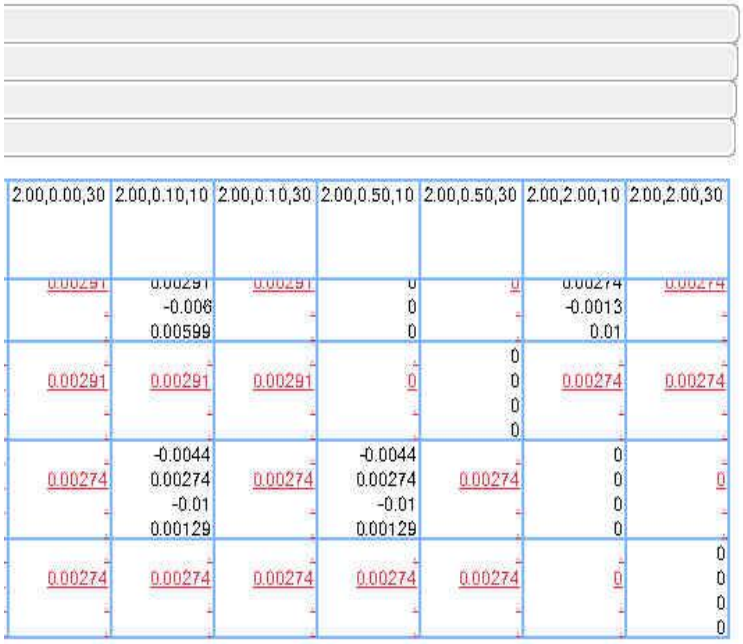
Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	0.00,0.00,10	0.00,0.00,30	0.00,0.10,10	0.00,0.10,30	0.00,0.50,10	0.00,0.50,30	0.00,2.00,10	0.00,2.00,30	2.00,0.00,10
	0.00353	0.00353 -0.0046 0.00995	0.00353	0.00353 -0.0017 0.01281	0.002	0.002 0.00141 0.00967	0.00338	0.00338 0.00294 0.01685	0.00291 -0.0088 0.00313
2.00,0.50,30	0.00353	0.00353	0.00353	0.00353	0.002	0.002	0.00338	0.00338	0.00291
2.00,2.00,10	0.00341	-0.0017 0.00341 -0.0087 0.00536	0.00341	0.00118 0.00341 -0.0058 0.00821	0.00341	0.00118 0.00341 -0.0058 0.00821	0.002	0.00554 0.002 0.00141 0.00967	-0.0072 0.00274 -0.0129 -0.0018
2.00,2.00,30	0.00341	0.00341	0.00341	0.00341	0.00341	0.00341	0.002	0.002	0.00274

Level						Least Sq Mean
0.00,0.00,10	A	B	C	D	E	
0.00,0.00,30	A	B		D		0.06969212
0.00,0.10,10	A	B	C	D	E	
0.00,0.10,30	A		C	D		0.06683498
0.00,0.50,10	A	B	C	D	E	
0.00,0.50,30	A	B	C			0.06683498
0.00,2.00,10	A	B	C	D	E	
0.00,2.00,30			C			0.06247845
2.00,0.00,10					E	0.07523091
2.00,0.00,30	A	B	C	D	E	
2.00,0.10,10		B			E	0.07237377
2.00,0.10,30	A	B	C	D	E	
2.00,0.50,10				D	E	0.07237377
2.00,0.50,30	A	B	C	D	E	
2.00,2.00,10	A	B		D		0.06801724
2.00,2.00,30	A	B	C	D	E	

Levels not connected by same letter are significantly different.

LS Means Plot





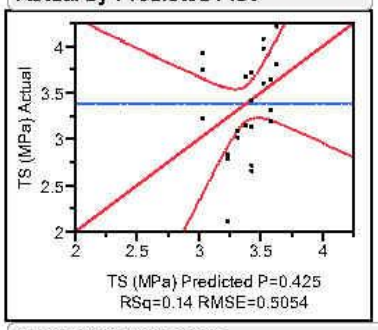
2.00,0.00,30	2.00,0.10,10	2.00,0.10,30	2.00,0.50,10	2.00,0.50,30	2.00,2.00,10	2.00,2.00,30
0.00291	0.00291 -0.006 0.00599	0.00291	0 0 0	0	0.00274 -0.0013 0.01	0.00274
0.00291	0.00291	0.00291	0	0 0 0 0	0.00274	0.00274
0.00274	-0.0044 0.00274 -0.01 0.00129	0.00274	-0.0044 0.00274 -0.01 0.00129	0.00274	0 0 0 0	0
0.00274	0.00274	0.00274	0.00274	0.00274	0	0 0 0 0

Least Squares Fit

Response TS (MPa)

Whole Model

Actual by Predicted Plot



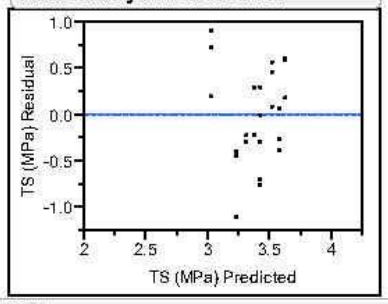
Parameter Estimates

Term		Estimate	Std Error	t Ratio	Prob>|t|
Intercept		3.386972	0.093676	36.16	<.0001*
MOE[0.00]	Biased	0.1022629	0.093123	1.10	0.2826
NP %[0.00]	Biased	-0.259506	0.163909	-1.58	0.1259
NP %[0.10]	Biased	0.0904942	0.163909	0.55	0.5858
NP %[0.50]	Biased	0.0290656	0.163909	0.18	0.8607
GLY[10]	Zeroed	0	0	.	.
MOE[0.00]*NP %[0.00]*GLY[10]	Zeroed	0	0	.	.
MOE[0.00]*NP %[0.10]*GLY[10]	Zeroed	0	0	.	.
MOE[0.00]*NP %[0.50]*GLY[10]	Zeroed	0	0	.	.

