MECHANICAL, BARRIER AND ANTIOXIDANT PROPERTIES OF EDIBLE FILMS MADE FROM WHEY PROTEIN ISOLATE INCORPORATED WITH GREEN TEA EXTRACT

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ABSTRACT

The aim of this research was to study the effect of adding green tea extract (GTE) on the mechanical, oxygen permeability, water vapor permeability and antioxidant properties of whey protein isolate edible films. Edible films incorporated with the alcoholic extract of green tea showed high antioxidant activity as compared with the standard antioxidant BHA (Butylated hydroxyl anisole), The scavenging values of the film with GTE and the control film were 65.22% and 9.5%, respectively. The mechanical tests, revealed a decreasing in thickness in the control film which was formed without GTE from 181 µm to 169 µm. The tensile strength has been reduced in the film to 7.87 MPa where it was in the control sample 8.29 MPa. However, the elongation rate increased to 117% in the film supported by the extract, which was higher than the elongation rate of the control protein film without GTE (107%). The permeability value of oxygen (OP) and water vapor permeability (WPV) were measured and the properties of film were evaluated. The OP value of the control film was 90.07 ml / m ^{2} * day, whereas the permeability of the film with (GTE) decreased to 72.45 ml / m ^{2} * day. The WVP value of the control film was 0.61(gm.mm / hr. m^2. mPa), while the permeability value of the film with GTE was 0.22 (gm.mm / hr. m^2. MPa). The water solubility of film with GTE was increased to 47.5% as compared to the control film which was 41.2%.In respect to the optical properieties the ∆E value of supported film samples was 29.09%,and (L, a, b) (28.3,-7.41, 51.01) respectively, while for the control Sample ∆E was 9.6% (L, a, b), (6.6,-9.27, 59.15) respectively.

Keywords: optical properties; tensile strength; Film solubility; oxygen permeability
Part of M.Sc. thesis of first author

دراسة الخواص الميكانيكية والحجزية والفعالية المضادة للاكسدة لاغشية معزول بروتينات الشرش القابلة للاكل المدعمة بمستخلص الشاي الاخضر

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المستخلص

هدف هذا البحث دراسة تاثير اضافة مستخلص الشاي الاخضر على الخصائص الميكانيكية والحجزية والفعالية المضادة للاكسدة للاغشية المعدة من معزول بروتينات الشرش ، اظهرت الفحوصات التي اجريت على الاغشية المدعمة بالمستخلص الكحولي للشاي الاخضر ان له فعالية مضادة للاكسدة عالية جدا خصوصا لدى مقارنتها مع التركيز الموصى به لمادة BHA اذ بلغت الفعالية (65.22% ،29.5%) على التوالي، اظهرت نتائج الفحوص الميكانيكية انخفاض في سمك غشاء نموذج السيطرة والذي شكل بدون مستخلص الشاي الاخصر من 181 µm الى169µm، كما انخفضت قوة الشد في الغشاء المدعم بالمستخلص الى 7.87 مكاباسكال حيث كانت في نموذج السيطرة 8.29 ميكاباسكال غير ان نسبة الاستطالة ارتفعت و بلغت 117% في الغشاء المدعم بالمستخلص بعد ان كانت 107% في عينة السيطرة، قيست قيم النفاذية لكل من الاوكسجين و بخار الماء لمعرفة وتقييم خصائص الاغشية الحجزية، حيث بلغت قيم نفاذية غشاء السيطرة للاوكسجين 90.07 (مل/م^2*يوم) في حين انخفضت النفاذية للاوكسجين لعينة الاغشية المدعمة بالمستخلص الى 72.45 (مل/م^2*يوم). اما نفاذية بخار الماء فقد كانت لنموذج السيطرة 0.61 (غم.ملم./ساعة.م^2.مكباسكال) بينما وصلت قيمة نفاذية بخار الماء لعينة الغشاء المدعم بالمستخلص الكحولي للشاي الاخضر 0.22 (غم.ملم./ساعة.م^2.مكباسكال)، كما لوحظ ازدياد ذائبية الاغشية في الماء عند اضافة المستخلص حيث وصلت ذائبية الاغشية الى 47.5% في حين كانت في نموذج السيطرة 41.2% اما بالنسبة الى الخصائص البصرية واللونية فقد بلغت قيمة ΔE لعينات الاغشية المدعمة 29.09 % و قيم (L,a,b) (28.3، 7.41-، 51.01)على التوالي اما عينات اغلفة السيطرة فكانت ΔE 9.6 % و (L,a,b) (6.6، 9.27-،59.15)على التوالي.

