Preparation of Edible Films Made from Chitosan with Pomegranate Peel Extract and Study Its Barrier, Mechanical and Antioxidant Properties

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Abstract. The goal of this work was to evaluate the effect of adding extract of Pomegranate peels (PPE) on the mechanical, reservation and antioxidant properties of edible chitosan films. The findings showed that the chitosan film, supported by pomegranate peels, possessed distinctive properties, as the thickness, tensile strength, elongation, oxygen permeability, and permeability of water vapor and solubility were 60 µm, 27.6 (Mpa), 18.3%, 30.13(ml / m²* day), 2.91(g.mm/day.m²KPa), 23.12% respectively, whereas in the control film they were 30 µm, 19.2 (Mpa), 10.6%, 60.47(ml / m²* day), 2.30(g.mm/day.m²KPa), 26.50% respectively. Edible films incorporated with Pomegranate peels extract showed an antioxidant activity of 66.42% compared to the standard antioxidant BHA (Butylated hydroxyl anisole) and Ascorbic acid which were 27.44% and 80.54%, respectively. In respect to the optical proprieties the ∆E value of control film samples was 8.7%, and (L, a, b) (26.46, 40.01, 2.02) respectively, while for the supported Sample ∆E was 26.37% (L, a, b) (72.24, 1.09, 5.30) respectively.

1. Introduction

Packaging technology plays an important role in the food processing chain, which is contained in containment and protection from chemical and microbial contamination from the environment surrounding food, physical and biological effects during food production and storage stages, marketing, transport and distribution stages until it reaches the last consumer without changes in sensory characteristics that may reach the limit. Damage [1]. Active food packaging is one of the new innovative technologies in the field of smart packaging that combines the food and packaging environment and their interactions in order to ensure the preservation of quality and to increase the shelf life of food by means of the presence of natural biological materials in natural polymers in order to protect the consumer and the environment through the preservation of food from pathological and food microorganisms. In addition to its biodegradability and environmental friendliness, the use of pomegranate husk extract to support chitosan film through the synergistic action of chitosan extract, which already has antimicrobial and antioxidant activity [2]. The objective of this study was to evaluate the antioxidant value and the mechanical barrier properties of film made from chitosan with Pomegranate peel extract

2. Materials and Methods

2.1. Pomegranate peel extract preparation

After drying the Pomegranate peel, the dried peel were grinded using an electric grinder and then the crushed leaves were sifted using a sieve (70/100 mesh) to obtain a green tea powder. Extraction was carried out according to the method cited by [3]. Extraction was carried out by preparing four types of extraction solutions; 50:50, 25:75, 0:100 and 100:0 (water: Methanol alcohol). The ground peels 15gm powder was mixed with 100ml water solution in conical flask. The flask was then placed in Magnetic stirred for 30 minutes. Then the extract was filtered through filter paper (Whatman no: 1) and evaporated using a rotary evaporator to reach the concentrated extract and then oven dried overnight at 40 °C to obtain the extract in a dried form. The yield of the extract was calculated as follows:

Yield (%) = (The weight of dried extract) / (Pomegranate peel powder weight) × 100
2.2. Preparation of film solution
The film forming solution was prepared using the method described by [4] with some modifications as follows:
In 1 percent of acetate acid, 2 g chitosan was dissolved during a 5 minute stirring. Then heated the solution for 3 hours to 40°C. Then cooling at room temperature left the solution. A plasticizer with the concentration of 1% was applied to the pomace at 10 minutes by removal of the air bubbles from the film solution for 10 minutes and the extract was added to the film solution with the concentration of 3%. Filtration with cloth was done to avoid bumps in the solution. To remove the air bubbles from the film solution the vacuum pump was used for 10 minutes and then the film solution was stored in the refrigerator in dark conditions to prevent oxidation.

2.3. Film Formation
According to the procedure reported by [5], portions of 8g of the film solution were casted on petri dishes (diameter of 8.5cm) and allowed to dry at 21 °C for 48hr. Finally films were removed from the dishes using a spatula, stored in polythene bags at 25°C and 50% RH until the necessary tests were carried out.