Effect Tests

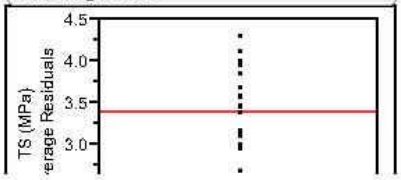
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F	
MOE	1	0	5.5511e-17	.	.	LostDFs
NP %	3	0	-1.735e-18	.	.	LostDFs
GLY	1	0	0	.	.	LostDFs
MOE*NP %*GLY	3	0	0	.	.	LostDFs

Residual by Predicted Plot



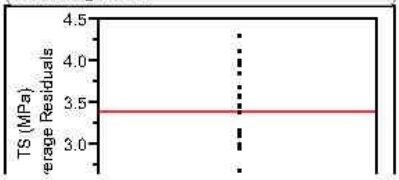
MOE

Leverage Plot



NP %

Leverage Plot



Least Squares Fit

Response TS (MPa)

MOE*NP %*GLY

Leverage Plot

LSMeans Differences Tukey HSD

α= 0.050 Q= 3.78936

LSMean[j]

LSMean[i]

Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	0.00,0.00,10	0.00,0.00,30	0.00,0.10,10	0.00,0.10,30	0.00,0.50,10	0.00,0.50,30	0.00,2.00,10	0.00,2.00,30	2.00,0.00,10
0.00,0.00,10	0 0 0 0	0	0.27017	0.27017	0.27017	0.27017	0.25474	0.25474	0.18625
0.00,0.00,30	0	0 0 0 0	0.27017	-0.35 0.27017 -1.3738 0.67378	0.27017	-0.2886 0.27017 -1.3124 0.73521	0.25474	-0.3995 0.25474 -1.3647 0.56584	0.20453 0.18625 -0.5012 0.91028
0.00,0.10,10	0.27017	0.27017	0 0 0 0	0	0.27017	0.27017	0.25474	0.25474	0.32815
0.00,0.10,30	0.27017	0.35 0.27017 -0.6738 1.37378	0	0 0 0 0	0.27017	0.06143 0.27017 -0.9624 1.08521	0.25474	-0.0495 0.25474 -1.0147 0.91584	0.55453 0.32815 -0.6889 1.798
0.00,0.50,10	0.27017	0.27017	0.27017	0.27017	0 0 0 0	0	0.25474	0.25474	0.32815
0.00,0.50,30	0.27017	0.28857 0.27017 -0.7352 1.31235	0.27017	-0.0614 0.27017 -1.0852 0.96235	0	0 0 0 0	0.25474	-0.1109 0.25474 -1.0762 0.85442	0.4931 0.32815 -0.7504 1.73657
0.00,2.00,10	0.25474	0.25474	0.25474	0.25474	0.25474	0.25474	0 0 0 0	0	0.31381
0.00,2.00,30	0.25474	0.39945 0.25474 -0.5658 1.36475	0.25474	0.04945 0.25474 -0.9158 1.01475	0.25474	0.11088 0.25474 -0.8544 1.07618	0	0 0 0 0	0.60398 0.31381 -0.5852 1.79313
2.00,0.00,10	0.18625	-0.2045 0.18625 -0.9103 0.50123	0.32815	-0.5545 0.32815 -1.798 0.68894	0.32815	-0.4931 0.32815 -1.7366 0.75037	0.31381	-0.604 0.31381 -1.7931 0.58517	0 0 0 0
2.00,0.00,30	0.18625	0.18625	0.32815	0.32815	0.32815	0.32815	0.31381	0.31381	0
2.00,0.10,10	0.32815	0.14547 0.32815 -1.098 1.38894	0.18625	-0.2045 0.18625 -0.9103 0.50123	0.32815	-0.1431 0.32815 -1.3866 1.10037	0.31381	-0.254 0.31381 -1.4431 0.93517	0.35 0.27017 -0.6738 1.37378
2.00,0.10,30	0.32815	0.32815	0.18625	0.18625	0.32815	0.32815	0.31381	0.31381	0.27017
2.00,0.50,10	0.32815	0.08405 0.32815 -1.1594 1.32752	0.32815	-0.266 0.32815 -1.5094 0.97752	0.18625	-0.2045 0.18625 -0.9103 0.50123	0.31381	-0.3154 0.31381 -1.5046 0.87374	0.28857 0.27017 -0.7352 1.31235
2.00,0.50,30	0.32815	0.32815	0.32815	0.32815	0.18625	0.18625	0.31381	0.31381	0.27017

Least Squares Fit

Response TS (MPa)

MOE*NP %*GLY

LSMeans Differences Tukey HSD

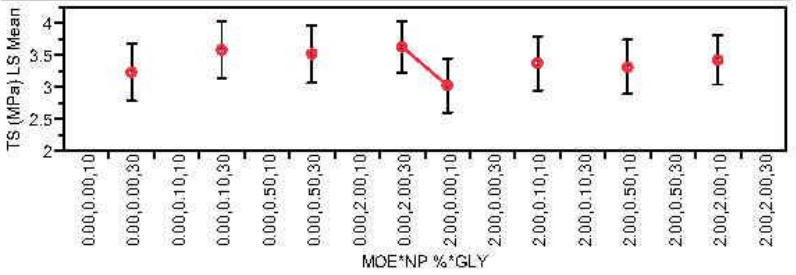
LSMean[j]

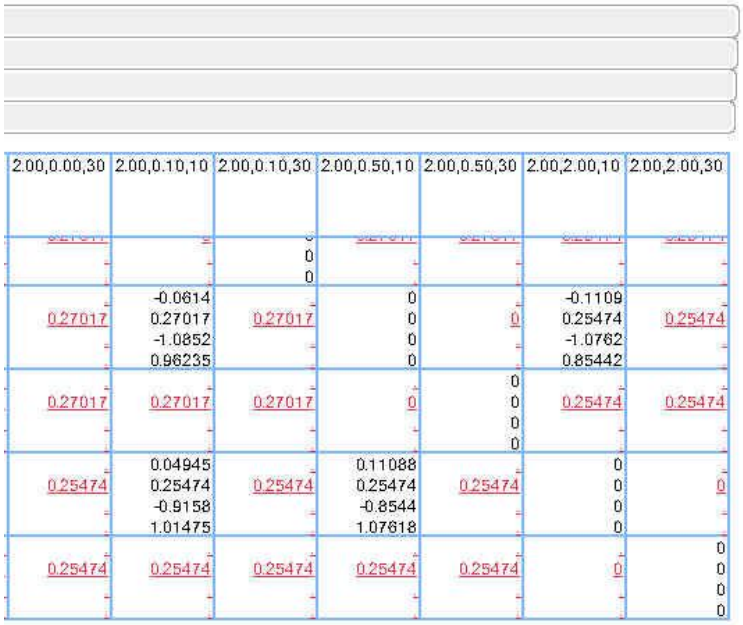
Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	0.00,0.00,10	0.00,0.00,30	0.00,0.10,10	0.00,0.10,30	0.00,0.50,10	0.00,0.50,30	0.00,2.00,10	0.00,2.00,30	2.00,0.00,10
2.00,0.50,10	0.32815	0.08405 0.32815 -1.1594 1.32752	0.32815	-0.266 0.32815 -1.5094 0.97752	0.18625	-0.2045 0.18625 -0.9103 0.50123	0.31381	-0.3154 0.31381 -1.5046 0.87374	0.28857 0.27017 -0.7352 1.31235
2.00,0.50,30	0.32815	0.32815	0.32815	0.32815	0.18625	0.18625	0.31381	0.31381	0.27017
2.00,2.00,10	0.3173	0.19493 0.3173 -1.0074 1.3973	0.3173	-0.1551 0.3173 -1.3574 1.0473	0.3173	-0.0936 0.3173 -1.296 1.10873	0.18625	-0.2045 0.18625 -0.9103 0.50123	0.39945 0.25474 -0.5658 1.36475
2.00,2.00,30	0.3173	0.3173	0.3173	0.3173	0.3173	0.3173	0.18625	0.18625	0.25474

Level		Least Sq Mean
0.00,0.00,10	A	
0.00,0.00,30	A	3.2297291
0.00,0.10,10	A	
0.00,0.10,30	A	3.5797291
0.00,0.50,10	A	
0.00,0.50,30	A	3.5183005
0.00,2.00,10	A	
0.00,2.00,30	A	3.6291810
2.00,0.00,10	A	3.0252032
2.00,0.00,30	A	
2.00,0.10,10	A	3.3752032
2.00,0.10,30	A	
2.00,0.50,10	A	3.3137746
2.00,0.50,30	A	
2.00,2.00,10	A	3.4246552
2.00,2.00,30	A	

Levels not connected by same letter are significantly different.

