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Effect of Microbial Transglutaminase on Physico-Chemical Properties of Blended *Nigella Sativa* Protein Concentrate/Pectin Based Edible Film

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Dedication

I am thankful for God Almighty, who gave me the ability and opportunity to complete this work.
I would like to dedicate my thesis to

My dear father **Bilal,** who deserves love and appreciation.

My beloved mother **Nadia,** who gave me all support to finish this study.

My brothers and sisters for their encouragement.

I also dedicate this work to my best friends and others who in one way or another shared their help and support.

I wish to benefit my Palestinian nation

Declaration

I certify that this thesis submitted for the degree of Master in Institute for Sustainable Development is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not be submitted for a higher degree to any other university or institution.

Liali B M Ali

Signature:

Date: 25/8/2021

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List of abbreviations

Abbreviation	Description
°C	Celsius
CH_4	Methane
CO_2	Carbon dioxide
_COOH	Carboxyl group
DW	Distilled water
EB	Elongation at break
FDA	Food and Drug Administration
FFS	Film forming solution
g	Gram
GLY	Glycerol
GRAS	Generally Recognized As Safe
h	Hour
HCl	Hydrochloric acid
HDPE	High-density polyethylene
H_2O	Water
Kg	Kilogram
LDPE	Low-density polyethylene
$Mg(NO_3)_2$: H_2O	Magnesium nitrate
mm	Millimeter
MPP	Marine plastic pollution
N	Normality
N_2	Nitrogen gas
NaOH	Sodium hydroxide
__NH_2	Amine group
NH_4	Ammonium
NSPC	Nigella sativa protein concentrate
PC	Protein concentrate
PEC	Pectin
pH	Power of hydrogen
RH	Relative humidity
_SH	Sulfhydryl group
SDG	Sustainable development goal
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
$	Dollar sign

TGase	Transglutaminase enzyme
TS	Tensile strength
U/g	Unit/gram
UV	Ultraviolet
YM	Young’s modulus
W/V	Weight for volume
w/w	Weight for weight

Abstract

Introduction: Plastics are produced about 350 million tons per year. It is widely used because it has several distinctive properties, which include being moldable, durable and cheap. But, plastic wastes are not biodegradable in the environment. Thus, this wastes accumulate in the seas, oceans and soils. Therefore, several methods were used to dispose of this wastes: landfilling wastes in the soil, but this negatively affects on the soil fertility because of accumulation of chemicals and toxins. Incineration of plastic wastes produce toxic gases that pollutes the environment and contributes to the aggravation of the global warming problem. Whereas, recycling of plastic wastes do not exceed 2% of total plastic wastes .

As it is known that the bulk of plastic wastes is from food packaging waste. Therefore, it was sought to develop edible films and wrappers made of natural and biodegradable materials that also provide protection for food and form barriers between food and the environment to maintain its shelf life, safety and quality. In general, edible films are generated from proteins, polysaccharides and fats. In addition, films can be injected with antimicrobial and antioxidant substances to maintain food safety and quality.

The edible based on protein have good mechanical properties. While, polysaccharides based on edible films provide films with good barrier for gases.

Although, using proteins is a creative option to create edible films in food industry, it requires improvements in its properties to become more resistant to be handled during the application in food packaging and to ensure protection of food products. Hence, blending protein solution with other bio-polymer such as pectin or transglutaminase enzymes or both to improve the properties of edible films-based protein.

Objectives: The objective of this study is to identify the proprieties of edible films of blending *Nigella sativa* protein concentrate (NSPC) and pectin (PEC). Also, to identify the effect of transglutaminase (TGase) enzyme on blending *Nigella sativa* protein concentrate/pectin based edible films characteristics, determine the water uptake and moisture content of *Nigella sativa* protein concentrate/pectin/TGase based edible films, and evaluate the biodegradability of *Nigella sativa* protein concentrate/pectin/TGase based edible films.

Materials and Methods: Protein percent was determined in *Nigella sativa* protein concentrate; four film forming solution-based *Nigella sativa* protein were prepared by its blending with pectin at different ratios (40:0, 40:6, 40:10, 40:40 w/w) at pH 7.5 to investigate influence of different concentrations of pectin's on mechanical properties of NSPC based edible films. Furthermore, the addition of TGase enzyme to NSPC/PEC solution at pH 7.5 was evaluated in two concentrations (20 U/g NSPC, 10 U/g NSPC), in terms of mechanical properties. Finally, NSPC/PEC (40:40 w/w)/TGase(10U/g NSPC) films with high tensile strength (TS) and adequate elongation at break (EB) were selected from prepared films and tested for biodegradability, water content and water uptake of film and compared with other films free of of TGase.

Results and Discussion: Blended NSPC/PEC (40:6 and 40:10 w/w) significantly increase the film thickness, whereas different concentrations of TGase have no significant differences on films thickness. Nevertheless, blended NSPC/PEC (40:6, 40:10 w/w) TGase (20U/g NSPC) has the tertiary synergistic effects on thickness values ($p<0.05$). Tensile strength was enhanced to about double as affected by pectin concentration (40:10 w/w). Low concentration of enzyme (10U/g NSPC) produced films with significantly higher tensile strength, especially when it was incorporated into NSPC/PEC (40:40 w/w), where films increased tensile strength to about 7 folds as compared to the NSPC films ($p<0.05$). Elastic films were obtained at ratios NSPC/PEC (40:6, 40:10 w/w) ,respectively. Moreover, the high concentration of TGase (20U/g NSPC) improved (EB) of films at ratios (40:6, 40:10 w/w) to 3 to 8 folds of these films in presence the low concentration of TGase, respectively. However, (10U/g NSPC) TGase has negative effects on elasticity (EB). Young's module (YM) was not significantly affected in presence PEC or TGase. But, crosslinked NSPC/PEC (40:40 w/w) with low concentration of enzyme significantly increase the YM and stiffness. Finally, low concentration of TGase enzyme responsible for significantly increasing water content and uptake, as well as decreasing of biodegradability of NSPC/PEC (40:40 w/w) films. Results demonstrated that TGase can improve properties of NSPC films when blending with PEC.

Chapter One: General Framework

1.1 Introduction

Today and worldwide, plastics are derived from fossil oil and gas, therefore, about 4% of fossil oil per year is converted directly into plastics (Hopewell et al., 2009). Plastic is a relatively inexpensive, durable and versatile materials, having properties resulted in a wide range of applications, which have brought many benefits to society (Thompson et al., 2009).

In contrast, plastic pollution is considered the major global and environmental challenge, because of growing demand on plastic through the last decades, and reaching about 350 million tons production per year (Corbari et al., 2020; Napper and Thompson, 2020).

Furthermore, plastic wastes pose a dangerous threat to the environment and society and can be discharged by different methods (Singh and Sharma, 2016; Ilyas et al., 2018).

Land filling plastics leads to abiotic and biotic degradation of the plastics (Gómez and Michel, 2013; Alabi et al., 2019). On the other hand, plastic feedstock is capable of leaching out toxic chemicals into the soil, underground water in addition to air pollution by releasing carbon dioxide and methane into the air (Alabi et al., 2019; He et al., 2019).

Microbial biodegradable plastics, may not decompose rapidly enough under ambient environmental conditions to avoid accumulation from continuous inputs (Gómez and Michel Jr, 2013). Open burning of plastics products, releases pollutants such as heavy metals, dioxins, polychlorinated biphenyls and furans that when inhaled, can cause health risks especially respiratory disorder, carcinogens and endocrine diseases (Raziyafathima et al., 2016). Many plastics can be recycled, but, this method is not fully utilized, due to difficulties with the collection and sorting of plastic wastes (Hopewell et al., 2009; Alabi et al., 2019).

Currently, with increasing concerns about plastic pollution, global warming, and oil depletion. A possible solution to reduce the consumption of the traditional plastics of petrochemical origin is "bio-plastics" which are polymers that can be easily degraded into CO_2, H_2O and inorganic compounds or biomass by the enzymatic action of microorganisms (Porta, 2019).So, it has been considered as an innovative eco-friendly alternative (Kumar and Thakur, 2017).

Biodegradable polymers are classified as agro-polymers (starch, chitin, protein) derived from plant or animal feedstock and bio polyesters (polyhydroxy-alkanoates, polylactic acid etc) produced by chemical synthesis from renewable sources or microorganisms (Ferreira et al., 2016). Moreover, producing bioplastic that is similar to traditional plastic in thermal and mechanical properties contributes to create high quality bioplastic, but it breaks down completely into eco-friendly products in the environment (Song et al., 2009).

However, edible film and coating that is defined as thin cover and primary packaging that contact directly with packaged food; is completely made from agro and bio-polymer (Aguirre-Joya et al., 2018). Edible films provides distinctive functions including barrier for gases and carrier for the antimicrobial and antioxidant to maintain safety, quality and nutritional value of food (Porta et al., 2016; Hammam, 2019).

Edible materials prepared in different forms and used for food packaging and coating. Bio-molecules can be used either individually or as a mixture to produce biodegradable films with desirable functional properties (Sabbah and Porta, 2017; Kocira et al., 2021).

Generally, lipids are utilized to decrease water permeability, polysaccharides can control gas permeability, but in contrast, proteins often provide good mechanical properties for edible packages(Chen et al., 2019).

Chemical, enzymatic modifications could be added for bioplastic products to support sustainable technologies and prevent plastic pollution (Porta et al., 2016; Sabbah and Porta, 2017).

Protein cross-linking approach can be formed by using chemical reagents or enzymes form covalent bonds between two or more protein molecules together to improve mechanical and barrier properties of edible films (Heck et al.,, 2013; Sabbah et al., 2019). Chemical cross linkers are toxic compounds or may produce dangerous by-products. So, it is undesirable for food applications, despite their commercially abundance and effectiveness (R Porta et al., 2011).

Therefore, interest has increased in introducing enzymes into the food industry as a safe alternatives to chemical ones (Heck et al., 2013). One of the common and cheap enzymes that are considered the most efficient for enhancing the features of protein based on films is

microbial (TGase) through generation peptide bonds between glutamines and lysines to develop suitable protein based biodegradable films (Sabbah et al., 2019).

In this study, biodegradable materials derived from food industry wastes especially protein and polysaccharide blended at different concentrations will be evaluated in the presence and absence of TGase enzyme as crosslinker, in order to produce innovative bioplastics/edible films with tailored properties.

1.2 Objectives

1. To identify the characteristics of edible films based on blending NSPC/PEC.
2. To identify the characteristics edible films based on blending NSPC/PEC/TGase
3. To determine the water uptake and water moisture of NSPC/PEC/TGase based edible films.
4. To evaluate thickness and mechanical properties of edible films under investigation in terms of (Tensile strength, Elongation at break and Young's modulus).
5. To evaluate the biodegradability of NSPC/PEC/TGase based edible films.

Chapter Two: Literature Review

2.1 Plastic & Sustainable Development Goals (SDG) of the United Nations (Global Goals for 2030)

Figure 2.1. The 17 UN sustainable development goals (https://www.un.org/sustainabledevelopment)

It is known that most of plastic accumulates and decomposes in the landfill and ocean that have serious impact on marine life and human health (Issifu and Sumaila, 2020).

Marine plastic pollution (MPP) could be limit the achievement of sustainable development goals (SDG). For instance, United Nations Environment Program reported about 51 trillion small fractions of plastic or microplastic particles that is broken down from large particles of plastic or maro-plastic in the ocean. Moreover, marine creatures are overlapping with maroplastic; this interferes with an achieved SDG 14 which aims to conserve and sustainably use the oceans, seas and marine resources. As well as micro-plastic can ingested by marine species; hence gets into food chain; this obstructs fulfillment of SDG 3 (Good Health and Well-Being). So, deterioration marine life causes loss of revenue for fisheries and the tourism sector, making carrying out SDG 12 (Decent Work and Economic Growth) difficulty (https://sustainabledevelopment.un.org; Issifu and Sumaila, 2020) (Figure 2.1 and 2.2).

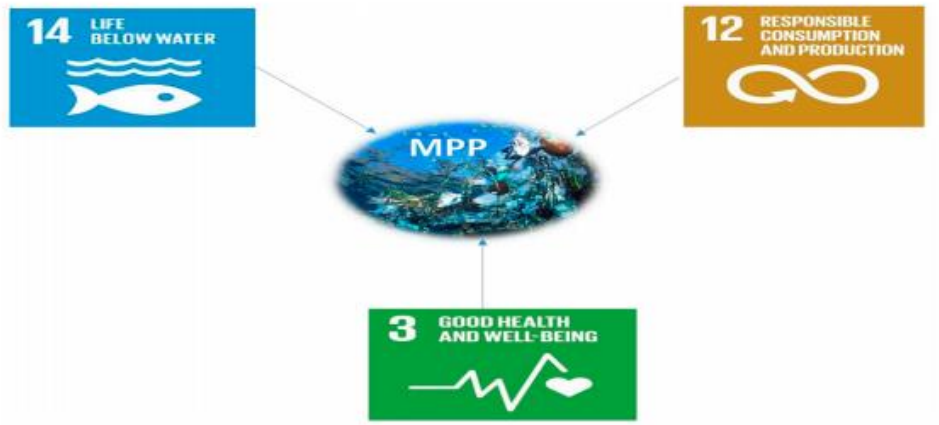


Figure 2.2. The relationship between marine plastic pollution and sustainable development goals (Issifu and Sumaila, 2020).

2.2 Disposal of plastic wastes methods and their impact on environment

The production of plastic reached 350 million tons annually. Moreover, the global population uses more than 700 thousand plastic shopping bags and 400 thousand plastic bottles per minute. But, recycling of plastic doesn't exceed 2% (Waring et al., 2018; Vimal and Kumar, 2019; Galloway et al., 2020).

Several methods are used for disposal of plastic wastes; more than 60% of plastic wastes are discharched in landfill worldwide (Hidayah, 2018). This discharge method of petroleum materials has negatively impact on various natural resource; alteration micro-flora within soil which causes loss of soil fertility and contamination of ground water (Chidambarampadmavathy et al., 2017).

Incineration of plastic wastes contributes to rise the carbon footprint as a result producing dangerous compounds (dioxins, polychlorinated biphenyls and furans into the atmosphere; thus, leading to global warming (Verma et al., 2016; Chidambarampadmavathy et al., 2017).

Although, recycling process is a green choice to dispose plastic because of limiting adversely impact of disposal of plastic. But not all plastic materials can be recycled with the same and high quality due to alteration chemical properties of substance during recycling process (North and Halden, 2013; Chidambarampadmavathy et al., 2017; Kehinde et al., 2020).

2.3 Bioplastic

Recently, bioplastic (consists of many biodegradable substances or mixture of them with synthetic ones) has received great attention in order to cover the increase in demand as eco-friendly solution that decreases serious effects discharge of non-biodegradable plastic that made of polylactates, polyhydroxyalkanoates and other of aliphatic polyesters (Chidambarampadmavathy et al., 2017; Shamsuddin et al., 2017; Vimal and Kumar, 2019).

For instance, production of one kilogram of plant resin or viscous substance releases about 0.49 kg carbon dioxide. While, emission of more than triple emissions of CO_2 of its analogues from petroleum products. Hence, bioplastic is decreasing the incidence of global warming by 80`% (Arikan and Ozsoy, 2015).

Although, the bioplastic industry is facing a serious cost challenge compared with the traditional plastic industry, its prices may be diminished with rapid progress of its researches and development of its technologies and production methods as well as increased awareness about serious costs of disposal of plastic. Also, raising supply of bioplastic; will reduces the price problem, but, petro-plastic industry reached to the summit of efficiency with approximately stability of prices (Arikan and Ozsoy, 2015).

2.4 Edible films

The master function of primary food packaging process is to ensure the protection of quality and safety of products from the surrounding environment during transportation, distribution, storage and final consumption (Marsh and Bugusu, 2007).

In other words, it is essential for increasing shelf life of product. Nevertheless, there are the minor functions include marketing, traceability and providing information about product (Kerry, 2012).

The greatest quantity of plastic wastes is derived from food packaging wastes. Its degradation requires about 200 years. Consequently, edible films came as green alternative to save environment from non-biodegradable packaging wastes that pollute it (Pavlath and Orts, 2009; Kerry, 2012). Since 1967, wax-based coatings and edible films have been applied by small companies on different types of fruits to limit moisture loss and become brighter nowadays (Debeaufort et al., 1998; Pavlath and Orts, 2009; Galus et al., 2020). Edible films industry

significantly increased in 1996 to 600 companies and used in large scale for various food products with earnings more than $100 million per year (Galus et al., 2020).Recently, food technology researches have sought to develop these innovation wrappings (edible and biodegradable films) that contribute in protection of the eco-system from contamination that derived from plastic packaging wastes with simultaneously ensuring the fulfillment of its main purpose that is mentioned above. Also, it can act as transporters for active compounds such as antimicrobial and antioxidant compound (Berk, 2009; Kour et al., 2013; Han, 2014b; Umaraw and Verma, 2017).