2.4. Determination of antioxidant activity
The antioxidant activity of the film sample was assessed using the adapted method DPPH (2, 2-diphenyl-1-picrylhydrazyl) - 10 mg of the film was momentarily dissolved into 10 ml of deionized water. At 10,000g for 10 minutes the mix was centrifuged. The mixture was vortexed and incubated in the dark for 30 minutes, mixing with a 2 ml of 0.2 mM Methane DPPH Solution, then centrifuged with 100,000g for five minutes. A spectrophotometer in three replicates was used to calculate the absorption. In the following equation, the proportion of free radical behavior was established:

\[
\text{scavenging activity \%} = \left( \frac{\text{ABS blank} - \text{ABS sample}}{\text{ABS blank}} \right) \times 100
\]

Where ABS is the absorbance value

2.5. Barrier properties
2.5.1. Permeation of oxygen
Oxygen permeability of the film sample was determined according to [6] using an oxygen permeability tester, O2 gas was released with a certain amount of nitrogen gas (N2) to arriving at a combination close to the composition of atmospheric. These gases are pushed under the pressure of 20 atmosphere on the film under examination, To calculate the permeability of gases passing through the film from the other side, This is done at 23°C and a relatively high humidity of 50 percent.

2.5.2. Water vapor permeability
Water vapor permeability of the experimental films was carried out in accordance with the modified method [7] of the weight cup of ASTM standard E96-95.A plot of weight gained versus time was used to determine the rate of transmission of the water vapor. The slope of the linear portion of this plot represented the steady-state volume of water vapor transmission through the film per unit time (g/h).The water vapor permeability was calculated according to the following equation:

\[
\text{WVP} = \frac{(W/t)}{(\Delta P / A)}
\]

(W/t)=water volume based on time measured by linear regression (R2> 0.99) by weight recorded within 7 days (g/day).
A= area exposed to permeability of the film (cm²)
X = film thickness (mm)
\Delta P = partial pressure which is different by film and size, as the following formula:

\[
\Delta P = S (R1 - R2)
\]

S= saturated vapor pressure at a temperature of 25 m (3166 Kpa)
R1= Relative humidity of desiccator (0.75) (estimated using RH Meter)
R2= Relative humidity under the film inside the cell (zero) (estimated based on the relative humidity of the salt used).
2.6. Film solubility in water
The method described [8] was used to measure film solubility. The film was cut into small pieces, 2 x 2 cm in size, and was dried at 100°C in an oven weighed to the nearest 0.01 g for the initial dry weight. The film was then placed in a beaker with 100 ml of distilled water and gently shaken for 24 hours. The solution was filtered through (whatman No.1) in order to recover the remaining undissolved film. The remaining parts of the film were dried at a constant weight of 100 (final dry weight). Water solubility (percent) was calculated using the following equation:

\[
\text{Solubility\%} = \frac{\text{initial dry weight} - \text{final dry weight}}{\text{initial dry weight}} \times 100
\]

Where ABS is the absorbance value

2.7. Mechanical tests
2.7.1. Film thickness assessment
The thickness of the film was measured using a digital micrometer to the close of 0.01 mm by[9]. Measuring was carried out at nine random positions for every film, measuring the mean values. Before performing the rest of the mechanical and barrier inspection, the film thickness was determined.

2.7.2. Determination of the tensile strength and elongation of the split
The tensile strength and elongation at breakage of the film samples were measured according to the method indicated [10] using Tensile strength device. The film sample was cut into strips (60mm×20mm). The crosshead speed set at 5 mm / s. The membrane was fixed between the handles of the device and the speed of pulling the models (5 mm / sec) estimated the tensile strength and elongation at cutting from the stress-strain curve drawn by the device for the samples.

2.8. Optical properties and surface color
The visual properties of the film were estimated using a Brightness and Color Meter [11]. Three readings were taken from the circumference of the film and its center. CIE standards were used for colors which expressed three symbols (B *, A *, L *) each one expresses a certain spectrum of colors, at the meeting the three values are defined and the color is determined by the value of total color deference (\( \Delta E \)). The symbol L * expresses the gradient from black to white and gives values from (0) to (100), and the symbol a * expresses the color gradient from green (when the value is low) to red (at high value +), and the symbol b * color of blue (when low value -) to yellow (at high value +). The value of the total color difference \( \Delta E \) was calculated by the following equation:

\[
\Delta E = \sqrt{(\Delta L *)^2 + (\Delta a *)^2 + (\Delta b *)^2}
\]

2.9. Fourier-transforming infrared spectroscopy (FTIR)
The infrared spectrum of extract-supported chitosan films was obtained using Fourier-transform infrared spectroscopy (FTIR, IRAffinity-1S, Shimadzu, Japan). Measurements were recorded over a range of (400-4000) cm(1).