LS Means Plot





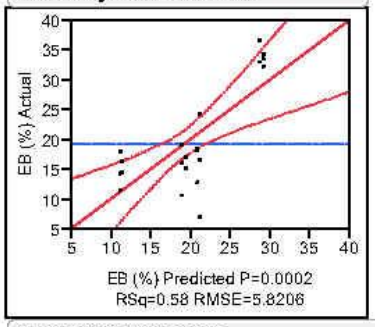
2.00,0.00,30	2.00,0.10,10	2.00,0.10,30	2.00,0.50,10	2.00,0.50,30	2.00,2.00,10	2.00,2.00,30
[illegible]	[illegible]	0 0 0	[illegible]	[illegible]	[illegible]	[illegible]
0.27017	-0.0614 0.27017 -1.0852 0.96235	0.27017	0 0 0 0	0	-0.1109 0.25474 -1.0762 0.85442	0.25474
0.27017	0.27017	0.27017	0	0 0 0 0	0.25474	0.25474
0.25474	0.04945 0.25474 -0.9158 1.01475	0.25474	0.11088 0.25474 -0.8544 1.07618	0.25474	0 0 0 0	0
0.25474	0.25474	0.25474	0.25474	0.25474	0	0 0 0 0

Least Squares Fit

Response EB (%)

Whole Model

Actual by Predicted Plot



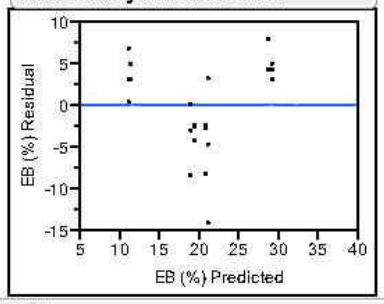
Parameter Estimates

Term		Estimate	Std Error	t Ratio	Prob>|t|
Intercept		20.098658	1.078748	18.63	<.0001*
MOE[0.00]	Biased	4.8768211	1.072384	4.55	0.0001*
NP %[0.00]	Biased	-3.843398	1.88754	-2.04	0.0525
NP %[0.10]	Biased	4.2066018	1.88754	2.23	0.0351*
NP %[0.50]	Biased	3.7180303	1.88754	1.97	0.0600
GLY[10]	Zeroed	0	0	.	.
MOE[0.00]*NP %[0.00]*GLY[10]	Zeroed	0	0	.	.
MOE[0.00]*NP %[0.10]*GLY[10]	Zeroed	0	0	.	.
MOE[0.00]*NP %[0.50]*GLY[10]	Zeroed	0	0	.	.

Effect Tests

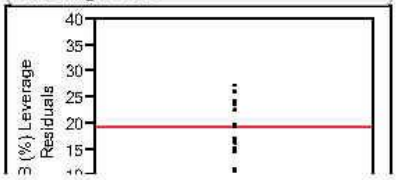
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F	
MOE	1	0	1.1369e-13	.	.	LostDFs
NP %	3	0	5.6843e-14	.	.	LostDFs
GLY	1	0	0	.	.	LostDFs
MOE*NP %*GLY	3	0	0	.	.	LostDFs

Residual by Predicted Plot



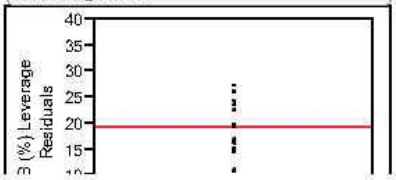
MOE

Leverage Plot



NP %

Leverage Plot

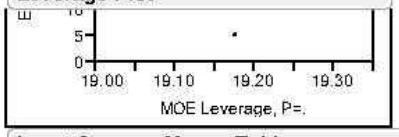


Least Squares Fit

Response EB (%)

MOE

Leverage Plot

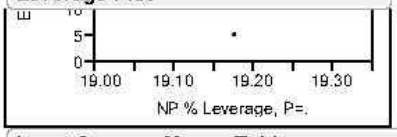


Least Squares Means Table

Level	Least Sq Mean		Std Error	Mean
0.00	.	NonEstimable	.	24.6615
2.00	.	NonEstimable	.	14.9818

NP %

Leverage Plot

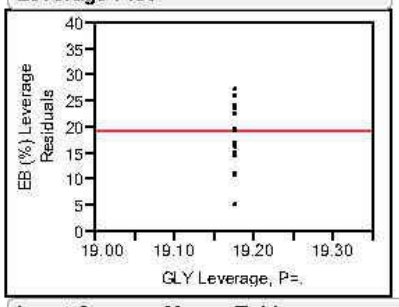


Least Squares Means Table

Level	Least Sq Mean		Std Error	Mean
0.00	.	NonEstimable	.	15.5586
0.10	.	NonEstimable	.	23.6086
0.50	.	NonEstimable	.	23.1200
2.00	.	NonEstimable	.	15.4756

GLY

Leverage Plot

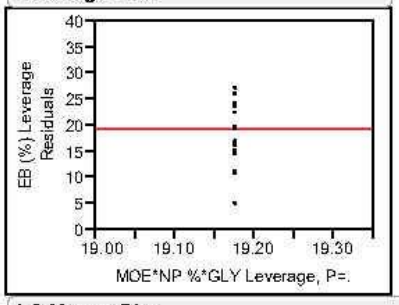


Least Squares Means Table

Level	Least Sq Mean		Std Error	Mean
10	.	NonEstimable	.	14.9818
30	.	NonEstimable	.	24.6615

MOE*NP %*GLY

Leverage Plot



LS Means Plot

Least Squares Fit

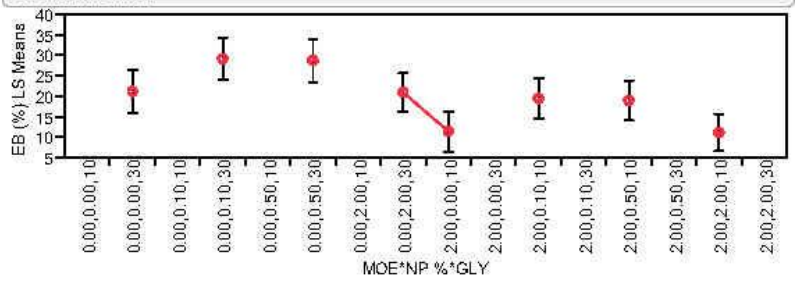
Response EB (%)

MOE*NP %*GLY

Leverage Plot

MOE*NP %*GLY Leverage, P=.