Edible films mean any thin material that has been created from renewable polymers such as proteins, polysaccharides and lipids which are used for covering food products to increase shelf life. Generally, it is mainly characterized by edibility and biodegradability (Porta et al., 2016; Hammam, 2019).

More specifically, polysaccharides provide good barrier for oxygen and carbon dioxide gases; fats block water transmission, but protein produces edible films with distinctive mechanical properties (Bourtoom, 2008; Pavlath and Orts, 2009; Nesic and Seslija, 2017).

In general, protein and polysaccharides can be used to form edible film individually, because of its polymeric nature, but lipids lack this structure (Campos et al., 2011).

However, studies indicated that, blending of two or more polymers have markedly developed physical, mechanical and barrier properties of edible wrappers more than the only single biopolymer (The et al., 2009; Galus et al., 2020).

2.4.1 Production methods of edible films

In general, formation of edible films has been made by two common processes:

2.4.1.1 Dry process

Dry process includes plasticizing of biopolymers by plasticizers (e.g glycerol) which decreases the glass transition temperature and cohesion within the polymer. In consequence, heating plasticized polymer to high temperature (120 and 170 °C) then polymer exposed to high pressure (170 − 350 bar) as well as cooling to produce homogeneous and soft film (García et al., 2016; Suhag et al., 2020).

Extrusion Process is the one of the most popular dry process and can be commercially used for films and packages production due to its cost-effectiveness, energy-efficiency and reduced time requirement (García et al., 2016; Suhag et al., 2020).

2.4.1.2 Casting method

Casting method (wet technique) is used to create edible film from biodegradable materials in laboratory. This method includes many steps; dissolving biopolymer in an appropriate solvent, casting or pouring solution /suspension on flat surface like petri dish and drying the solution to evaporate solvent by air dryer to produce dried film that should have cohesion without any mechanical damage. Also, it is distinguished by its low cost, but it requires long time (Figure 2.3) (Suhag et al., 2020).

Commonly, temperature, relative humidity, thickness and structure of the casting solution are the major factors that have effect on properties of prepared film. So, the weak and fissure film is generated by formation film from only one polymer so it should add plasticizers; or drying at high temperature (rapid drying) that decreases intermolecular interactions which lead to limit movement of polymer molecules when the concentration of solvent is diminished that creating inconsistent and heterogeneous film (Campos et al., 2011; Skurtys et al., 2014; Galus et al., 2020; Suhag et al., 2020).

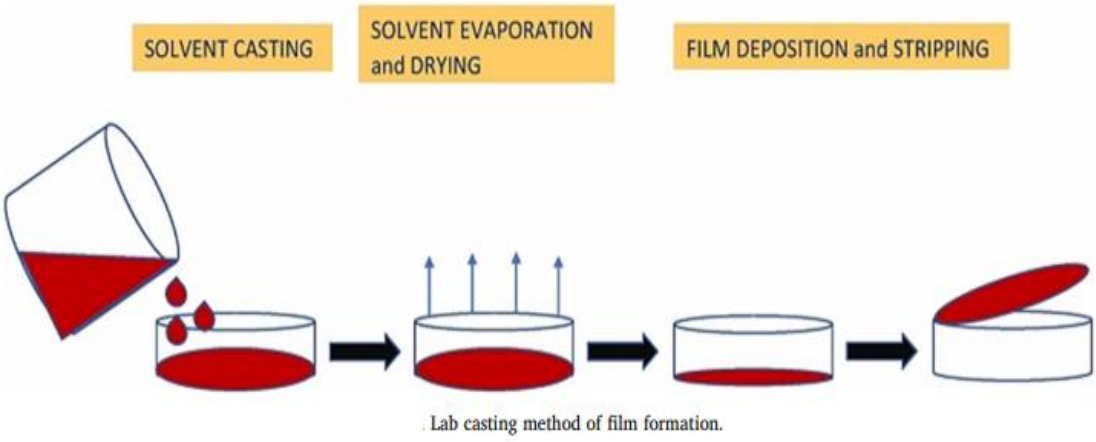


Figure 2.3. Lab casting method (Suhag et al., 2020).

However, to produce edible films with idealistic characteristics, it should have many features.

The features are safety, digestibility, non-allergic, mechanical stability, maintaining the moisture content of the product, preserving the nutritional and sensory properties (colour, taste and flavor), biochemical stability, protecting from microbial deterioration, controlling gases exchange and economically feasible(Pavlath and Orts, 2009; García et al., 2016).

2.5 Protein based edible films

Individual protein is made of many amino acid molecules that generates multi structures of proteins with various properties. Also, denaturation of protein with different methods such as heat, acid/base or solvent can be produced extended structures. As well as protein has a high potential to form various bonds and crosslinkes due to amino-acids reactive sites that including amine groups ($–NH_2$), carboxyl (–COOH) groups and sulfhydryl (–SH) groups. This properties of protein allow it to be suitable choice to produce edible films. Moreover, protein based edible film is effective for transport active compounds, restriction of (oxygen, carbon dioxide and ethylene) gases transportation, prevention dehydration of wrapped product and fertilization of soil because of its nitrogen content when it is degraded (Wittaya, 2012; Benbettaïeb et al., 2016; Chen et al., 2019).

Even though, the main undesirable feature of protein its permeability to humidity. But addition of other biopolymer (such as fat) can be a good solution for this limitation. Furthermore, protein based edible film has poor mechanical properties which can be solved by using plasticizer to increase elasticity through reducing intermolecular forces and improving movement of chain, enzymatic, chemical or physical treatments (Bourtoom, 2009; Wittaya, 2012; Benbettaïeb et al., 2016; Chen et al., 2019).

Many plant crops contain high protein content. For example, the percent of protein in soybeans (38% – 44%), sunflower seeds (28% – 42%), peas (22% – 28%) and cereal grains (8% – 15%) (Vimal and Kumar, 2019).

Particularly, *nigella sativa* (Black cumin) plant is a member of Ranunculaceae family; it is native plant in southern Europe and southeast and southwest Asia. Basically, black cumin ingredients are 35% fat (mainly unsaturated), 29% carbohydrates, 21% proteins, 6% crude fibers, 5% moisture, 4% ash as well as bio active compounds (Alanazi et al., 2016).

A significant amounts of by-products of nigella sativa is produced after oil extraction process; these residues are used to feed animals because of its high protein content. Recently, reports

proved that nigella can be used as defatted cake to extract protein in order to make protein based edible films for food wrapping (Sabbah et al., 2020).

Additionally, nigella sativa protein concentrates-based film characterizes with black color that can maintain coated food and drugs from degradation and damage in the presence of oxygen or/and (UV or artificial light) (Sabbah et al., 2020).

2.6 Polysaccharides based edible films

Polysaccharides are derived from plant, marine and microbial sources (starch, cellulose, pectin and their derivatives, pullulan, alginates and chitosan). In recent times, it has been widely studied for biodegradable food wrappings production (Sothornvit and Krochta, 2005; Nešić et al., 2020). The basic functions of polysaccharide based edible films are oxygen and carbon dioxide barrier because of strength of hydrogen bonds network, as well as reducing dehydration, darkening of the surface and oxidation of fat and acting as carriers for some organic acids to prevent growth of bad bacteria (*Listeria monocytogenes*, *Salmonella spp*., and *Escherichia coli*). Moreover, polysaccharide based edible films is considered as antioxidant and functional supplements to improve food products (Hassan et al., 2018; Kocira et al., 2021).

Nevertheless, strong water vapor permeability is the major disadvantage of polysaccharide polymers due to their high affinity for water and weak mechanical properties. Although, it could be controlled through blending with another polysaccharide, lipids and active components such as plasticizers and emulsifiers (Espitia et al., 2014; Cazón et al., 2017; Nešić et al., 2020).

Pectin (PEC) is mainly extracted from cell wall of apple pomace and citrus peels so it's easily obtainable. Pectin used in food technology as gelling, stabilizing, or thickening agents for improving the properties of products like yoghurt drinks and jams. Pectin is consisting of 1, 4-D-galacturonic acids with difference degree of esterification of methyl carboxyl groups(Figure 2.4) (Thakur et al., 1997).

Additionally, polysaccharides have been used for edible film formulation. It is considered as generally recognized as safe (GRAS) by the Food and Drugs Administration (FDA) (Espitia et al., 2014; Chakravartula et al., 2019; Mohamed et al., 2020).

PEC based edible film prevents dehydration because of its ability to absorb moisture, and providing distinctive mechanical properties (through many factors such as low pH or presence of other solutes or polyvalent cations such as calcium that allow gel-forming) and blocks transmission oil, aroma and oxygen. Fruits are susceptible to deterioration and spoilage during a very short period after harvesting, therefore, PEC has been used for fresh vegetables and fruits wrapping, such as apple, apricot, avocado, berries, guava, chestnuts, melon, peach, walnuts, papaya, tomato and carrot (Thakur et al., 1997; Espitia et al., 2014; Chakravartula et al., 2019; Al-Asmar et al., 2020; Mohamed et al., 2020).

Recently, Al-Asmar et al. (2020), observed that pectin-based films prepared with mesoporous silica nanoparticles and glycerol could increase shelf life of strawberries for 8 days at refrigerated temperature.

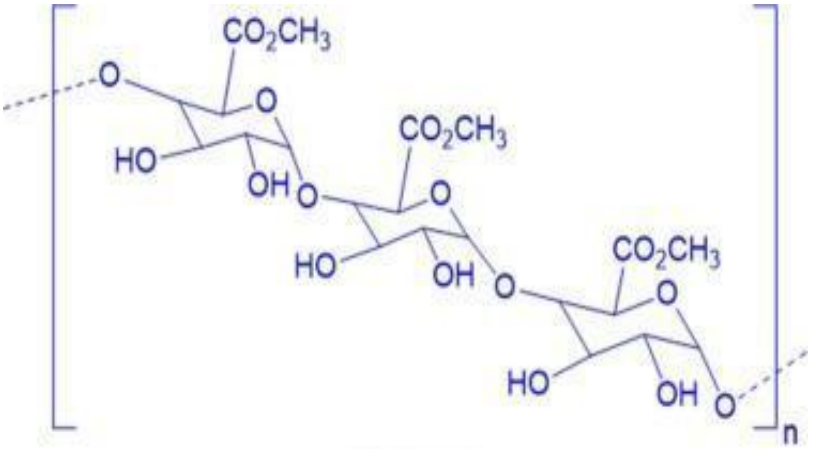


Figure 2.4. Chemical structure of pectin (Mohamed et al., 2020).

2.7 Plasticizers

Plasticizers are materials that widely used as industrial polymer additives, have low molecular weight and doesn't evaporate quickly. It could be able to decrease intermolecular forces (such as hydrogen bonding, van der Waals forces) among polymer chains due to low molecular weight by taking over intermolecular spaces. Consequently, energy for molecular motion is lowered. Commonly, it used to minimize rigidity of polymer chains and increase resistance of fracture in order to enhance its flexibility (Vieira et al., 2011; Epure et al., 2011).

Generally, both the following essential theories illustrate the effect of plasticizers in edible films and polymers are clearly shown in (Figure 2.5):

Firstly, gel theory suggested that the plasticizers break polymer-polymer attachments by getting in between the chains to allow polymer molecules movement easily. This makes gel structure more flexible and less hardness.

Secondly, free volume theory explained the glass transition temperature that measures polymer mobility can be diminished by plasticizers, which greatly increases an internal empty space of the polymer. Therefore, polymer transforms from rigid substance to elastic one (Sothornvit and Krochta, 2005; Foroughi-Dahr et al., 2017; Epure et al., 2011).

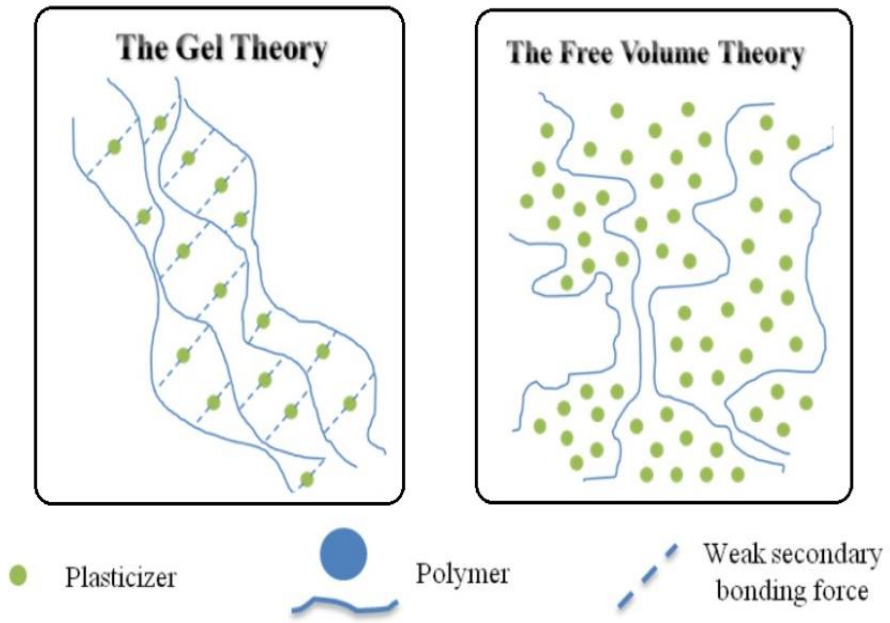


Figure 2.5. Mechanisms the main of theories of plasticizers (Bocqué et al., 2016).

Different kinds of plasticizers such as glycerol, sorbitol, propylene glycol or polyethylene glycol have significant role in increasing flexibility and modifying mechanical properties. But glycerol is considered the popular plasticizers because it is effective, cheap and abundant. However, the influence of plasticizers on physical, mechanical, thermal and barrier features of

films can be determined according to its type and concentration (Sothornvit and Krochta, 2005; Sanyang et al., 2015).

It has been proved that 30 w/w% glycerol give better mechanical properties compared with (15,20,30 and 40 w/w%) (Epure et al., 2011).

2.8 Transglutaminase enzyme (TGase)

Obviously, TGase enzyme is abundant in the environment; in many invertebrates, mammals, and microorganisms. However, microbial species (*Streptoverticillium cinnamoneum* subsp., *Streptoverticillium griseocarneum*, *Streptoverticillium ladakanum*, *Streptomyces netropsis*, and *Streptomyces lydicus*) can provide cheap, easy obtainable and stable TGase enzymes in order to develop biotechnology applications (Chambi and Grosso, 2006; Benbettaïeb et al., 2016; Vimal and Kumar, 2019).

Fortunately, TGase was classified by Food and Drug Administration (FDA) as "generally recognized as safe" (GRAS)) for human consumption. So, it can be used in food industry (Vimal and Kumar, 2019; Duarte et al., 2020). The optimal conditions to activate TGase are pH (5-8), and temperatures (55-70°C); where the most favorable temperature of enzyme is 55°C but its activity diminishing with time at this temperature (Vimal and Kumar, 2019; Duarte et al.,2020).

Sabbah et al. (2020), concluded that NSPC film containing 20% glycerol at pH 8 in presence of TGase showed more homogenous, resistant and flexible films after studying effects of different pH and glycerol concentrations.

Cross linkages can be created in protein for modification and improvement properties of protein based food products by various methods. The first method; chemical agents (including amoung others glutaraldehyde, glyceraldehyde, formaldehyde, glyoxal) but its harmful for humane consuming; it's not suitable for producing edible wrappers. The second method; physical agents (like UV- and c-irradiation). The third method; enzymes such as peroxidase, tyrosinase and TGases (Chambi and Grosso, 2006; Porta et al., 2011; Benbettaïeb et al.,, 2016; Vimal and Kumar, 2019).

Protein based films has a weak mechanical property due to its hydrophilic nature; but cross linkers can provide a solution to these problem (Chambi and Grosso, 2006; Porta et al.,, 2011).

In fact, TGase can introduce iso-peptide bonds between glutamine amino-acids (acyl donor) and Lysine amino-acids (acyl acceptor) to form intra- and inter-molecular crosslinks in the proteins sequences (as shown in Figure 2.6) provided that proteins give acyl donors and/or acceptor substrates of the enzyme. Thus, producing these cross linkages make films more resistance in addition to decrease free volume within films; this will significantly reduce transmission of gasses (oxygen and carbon dioxide) into films (Chambi and Grosso, 2006; Porta et al., 2011; Porta et al., 2016; Vimal and Kumar, 2019; Giosafatto et al., 2020). TGase cross linkages are commonly detected by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis through increasing molecular weight of protein (Benbettaïeb et al., 2016).