3. Results and Discussion
3.1. Pomegranate peel extract
The results shown in Figure (1) showed that the percentages of extraction have reached (22.9, 56.8, 27.8)% of methyl alcohol at concentrations of (100, 75 and 50)% respectively, and the percentage of aqueous extract was (17.3) %. The higher percentage of alcohol extraction compared to water extraction may be due to the quantity of the phenolic compounds in PPE that dissolved in alcohol than in water [12].
3.2. Antioxidant effectiveness of films
The results of the DPPH efficacy evaluation showed that the chitosan film alone without adding pomegranate peels gave antioxidant activity reached 35.13%. This activity increased significantly to 66.42% as a result of adding pomegranate peels alcohol extract to the chitosan films as shown in (Table 1). [13] reported that the antioxidant activity of the chitosan casing was 37.21%, Whereas, the efficacy of chitosan supported by (Pistacia terebinthus) leaf extract increased to 93.98%. The antioxidant activity of pomegranate peels is attributed to large amounts of antioxidants such as phenolic compounds such as flavonoids, anthocyanins, catechins, other flavonoids as well as tannins such as punicalin, pedunculagin, punicalagin, gallic acid, ellagic acid., where the phenolic compounds to curb free radicals based on the donating groups of the electron, which leads to prevent the emergence of the root chain [14]

Table 1. Antioxidant efficacy values of different film samples

| Sample          | Antioxidant activity % |
|-----------------|------------------------|
| Chitosan film   | 35.13                  |
| Chitosan + 3% PPE | 66.42                |
| BHA 0.02%       | 27.44                  |
| Ascorbic acid   | 80.54                  |

3.3. Barrier properties
3.3.1. Film permeability of oxygen
Membranes with good oxygen retention properties contribute to improved food quality and extend food shelf life [15]. The addition of the Pomegranate peels extract of chitosan film was found to result in an increase in the permeability of the film to oxygen compared to the control film sample without extract. The oxygen permeability value of the chitosan film (control) was 30.13 (ml/m2/day). The oxygen permeability value of the film sample supported by the extract was 60.47 ml/m2/day. (Fig 2). [16]; [17] indicated that the permeability value of chitosan membranes was high, possibly due to the
water absorption capacity of chitosan as well as the presence of the plasticizer that led to the opening of the cellular tissue of the membranes. The hydrophobic polymer films exhibit good gas blocking properties [18]. [19] also reported that the carbohydrate films of a hydrophilic nature have a high capacity to reserve carbon dioxide and oxygen.

![Figure 2. Oxygen permeability of chitosan film](image)

3.3.2. Film permeability of water vapor

The permeability to water vapor of the chitosan film for the control (T1) reached 2.30 (g. Mm. / Hr. M². MPa). On the other hand, the permeability of the film sample supported by the extract was slightly increased. (T2), reaching 2.91 (g. Mm. / Hour). M².MicPascal (Fig 3), [10] argued that chitosan films are highly permeable to water vapor due to their hydrophilic existence. [20] suggested that the chitosan film's water vapor permeability value was 1.45 (g. Mm. / Hr. M2. MPa), while the chitosan film's water vapor permeability value supported by mango leaf extract was 2.23 (g. Mm. / Hrs. M2.Mpa). Perhaps the reason for this differentiation in water vapor permeability values is due to the design of the extract used. [21] reported that the water vapor permeability value of the chitosan film was 0.8172 (g. Mm / hr. M². MPa) and that the permeability of the chitosan film supported with essential oils (cinnamon, clove, thyme) was 1.1344 g. / H. M²) , This may be due to the irregular membrane structure with the possibility of air bubbles and oil droplets in the membrane which may lead to a weakening of the interactions between the polymer molecules, resulting in an open structure and increasing the permeability of water vapor across the membranes, followed by an increase in the value of WVP.

![Figure 3. Chitosan film water vapor permeability](image)
3.3.3. Water solubility of films

Water solubility is an important factor in the film. The results showed that the water solubility of the film decreased when the extract was applied to the film, while the water solubility of the control film (T1) decreased by 26.50 per cent. While the percentage solubility of extract (T2) enriched Chitosan films is 23.12 percent. These results are largely consistent with the results confirmed by [20] Where the water solubility values for chitosan film were 27.35, this value decreases with the addition of the supporting substances. This may be due to the strength of the interaction between the phenolic compounds in the granate peel extract and the polysaccharide chain of chitosan, which may lead to a decrease in the availability of the polymer amine and hydroxyl groups to interact with water, and then the decrease in the water solubility of the film supported by the extract indicates the strength of the cohesion between the peel extract of the pomegranate and chitosan [22].