الكلمات المفتاحية: الخواص البصرية، قوة الشد، ذوبانية الاغشية، نفاذية الاوكسجين.
بحث مستل من رسالة ماجستير للباحث الاول

INTRODUCTION

The development of packaging and packaging of food has a large area of research and modern methods focus on the creation of an effective packaging made from biodegradable materials to reduce the waste and the use of recyclable materials, while the active packaging against microbes (antimicrobial) is one of the most important scientific packaging that effect on food security(9,23). In fact, edible coatings that surround the surface of the food product may lead to increase the shelf life by reducing the permeability of moisture and soluble substances. Positive effects that can be obtained from edible packaging could affect the properties of coated materials and the physical and chemical properties of the components of the coated materials (23). The whey protein isolate has been widely use in formation of edible film for the properties that make them an important source in the manufacture of film and these characteristics are the transparency of films, no smell or taste its poor oxygen and fat permeability (26). Green tea extract is commonly used as food additives for food products (25). Where the aim of this study was to evaluate the antioxidant value and the mechanical, barrier properties for film made from whey protein isolate with green tea extract.

MATERIALS AND METHODS

Green tea extract preparation: After drying the green tea leaves, the dried leaves 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 (8). Extraction was carried out by preparing four types of extraction solutions; 50:50, 25:75, 0: 100 and 100: 0 (water: ethyl alcohol). The ground leaves powder was mixed with the water solution in a 500 mL conical flask with 10/100(weight / weight). The flask was then placed in a shaking water bath at 250 rpm and 60°C for 240 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 50 °C to obtain the extract in a dried form. The yield of the extract was calculated as follow.

$$\textbf{Yield (\%)} = \frac{\textbf{The weight of dried extract}}{\textbf{Green tea leaves powder weight}} \times \mathbf{100}$$

Preparation of film solution

Film forming solution was prepared according to the method described by Roy (24) with some modifications as follows:

10 g of whey proteins isolate was dissolved in 100 ml of deionized water under stirring for 5 minutes. Then the solution was heated to 90°C for 30 minutes under stirring. Then, the solution left to cool at room temperature. Filtration with cloth was performed to avoid any lumps in the solution; pH was adjusted to 7 by NaOH 0.01N .Glycerol was added as a plasticizer at the concentration of 5%, and then the extract of green tea was added to the film solution with a concentration of 3%. 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.

Film Formation

According to the procedure reported by Roy (28), portions of 8g of the film solution were casted on petri dishes (diameter of 8.5cm) and allowed to dry at 21 °C for 48 hr. 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.

Determination of antioxidant activity

The antioxidant activity of the film sample was evaluated using DPPH (2, 2-diphenyl-1-picrylhydrazyl) according to the modified method (29) .Briefly 10mg of the film was dissolved in 10 ml deionized water. The mixture was centrifuged at 10,000g for 10min. 0.2ml of the film extract solution was mixed with 2ml of 0.2mM methanolic solution of DPPH the mixture was vortexed and incubated in the dark for 30 min .Then it was centrifuged at 10,000g for 5min.===The absorbance was measured using a spectrophotometer at 517 nm in three replicates. The percentage of free radical scavenging activity was determined using the following equation:

$$\textbf{scavenging activity}\% = \frac{\textbf{ABS blank} - \textbf{ABS sample}}{\textbf{ABS blank}} \times \mathbf{100}$$

Where ABS is the absorbance value

Determination of the film thickness

The film thickness was measured by Arham method (4) using a digital micrometer to the nearest 0.01 mm. Measurements was performed at nine different random positions for each film, Mean values were calculated. The thickness of the film was calculated before conducting the rest of the mechanical and barrier assay.