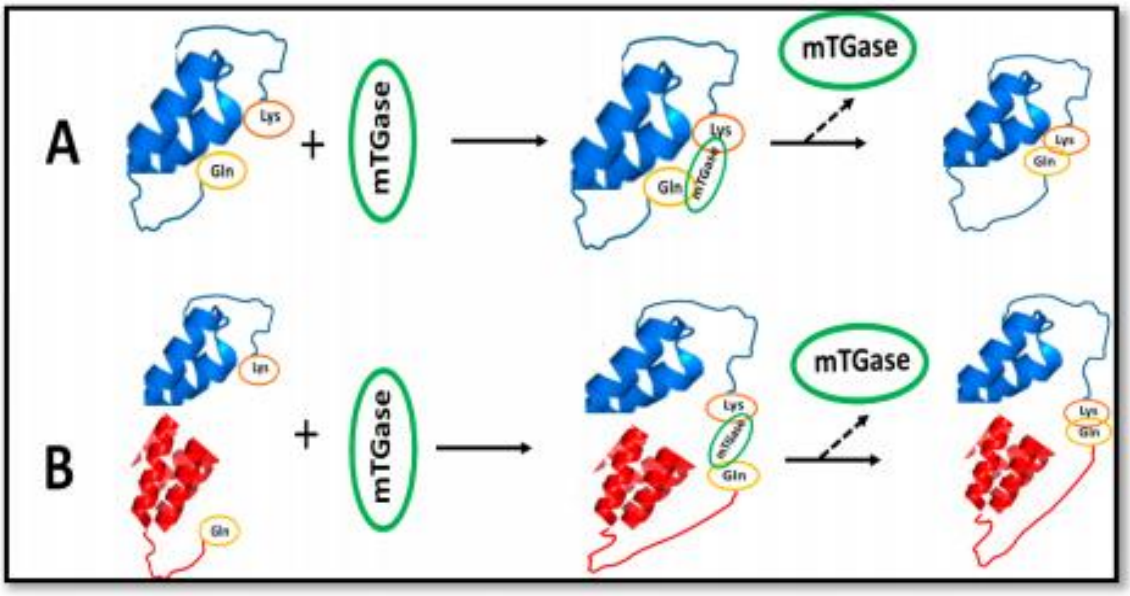


Figure 2.6. Intra-(A) and inter-(B) iso-peptide bond between glutamine and lysine created by TGase (Giosafatto et al., 2020).

It was reported that, the coated apple with whey protein/PEC/TGase edible film can reduce weight loss of apple fruit by 80% within 10 days during storage because it acts as barrier against water vapour and gasses (Marquez et al., 2017; Duarte et al., 2020).

Other investigators, reported that concentration of TGase enzymes (20 U/g) is an effective concentration to improve tensile strength (TS) property and barriers of water vapour and oxygen gas of soy protein films (Guocheng et al., 2007; Su et al., 2007; Benbettaïeb et al., 2016).

There are other applications for TGase, as known that frying process for carbohydrates rich food at high temperature creates acrylamide chemical that is considered serious and carcinogenic for human health. According to the (Al-Asmar et al., 2020), acrylamide content diminished in fried kobbah coated with grass pea flour modified TGase films in the presence of nanoparticles (Mesoporous silica nanoparticles or chitosan nanoparticles) 41.0% and 47.5%, respectively; through decreasing oil content and increasing water content inside the Kobbah by film that lead to low acrylamide formation .

2.9 Mechanical properties

Mechanical properties are the properties of a material after being subjected to a force (tension). Industrially, it should be studied to know the behavior of material such as (firmness and resistance during processing, shipping, and storage) when exposed to external forces (Foegeding and Drake, 2007; Younis and Zhao, 2019). The essential indicators that have a significant role in describing the mechanical behavior of substance are YM and TS (Farsi et al., 2017).

In detail, the ultimate strength that can cause breaking of substance is called TS. The below curve (stress-strain) shows two areas (elastic and plastic deformation areas). The elastic area means the material has still possessed original shape without any rupture after applying load. While plastic area is defined as fracture of material with loss of its primary shape when exposed to force, as shown in (Figure 2.7).

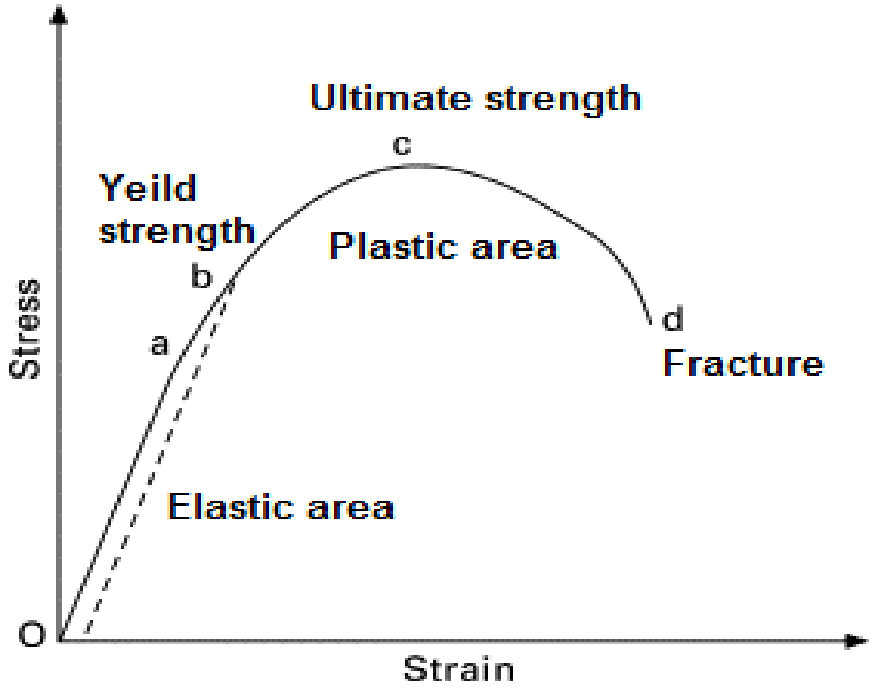


Figure 2.7. Curve of (stress-strain) (Maruyama, 2010).

In addition, TS is influenced by several factors including the chemical structures, microstructures, concentration and processing conditions (Maruyama, 2010; Chakravartula et al., 2019). However, the difference between the length of sample after conducting the TS test and the original length is known as EB property (Fan and Fu, 2016). Often, elevation TS of film accompanied by lowering EB property (Chakravartula et al., 2019). Also, there are many definitions for YM. The first one, it is the proportion between stress and strain, the second one, it is obtained from the slope of the strain–stress diagram for the matter, and the third one can measure the hardness of material (Bell, 2005; Azammi et al., 2020).

In general, edible films have owned lower TS compared to non-biodegradable films (plastic). But, EB for edible films are very much like petroleum films such as soy protein isolate/fatty acids/glycerol-based film has EB as its counterpart from popular plastic types low-density polyethylene (LDPE) and high-density polyethylene (HDPE) (Han, 2014a). Additionally, previous studies concluded that NSPC/GLy/TGase based films provide elastic, resistance and biodegradable packages (Sabbah et al., 2020).

Another research by Porta et al. (2016) indicated TGase has obvious effects on improvement

of edible film properties; double TS in the bitter vetch protein/pectin films but it's become treble in the presence of enzyme. Also, films containing TGase and pectin had elevated EB that forms more flexible films. Mariniello et al., 2007, found that fennel residues / phasolin / TGase has mechanical properties and water vapor permeability similar to the commercial films Ecoflex and Mater-Bi.

2.10 Biodegradation

Plastic has been classified as strength and more resistant material which leading to very difficult and slow degradation extending for decades or hundreds of years, especially in marine life where absence of oxygen and lower temperature considered helpers in decomposition process (Webb et al., 2013). On the other hand, biopolymers are naturally disassembled by microorganisms (bacteria, fungi, yeast, algae, and other organisms) to by-products residues such as carbon dioxide (CO_2), water (H_2O), ammonium (NH_4^+), nitrogen (N_2), and methane (CH_4)) that benefiting other living organisms (Folino et al., 2020).

Bio-deterioration is very useful for environment due to their short end of life cycle material and determining the fate of contaminants and their toxicity (Eskander and Saleh, 2017). In addition, the factors which have impact on biodegradation process and its rate are moisture, temperature, oxygen content, pH, time, UV radiation, hydrophobic or hydrophilic material structure, nutrient uptake by microorganisms and their availability (Gu, 2016; Isroi et al., 2018). Furthermore, increasing complexity of material needs more microorganisms to breakdown substance. Hence, biodegradability rate is low (Folino et al., 2020).

Aerobic biodegradation means that oxygen is consumed via microorganisms to convert organic matter into (CO_2), (H_2O) and accompanied by raising the numbers of microorganisms (Eskander and Saleh, 2017). Anaerobic biodegradation means the degradation of biological compounds with absence of oxygen, so microorganisms can be catalyzed through inorganic compounds such as (nitrate, sulfate, and iron) for breaking down organic material. The resulted byproducts of anaerobic biodegradation (nitrogen gas, hydrogen sulfide, and methane) rely on the inorganic chemical type (Joutey et al., 2013; Eskander and Saleh, 2017).

The common indicators of biodegradability of bioplastic include monitoring the changes in weight (reduction of weight) and morphology of the substance, measuring the emissions of (CO_2 and/or CH_4), and quantifying the biological oxygen demand or lowering total carbon (Di Bartolo et al.,, 2021). (Mariniello et al., 2007), conducted an experiment to develop renewable mulching films in agriculture by creation films from fennel residues with/without phaseolin in the absence and in the presence enzyme TGase. The findings elucidated that enzyme provide more resistance film after 21-day soil burial tests. Nonetheless, the ideal evidence to evaluate bio-decomposition and microbial activity has been proved estimating (CO_2 and/or CH_4) production or O_2 depletion because weight reduction is not constantly meaning decomposition polymer to monomers (Arcos-Hernandez et al., 2012; Tosin et al., 2012; Harrison et al., 2018; Folino et al., 2020).

2.11 Antimicrobial agents

Food is considered as attractive medium for growth of pathogenic and spoilage microorganisms, particularly *Listeria monocytogenes*, *Salmonella enteritidis*, and *Escherichia coli* O157:H7 which threatens consumer safety and reputation of food industries, and also their shelf life in the market (García et al., 2016; Huang et al., 2019). Recently, researchers have found the active packaging materials that can be used as innovative tool to enhance safety and quality of food products through limit harmful microorganisms (Huang, et al., 2019).

Active packaging materials also provide new and desirable function (Limbo and Khaneghah, 2015); it can control many processes to improve shelf life including overcoming on breathing of fresh fruit and vegetables, oxidation of fats and harmful microbiological growth by incorporation active materials such as antioxidant and antimicrobial agents (Wyrwa and Barska, 2017). However, antimicrobial edible films can be generated through using biodegradable polymer itself is an anti-microbial agent such as chitosan and/or incorporation natural antimicrobial agents like organic acids or bacteriocins (Campos et al., 2011; Kapetanakou et al., 2014; García et al., 2016).

As example, incorporation of oregano extract as antimicrobial agent (0.1-1%) into starch-chitosan film is an effective agent in inhibiting growth of *Bacillus Cereus* (Pelissari et al., 2009; García et al., 2016). Antimicrobial compounds within hydrocolloid films are liberated into food matrix for suppression or restriction growth of bacteria during storage period (Mastromatteo et al., 2009).

Hence, the choice of antimicrobial type is mainly dependent on the target microorganisms and their interactions with food components, as well as components of edible films, and ingredients of food product (Campos et al., 2011; Kapetanakou et al., 2014; García et al., 2016). Additionally, various factors control the effectiveness of antimicrobial activity in edible films including product properties, water activity, pH and fat content, surrounding conditions of the antimicrobial edible films and coatings, relative humidity, antimicrobial–hydrocolloid interactions, and structural changes due to the presence of antimicrobial (Mastromatteo et al., 2009; García et al., 2016).

2.12 Antioxidant agents

Natural antioxidants like essential oils, plant extracts, ascorbic acid and α-tocopherol delay the oxidation process of foods that causes damage, color changes due to enzymatic browning, and rancidity (Martín-Belloso et al., 2009; Senturk Parreidt et al., 2018). For example, previous research concluded that chitosan coatings with oleoresins can significantly inhibit browning reaction of cut-fresh fruit which reduces discoloration (Eça et al., 2014). However, the efficiency of antioxidant agent in films within storage time is related to the concentration of antioxidant in the film, light, relative humidity, and temperature of storage conditions (Lee et al., 2014).

Chapter Three: Materials and Methods

3.1 Study Overview

In this study, NSPC% was examined in order to estimate concentration of protein in stock solution. Furthermore, NSPC based films were prepared and blended with different concentrations of PEC, and evaluated in different ratios (40:0, 40:6, 40:10, 40:40w/w) in order to study its effect on mechanical properties of NSPC edible films. Moreover, the addition of TGase enzyme with two concentrations (10 U/g NSPC, 20 U/g NSPC) to determine its effect on NSPC/PEC based films. Finally, choosing film with highest TS and medium EB from prepared NSPC/PEC/TGase and was evaluated via many tests (biodegradability test, water content and water uptake of film) and were compared with other prepared film without the presence of TGase.

3.2 Materials

NSPC was provided by Al Hethnawi General Trade Co. (Jenin, Palestine). TGase (Activa WM), derived from *Streptoverticillium* sp., was purchased by Prodotti Gianni SpA (Milano, Italy). GLY (about 87%) was purchased from Merck Chemical Company (Darmstadt, Germany). Protease isolated from Bacillus Licheniformis (≥2.4 U g-1) and Low methoxyl pectin (Galacturonic acid ≥74.0 %, dried basis) were provided from Sigma-Aldrich, Co., 3050 (Denmark). All other chemicals and reagents were used of analytical-grade commercial products.

3.3 Extraction of protein and estimation of protein concentrate percentage

Extraction of PC from NS defatted seeds cake is prepared as previously described (Sabbah et al., 2020) with a few modifications. *Nigella Sativa* seeds were grinded by a household coffee grinder for 5 min and dissolved in distilled water (DW) (1:10, w/v), the pH was adjusted to 12 with 1 N NaOH to solubilize protein and stirred at medium speed for 2 hours at ambient temperature. Then the previous solution was centrifuged at 3800 rpm for 20 min, the supernatant was collected, and adjustment to pH 5.4 by 1 N HCl to produce a precipitate with centrifugation at 3800 rpm for 20 min. The pellet was evenly distributed on a plate made from tin paper and dried in the oven at 37℃. The PC percentage was determined by Kjeldahl

method (http://methods.aaccnet.org) using a nitrogen conversion factor of 6.25.

3.4 PEC preparation

Two PEC concentrations were prepared (1% and 2%) through dissolving PEC in DW until solubilization the entire of PEC solution (Porta et al., 2016).

3.5 TGase preparation

TGase was prepared according to Porta et al., 2016 with some modification by dissolving 0.1g of TGase in 1 ml DW and shacked by vortex for 2 min.

3.6 Stock NSPC preparation

4 g of NSPC powder were added to 100 ml of DW, under constant stirring and 1 N NaOH was used to adjust the pH to 12 up to complete protein solubilization, as previously described by Sabbah et al., 2020.

3.7 NSPC/PEC/GLY film preparation

Four kinds of FFS were produced to detect the effect of the concentration of PEC in NSPC film. NSPC FFS containing PEC in a ration of (40:0, w/w), (40:6, w/w), (40:10, w/w) and (40:40, w/w) respectively were selected from primary scenario of research containing NSPC : PEC FFS (40:0, 40:1, 40:2, 40:4, 40:6, 40:8, 40:10, 40:20, 40:30 and 40:40, w/w).

20 ml NSPC was added with PEC in different concentrations (0, 60, 100, 400) mg from 1% stock solution of PEC, while (40:40, w/w) film which prepared from 2% stock solution of PEC. The pH was adjusted to 7.5 by 1 N HCl and the previous solution was stirred for 30 min. 30% W/W protein GLY was added and the solution was stirred for another 30min. The films were incubated at 37℃ for 2 h in water bath

NSPC/PEC/GLY FFS were poured on polystyrene Petri dishes (8 mg proteins/cm^2) at 37°C overnight. Dried intact films were peeled from the casting surface and put at 25°C and 53% RH for 2 h, inside a desiccator that saturated with solution of $Mg(NO_3)_2 \cdot 6H_2O$, before being tested. All procedures were carried out according to Porta et al., 2016) with some modifications.

3.8 NSPC/PEC/GLY/TGase film preparation

20 ml NSPC was poured into a glass beaker. Then, PEC was added in different concentrations

(0, 60, 100, 400) mg from 1% stock solution of PEC, while (40:40, w/w) film was prepared from 2% stock solution of PEC. The pH of FFS was adjusted to 7.5 by 1 N HCl and the FFS was stirred for 30min. GLY (30% w/w protein) was added and FFS was stirred for 30min. TGase was added in two different concentration (10 and 20 U/g NSPC). The films were incubated at 37℃ for 2 h in water bath. FFS were poured on polystyrene Petri dishes (8 mg proteins/cm^2) at 37°C overnight. Dried intact films were peeled from the casting surface and put at 25°C and 53% RH for 2 h, inside a desiccator that saturated with a solution of Mg $(NO_3)_2 \cdot 6H_2O$, before being tested. All procedures were carried out according to Porta et al., 2016 with some modifications.