3.3.4. Mechanical tests

It is important to evaluate the mechanical properties of the edible packaging because it indicates the strength and durability of the edible packaging and its ability to maintain food safety during transport, shipping and storage [23]. In food packaging applications it is essential that the films be strong, tensile and flexible [24]. The film thickness is an important mechanical feature in food packaging applications and one of the main criteria for determining the physical properties of the film. The thickness of the control film (T1) reached 30 μm and the addition of the film extract (T2) increased the membrane thickness by 60 μm. As for the tensile strength, it was found that when the film was incorporated with pomegranate extract, the film's tensile strength was increased to 27.6 MPa, as it was 19.2 MPa for control. [25] indicated that the tensile strength of the chitosan film was 20.8 MPa while the tensile strength of the chitosan film increased when the pomegranate extract was added to the film was 29.9 MPa. The increase in tensile strength and elongation of chitosan membranes fortified with pomegranate peel extract may be due to phenolic compounds containing a number of OH groups forming hydrogen bonds with chitosan, and there has been an increase in tensile strength and interaction between polyphenolic compounds of pomegranate peel extract with tissue. Membrane of chitosan, to some extent, the increase in the thickness of the membrane may improve the tensile strength of the chitosan membrane supported by the extract [26]. With regard to the elongation rate, it was 18.3% in the film supported by the extract, which was higher than the elongation rate of the chitosan film with an extract of 10.6 per cent. Increased tensile strength and elongation of chitosan films fortified with pomegranate peel extract may be due to the fact that the phenolic compounds contain a number of OH groups that form hydrogen bonds with chitosan and then there was an increase in the tensile strength and because of the interaction between polyphenolic compounds of pomegranate peel extract with tissue. the increase in the thickness of the film may improve the tensile strength of the chitosan film supported by the extract [20].

Table 2. Thickness, tensile strength and elongation at break film samples

| Sample               | Tensile strength (MPa) | Elongation at break % | films thickness(μm) |
|----------------------|------------------------|-----------------------|---------------------|
| Chitosan film (control) | 19.2                   | 10.6                  | 30                  |
| Chitosan + 3% PPE (extract) | 27.6                   | 1.3                   | 60                  |
3.3.5. Optical color characteristics

The opacity of the material is evidence of the amount of light passing through it. This evidence is important for controlling the entry of light through food products affecting photooxidation. It also has its role in blocking the passage of light through the membrane which plays a role in lipid oxidation. In the color system (~a*, b*, L~), L~ represents the intensity of light, a* and b* represent the consistency of colors, the L~ value is low indicating that the color is dark, the high L~ value indicates that the color is light, either if a* + is toward red, if a* - is towards green, b* + is towards yellow, b* - is toward blue shown in Table (5). The results showed an increase in opacity with the addition of pomegranate husk extract to chitosan membranes and discolored yellowish-red color. This is a better color characteristic than the non-extractive control film, which is transparent due to the reduction of photosynthetic oxidation of food.

Table 3. Optical and color properties of film samples

| Films                        | ΔE  | L*  | a*    | b*    |
|------------------------------|-----|-----|-------|-------|
| Chitosan film (control)      | 8.7 % | 26.46 | 40.01 | 2.02  |
| Chitosan + 3% PPE (extract)  | 26.37 % | 72.24 % | 1.09  | 5.30  |

3.3.6. Fourier-transform infrared spectroscopy

Figure (4) shows the results of the diagnosis of the active groups and the appearance of the measurements in the form of peaks starting at a wavelength of 3404.36 cm⁻¹ due to the oscillation of the hydroxyl groups OH and the bundles of the N-H amine groups, which are intertwined with the hydroxyl groups, which appear within the same area and the intensity of this beam varies according to type The reactants and the intensity of the reaction, which express an increase in the hydrogen bond between the components of the chitosan film, While small beams were observed at the wavelengths 2997.38 and 2883.58 cm⁻¹ due to the amplitude oscillation of the CH group and the aliphatic CH₂ and the wavelengths 1695.43 cm⁻¹, 1581.63 cm⁻¹ and 1384.89 cm⁻¹ bands, the amplitude oscillation of the carbonyl group C = O of amide I and the NH group of amide II has been shown to indicate the formation of chitosan as it contains the NH₂ group. [27], and the CN group of amide III (amide III) Respectively, the wavelength 1456.26 cm⁻¹ is due to the CH₂ group and the wavelength 1383.60 cm⁻¹ is related to the CH₃ groups, while the wavelengths between 1151.50 and 1035.77 it can be attributed to the bending vibration of the CO group resulting from the addition of glycerol as a plasticizer [23]. as FTIR is one of the important technologies used to diagnose active groups of chitosan membranes fortified with pomegranate peel extract as an active substance.

4. Conclusion

The present study showed that by pomegranate peel extract rich in polyphenols was successfully incorporated into the chitosan films. The prepared films have proven good mechanical and antioxidant properties and low oxygen permeability, making them suitable for use as packaging materials for preservation purposes.
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Figure 4. Infrared spectrum of chitosan film supported by pomegranate peel extract by FT

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