Determination of tensile strength and elongation at break

The tensile strength and elongation at break of the film samples were measured according to the method mentioned by Yuan (31) using Tensile strength device. The film sample was cut into strips (60mm×20mm).The crosshead speed set at 5 mm / s.

Oxygen permeability

Oxygen permeability of the film sample was determined according to Bonilla (6) using an oxygen permeability tester, O_2 gas was released with a certain amount of nitrogen gas (N_2) 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 process is performed at a temperature of 23°C and a relative humidity of 50%.

Water vapor permeability

Water vapor permeability of the experimental films was carried out according to the modified method (14) of weight cup of ASTM standard E96-95.A plot of weight gained versus time was used to determine the water vapor transmission rate .The slope of the linear portion of this plot represented the steady state amount of water vapor transmission through the film per unit time (g/h).The water vapor permeability was calculated according to the following equation:

$$\mathbf{WVP} = (\mathbf{W} / \mathbf{t}).(\mathbf{X}.\Delta \boldsymbol{P}.\boldsymbol{A})$$

(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 (cm^2)

X = film thickness (mm)

ΔP = partial pressure which is different by film and size, as the following formula:

$$\Delta \boldsymbol{P} = \boldsymbol{S}(\boldsymbol{R1} - \boldsymbol{R2})$$

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)

Film solubility in water

The method that Ramos described (22) was used to measure the film solubility. The film was cut into small pieces and was dried at 100°C in an oven weighed to the nearest 0.01 g for initial dry weight. Then, the film was placed in beaker with100 ml of distilled water and shaken gently for 24 h. The solution was filtered through (whatman No.1) to recover the remaining undissolved film. The remaining pieces of the film were dried at 100 to constant weight (final dry weight). Solubility in water (%) was calculated by using the following equation:

$$\textbf{Solubility}\% = \frac{\textbf{initial dry weight} - \textbf{final dry weight}}{\textbf{initial dry weight}} \times \mathbf{100}$$

Optical properties and surface color

The visual properties of the film were estimated using a Brightness and Color Meter (1). 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 (ΔE). 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 according to the following equation:

$$\Delta \boldsymbol{E} = \sqrt{(\Delta \boldsymbol{L}*)^2 + (\Delta \boldsymbol{a}*)^2 + (\Delta \mathbf{b}*)^2}$$

RESULTS AND DISCUSSION

Green tea extract: the percentages of GTE extract when obtained by different concentration of extract solutions (75% alcoholic - 50% alcoholic - 25% alcoholic - water 100%) have reached 10.8%, 10.4%, 9.1% and 7.5% respectively, The higher percentage of alcohol extraction compared to water extraction may be due to the quantity of the phenolic compounds that dissolved in alcohol than in water (27)(Fig.l).