3.9 Film thickness

Film thickness was measured with a micrometer (non- digital micrometer, measuring range 0-25mm, its accuracy rated within ±0.0001 inch) at different positions for each film sample. At least five measurements were taken on each film sample and the thickness mean values were considered in the different tests.

3.10 Mechanical properties

Films TS, EB and YM were determined by using CT3 Texture Analyzer that was set at these parameters ((Test type (tension), test target (distance) with target value 100 mm, and general test parameters (Trigger load 5g with test speed 1 mm/s). Film samples strips (10−11 mm wide and 35 mm long), obtained by using a sharp scissors, were equilibrated for 2 h at 50% RH and 25°C in an environmental chamber containing Mg $(NO_3)_2 \cdot 6H_2O$ solution, and five samples of each film type were tested at least.

TS, EB, YM were calculated after recording thickness, load and extension for each samples according to IPC-TM-650 (2.4.18.3) (https://www.ipc.org), as the following:

$$TS = \frac{load\ at\ break}{(original\ width)(original\ thickness)} \qquad EB = \frac{(elongation\ at\ break)}{(initial\ gage\ length)}$$

$$YM = \frac{(load\ at\ point\ on\ tangent)}{\frac{(original\ width)(original\ thickness)}{\frac{(elongation\ at\ poit\ on\ tangent)}{(initial\ gage\ length)}}}$$

3.11 Moisture content

Analysis of moisture content was performed according to Giosafatto et al., (2019), with triplicate samples (1.5*1.5) cm of NSPC/PEC (40:40, w/w) films containing TGase (10U/g NSPC) and dried in hot air oven, at105°C , overnight, and calculated as

$$\text{Moisture content (\%)} = (W1 - W2)/W1 \times 100$$

Where, W1 initial weight of the sample, W2 sample weight after drying.

3.12 Water uptake

Moisture uptake of films were measured as previously described Giosafatto et al., (2019) , where triplicate samples of NSPC/PEC films containing TGase (40:40w/w, 10U/g NSPC) were weighed. Then, samples were dried at 105 °C for 24 h and placed it in desiccator at RH 50% (saturated solution of Mg $(NO_3)_2$ for other 24 h.

$$\text{Film moisture uptake (\%)} = (W3 - W2)/W3 \times 100$$

Where W2: weight of sample after drying, W3: weight of wet films.

3.13 Film biodegradation

Film biodegradation test was carried out as previously described (Qazanfarzadeh et al., 2021) with slight modifications. Each films sample (2 × 2 cm) were weighed (NSPC/PEC films containing TGase (40:40w/w, 10U/g NSPC) (W1) after overnight drying at 105°C and added to aqueous solution containing 600 mg protease that isolated from *Bacillus licheniformis* and 20ml buffer solution at pH 8. Then, put previous solution in a shaker at 23°C for overnight, finally weighing (W2) the samples after drying at 105°C another 24 h to calculate biodegradability of films as following

$$\text{Biodegradability of films \%} = (W1-W2)/W1 \times 100$$

3.14 Statistical Analysis

JMP software 10.0 (SAS Institute, Cary, NC, USA), two-way ANOVA, and the least means differences Tukey HSD were used. Differences were considered to be significant at $p < 0.05$.

Chapter Four: Results and Discussion

4.1 Blended NSPC films in the presence or absence of different concentration of TGase

The protein percentage was determined by Kjeldahl's method in the extracted NSPC, and was found 50% by using a nitrogen conversion factor of 6.25.

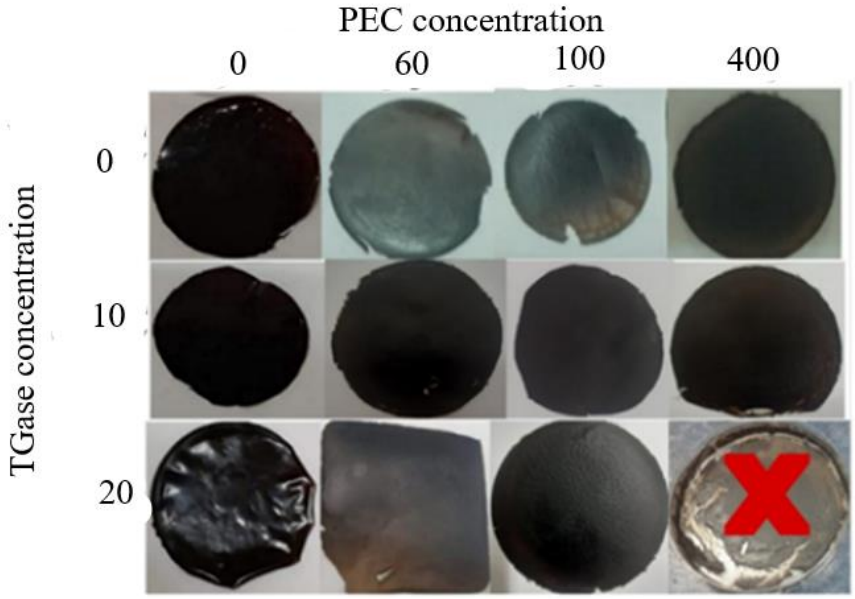


Figure 4.1. Images of NSPC and PEC were blended with different ration (40:0, 40:6, 40:10, 40:40w/w) and different concentrations of TGase (10, 20U/g NSPC) at pH 7.5.

Figure 4.1 presents images of films surface morphology that derived from blended NSPC solution with different concentration of PEC and different concentrations of TGase at pH 7.5. Generally, all produced films were homogenous except crosslinked NSPC/PEC (40:40 w/w) solutions with (20U/g NSPC) wasn't produce handleable and measurable film as shown and marked with red cross sign. But, NSPC films containing PEC in presence (10U/g NSPC) TGase provide more homogeneous distribution and smoother films. Furthermore, NSPC/PEC (40:40 w/w) films with/without TGase exhibit more compact matrixes of films. Finally, more brightness film was created by NSPC based film in presence of (20U/g NSPC) TGase.

4.2 Mechanical properties

Maintenance of edible films integrity and its resistance to environmental forces during food packaging application are achieved if it have a sufficient mechanical properties such as strength and flexibility (Masamba et al., 2016).

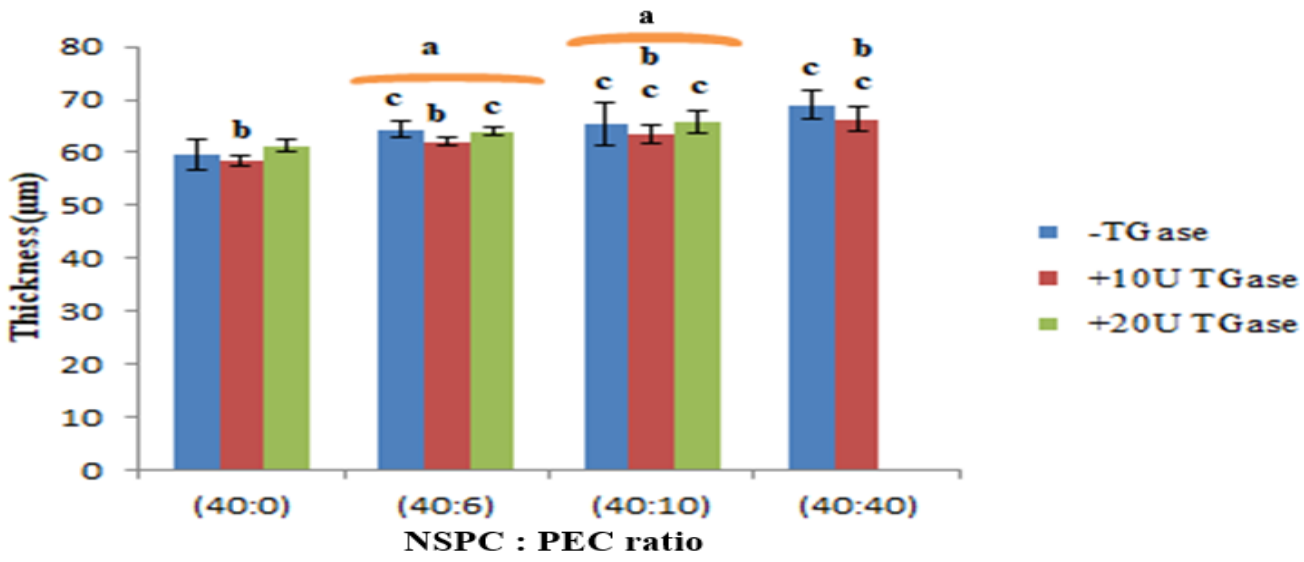


Figure 4.2. Effect of blended NSPC and PEC with different ratio (40:0, 40:6, 40:10, 40:40 w/w) and different concentrations of TGase (0, 10, 20U/g NSPC) on thickness of NSPC based films. According to pectin concentration, the values significantly different were reported by (a), whereas depending on TGase concentration, the values significantly different were indicated by (b). The values significantly different were reported by (c) in presence of both PEC and TGase at $p<0.05$.

Figure (4.2) shows that based on PEC concentration, the values of thickness are within range from (59.7±2.87 to 68.89±2.69 µm), the results explained that increasing PEC concentrations (0, 60, 100, 400 mg) in NSPC films produce thicker films due to addition more solids concentration (Mendes et al., 2020).

But the thickness property was significantly improved at 60 and 100 mg concentrations of PEC ($p<0.05$) in comparison with NSPC films alone. Lozano-Grande et al., 2016, previously observed high thickness values were generated by increasing concentrations of pectin. Similar findings were also concluded by Galus et al., (2012) higher content of pectin produces higher thickness values.

The presence of 10Ug/ NSPC TGase significantly reduces the thickness values (58.4±1.02, 62.11±0.74, 63.5±1.61, 66.29±2.25 µM) of blended NSPC/PEC films. While, no significant effect of 20U/g NSPC TGase on thickness of films.

These findings related to TGase concentrations similar to results obtained by Masamba et al., (2016) who found no significant effect of increasing TGase concentrations on improving thickness values of films. But, the results are contrary to results by other authors who reported

thickness of films was positively increased with increasing concentration of TGase because of high concentration of material Tang et al., (2011); Yayli et al., (2017). However, synergistic effects of the tertiary blends of NSPC/PEC (40:6, 40:10 w/w) /TGase (20U/g NSPC) have positive significantly impact on thickness (63.88±0.78, 65.78±2.04) respectively compared with NSPC films thickness (59.6 ± 2.87) ($p<0.05$).

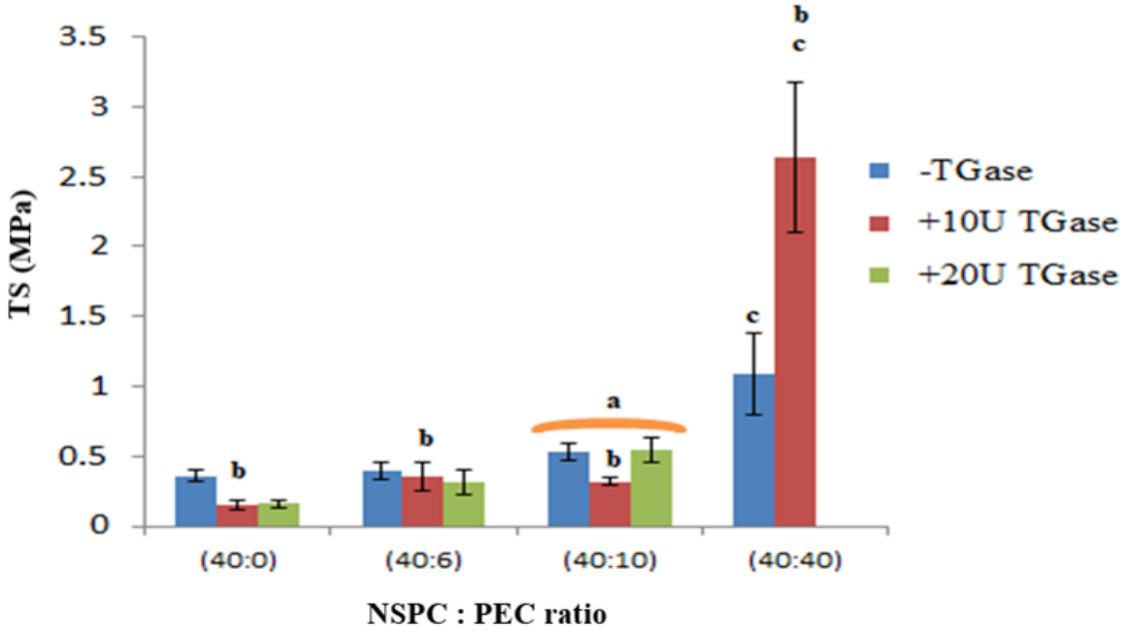


Figure 4.3. Effect of blended NSPC and PEC with different ratio (40:0, 40:6, 40:10, 40:40 w/w) and different concentrations of TGase (0, 10, 20U/g NSPC) on TS of NSPC based films.

According to pectin concentration, the values significantly different were reported by (a), whereas depending on TGase concentration, the values significantly different were indicated by (b). The values significantly different were reported by (c) in presence of both PEC and TGase at $p<0.05$.

Figure 4.3 showed that blended NSPC/PEC (40:10 w/w) films significantly enhance TS to about double TS of NSPC films from 0.22±0.04 to 0.464 ±0.06. Also, significantly increasing in TS of films in presence of (10U/g NSPC) TGase. But, no significant differences in the films containing (20U/g NSPC). These results correspond with results concluded by Tang et al., (2011); TS was improved at low concentration of TGase. Similar to these findings were reported by Yayli et al., (2017); Kołodziejska et al., (2004); Tang et al., 2011, that high concentration TGase increases cross-links that lead to reduce protein molecules mobility, hence provide unsuitable mechanical properties; this also shows in Figure 4.1 in NSPC/PEC

(40:40 w/w) film with (20U/g NSPC) TGase which is unusable that was marked with red cross sign. However, Yilmaz et al., (2020) previously concluded that increasing TS value (0.98–1.96 MPa) when increasing TGase concentration from 0 to 5 %.

Figure 4.3. additionally showed that crosslinked-NSPC/PEC (40:40 w/w) with 10U/g NSPC TGase markedly developed TS to about 7 times (2.64 MPa) compared to the NSPC films without PEC / TGase ($p<0.05$). As previously reported by Porta et al., (2016), the double TS was observed in prepared bitter vetch protein films with PEC because of catalyzing ionic bonds between bitter vetch protein and PEC, but in presence of TGase lead to the highest TS due to its formation of crosslinks.

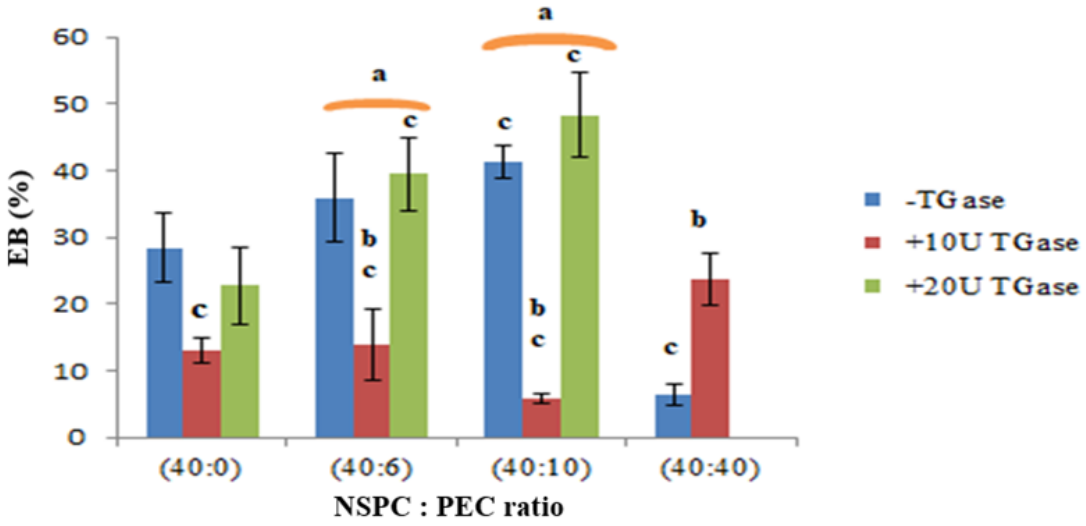


Figure 4.4. Effect of blended NSPC and PEC with different ratio (40:0, 40:6, 40:10, 40:40 w/w) and different concentrations of TGase (0, 10, 20U/g NSPC) on EB of NSPC based films. According to pectin concentration, the values significantly different were reported by (a), whereas depending on TGase concentration, the values significantly different were indicated by (b). The values significantly different were reported by (c) in presence of both PEC and TGase at $p<0.05$.