LS Means Plot



LSMeans Differences Tukey HSD

α= 0.050 Q= 3.78936

LSMean[j]

Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	0.00,0.00,10	0.00,0.00,30	0.00,0.10,10	0.00,0.10,30	0.00,0.50,10	0.00,0.50,30	0.00,2.00,10	0.00,2.00,30	2.00,0.00,10
0.00,0.00,10	0 0 0 0	0	3.11125	3.11125	3.11125	3.11125	2.93351	2.93351	2.14477
0.00,0.00,30	0	0 0 0 0	3.11125	-8.05 3.11125 -19.84 3.73966	3.11125	-7.5614 3.11125 -19.351 4.22823	2.93351	0.23784 2.93351 -10.878 11.354	9.75364 2.14477 1.62633 17.881
0.00,0.10,10	3.11125	3.11125	0 0 0 0	0	3.11125	3.11125	2.93351	2.93351	3.77888
0.00,0.10,30	3.11125	8.05 3.11125 -3.7397 19.8397	0	0 0 0 0	3.11125	0.48857 3.11125 -11.301 12.2782	2.93351	8.28784 2.93351 -2.8283 19.404	17.8036 3.77888 3.4841 32.1232
0.00,0.50,10	3.11125	3.11125	3.11125	3.11125	0 0 0 0	0	2.93351	2.93351	3.77888
0.00,0.50,30	3.11125	7.56143 3.11125 -4.2282 19.3511	3.11125	-0.4886 3.11125 -12.278 11.3011	0	0 0 0 0	2.93351	7.79926 2.93351 -3.3169 18.9154	17.3151 3.77888 2.99553 31.6346
0.00,2.00,10	2.93351	2.93351	2.93351	2.93351	2.93351	2.93351	0 0 0 0	0	3.61379
0.00,2.00,30	2.93351	-0.2378 2.93351 -11.354 10.8783	2.93351	-8.2878 2.93351 -19.404 2.82831	2.93351	-7.7993 2.93351 -18.915 3.31689	0	0 0 0 0	9.51581 3.61379 -4.1782 23.2098
2.00,0.00,10	2.14477	-9.7536 2.14477 -17.881 -1.6263	3.77888	-17.804 3.77888 -32.123 -3.4841	3.77888	-17.315 3.77888 -31.635 -2.9955	3.61379	-9.5158 3.61379 -23.21 4.17817	0 0 0 0
2.00,0.00,30	2.14477	2.14477	3.77888	3.77888	3.77888	3.77888	3.61379	3.61379	0

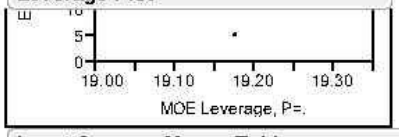
LSMean[i]

Least Squares Fit

Response EB (%)

MOE

Leverage Plot

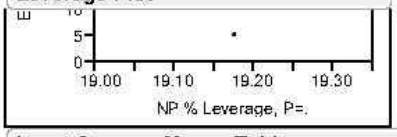


Least Squares Means Table

Level	Least Sq Mean		Std Error	Mean
0.00	.	NonEstimable	.	24.6615
2.00	.	NonEstimable	.	14.9818

NP %

Leverage Plot

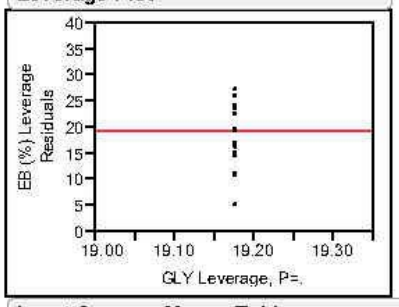


Least Squares Means Table

Level	Least Sq Mean		Std Error	Mean
0.00	.	NonEstimable	.	15.5586
0.10	.	NonEstimable	.	23.6086
0.50	.	NonEstimable	.	23.1200
2.00	.	NonEstimable	.	15.4756

GLY

Leverage Plot

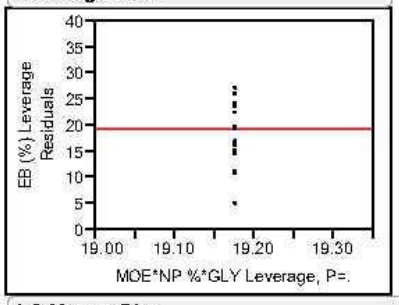


Least Squares Means Table

Level	Least Sq Mean		Std Error	Mean
10	.	NonEstimable	.	14.9818
30	.	NonEstimable	.	24.6615

MOE*NP %*GLY

Leverage Plot



LS Means Plot

Least Squares Fit

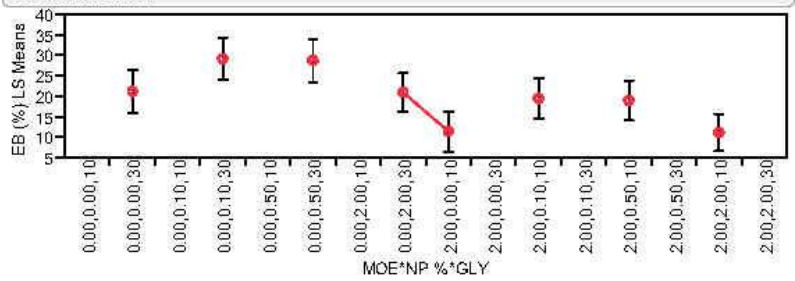
Response EB (%)

MOE*NP %*GLY

Leverage Plot

MOE*NP %*GLY Leverage, P=.