Figure 4.4 presented that the addition of PEC in NSPC based films may have a role in improving flexibility of films (29.7% and 31.8%) as shown at concentration of PEC (60 and 100 mg), respectively ($p<0.05$). But it was greatly reduced in presence of TGase (10U/g NSPC) (14.07%) to about half EB of films without TGase (28.0%). Conversely, 20U/g NSPC TGase- crosslinked NSPC/PEC complexes significantly contributes in increasing EBto about

3, 8 folds (39.5%, 48.36%) at concentrations of PEC 60, 100mg, respectively in comparison with NSPC films at different concentrations of PEC in presence (10U/g NSPC) TGase. In another words, crosslinked NSPC/PEC (40:10 w/w) films with 20U/g NSPC TGase provide more extensible films. On other hand, Tang et al., (2011) summarized that low concentration of TGase increased EB. This was illustrated through the following explanation that formation of low energy intermolecular interactions by low enzyme concentration between proteins, improving elasticity of films. On the contrary, . Yilmaz et al., (2020) reported that increasing TGase concentration from 0 to 5 % lead to reducing EB value (159.84–22.47 %).

In addition, Di Pierro et al., (2013) observed that film-based whey protein containing TGase significantly decreased in elongation at break, on account of creating covalent bonds among single whey protein polymers, but formation covalent bonds by TGase in film containing both whey protein/pectin; this improvement of EB referred to improved superamolecular structural network (large formula) that increases elasticity (EB) to about 6 folds compared with whey protein /PEC without TGase at pH 5.1.

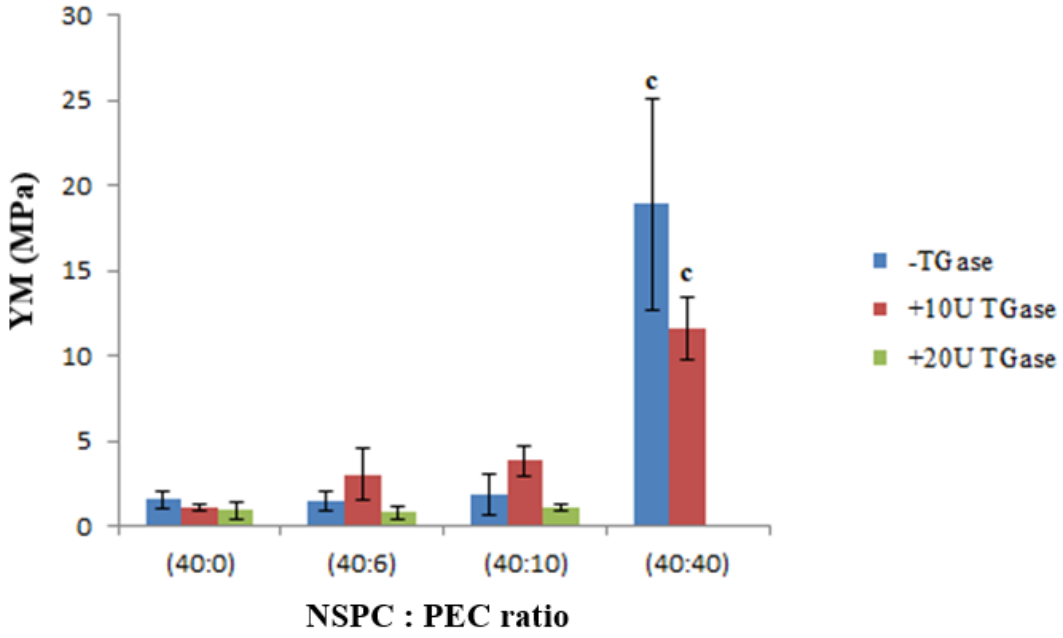


Figure 4.5. Effect of blended NSPC and PEC with different ratio (40:0, 40:6, 40:10, 40:40 w/w) and different concentrations of TGase (0, 10, 20U/g NSPC) on YM of NSPC based films. According to pectin concentration, the values significantly different were reported by (a), whereas depending on TGase concentration, the values significantly different were indicated by (b). The values significantly different were reported by (c) in presence of both PEC and TGase at $p<0.05$.

Figure 4.5 indicated that no significant differences in YM values observed with increasing concentrations of PEC and TGase. Nevertheless, prepared NSPC films with the addition both PEC (400 mg) and TGase (10U/g NSPC) provide significantly high YM and stiffness (11.58 MPa) in comparison with NSPC based films (1.5 MPa) ($p<0.05$). In the same time, NSPC films in presence of PEC (100 mg) and TGase (20U/g NSPC) exhibited significantly low value of YM (1.12 MPa) with high elasticity (48.36%) as shown in Figure 4.4 and 4.5 as compared to NSPC films containing PEC (400 mg) and TGase(10U/ NSPC) . In the previous study of Di Pierro et al., (2013), investigators concluded the possibility to produce films with low YM and stiffness by prepared whey protein /PEC with TGase (8U/g whey protein) films.

4.3 Water content

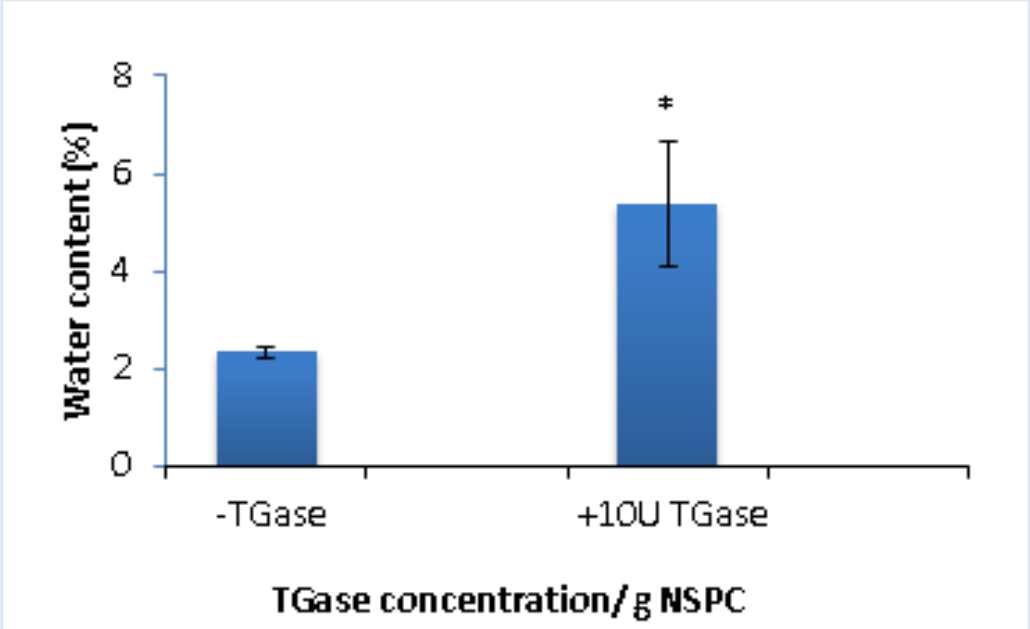


Figure 4.6. Effect of TGase (10U/g NSPC) on % water content of NSPC/PEC (40:40 w/w) based films. Values indicated by (*) was significantly different compared to the same film in the absence of TGase ($p \leq 0.05$).

Determination of behavior of edible package depend on its moisture content, because water can transfer across the film that have effect on shelf life and integrity of packaged food product and properties of film Othman et al., (2017); Yayli, et al. (2017); Masamba et al., (2016); Giosafatto et al., (2019).

The results shown in (Figure 4.6) reported that, prepared TGase-modified film at pH 7.5 could significantly increase ($p<0.05$) the water content of NSPC/PEC (40:40 w/w) 5.40% ± 1.28. In contrast, water content of film without TGase is 2.35% ± 0.11. These results are corresponding with Masamba et al., (2016) who demonstrated enzymatic treatment increased moisture content of edible films. Despite that, incorporating TGase into zein (protein found in maize) films and red bean protein films decreased moisture content was reported by Tang et al., (2011); Yayli et al., (2017). Generally, values of moisture content of films depend on various factors such as the type of solvents, type of proteins and enzyme concentrations (Masamba et al., 2016). However, several researchers concluded that the introduction of hydrophobic materials like oils decreases moisture content because of increasing hydrophobic chains in the polymeric matrix (Masamba et al., 2016; Giosafatto et al., 2019)

4.4 Water uptake

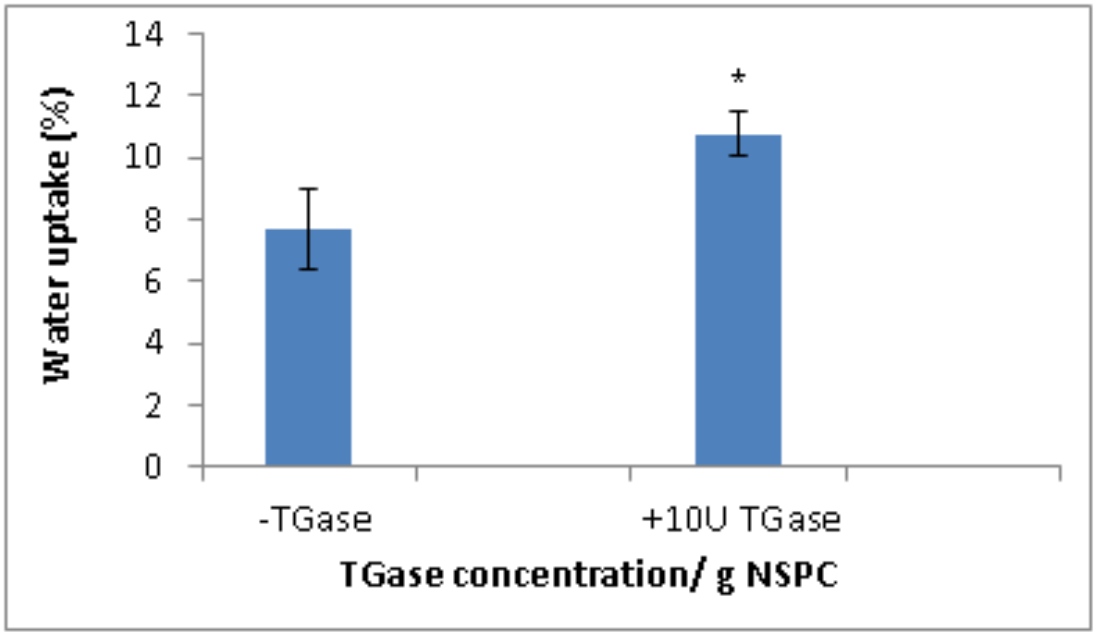


Figure 4.7. Effect of TGase (10U/g NSPC) on % water uptake of NSPC/ PEC(40:40 w/ w) based films. Values indicated by (*) was significantly different compared to the same film in the absence of TGase ($p \leq 0.05$).

One of the most important features of edible films is water uptake or water solubility. In fact, film's morphology and its hydrophilic nature are factors control water uptake Giosafatto et al., (2019). As illustrated in Figure 4.7; the observations explain the effect of TGase (10U/g

NSPC) on water uptake of NSPC/PEC (40:40 w/w) based films in desiccator at RH 50% (saturated solution of Mg $(NO_3)_2$ during 24 hours. The water uptake of NSPC/PEC (40:40 w/w) films in the presence of (10U/g NSPC) reached to 10.77% ± 0.74. But, in the absence of TGase, the absorption of moisture was 7.68% ± 1.28 only. Therefore, enzyme modification (10U/g NSPC) has significantly impact ($p<0.05$) on increasing the water uptake by films. In our opinion, some applications require edible films with high water uptake because it may help reducing moisture in dry foods and maintain its quality. However, it needs more research. Conversely, Yayli et al., (2017) reported water uptake was diminished by TGase modification into mechanically deboned chicken meat proteins-based films because of enzyme treatment creates covalent bonds and cross-linking that lead to increase molecular weight of polymer in films; hence reducing water uptake.

4.5 Biodegradability

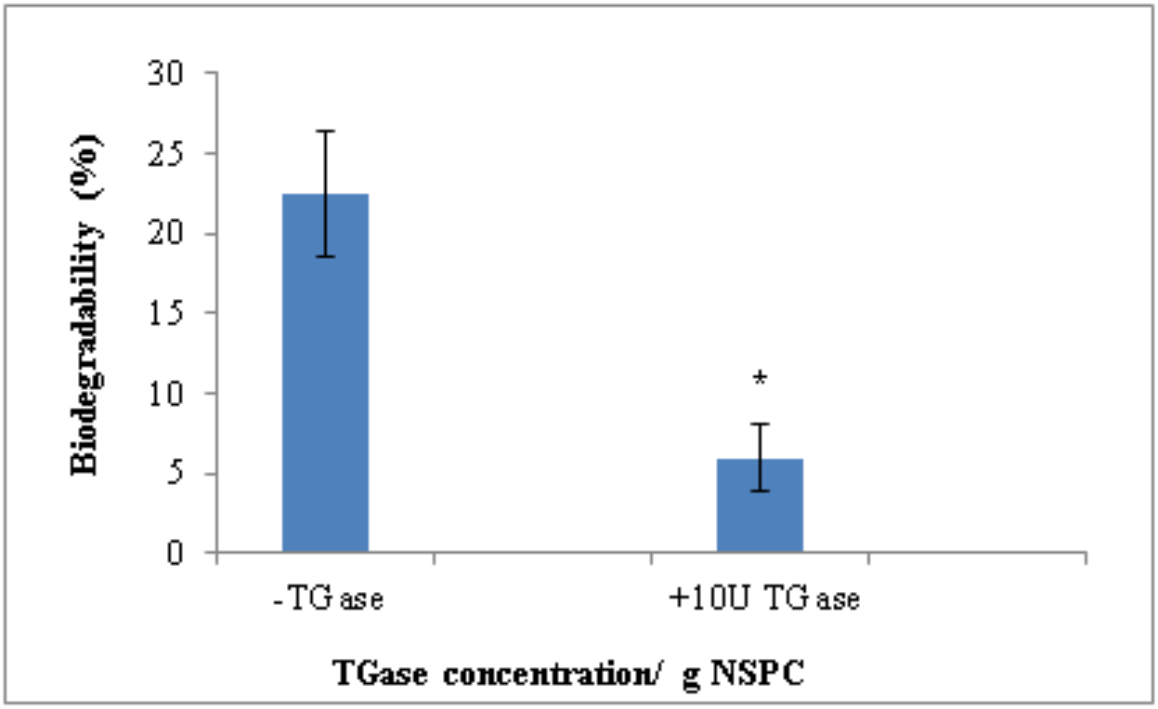


Figure 4.8. Effect of TGase (10U/g NSPC) on % biodegradability of NSPC/PEC(40:40 w/w) based films. Values indicated by (*) was significantly different compared to the same film in the absence of TGase ($p \leq 0.05$).

Petroleum plastic is characterized by its non-biodegradable in soil and toxic, thus it is

considered a dangerous threat to the ecosystem. On the other hand, edible films are prepared by natural and biodegradable materials. So, it is an eco-friendly. However, edible films are required to be more stable and enduring during its using and storage. After that, it can biodegrade effectively. Therefore, researchers found that cross linkage into protein based films matrix; it may reduce rate of biodegradability by increasing its molecular weight Li and Chen, (2000), Issifu and Sumaila, (2020).

Figure. 4.8., showed results of biodegradability test that carried out in presence of protease isolated from *Bacillus Licheniformis* at 25℃ and pH 8. The biodegradability of NSPC/PEC (40:40 w/w) in presence of (10U/g NSPC) TGase was 5.93% ± 2.07 whereas without TGase was 22.48% ± 3.88 after 24 hours. The modification of film by 10U/g NSPC TGase significantly ($p<0.05$) reduces the rate of biodegradability and increases its resistance to the enzymatic test, because, TGase created crosslinks. This is similar to Massardier-Nageotte et al.,(2006), Folino et al., (2020) reported that increased complexity of polymer requires more microorganisms to degrade it. So, biodegradability rate is low.

Recently, Qazanfarzadeh et al., (2021) have reported films based secalin (major protein of the rye grain) lose about 95% of initial weight, whereas secalin/zein films degraded by 89%. Hence, the films containing zein are more strength and standing against enzymatic test**.**

Chapter Five: Conclusions and Recommendations

The findings of study indicated that the different concentrations of both the pectin and/or TGase have significantly effect on improving mechanical properties on NSPC based films. Crosslinked NSPC/PEC (40:40 w/w) with low TGase concentration generates films with high tensile strength values significantly with the control film. However, crosslinked NSPC/PEC with high TGase concentration forms films with high elongation at break values except high concentration of pectin. Moreover, low concentration of enzyme increases water content and uptake of films and also, it decreases biodegradability rate that means film more resistance. Promising blended edible films from low price materials for food packaging was successfully obtained in the presence of TGase enzyme.

Recommendations

- Based on results and conclusions, the researchers have recommended applying NSPC/PEC (40:40 w/w) based edible films with low concentration of TGase on food product to evaluate its effect on shelf life and food quality.
- Studying the effect of different concentration of glycerol on NSPC/PEC (40:40 w/w) with low concentration of TGase properties.

References

Abdalrazeq, M., Giosafatto, C. V. L., Esposito, M., Fenderico, M., Di Pierro, P., & Porta, R. (2019). Glycerol-plasticized films obtained from whey proteins denatured at alkaline pH. *Coatings, 9*(5), 322.

Aguirre-Joya, J. A., De Leon-Zapata, M. A., Alvarez-Perez, O. B., Torres-León, C., Nieto-Oropeza, D. E., Ventura-Sobrevilla, J. M., . . . Ramos-Aguiñaga, M. E. (2018). Basic and applied concepts of edible packaging for foods. In *Food packaging and preservation* (pp. 1-61): Elsevier.

Al-Asmar, A., Giosafatto, C. V. L., Sabbah, M., & Mariniello, L. (2020). Hydrocolloid-based coatings with nanoparticles and transglutaminase crosslinker as innovative strategy to produce healthier fried kobbah. *Foods, 9*(6), 698.

Al-Asmar, A., Giosafatto, C. V. L., Sabbah, M., Sanchez, A., Villalonga Santana, R., & Mariniello, L. (2020). Effect of mesoporous silica nanoparticles on the physicochemical properties of pectin packaging material for strawberry wrapping. *Nanomaterials, 10*(1), 52.

Alabi, O., Ologbonjaye, K., Awosolu, O., & Alalade, O. (2019). Public and environmental health effects of plastic wastes disposal: a Review. *J Toxicol Risk Assess, 5*, 021.

Alanazi, I. O., Benabdelkamel, H., Alfadda, A. A., AlYahya, S. A., Alghamdi, W. M., Aljohi, H. A., . . . Masood, A. (2016). Proteomic analysis of the protein expression profile in the mature Nigella sativa (black seed). *Applied biochemistry and biotechnology, 179*(7), 1184-1201.

Arcos-Hernandez, Monica V., Laycock, Bronwyn, Pratt, Steven, Donose, Bogdan C., Nikolić, Melissa A. L., Luckman, Paul, Werker, Alan, and Lant, Paul A (2012). Biodegradation in a soil environment of activated sludge derived polyhydroxyalkanoate (PHBV). *Polymer degradation and stability, 97*(11), 2301-2312.

Arikan, E. B., & Ozsoy, H. D. (2015). A review: investigation of bioplastics. *J. Civ. Eng. Arch, 9*, 188-192.

Azammi, A. N., Ilyas, R., Sapuan, S., Ibrahim, R., Atikah, M., Asrofi, M., & Atiqah, A. (2020). Characterization studies of biopolymeric matrix and cellulose fibres based composites related to functionalized fibre-matrix interface. In *Interfaces in Particle*

and Fibre Reinforced Composites (pp. 29-93): Elsevier.

Bell, F. (2005). ENGINEERING GEOLOGY| Problematic Soils.

Benbettaïeb, N., Gay, J. P., Karbowiak, T., & Debeaufort, F. (2016). Tuning the functional properties of polysaccharide–protein bio-based edible films by chemical, enzymatic, and physical cross-linking. *Comprehensive Reviews in Food Science and Food Safety, 15*(4), 739-752.

Berk, Z. (2009). *Food process engineering and technology* (0123736609). Retrieved from

Bocqué, M., Voirin, C., Lapinte, V., Caillol, S., & Robin, J. J. (2016). Petro-based and bio-based plasticizers: Chemical structures to plasticizing properties. *Journal of Polymer Science Part A: Polymer Chemistry, 54*(1), 11-33.

Bourtoom, T. (2008). Edible films and coatings: characteristics and properties. *International food research journal, 15*(3), 237-248.

Bourtoom, T. (2009). Edible protein films: properties enhancement. *International Food Research Journal, 16*(1), 1-9.

Campos, C. A., Gerschenson, L. N., & Flores, S. K. (2011). Development of edible films and coatings with antimicrobial activity. *Food and bioprocess technology, 4*(6), 849-875.

Cazón, P., Velazquez, G., Ramírez, J. A., & Vázquez, M. (2017). Polysaccharide-based films and coatings for food packaging: A review. *Food Hydrocolloids, 68*, 136-148.

Chakravartula, S. S. N., Soccio, M., Lotti, N., Balestra, F., Dalla Rosa, M., & Siracusa, V. (2019). Characterization of composite edible films based on pectin/alginate/whey protein concentrate. *Materials, 12*(15), 2454.

Chambi, H., & Grosso, C. (2006). Edible films produced with gelatin and casein cross-linked with transglutaminase. *Food Research International, 39*(4), 458-466.

Chen, H., Wang, J., Cheng, Y., Wang, C., Liu, H., Bian, H., . . . Han, W. (2019). Application of protein-based films and coatings for food packaging: A Review. *Polymers, 11*(12), 2039.

Chidambarampadmavathy, K., Karthikeyan, O. P., & Heimann, K. (2017). Sustainable bio-plastic production through landfill methane recycling. *Renewable and Sustainable Energy Reviews, 71*, 555-562.

Corbari, L., Maltese, A., Capodici, F., Mangano, M., Sarà, G., & Ciraolo, G. (2020). Indoor spectroradiometric characterization of plastic litters commonly polluting the

Mediterranean Sea: toward the application of multispectral imagery. *Scientific Reports, 10*(1), 1-12.

Debeaufort, F., Quezada-Gallo, J.-A., & Voilley, A. (1998). Edible films and coatings: tomorrow's packagings: a review. *Critical Reviews in food science, 38*(4), 299-313.

Di Bartolo, A., Infurna, G., & Dintcheva, N. T. (2021). A review of bioplastics and their adoption in the circular economy. *Polymers, 13*(8), 1229.

Di Pierro, P., Rossi Marquez, G., Mariniello, L., Sorrentino, A., Villalonga, R., & Porta, R. (2013). Effect of transglutaminase on the mechanical and barrier properties of whey protein/pectin films prepared at complexation pH. *Journal of agricultural and food chemistry, 61*(19), 4593-4598.

Duarte, L., Matte, C. R., Bizarro, C. V., & Ayub, M. A. Z. (2020). Review transglutaminases: part II—industrial applications in food, biotechnology, textiles and leather products. *World Journal of Microbiology and Biotechnology, 36*(1), 1-20.

Eça, K. S., Sartori, T., & Menegalli, F. C. (2014). Films and edible coatings containing antioxidants-a review. *Brazilian Journal of Food Technology, 17*(2), 98-112.

Epure, V., Griffon, M., Pollet, E., & Avérous, L. (2011). Structure and properties of glycerol-plasticized chitosan obtained by mechanical kneading. *Carbohydrate Polymers, 83*(2), 947-952.

Espitia, P. J. P., Du, W.-X., de Jesús Avena-Bustillos, R., Soares, N. d. F. F., & McHugh, T. H. (2014). Edible films from pectin: Physical-mechanical and antimicrobial properties-A review. *Food Hydrocolloids, 35*, 287-296.

Fan, M., & Fu, F. (2016). *Advanced high strength natural fibre composites in construction*: Woodhead Publishing.

Farsi, A., Pullen, A., Latham, J., Bowen, J., Carlsson, M., Stitt, E., & Marigo, M. (2017). Full deflection profile calculation and Young's modulus optimisation for engineered high performance materials. *Scientific reports, 7*, 46190.

Ferreira, A. R., Alves, V. D., & Coelhoso, I. M. (2016). Polysaccharide-based membranes in food packaging applications. *Membranes, 6*(2), 22.

Foegeding, E., & Drake, M. (2007). Invited review: Sensory and mechanical properties of cheese texture. *Journal of dairy science, 90*(4), 1611-1624.

Folino, A., Karageorgiou, A., Calabrò, P. S., & Komilis, D. (2020). Biodegradation of wasted

bioplastics in natural and industrial environments: A Review. *Sustainability, 12*(15), 6030.

Foroughi-Dahr, M., Mostoufi, N., Sotudeh-Gharebagh, R., & Chaouki, J. (2017). Particle coating in fluidized beds. In *Reference module in chemistry, molecular sciences and chemical engineering*: Elsevier.

Galloway, T., Haward, M., Mason, S., Babayemi, J., Hardesty, B., Krause, S., Horton, A. (2020). Science-based solutions to plastic pollution. *One Earth, 2*, 5-7.

Galus, S., Arik Kibar, E. A., Gniewosz, M., & Kraśniewska, K. (2020). Novel materials in the preparation of edible films and coatings—A review. *Coatings, 10*(7), 674.

Galus, S., Turska, A., & Lenart, A. (2012). Sorption and wetting properties of pectin edible films. *Czech Journal of Food Sciences, 30*(5), 446-455.

García, M. P. M., Gómez-Guillén, M. C., López-Caballero, M. E., & Barbosa-Cánovas, G. V. (2016). *Edible films and coatings: fundamentals and applications*: Crc Press.

Giosafatto, C. V. L., Fusco, A., Al-Asmar, A., & Mariniello, L. (2020). Microbial transglutaminase as a tool to improve the features of hydrocolloid-based bioplastics. *International Journal of Molecular Sciences, 21*(10), 3656.

Giosafatto, C. V. L., Sabbah, M., Al-Asmar, A., Esposito, M., Sanchez, A., Villalonga Santana, R., . . . Porta, R. (2019). Effect of mesoporous silica nanoparticles on glycerol-plasticized anionic and cationic polysaccharide edible films. *Coatings, 9*(3), 172.

Gómez, E. F., & Michel, F. C. (2013). Biodegradability of conventional and bio-based plastics and natural fiber composites during composting, anaerobic digestion and long-term soil incubation. *Polymer Degradation and Stability, 98*(12), 2583-2591.Gu, J.-D. (2016). *Applied Environmental Biotechnology, 1*(1), 92-95.

Hammam, A. R. (2019). Technological, applications, and characteristics of edible films and coatings: A review. *SN Applied Sciences, 1*(6), 1-11.

Han, J. H. (2014a). Edible films and coatings: a review. *Innovations in food packaging*, 213-255.

Han, J. H. (2014b). A review of food packaging technologies and innovations. In *Innovations in food packaging* (pp. 3-12): Elsevier.

Harrison, J. P., Boardman, C., O'Callaghan, K., Delort, A.-M., & Song, J. (2018).

Biodegradability standards for carrier bags and plastic films in aquatic environments: a critical review. *Royal Society open science, 5*(5), 171792.

Hassan, B., Chatha, S. A. S., Hussain, A. I., Zia, K. M., & Akhtar, N. (2018). Recent advances on polysaccharides, lipids and protein based edible films and coatings: A review. *International Journal of Biological Macromolecules, 109*, 1095-1107.

He, P., Chen, L., Shao, L., Zhang, H., & Lü, F. (2019). Municipal solid waste (MSW) landfill: A source of microplastics?-Evidence of microplastics in landfill leachate. *Water research, 159*, 38-45.

Heck, T., Faccio, G., Richter, M., & Thöny-Meyer, L. (2013). Enzyme-catalyzed protein crosslinking. *Applied microbiology and biotechnology, 97*(2), 461-475.

Hidayah, N. (2018). *A review on landfill management in the utilization of plastic waste as an alternative fuel.* Paper presented at the E3S Web of Conferences.

Hopewell, J., Dvorak, R., & Kosior, E. (2009). Plastics recycling: challenges and opportunities. *Philosophical Transactions of the Royal Society B: Biological Sciences, 364*(1526), 2115-2126.

Huang, T., Qian, Y., Wei, J., & Zhou, C. (2019). Polymeric antimicrobial food packaging and its applications. *Polymers, 11*(3), 560.

Ilyas, M., Ahmad, W., Khan, H., Yousaf, S., Khan, K., & Nazir, S. (2018). Plastic waste as a significant threat to environment–a systematic literature review. *Reviews on environmental health, 33*(4), 383-406.

Isroi, G. S., Eris, D. D., & Cahyaningtyas, A. A. (2018). Biodegradability of cassava edible bioplastics in landfill and plantation soil. *Kimia dan Kemasan, 40*(2), 129-140.

Issifu, I., & Sumaila, U. R. (2020). A review of the production, recycling and management of marine plastic pollution. *Journal of Marine Science and Engineering, 8*(11), 945.

Joutey, N. T., Bahafid, W., Sayel, H., & El Ghachtouli, N. (2013). Biodegradation: involved microorganisms and genetically engineered microorganisms. *Biodegradation-life of science*, 289-320.

Kapetanakou, A. E., Manios, S. G., & Skandamis, P. N. (2014). Application of edible films and coatings on food. *Novel Food Preservation and Microbial Assessment Techniques*, 237-273.

Kehinde, O., Ramonu, O., Babaremu, K., & Justin, L. (2020). Plastic wastes: environmental

hazard and instrument for wealth creation in Nigeria. *Heliyon, 6*(10), e05131.

Kerry, J. P. (2012). *Advances in meat, poultry and seafood packaging*: Elsevier.

Koçaker, B. (2003). *Product properties prediction after forming process sequence.* METU.

Kocira, A., Kozłowicz, K., Panasiewicz, K., Staniak, M., Szpunar-Krok, E., & Hortyńska, P. (2021). Polysaccharides as edible films and coatings: characteristics and influence on fruit and vegetable quality—A Review. *Agronomy, 11*(5), 813.

Kołodziejska, I., Kaczorowski, K., Piotrowska, B., & Sadowska, M. (2004). Modification of the properties of gelatin from skins of Baltic cod (Gadus morhua) with transglutaminase. *Food Chemistry, 86*(2), 203-209.

Kumar, S., & Thakur, K. (2017). Bioplastics-classification, production and their potential food applications. *Journal of Hill Agriculture, 8*(2), 118-129.

Lee, Y. S., Shin, H. S., Han, J. K., Lee, M., & Giacin, J. R. (2004). Effectiveness of antioxidant-impregnated film in retarding lipid oxidation. *Journal of the Science of Food and Agriculture, 84*(9), 993-1000.

Li, J., & Chen, H. (2000). Biodegradation of whey protein-based edible films. *Journal of Polymers and the Environment, 8*(3), 135-143.

Limbo, S., & Khaneghah, A. M. (2015). Active packaging of foods and its combination with electron beam processing *Electron Beam Pasteurization and Complementary Food Processing Technologies* (pp. 195-217): Elsevier.

Lozano-Grande, M. A., Valle-Guadarrama, S., Aguirre-Mandujano, E., Lobato-Calleros, C. S., & Huelitl-Palacios, F. (2016). Films based on hawthorn (*Crataegus spp.*) fruit pectin and candelilla wax emulsions: characterization and application on *Pleurotus ostreatus*. *Agrociencia, 50*(7), 849-866.

Mariniello, L., Giosafatto, C., Moschetti, G., Aponte, M., Masi, P., Sorrentino, A., & Porta, R. (2007). Fennel waste-based films suitable for protecting cultivations. *Biomacromolecules, 8*(10), 3008-3014.

Marquez, G. R., Di Pierro, P., Mariniello, L., Esposito, M., Giosafatto, C. V., & Porta, R. (2017). Fresh-cut fruit and vegetable coatings by transglutaminase-crosslinked whey protein/pectin edible films. *LWT, 75*, 124-130.

Martín-Belloso, O., Rojas-Graü, M. A., & Soliva-Fortuny, R. (2009). Delivery of flavor and active ingredients using edible films and coatings *Edible films and coatings for food*

applications (pp. 295-313): Springer.

Marsh, K., & Bugusu, B. (2007). Food packaging—roles, materials, and environmental issues. *Journal of food science, 72*(3), R39-R55.

Maruyama, N. (2010). Mechanical testing of metallic biomaterials. In *Metals for Biomedical Devices* (pp. 157-177): Elsevier.

Massardier-Nageotte, V., Pestre, C., Cruard-Pradet, T., & Bayard, R. (2006). Aerobic and anaerobic biodegradability of polymer films and physico-chemical characterization. *Polymer Degradation and Stability, 91*(3), 620-627.

Masamba, K., Li, Y., Hategekimana, J., Zehadi, M., Ma, J., & Zhong, F. (2016). Evaluation of mechanical and water barrier properties of transglutaminase cross-linked zein films incorporated with oleic acid. *International Journal of Food Science & Technology, 51*(5), 1159-1167.

Mastromatteo, M., Barbuzzi, G., Conte, A., & Del Nobile, M. (2009). Controlled release of thymol from zein based film. *Innovative Food Science & Emerging Technologies, 10*(2), 222-227.

Mendes, J., Norcino, L., Manrich, A., Pinheiro, A., Oliveira, J., & Mattoso, L. (2020). Characterization of pectin films integrated with cocoa butter by continuous casting: physical, thermal and barrier properties. *Journal of Polymers and the Environment, 28*, 2905-2917.

Mohamed, S. A., El-Sakhawy, M., & El-Sakhawy, M. A.-M. (2020). Polysaccharides, protein and lipid-based natural edible films in food packaging: A review. *Carbohydrate Polymers*, 116178.

Napper, I. E., & Thompson, R. C. (2020). Plastic debris in the marine environment: History and future challenges. *Global Challenges, 4*(6), 1900081.