LS Means Plot



LSMeans Differences Tukey HSD

α= 0.050 Q= 3.78936

LSMean[j]

Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	0.00,0.00,10	0.00,0.00,30	0.00,0.10,10	0.00,0.10,30	0.00,0.50,10	0.00,0.50,30	0.00,2.00,10	0.00,2.00,30	2.00,0.00,10
0.00,0.00,10	0 0 0 0	. 0	. 3.11125	. 3.11125	. 3.11125	. 3.11125	. 2.93351	. 2.93351	. 2.14477
0.00,0.00,30	. 0	0 0 0 0	. 3.11125	-8.05 3.11125 -19.84 3.73966	. 3.11125	-7.5614 3.11125 -19.351 4.22823	. 2.93351	0.23784 2.93351 -10.878 11.354	9.75364 2.14477 1.62633 17.881
0.00,0.10,10	. 3.11125	. 3.11125	0 0 0 0	. 0	. 3.11125	. 3.11125	. 2.93351	. 2.93351	. 3.77888
0.00,0.10,30	. 3.11125	8.05 3.11125 -3.7397 19.8397	. 0	0 0 0 0	. 3.11125	0.48857 3.11125 -11.301 12.2782	. 2.93351	8.28784 2.93351 -2.8283 19.404	17.8036 3.77888 3.4841 32.1232
0.00,0.50,10	. 3.11125	. 3.11125	. 3.11125	. 3.11125	0 0 0 0	. 0	. 2.93351	. 2.93351	. 3.77888
0.00,0.50,30	. 3.11125	7.56143 3.11125 -4.2282 19.3511	. 3.11125	-0.4886 3.11125 -12.278 11.3011	. 0	0 0 0 0	. 2.93351	7.79926 2.93351 -3.3169 18.9154	17.3151 3.77888 2.99553 31.6346
0.00,2.00,10	. 2.93351	. 2.93351	. 2.93351	. 2.93351	. 2.93351	. 2.93351	0 0 0 0	. 0	. 3.61379
0.00,2.00,30	. 2.93351	-0.2378 2.93351 -11.354 10.8783	. 2.93351	-8.2878 2.93351 -19.404 2.82831	. 2.93351	-7.7993 2.93351 -18.915 3.31689	. 0	0 0 0 0	9.51581 3.61379 -4.1782 23.2098
2.00,0.00,10	. 2.14477	-9.7536 2.14477 -17.881 -1.6263	. 3.77888	-17.804 3.77888 -32.123 -3.4841	. 3.77888	-17.315 3.77888 -31.635 -2.9955	. 3.61379	-9.5158 3.61379 -23.21 4.17817	0 0 0 0
2.00,0.00,30	.	.	.	.	.	.	.	.	.

LSMean[i]

2.00,0.00,30	2.00,0.10,10	2.00,0.10,30	2.00,0.50,10	2.00,0.50,30	2.00,2.00,10	2.00,2.00,30
2.14477	3.77888	3.77888	3.77888	3.77888	3.65398	3.65398
2.14477	1.70364 3.77888 -12.616 16.0232	3.77888	2.19221 3.77888 -12.127 16.5118	3.77888	9.99148 3.65398 -3.8548 23.8377	3.65398
3.77888	2.14477	2.14477	3.77888	3.77888	3.65398	3.65398
3.77888	9.75364 2.14477 1.62633 17.881	2.14477	10.2422 3.77888 -4.0773 24.5618	3.77888	18.0415 3.65398 4.19522 31.8877	3.65398
3.77888	3.77888	3.77888	2.14477	2.14477	3.65398	3.65398
3.77888	9.26507 3.77888 -5.0545 23.5846	3.77888	9.75364 2.14477 1.62633 17.881	2.14477	17.5529 3.65398 3.70685 31.3992	3.65398
3.61379	3.61379	3.61379	3.61379	3.61379	2.14477	2.14477
3.61379	1.46581 3.61379 -12.228 15.1598	3.61379	1.95438 3.61379 -11.74 15.6484	3.61379	9.75364 2.14477 1.62633 17.881	2.14477
0	-8.05 3.11125 -19.84 3.73966	3.11125	-7.5614 3.11125 -19.351 4.22823	3.11125	0.23784 2.93351 -10.878 11.354	2.93351
0						

Least Squares Fit

Response EB (%)

MOE*NP %*GLY

LSMeans Differences Tukey HSD

LSMean[j]

Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	0.00,0.00,10	0.00,0.00,30	0.00,0.10,10	0.00,0.10,30	0.00,0.50,10	0.00,0.50,30	0.00,2.00,10	0.00,2.00,30	2.00,0.00,10
		-17.881 -1.6263		-32.129 -3.4841		-31.633 -2.9955		-23.21 4.17817	0 0
2.00,0.00,30	. 2.14477 .	. 2.14477 .	. 3.77888 .	. 3.77888 .	. 3.77888 .	. 3.77888 .	. 3.61379 .	. 3.61379 .	. 0 .
2.00,0.10,10	. 3.77888 .	-1.7036 3.77888 -16.023 12.6159	. 2.14477 .	-9.7536 2.14477 -17.881 -1.6263	. 3.77888 .	-9.2651 3.77888 -23.585 5.05447	. 3.61379 .	-1.4658 3.61379 -15.16 12.2282	8.05 3.11125 -3.7397 19.8397
2.00,0.10,30	. 3.77888 .	. 3.77888 .	. 2.14477 .	. 2.14477 .	. 3.77888 .	. 3.77888 .	. 3.61379 .	. 3.61379 .	. 3.11125 .
2.00,0.50,10	. 3.77888 .	-2.1922 3.77888 -16.512 12.1273	. 3.77888 .	-10.242 3.77888 -24.562 4.07733	. 2.14477 .	-9.7536 2.14477 -17.881 -1.6263	. 3.61379 .	-1.9544 3.61379 -15.648 11.7396	7.56143 3.11125 -4.2282 19.3511
2.00,0.50,30	. 3.77888 .	. 3.77888 .	. 3.77888 .	. 3.77888 .	. 2.14477 .	. 2.14477 .	. 3.61379 .	. 3.61379 .	. 3.11125 .
2.00,2.00,10	. 3.65398 .	-9.9915 3.65398 -23.838 3.85478	. 3.65398 .	-18.041 3.65398 -31.888 -4.1952	. 3.65398 .	-17.553 3.65398 -31.399 -3.7066	. 2.14477 .	-9.7536 2.14477 -17.881 -1.6263	-0.2378 2.93351 -11.354 10.8783
2.00,2.00,30	. 3.65398 .	. 3.65398 .	. 3.65398 .	. 3.65398 .	. 3.65398 .	. 3.65398 .	. 2.14477 .	. 2.14477 .	. 2.93351 .

Level							Least Sq Mean
0.00,0.00,10	A	B	C	D	E	F	.
0.00,0.00,30	A	B		D	E		21.132081
0.00,0.10,10	A	B	C	D	E	F	.
0.00,0.10,30	A			D			29.182081
0.00,0.50,10	A	B	C	D	E	F	.
0.00,0.50,30	A	B					28.693510
0.00,2.00,10	A	B	C	D	E	F	.
0.00,2.00,30	A	B	C	D			20.894246
2.00,0.00,10			C			F	11.378439
2.00,0.00,30	A	B	C	D	E	F	.
2.00,0.10,10		B	C		E	F	19.428439
2.00,0.10,30	A	B	C	D	E	F	.
2.00,0.50,10			C	D	E	F	18.939868
2.00,0.50,30	A	B	C	D	E	F	.
2.00,2.00,10					E	F	11.140603
2.00,2.00,30	A	B	C	D	E	F	.

Levels not connected by same letter are significantly different.