Nešić, A., Cabrera-Barjas, G., Dimitrijević-Branković, S., Davidović, S., Radovanović, N., & Delattre, C. (2020). Prospect of polysaccharide-based materials as advanced food packaging. *Molecules, 25*(1), 135.

Nesic, A. R., & Seslija, S. I. (2017). The influence of nanofillers on physical–chemical properties of polysaccharide-based film intended for food packaging. In *Food packaging* (pp. 637-697): Elsevier.

North, E. J., & Halden, R. U. (2013). Plastics and environmental health: the road ahead. *Reviews on environmental health, 28*(1), 1-8.

Othman, S. H., Edwal, S. A. M., Risyon, N. P., Basha, R. K., & A TALIB, R. (2017). Water sorption and water permeability properties of edible film made from potato peel waste. *Food Science and Technology, 37*, 63-70.

Pavlath, A. E., & Orts, W. (2009). Edible films and coatings: why, what, and how? In *Edible films and coatings for food applications* (pp. 1-23): Springer.

Pelissari, F. M., Grossmann, M. V., Yamashita, F., & Pineda, E. A. G. (2009). Antimicrobial, mechanical, and barrier properties of cassava starch− chitosan films incorporated with oregano essential oil. *Journal of agricultural and food chemistry, 57*(16), 7499-7504.

Porta, R. (2019). The plastics sunset and the bio-plastics sunrise. In: Multidisciplinary Digital Publishing Institute.

Porta, R., Di Pierro, P., Sabbah, M., Regalado-Gonzales, C., Mariniello, L., Kadivar, M., & Arabestani, A. (2016). Blend films of pectin and bitter vetch (*Vicia ervilia*) proteins: Properties and effect of transglutaminase. *Innovative Food Science & Emerging Technologies, 36*, 245-251.

Porta, R., Di Pierro, P., Sorrentino, A., & Mariniello, L. (2011). Promising perspectives for transglutaminase in "bioplastics" production. *J Biotechnol Biomaterial, 1*, 1-4

Porta, R., Mariniello, L., Di Pierro, P., Sorrentino, A., & Giosafatto, C. V. L. (2011). Transglutaminase crosslinked pectin-and chitosan-based edible films: A review. *Critical reviews in food science and nutrition, 51*(3), 223-238.

Qazanfarzadeh, Z., Kadivar, M., Shekarchizadeh, H., & Porta, R. (2021). Rye secalin characterisation and use to improve zein-based film performance. *International Journal of Food Science & Technology, 56*(2), 742-752.

Raziyafathima, M., Praseetha, P., & Rimal Isaac, R. (2016). Microbial degradation of plastic waste: a review. *J Pharm Chem Biol Sci, 4*(2), 231-242.

Sabbah, M., Altamimi, M., Di Pierro, P., Schiraldi, C., Cammarota, M., & Porta, R. (2020). Black edible films from protein-containing defatted cake of *Nigella sativa* seeds. *International Journal of Molecular Sciences, 21*(3), 832.

Sabbah, M., Giosafatto, C. V. L., Esposito, M., Di Pierro, P., Mariniello, L., & Porta, R. (2019). Transglutaminase cross-linked edible films and coatings for food applications.

In *Enzymes in food biotechnology* (pp. 369-388): Elsevier.

Sabbah, M., & Porta, R. (2017). Plastic pollution and the challenge of bioplastics. *J. Appl. Biotechnol. Bioeng, 2*(3), 00033.

Sanyang, M. L., Sapuan, S. M., Jawaid, M., Ishak, M. R., & Sahari, J. (2015). Effect of plasticizer type and concentration on tensile, thermal and barrier properties of biodegradable films based on sugar palm (*Arenga pinnata*) starch. *Polymers, 7*(6), 1106-1124.

Senturk Parreidt, T., Müller, K., & Schmid, M. (2018). Alginate-based edible films and coatings for food packaging applications. *Foods, 7*(10), 170.

Shamsuddin, I. M., Jafar, J. A., Shawai, A. S. A., Yusuf, S., Lateefah, M., & Aminu, I. (2017). Bioplastics as better alternative to petroplastics and their role in national sustainability: a review. *Advances in Bioscience and Bioengineering, 5*(4), 63-70.

Singh, P., & Sharma, V. (2016). Integrated plastic waste management: environmental and improved health approaches. *Procedia Environmental Sciences, 35*, 692-700.

Skurtys, O., Acevedo, C., Pedreschi, F., Enronoe, J., Osorio, F., & Aguilera, J. (2014). *Food hydrocolloid edible films and coatings*: Nova Science Publishers, Incorporated Hauppauge, New York, USA.

Song, J., Murphy, R., Narayan, R., & Davies, G. (2009). Biodegradable and compostable alternatives to conventional plastics. *Philosophical Transactions of the Royal Society B: Biological Sciences, 364*(1526), 2127-2139.

Sothornvit, R., & Krochta, J. M. (2005). Plasticizers in edible films and coatings. In *Innovations in food packaging* (pp. 403-433): Elsevier.

Su, G., Cai, H., Zhou, C., & Wang, Z. (2007). Formation of edible soybean and soybean-complex protein films by a cross-linking treatment with a new Streptomyces transglutaminase. *Food Technology and Biotechnology, 45*(4), 381-388.

Suhag, R., Kumar, N., Petkoska, A. T., & Upadhyay, A. (2020). Film formation and deposition methods of edible coating on food products: A review. *Food Research International, 136*, 109582.

Tang, C. H., Xiao, M. L., Chen, Z., & Yang, X. Q. (2011). Properties of transglutaminase-treated red bean protein films. *Journal of Applied Polymer Science, 122*(2), 789-797.

Thakur, B. R., Singh, R. K., Handa, A. K., & Rao, M. (1997). Chemistry and uses of pectin- a

review. *Critical Reviews in Food Science & Nutrition, 37*(1), 47-73.

The, D. P., Debeaufort, F., Voilley, A., & Luu, D. (2009). Biopolymer interactions affect the functional properties of edible films based on agar, cassava starch and arabinoxylan blends. *Journal of Food Engineering, 90*(4), 548-558.

Thompson, R. C., Moore, C. J., Vom Saal, F. S., & Swan, S. H. (2009). Plastics, the environment and human health: current consensus and future trends. *Philosophical transactions of the royal society B: biological sciences, 364*(1526), 2153-2166Tosin, M., Weber, M., Siotto, M., Lott, C., & Degli-Innocenti, F. (2012). Laboratory test methods to determine the degradation of plastics in marine environmental conditions. *Frontiers in microbiology, 3*, 225.

Umaraw, P., & Verma, A. K. (2017). Comprehensive review on application of edible film on meat and meat products: An eco-friendly approach. *Critical Reviews in Food Science and Nutrition, 57*(6), 1270-1279.

Verma, R., Vinoda, K., Papireddy, M., & Gowda, A. (2016). Toxic pollutants from plastic waste-a review. *Procedia Environmental Sciences, 35*, 701-708.

Vieira, M. G. A., da Silva, M. A., dos Santos, L. O., & Beppu, M. M. (2011). Natural-based plasticizers and biopolymer films: A review. *European Polymer Journal, 47*(3), 254-263.

Vimal, A., & Kumar, A. (2019). Enzymes in food biotechnology: production, applications, and future prospects: Amsterdam (Netherlands): Elsevier.

Waring, R. H., Harris, R. M., & Mitchell, S. C. (2018). Plastic contamination of the food chain: A threat to human health? *Maturitas, 115*, 64-68.

Webb, H. K., Arnott, J., Crawford, R. J., & Ivanova, E. P. (2013). Plastic degradation and its environmental implications with special reference to poly (ethylene terephthalate). *Polymers, 5*(1), 1-18.

Wittaya, T. (2012). Protein-based edible films: Characteristics and improvement of properties. *Structure and function of food engineering*, 43-70.

Wyrwa, J., & Barska, A. (2017). Innovations in the food packaging market: active packaging. *European Food Research and Technology, 243*(10), 1681-1692.

Yayli, D., Turhan, S., & Saricaoglu, F. T. (2017). Edible packaging film derived from mechanically deboned chicken meat proteins: Effect of transglutaminase on

physicochemical properties. *Korean journal for food science of animal resources, 37*(5), 635.

Yilmaz, K., Turhan, S., Saricaoglu, F. T., & Tural, S. (2020). Improvement of physicochemical, mechanical, thermal and surface properties of anchovy by-product protein films by addition of transglutaminase, and the correlation between secondary structure and mechanical properties. *Food Packaging and Shelf Life, 24*, 100483.

Younis, H. G., & Zhao, G. (2019). Physicochemical properties of the edible films from the blends of high methoxyl apple pectin and chitosan. *International Journal of Biological Macromolecules, 131*, 1057-1066.

Appendices

Appendices A:

All results of mechanical properties, water content%, water uptake%, and biodegradability% values in laboratory

PEC	TGase	Thickness µM	TS (MPa)	EB (%)	YM (MPa)
0	0	55	0.31	28.86	1.37
0	0	62	0.31	32.26	1.2
0	0	63	0.38	35.2	1.2
0	0	60	0.37	26	1.5
0	0	58	0.41	20	2.5
		59.6	0.356	28.464	1.554
		2.870540019	0.039799	5.2469176	0.48628
0	10	59	0.2	14.54	1.37
0	10	60	0.1	9.33	1.05
0	10	58	0.13	12.72	0.986
0	10	58	0.18	13.86	0.986
0	10	57	0.15	14.125	1.09
		58.4	0.152	12.915	1.0964
		1.019803903	0.03544	1.8914545	0.14243
6	10	62	0.33	12.5	5.6
6	10	63	0.3	13.9	2.12
6	10	61	0.26	21.13	1.27
6	10	63	0.27	13.88	1.27
6	10	63	0.3	10.1	3.01
6	10	62	0.54	11.63	4.62
6	10	61	0.5	24.8	2.01
6	10	62	0.32	10.37	3.04
6	10	62	0.29	6.3	4.63
		62.11111111	0.345556	13.845556	3.06333
		0.737027731	0.09593	5.4040152	1.48126

10	10	66	0.25	5.5	4.54
10	10	63	0.33	7.5	4.46
10	10	65	0.32	5.31	6.102
10	10	63	0.33	6.15	5.31
10	10	63	0.33	5.27	6.19
10	10	61	0.35	5.25	6.88
		63.5	0.318333	5.83	5.58033
		1.607275127	0.031842	0.8083522	0.88915
40	10	70	3.14	23.81	13.17
40	10	65	3.5	24.6	14.2
40	10	69	3.03	28.5	10.6
40	10	65	2.04	24.46	10.6
40	10	66	2.41	23	10.46
40	10	66	2.04	15.38	13.22
40	10	63	2.33	26.22	8.87
		66.28571429	2.641429	23.71	11.5886
		2.249716535	0.535949	3.789116	1.79686
0	20	63	0.15	17.94	1.92
0	20	60	0.11	20.14	0.527
0	20	61	0.13	30.25	0.533
0	20	62	0.21	29.22	0.7
0	20	60	0.16	24.13	0.6
0	20	62	0.17	14.63	1.19
		61.33333333	0.155	22.718333	0.91167
		1.105541597	0.031491	5.7138995	0.50456

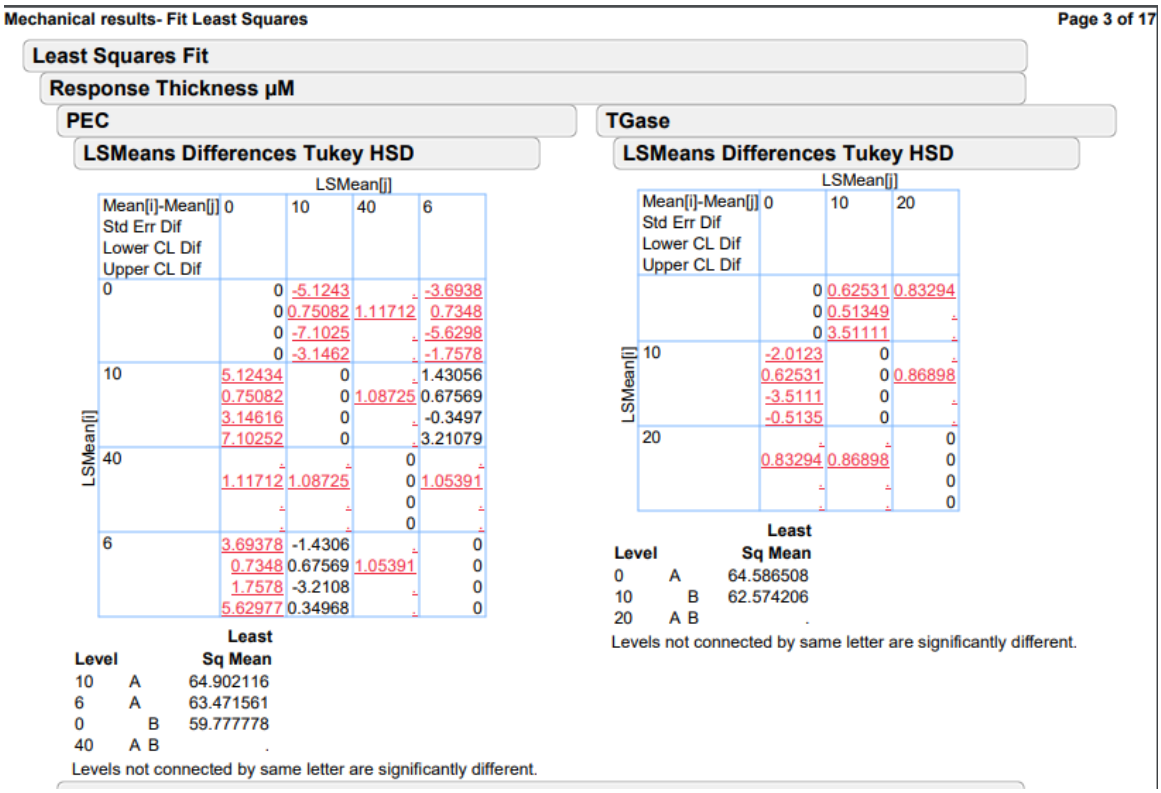
6	20	65	0.48	46.53	1.8
6	20	63	0.25	42.5	0.585
6	20	64	0.42	46.5	0.585
6	20	64	0.26	38.11	0.562
6	20	63	0.26	39.26	0.673
6	20	63	0.23	32.04	0.704
6	20	64	0.22	31	0.716
6	20	65	0.32	40.18	0.788
		63.875	0.305	39.515	0.8016
		0.78062475	0.08944	5.451752	0.3843
10	20	65	0.48	48.8	1.3
10	20	63	0.4	45.8	0.88
10	20	67	0.64	54.117	0.895
10	20	70	0.64	55.5	1.148
10	20	67	0.6	45	1.33
10	20	67	0.49	51.8	0.945
10	20	64	0.47	41.7	1.118
10	20	64	0.48	36.2	1.3
10	20	65	0.68	56.4	1.2
		65.7777778	0.54222	48.36856	1.124
		2.04275292	0.09271	6.4192	0.1683
6	0	65	0.36	44.19	0.81
6	0	62	0.41	40.36	0.97
6	0	67	0.47	28.5	1.65
6	0	64	0.45	40.36	1.65
6	0	65	0.43	26.9	1.8
6	0	64	0.33	41	2.6
6	0	64	0.29	30	0.85
		64.4285714	0.39143	35.90143	1.4757
		1.39970842	0.06151	6.60181	0.6

PEC	TGase	Water content (%)
40	0	2.5
40	0	2.4
40	0	2.2
40	0	2.3
		2.35
		0.11
40	10	3.6
40	10	6.1
40	10	6.5
		5.40
		1.28

PEC	TGase	Water uptake (%)
40	0	9.3
40	0	6.8
40	0	6.1
40	0	8.5
		7.68
		1.28
40	10	11.6
40	10	9.8
40	10	10.9
		10.77
		0.74

PEC	TGase	Biodegradability (%)
40	0	24.5
40	0	20
40	0	17.7
40	0	27.7
		22.48
		3.88
40	10	4
40	10	5
40	10	8.8
		5.93
		2.07

Appendices B: Statistical analysis for all above results in study



Mechanical results- Fit Least Squares — Page 3 of 17

Least Squares Fit

Response Thickness µM

PEC

LSMeans Differences Tukey HSD

LSMean[j]

Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	0	10	40	6
0	0	-5.1243	.	-3.6938
	0	0.75082	1.11712	0.7348
	0	-7.1025	.	-5.6298
	0	-3.1462	.	-1.7578
10	5.12434	0	.	1.43056
	0.75082	0	1.08725	0.67569
	3.14616	0	.	-0.3497
	7.10252	0	.	3.21079
40	.	.	0	.
	1.11712	1.08725	0	1.05391
	.	.	0	.
	.	.	0	.
6	3.69378	-1.4306	.	0
	0.7348	0.67569	1.05391	0
	1.7578	-3.2108	.	0
	5.62977	0.34968	.	0

LSMean[i]

Level		Least Sq Mean
10	A	64.902116
6	A	63.471561
0	B	59.777778
40	A B	.