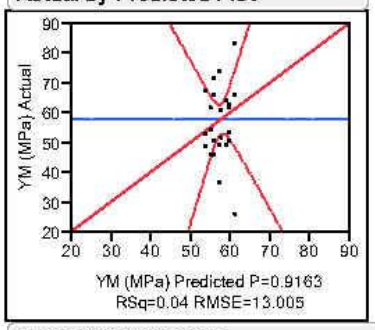
2.00,0.00,30	2.00,0.10,10	2.00,0.10,30	2.00,0.50,10	2.00,0.50,30	2.00,2.00,10	2.00,2.00,30
.	3.73966	.	4.22823	.	11.354	.
0 0 0 0	3.11125	3.11125	3.11125	3.11125	2.93351	2.93351
3.11125	0 0 0 0	0	0.48857 3.11125 -11.301 12.2782	3.11125	8.28784 2.93351 -2.8283 19.404	2.93351
3.11125	0	0 0 0 0	3.11125	3.11125	2.93351	2.93351
3.11125	-0.4886 3.11125 -12.278 11.3011	3.11125	0 0 0 0	0	7.79926 2.93351 -3.3169 18.9154	2.93351
3.11125	3.11125	3.11125	0	0 0 0 0	2.93351	2.93351
2.93351	-8.2878 2.93351 -19.404 2.82831	2.93351	-7.7993 2.93351 -18.915 3.31689	2.93351	0 0 0 0	0
2.93351	2.93351	2.93351	2.93351	2.93351	0	0 0 0 0

Least Squares Fit

Response YM (MPa)

Whole Model

Actual by Predicted Plot



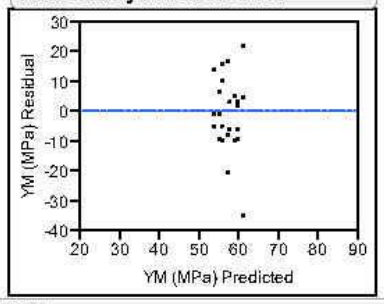
Parameter Estimates

Term		Estimate	Std Error	t Ratio	Prob>|t|
Intercept		57.434714	2.410158	23.83	<.0001*
MOE[0.00]	Biased	-1.987705	2.395939	-0.83	0.4146
NP %[0.00]	Biased	1.7298992	4.217174	0.41	0.6852
NP %[0.10]	Biased	-0.371529	4.217174	-0.09	0.9305
NP %[0.50]	Biased	-1.677244	4.217174	-0.40	0.6942
GLY[10]	Zeroed	0	0	.	.
MOE[0.00]*NP %[0.00]*GLY[10]	Zeroed	0	0	.	.
MOE[0.00]*NP %[0.10]*GLY[10]	Zeroed	0	0	.	.
MOE[0.00]*NP %[0.50]*GLY[10]	Zeroed	0	0	.	.

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F	
MOE	1	0	2.8422e-14	.	.	LostDFs
NP %	3	0	-7.105e-15	.	.	LostDFs
GLY	1	0	0	.	.	LostDFs
MOE*NP %*GLY	3	0	0	.	.	LostDFs

Residual by Predicted Plot

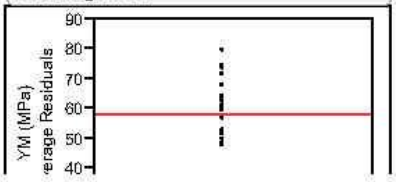


MOE

Leverage Plot

NP %

Leverage Plot

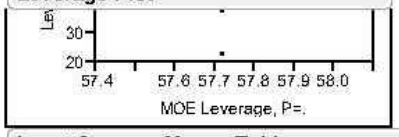


Least Squares Fit

Response YM (MPa)

MOE

Leverage Plot

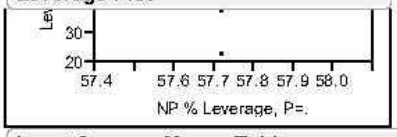


Least Squares Means Table

Level	Least Sq Mean		Std Error	Mean
0.00	.	NonEstimable	.	55.4715
2.00	.	NonEstimable	.	59.4412

NP %

Leverage Plot

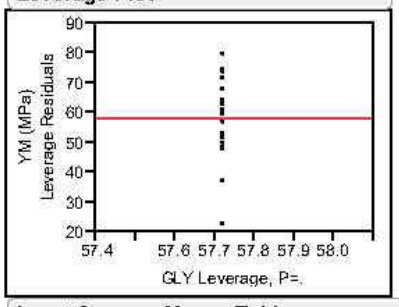


Least Squares Means Table

Level	Least Sq Mean		Std Error	Mean
0.00	.	NonEstimable	.	59.4486
0.10	.	NonEstimable	.	57.3471
0.50	.	NonEstimable	.	56.0414
2.00	.	NonEstimable	.	57.9744

GLY

Leverage Plot

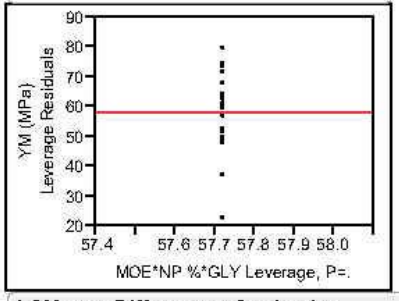


Least Squares Means Table

Level	Least Sq Mean		Std Error	Mean
10	.	NonEstimable	.	59.4412
30	.	NonEstimable	.	55.4715

MOE*NP %*GLY

Leverage Plot



LSMeans Differences Student's t

α= 0.050 t= 2.05954

	LSMean[j]								
Mean[i]-Mean[j]	0.00,0.00,10	0.00,0.00,30	0.00,0.10,10	0.00,0.10,30	0.00,0.50,10	0.00,0.50,30	0.00,2.00,10	0.00,2.00,30	2.00,0.00,10
Std Err Dif									
Lower CL Dif									
Upper CL Dif									

Least Squares Fit

Response YM (MPa)

MOE*NP %*GLY

Leverage Plot

LSMeans Differences Student's t

α= 0.050 t= 2.05954

LSMean[j]

LSMean[i] Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	0.00,0.00,10	0.00,0.00,30	0.00,0.10,10	0.00,0.10,30	0.00,0.50,10	0.00,0.50,30	0.00,2.00,10	0.00,2.00,30	2.00,0.00,10
0.00,0.00,10	0 0 0 0	0	6.95121	6.95121	6.95121	6.95121	6.5541	6.5541	4.79188
0.00,0.00,30	0	0 0 0 0	6.95121	2.10143 6.95121 -12.215 16.4177	6.95121	3.40714 6.95121 -10.909 17.7234	6.5541	1.41103 6.5541 -12.087 14.9095	-3.9754 4.79188 -13.844 5.89365
0.00,0.10,10	6.95121	6.95121	0 0 0 0	0	6.95121	6.95121	6.5541	6.5541	8.44283
0.00,0.10,30	6.95121	-2.1014 6.95121 -16.418 12.2149	0	0 0 0 0	6.95121	1.30571 6.95121 -13.011 15.622	6.5541	-0.6904 6.5541 -14.189 12.808	-6.0768 8.44283 -23.465 11.3115
0.00,0.50,10	6.95121	6.95121	6.95121	6.95121	0 0 0 0	0	6.5541	6.5541	8.44283
0.00,0.50,30	6.95121	-3.4071 6.95121 -17.723 10.9091	6.95121	-1.3057 6.95121 -15.622 13.0106	0	0 0 0 0	6.5541	-1.9961 6.5541 -15.495 11.5023	-7.3826 8.44283 -24.771 10.0058
0.00,2.00,10	6.5541	6.5541	6.5541	6.5541	6.5541	6.5541	0 0 0 0	0	8.074
0.00,2.00,30	6.5541	-1.411 6.5541 -14.909 12.0874	6.5541	0.6904 6.5541 -12.808 14.1888	6.5541	1.99612 6.5541 -11.502 15.4945	0	0 0 0 0	-5.3864 8.074 -22.015 11.2423
2.00,0.00,10	4.79188	3.97541 4.79188 -5.8936 13.8445	8.44283	6.07684 8.44283 -11.311 23.4652	8.44283	7.38255 8.44283 -10.006 24.7709	8.074	5.38643 8.074 -11.242 22.0151	0 0 0 0
2.00,0.00,30	4.79188	4.79188	8.44283	8.44283	8.44283	8.44283	8.074	8.074	0
2.00,0.10,10	8.44283	1.87398 8.44283 -15.514 19.2623	4.79188	3.97541 4.79188 -5.8936 13.8445	8.44283	5.28112 8.44283 -12.107 22.6695	8.074	3.28501 8.074 -13.344 19.9137	-2.1014 6.95121 -16.418 12.2149
2.00,0.10,30	8.44283	8.44283	4.79188	4.79188	8.44283	8.44283	8.074	8.074	6.95121
2.00,0.50,10	8.44283	0.56827 8.44283 -16.82 17.9566	8.44283	2.6697 8.44283 -14.719 20.058	4.79188	3.97541 4.79188 -5.8936 13.8445	8.074	1.97929 8.074 -14.649 18.608	-3.4071 6.95121 -17.723 10.9091
2.00,0.50,30	8.44283	8.44283	8.44283	8.44283	4.79188	4.79188	8.074	8.074	6.95121

2.00,0.00,30	2.00,0.10,10	2.00,0.10,30	2.00,0.50,10	2.00,0.50,30	2.00,2.00,10	2.00,2.00,30
4.79188	8.44283	8.44283	8.44283	8.44283	8.16378	8.16378
4.79188	-1.874 8.44283 -19.262 15.5144	8.44283	-0.5683 8.44283 -17.957 16.8201	8.44283	-2.5644 8.16378 -19.378 14.2492	8.16378
8.44283	4.79188	4.79188	8.44283	8.44283	8.16378	8.16378
8.44283	-3.9754 4.79188 -13.844 5.89365	4.79188	-2.6697 8.44283 -20.058 14.7186	8.44283	-4.6658 8.16378 -21.479 12.1478	8.16378
8.44283	8.44283	8.44283	4.79188	4.79188	8.16378	8.16378
8.44283	-5.2811 8.44283 -22.669 12.1072	8.44283	-3.9754 4.79188 -13.844 5.89365	4.79188	-5.9715 8.16378 -22.785 10.8421	8.16378
8.074	8.074	8.074	8.074	8.074	4.79188	4.79188
8.074	-3.285 8.074 -19.914 13.3437	8.074	-1.9793 8.074 -18.608 14.6494	8.074	-3.9754 4.79188 -13.844 5.89365	4.79188
0	2.10143 6.95121 -12.215 16.4177	6.95121	3.40714 6.95121 -10.909 17.7234	6.95121	1.41103 6.5541 -12.087 14.9095	6.5541
0 0 0 0	6.95121	6.95121	6.95121	6.95121	6.5541	6.5541
6.95121	0 0 0 0	0	1.30571 6.95121 -13.011 15.622	6.95121	-0.6904 6.5541 -14.189 12.808	6.5541
6.95121	0	0 0 0 0	6.95121	6.95121	6.5541	6.5541
6.95121	-1.3057 6.95121 -15.622 13.0106	6.95121	0 0 0 0	0	-1.9961 6.5541 -15.495 11.5023	6.5541
6.95121	6.95121	6.95121	0	0 0	6.5541	6.5541

Least Squares Fit

Response YM (MPa)

MOE*NP %*GLY

LSMeans Differences Student's t

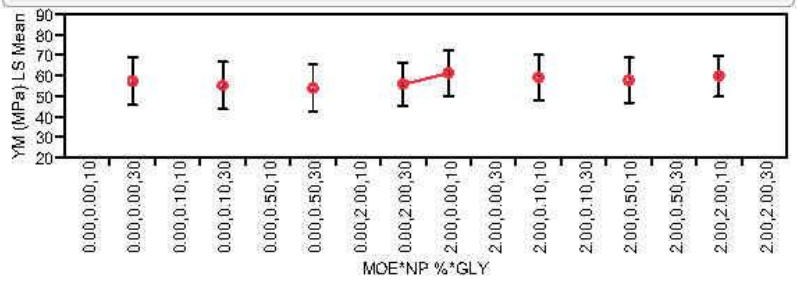
LSMean[j]

Mean[i]-Mean[j] / Std Err Dif / Lower CL Dif / Upper CL Dif	0.00,0.00,10	0.00,0.00,30	0.00,0.10,10	0.00,0.10,30	0.00,0.50,10	0.00,0.50,30	0.00,2.00,10	0.00,2.00,30	2.00,0.00,10
2.00,0.50,10		0.56827		2.6697		3.97541		1.97929	-3.4071
	8.44283	8.44283	8.44283	8.44283	4.79188	4.79188	8.074	8.074	6.95121
		-16.82		-14.719		-5.8936		-14.649	-17.723
		17.9566		20.058		13.8445		18.608	10.9091
2.00,0.50,30									
	8.44283	8.44283	8.44283	8.44283	4.79188	4.79188	8.074	8.074	6.95121
2.00,2.00,10		2.56438		4.66581		5.97153		3.97541	-1.411
	8.16378	8.16378	8.16378	8.16378	8.16378	8.16378	4.79188	4.79188	6.5541
		-14.249		-12.148		-10.842		-5.8936	-14.909
		19.378		21.4794		22.7852		13.8445	12.0874
2.00,2.00,30									
	8.16378	8.16378	8.16378	8.16378	8.16378	8.16378	4.79188	4.79188	6.5541

Level		Least Sq Mean
0.00,0.00,10	A	
0.00,0.00,30	A	57.176909
0.00,0.10,10	A	
0.00,0.10,30	A	55.075480
0.00,0.50,10	A	
0.00,0.50,30	A	53.769766
0.00,2.00,10	A	
0.00,2.00,30	A	55.765884
2.00,0.00,10	A	61.152318
2.00,0.00,30	A	
2.00,0.10,10	A	59.050890
2.00,0.10,30	A	
2.00,0.50,10	A	57.745175
2.00,0.50,30	A	
2.00,2.00,10	A	59.741293
2.00,2.00,30	A	

Levels not connected by same letter are significantly different.