Levels not connected by same letter are significantly different.

TGase

LSMeans Differences Tukey HSD

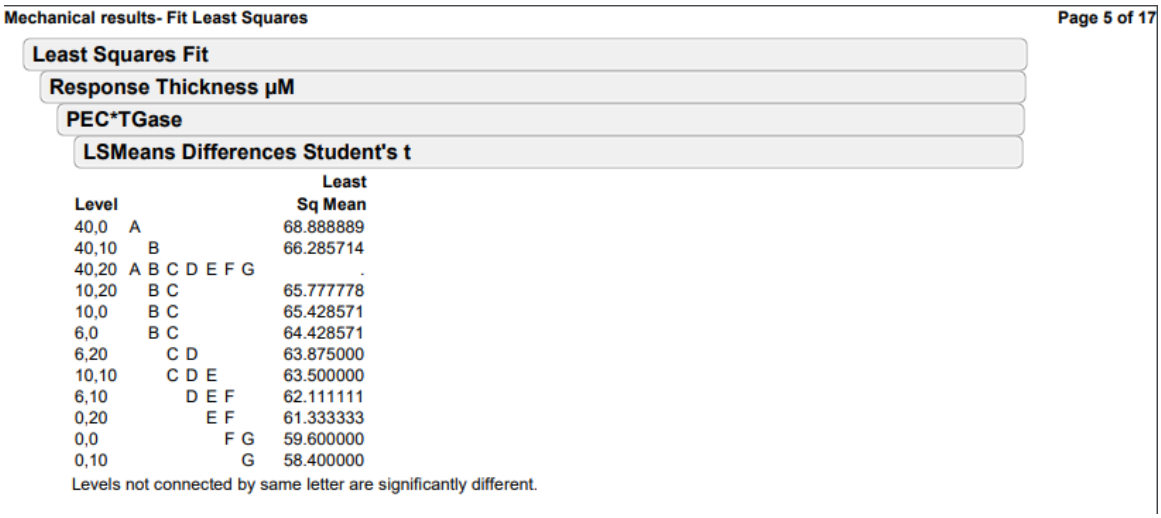
LSMean[j]

Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	0	10	20
	0	0.62531	0.83294
	0	0.51349	.
	0	3.51111	.
10	-2.0123	0	.
	0.62531	0	0.86898
	-3.5111	0	.
	-0.5135	0	.
20	.	.	0
	0.83294	0.86898	0
	.	.	0
	.	.	0

LSMean[i]

Level		Least Sq Mean
0	A	64.586508
10	B	62.574206
20	A B	.

Levels not connected by same letter are significantly different.



Mechanical results- Fit Least Squares — Page 5 of 17

Least Squares Fit

Response Thickness µM

PEC*TGase

LSMeans Differences Student's t

Level								Least Sq Mean
40,0	A							68.888889
40,10		B						66.285714
40,20	A	B	C	D	E	F	G	.
10,20		B	C					65.777778
10,0		B	C					65.428571
6,0		B	C					64.428571
6,20			C	D				63.875000
10,10			C	D	E			63.500000
6,10				D	E	F		62.111111
0,20					E	F		61.333333
0,0						F	G	59.600000
0,10							G	58.400000

Levels not connected by same letter are significantly different.

Response TS (MPa)

PEC

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
6	0.34732804	0.04389729	0.34542

LSMeans Differences Tukey HSD

α= 0.050 Q= 2.6347

LSMean[i]	Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	LSMean[j] 0	10	40	6
0		0	-0.2435	.	-0.1263
		0	0.07085	0.10542	0.06934
		0	-0.4301	.	-0.309
		0	-0.0568	.	0.05637
10		0.24347	0	.	0.11714
		0.07085	0	0.1026	0.06376
		0.05679	0	.	-0.0509
		0.43015	0	.	0.28514
40		.	.	0	.
		0.10542	0.1026	0	0.09946
		.	.	0	.
		.	.	0	.
6		0.12633	-0.1171	.	0
		0.06934	0.06376	0.09946	0
		-0.0564	-0.2851	.	0
		0.30902	0.05086	.	0

Level		Least Sq Mean
10	A	0.46447090
6	A B	0.34732804
0	B	0.22100000
40	A B	.

Levels not connected by same letter are significantly different.

TGase

Least Squares Means Table

LSMeans Differences Tukey HSD

α= 0.050 Q= 2.39689

LSMean[i]	Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	LSMean[j] 0	10	20
0		0	-0.2726	.
		0	0.05901	0.0786
		0	-0.414	.
		0	-0.1312	.
10		0.27259	0	.
		0.05901	0	0.082
		0.13115	0	.
		0.41403	0	.
20		.	.	0
		0.0786	0.082	0
		.	.	0
		.	.	0

Level		Least Sq Mean
10	A	0.86432937
0	B	0.59173810
20	A B	.

Levels not connected by same letter are significantly different.

Least Squares Fit

Response TS (MPa)

PEC*TGase

LSMeans Differences Student's t

Level		Least Sq Mean
40,10	A	2.6414286
40,0	B	1.0866667
40,20	A B C D	.
10,20	C	0.5422222
10,0	C	0.5328571
6,0	C D	0.3914286
0,0	C D	0.3560000
6,10	C D	0.3455556
10,10	C D	0.3183333
6,20	D	0.3050000
0,20	D	0.1550000
0,10	D	0.1520000

Levels not connected by same letter are significantly different.

Least Squares Fit

Response EB (%)

PEC*TGase

LSMeans Differences Tukey HSD

Level							Least Sq Mean
10,20	A						48.368556
10,0	A	B					41.311429
6,20		B					39.515000
6,0		B	C				35.901429
0,0			C	D			28.464000
40,10				D			23.710000
40,20	A	B	C	D	E	F	.
0,20				D	E		22.718333
6,10					E	F	13.845556
0,10					E	F	12.915000
40,0						F	6.388889
10,10						F	5.830000

Levels not connected by same letter are significantly different.

Least Squares Fit

Response YM (MPa)

PEC

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
6	1.7802242	0.50824690	1.8464

LSMeans Differences Tukey HSD

α= 0.050 Q= 2.6347

LSMean[i]	Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	LSMean[j] 0	10	40	6
0		0	-1.6617	.	-0.5929
		0	0.82035	1.22058	0.80285
		0	-3.8231	.	-2.7081
		0	0.49966	.	1.5224
10		1.66171	0	.	1.06884
		0.82035	0	1.18794	0.73826
		-0.4997	0	.	-0.8763
		3.82308	0	.	3.01394
40		.	.	0	.
		1.22058	1.18794	0	1.15151
		.	.	0	.
		.	.	0	.
6		0.59287	-1.0688	.	0
		0.80285	0.73826	1.15151	0
		-1.5224	-3.0139	.	0
		2.70814	0.87626	.	0

Level		Least Sq Mean
10	A	2.8490635
6	A	1.7802242
0	A	1.1873556
40	A	.

TGase

Least Squares Means Table

LSMeans Differences Tukey HSD

α= 0.050 Q= 2.39689

LSMean[i]	Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	LSMean[j] 0	10	20
0		0	0.61098	.
		0	0.68322	0.91007
		0	-1.0266	.
		0	2.24859	.
10		-0.611	0	.
		0.68322	0	0.94946
		-2.2486	0	.
		1.02663	0	.
20		.	.	0
		0.91007	0.94946	0
		.	.	0
		.	.	0

Level		Least Sq Mean
0	A	5.9431429
10	A	5.3321595
20	A	.

Levels not connected by same letter are significantly different.

Least Squares Fit

Response YM (MPa)

PEC*TGase

LSMeans Differences Tukey HSD

Level					Least Sq Mean
40,0	A				18.900000
40,10		B			11.588571
40,20	A	B	C	D	.
10,10			C		5.580333
6,10			C	D	3.063333
10,0			C	D	1.842857
0,0			C	D	1.554000
6,0			C	D	1.475714
10,20				D	1.124000
0,10			C	D	1.096400
0,20			C	D	0.911667
6,20				D	0.801625

Levels not connected by same letter are significantly different.

Least Squares Fit

Response Water content (%)

PEC*TGase

LSMeans Differences Student's t

Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	LSMean[j] 40,0	 40,10
LSMean[i] 40,0	0 0 0 0	-3.05 0.763 -5.0114 -1.0886
LSMean[i] 40,10	3.05 0.763 1.08865 5.01135	0 0 0 0

Level		Least Sq Mean
40,10	A	5.4000000
40,0	B	2.3500000

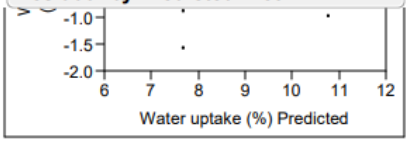
Levels not connected by same letter are significantly different.

Least Squares Fit

Response Water uptake (%)

Whole Model

Residual by Predicted Plot



TGase

LSMeans Differences Student's t

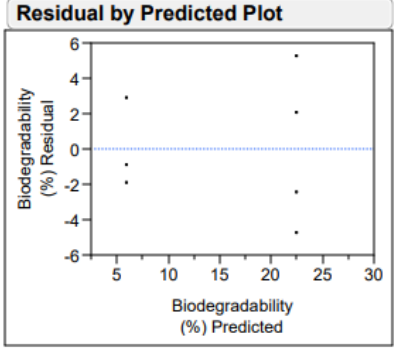
		LSMean[j]	
	Mean[i]-Mean[j]	0	10
	Std Err Dif		
	Lower CL Dif		
	Upper CL Dif		
LSMean[i]	0	0	-3.0917
		0	0.97894
		0	-5.6081
		0	-0.5752
	10	3.09167	0
		0.97894	0
		0.57523	0
		5.60811	0

Level		Least Sq Mean
10	A	10.766667
0	B	7.675000

Levels not connected by same letter are significantly different.

TGase Leverage, P=0.0024

Residual by Predicted Plot



Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
0	22.475000	1.9125136	22.4750
10	5.933333	2.2083805	5.9333

LSMeans Differences Student's t

α= 0.050 t= 2.57058

		LSMean[j]	
	Mean[i]-Mean[j]	0	10
	Std Err Dif		
	Lower CL Dif		
	Upper CL Dif		
LSMean[i]	0	0	16.5417
		0	2.92141
		0	9.03194
		0	24.0514
	10	-16.542	0
		2.92141	0
		-24.051	0
		-9.0319	0

Level		Least Sq Mean
0	A	22.475000
10	B	5.933333

Levels not connected by same letter are significantly different.

تأثير الانزيم الميكروبي ترانس جلوتاميناز على الخصائص الفيزيائية و الكيميائية للأغلفة المحضرة من خلط بروتين القزحة المركز مع البكتين

اعداد: ليالي بلال محمد علي

المخلص:

ينتج العالم من البلاستيك حوالي 350 مليون طن سنويًا. حيث يستخدم البلاستيك على نطاق واسع لأنه يحتوي على العديد من الخصائص المميزة مثل القابلية للتشكيل ودائم ورخيص الثمن. لكن النفايات البلاستيكية غير قابلة للتحلل في البيئة، وهذا يسبب تراكم هذه النفايات في البحار والمحيطات والتربة. لذلك، تم استخدام عدة طرق للتخلص من هذه المخلفات وهي: دفن النفايات في التربة ولكن هذا يؤثر سلباً على خصوبة التربة بسبب تراكم المواد الكيميائية والسموم. اما طريقة حرق النفايات البلاستيكية تنتج غازات سامة تلوث البيئة وتساهم في تفاقم مشكلة الاحتباس الحراري. بينما إعادة تدوير المخلفات البلاستيكية لا تتجاوز 2٪ من إجمالي المخلفات البلاستيك.

كما هو معروف أن الجزء الأكبر من نفايات البلاستيك ناتج عن نفايات تغليف المواد الغذائية. لذلك ، تم السعي إلى تطوير أغشية وأغلفة صالحة للأكل مصنوعة من مواد طبيعية وقابلة للتحلل في البيئة و توفر أيضًا الحماية للأغذية و كما تشكل حواجز بين الغذاء والبيئة للحفاظ على صلاحيتها وسلامتها وجودتها. بشكل عام ، يتم إنتاج الأغلفة الصالحة للأكل من البروتينات والسكريات والدهون. بالإضافة إلى ذلك ، يمكن حقن الأغلفة بمواد مضادة للميكروبات ومضادات الأكسدة للحفاظ على سلامة الأغذية وجودتها.

تمتلك الاغلفة الصالحة للأكل المصنعة من البروتين خواص ميكانيكية جيدة. بينما المصنعة من السكريات المتعددة تنتج أغلفة صالحة للأكل تمنع دخول الغازات للمنتج الغذائي التي قد تؤثر سلبا عليه.

مؤخرا, أثبت الباحثون ان عملية مزج البوليمرات الطبيعية قد توفر أغلفة صالحة للأكل بخواص أفضل ميكانيكيا ولها القدرة على الثبات في البيئة المحيطة اعتمادا على خاصية النفاذية للغازات. لذا تم في هذا البحث استخدام بروتين القزحة الذي تم استخلاصه من متبقيات استخلاص زيت القزحة من بذوره ليتم استغلالها نظرا لأنها متوافره بكميات كبيرة وتحتوي على كمية وافرة من البروتين المركز.

وكما ذكرنا سابقا أن البروتينات تعد خيارًا مميزاً لتصنيع أغلفة صالحة للأكل. الا أن العديد من الدراسات تشير بأن خصائص هذه الاغلفة تحتاج الى تطوير لتصبح أكثر مقاومة, وتمتلك خواص ميكانيكية أفضل

يمكن التعامل معها أثناء تطبيقها على تغليف المواد الغذائية, وكذلك لحماية المنتجات الغذائية. وبالتالي، فإن مزج محلول البروتين مع بوليمر حيوي آخر مثل البكتين وبوجود إنزيم ترانس جلوتاميناز قد يكون له دور فعال في تطوير الأغلفة الصالحة للأكل التي تعتمد في تصنيعها على البروتين.

يهدف هذا البحث لدراسة تأثير تراكيز مختلفة من انزيم ترانس جلوتاميناز والبكتين على الخصائص الميكانيكية للأغلفة المحضرة من البروتين، وتأثيرها على محتوى الماء فيها، و قدرتها على امتصاص الرطوبة من البيئة المحيطة، ومعدل تحلل الغلاف.

تقوم منهجية الدراسة على التجربة والبحث, حيث تم تحضير أربعة أغلفة من بروتين القزحة المركز مع تراكيزمختلفة من البكتين (0, 60, 100, 400)ملغم وضبطهم عند درجة الحموضة 7.5 للتحقيق في تأثير تراكيز البكتين على الخواص الميكانيكية للأغلفة الصالحة للأكل التي صنعت من بروتين القزحة المركز. ثم تم دراسة تأثير إضافة إنزيم ترانس جلوتاميناز بعدة تراكيز (20,10,0 وحدة/غم بروتين) إلى محاليل المصنعة من بروتين القزحة المركز بتراكيز بكتين مختلفة وضبطهم عند درجة الحموضة 7.5 .بعد ذلك, تم اجراء فحص الخصائص الميكانيكية والسماكة على الاغلفة المنتجة. أخيرًا ، اختير الغلاف المحضر من بروتين القزحة المركز مع تركيز بكتين 400ملغم بوجود الانزيم منخفض التركيز (10وحدة/غم بروتين) لأنه ذات خصائص ميكانيكية مناسبة من أجل اجراء فحص التحلل البيولوجي ,ومحتوى الماء, وامتصاص الغلاف للماء , ومقارنتها مع نفس الغلاف لكن بغياب انزيم ترانس جلوتاميناز.

اشارت نتائج الدراسة الى ان اضافة تراكيز بكتين (60, 100 ملغم) الى محلول بروتين القزحة المركز حسنت من سماكة ومرونة الاغلفة المحضرة بشكل واضح. كما أوضحت النتائج انه عند اضافة الانزيم بتركيز منخفض زادت قيم قوة الشد للأغلفة في حين قيم المرونة والاستطالة قلت بينما زادت المرونة عند ارتفاع تركيز انزيم باستثناء عند أعلى تركيز للبكتين لم يتكون غلاف متماسك , حيث كان غير قابل للتعامل معه او قياسه أودراسة خصائصه. أشارت النتائج الى انه لم يحدث فرق احصائي في معامل المرونة للغلاف الا عند اضافة بكتين بتركيز 400 ملغم بوجود اعلى تركيز مستخدم للأنزيم الى محلول بروتين القزحة المركز.

أما من حيث محتوى الرطوبة للفيلم وقدرته على امتصاص الرطوبة , فان النتائج بينت ان الانزيم لديه تأثير واضح على زيادة محتوى الرطوبة للأغلفة وزيادة قدرتها على امتصاص الرطوبة ايضا. كما للإنزيم دور مباشر في تقليل معدل التحلل للغلاف.

يمكن الاستنتاج من هذه النتائج، أن للإنزيم دور فعال في تحسين الخصائص الميكانيكية للأغلفة المعتمدة على بروتين القزحة عند مزجها مع البكتين.