LS Means Plot



2.00,0.00,30	2.00,0.10,10	2.00,0.10,30	2.00,0.50,10	2.00,0.50,30	2.00,2.00,10	2.00,2.00,30
[illegible]	[illegible]	0 0	[illegible]	[illegible]	[illegible]	[illegible]
6.95121	-1.3057 6.95121 -15.622 13.0106	6.95121	0 0 0 0	0	-1.9961 6.5541 -15.495 11.5023	6.5541
6.95121	6.95121	6.95121	0	0 0 0 0	6.5541	6.5541
6.5541	0.6904 6.5541 -12.808 14.1888	6.5541	1.99612 6.5541 -11.502 15.4945	6.5541	0 0 0 0	0
6.5541	6.5541	6.5541	6.5541	6.5541	0	0 0 0 0

جـامعـــة النجاح الوطنية

كليـــة الدراســـات العليـــا

تأثير استخدام جزيئات النانو على الاغلفة الحيوية القابلة للأكل والمصنوعة من مخلفات عمليات التصنيع وتطبيقه على عمليات التغليف للأغدية

إعداد

نسرين وجيه سيد منصور

إشراف

د. محمد صباح

قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في التغذية وتكنولوجيا الغذاء، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس– فلسطين.

2022

تأثير استخدام جزيئات النانو على الاغلفة الحيوية القابلة للأكل والمصنوعة من مخلفات عمليات التصنيع وتطبيقه على عمليات التغليف للأغدية

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الملخص

الهدف من هذه الدراسة هو دراسة تأثير جزيئات النانو على خصائص الأغلفة الحيوية القابلة للتحلل، حيث كانت المواد الخام المستخدمة في اجراء هذه الدراسة من مخلفات عمليات تصنيع الأغذية، اذ تم استخدام البروتين المستخلص من متبقيات استخلاص زيت القزحة, كماده أساسيه لتكوين البلاستيك الحيوي بالإضافة الى استخدام متبقيات استخلاص البروتين و الدهون من نبات جوز الهند لتصنيع جزيئات النانو المضافة والتي تم اضافتها لدراسة تأثرها في تدعيم الخصائص الميكانيكية والفيزيائية مواد التغليف القابل للتحلل. وتم تصنيع تلك المواد بوجود الجليسيرول بنسبة 30%، وايضا تمت اضافة زيت بذور المورينغا بنسبة 2% منفردا وايضا بعد دمجها مع جزيئات نانو سيليلوز من اجل دراسة تأثيره على الاغلفة القابلة للتحلل من حيث الصفات الميكانيكية والفيزيائية ومن حيث قدرته على تدعيم خصائص اخرى مثل مضادات الأكسدة ومقاومته لنمو البكتيريا.

وكانت نتيجة الدراسة انه عند استخدام تراكيز منخفضه من جزيئات النانو سيليلوز على الاغلفة القابلة للتحلل لم يكن هناك فرق كبير ذو دلاله معنويه على سماكة الاغلفة و بالمقارنة بالتراكيز العالية التي كان لها تثير سلبي على سماكته. بينما أدت اضافتها إلى زياده في الخصائص الميكانيكية المتمثلة بقوة الشد

والمرونة، بينما نسبة الاستطالة كان تأثير إضافة جزيئات النانو بتراكيز قليله (0.1الى 0.5 %) إيجابيا بحيث كانت هناك زيادة في نسبتها بينما كان التأثير عكسيا عند إضافة تراكيز عالية من جزيئات النانو من 1 الى 3 %)، أما من حيث نسبة الماء في الأغلفة القابلة للتحلل فقد كانت النسبة مماثله للغلاف الخالي من جزيئات النانو (هذا بالنسبة لتراكيز من 0.1 الى 0.5) مع انخفاض ضئيل، لكن عند مقارنة التراكيز العالية ب الغلاف الخالي من جزيئات النانو لوحظ انخفاض واضح في نسبة احتوائها على الماء وبالمثل ايضا لدى مقارنتها بالتراكيز الاقل.

ومن حيث مقارنة الخصائص الميكانيكية للغلاف القابل للتحلل بوجود زيت المورينغا وإضافة جزيئات النانو لوحظ عدم تأثر قوة الشد والمرونة بإضافة الزيت للمزيج حيث أنه حافظ الغلاف القابل للتحلل على صفاته الأولى بوجود جزيئات النانو قبيل اضافة الزيت حيث ان التحليل الاحصائي أثبت ذلك، أن الفروقات ليست ذات دلاله معنويه، إلا أن نسبة الاستطالة عند كانت ذات دلاله معنويه حيث أن وجود الزيت أدى لانخفاض نسبة الاستطالة في الغلاف القابل للتحلل، بينما أدى وجود الزيت إلى زياده في السماكة وكانت إحصائيا مثبته بدلاله معنويه.

أما من حيث مقارنة نسبة وجود الماء بينهما فقد لوحظ انخفاض نسبة الماء بوجود جزيئات النانو لكل التراكيز المستخدمة مع وجود الزيت او بدون اضافته لدى مقارنتها الغلاف القابل للتحلل الخالي من كليهما، وبالنسبة لقدرتها على امتصاص الماء من المحيط فقد لوحظ انخفاضها بشكل عام بوجود جزيئات النانو إلا أن وجود تركيز 0.5 % منها كان ذو دلاله معنويه لدى مقارنته بعينه بدون وجود الزيت لنفس التركيز.

كما أن إضافة الزيت أدت إلى زيادة القدرة على مقاومة الأكسدة لدى الغلاف القابل للتحلل بشكل عام عند مقارنتها بالعينة التي لا تحتوي الزيت ولكن التركيز العالي من جزيئات النانو كانت نسبة مضادات الأكسدة لديه اقل من التراكيز الاقل. أما من حيث مقاومتها لنمو البكتيريا فقد تم دراسة نوعين من البكتيريا موجبة

جرام وسالبة جرام ولوحظت فعالية جيده لتثبيط نمو بكتيريا موجبة غرام عند دراسة قدرة الزيت على ذلك وعند اضافة الزيت لعينات الغلاف القابل للتحلل لوحظت ايضا فاعلية ضد بكتيريا موجبة جرام وفاعليه منخفضه ضد سالبة جرام كما ظهر تحت الفحص الميكروسكوبي للعينات. كما تمت تجربة قابلية استخدام المنتج لتغليف الطعام عن طريق التغليف المباشر للزبدة او صنع اكياس صغيره تحتوي على كميه من الزيت الزيتون.