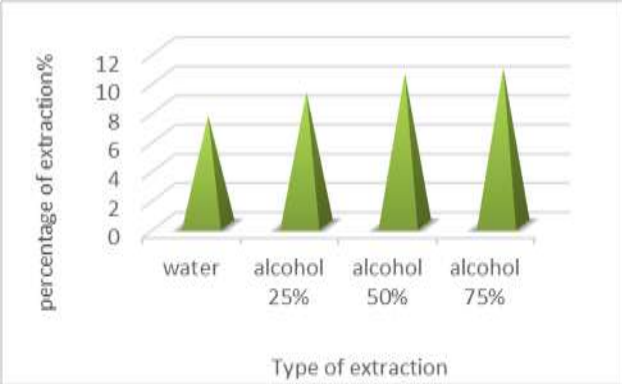

Figure 1. Extraction percentages using different Extraction solution

Antioxidant effectiveness of films

The results of the DPPH efficacy evaluation showed that the whey protein isolate film alone without adding tea extract gave antioxidant activity reached 20.2%. This activity increased from 20.2% to 53.25% as a result of adding 50% green tea alcohol extract to the whey protein isolate-based films as shown in. (Table 1) Hernondez-ledesma and Dóvalos (17). mentioned that the α-lactalbumin and β-lacto globulin had an antioxidant activity.Marquez et al. (21) found that the antioxidant activity of the films of the whey proteins was (15%). This study results is also consistent with Barzegaran et al. (5) Findings in studying the effect of adding green tea extract on the properties of polyamide films, they have reported that the antioxidant activity increased by increasing the concentration of green tea extract from 2.5 to 20% and reached 67.2 - 88.75%, respectively. Mangmee & Homthawornchoo (20) also found an increase in the scavenging activity when the green tea extract was added to rice-starch chitosan films mix and increased by increasing the concentration of the extract. In estimating the effectiveness of the BHA, which is used in food at a concentration of 0.02% the antioxidant activity was (29.85%) which showed the superiority of the films containing GTE in terms of efficacy. The antioxidant activity of green tea extract is attributed to its phenolic compounds, especially catechins and Gallic acid (ECG), (EGCG), (GCC), (GC), (GCG), In addition to its contents of carotenoids, tocopherolates, vitamin C and minerals such as chrom, manganese, selenium and zinc. Phenolic compounds inhibit free radicals based on the aggregates of the electron and thus prevent the formation of the root chain, and also because of the association with the transitional ion catalysts and then the formation of bonds with the free radicals through the interaction of them to induce inhibition of oxidation process (28).

Table 1. Antioxidant efficacy values of different film samples before and after addition GTE at different concentration

Sample	Antioxidant activity
Whey film	**% 20.2**
Whey + 2% GTE	**% 53.25**
Whey + 3% GTE	**% 65.22**
BHA 0.02%	**% 29.55**

Mechanical tests

The mechanical properties of any edible film are very important because it determines how the film behaves during circulation and storage, It is one of the criteria that determines the durability of the film and its ability to enhance food safety, In food packaging applications it is essential that the films be strong, tensile and flexible (13). The first mechanical property of the film was the thickness of the film (Table 2). It was found that when the extract was added to the film, the thickness of the control film (which was formed without the extract) decreased from 181 µm to 169 µm .This is consistent with Zinoviadou *et al*. finding (35) when oregano oil add to films the thickness decreased to 168.7 µm. The reason may be due to the increase in the bonds between the proteins and tea extract (16). As for tensile strength, it was found that when the film was incorporated with 3% tea extract, the tensile strength of the film was reduced to 7.87 MPa as it was 8.29 MPa for control. Galus & Kadzińska (12) have reported that the tensile force of the whey films were 7.1 Mpa . Javanmard and Golestan (18) reported that the tensile strength of the whey proteins film decreased when olive oil was added at different concentrations, starting with 1.944 MPa and decreasing to 0.904 MPa. With regard to the elongation rate, it was 117% in the film supported by the extract, which was higher than the elongation rate of the protein film without the extract, which was 107%. Yoshida and Antunes (30) found that the elongation rate of whey protein films was 70%. The reduction in the tensile strength and the increase in the elongation of films incorporated with the green tea extract compared with the control film may be due to the reduction of cross-linkages between the polymer chains and the same chains due to the presence of tea compounds. More restricted and non-specific motion on the vertical and horizontal axes and along the polymer unlike the control models (3), (2). There are many other factors affecting the mechanical properties of films, such as temperature and pH of film forming solutions. Heating time is less important factors than previous (7).

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

Samples	Elongation at break %	Tensile strength (MPa)	films thickness(μm)
Film control (without extract)	107	8.29	181
Film + 3% extract	117	7.87	169

Film permeability of oxygen

The permeability of films to oxygen is one of the most important properties as it provides protection from oxygen permeability and passage into food content (7).It was found that the addition of the green tea extract 3% whey proteins isolate films resulted in reduced permeability of the films to oxygen as compared with the control film sample without extract, The oxygen permeability value of the whey proteins isolate film (control) was 90.07 (ml / m^2 / day).The oxygen permeability value of the films sample supported by the extract was 72.45 ml / m^2 / day. (Fig2). These results are similar to those indicated by Ramos (25). The value of permeability of the film from whey proteins was 76.1 (ml / m^2 * day). The decrease in permeability values could be due to the polymer's crystalline change, whereas the presence of non-amorphous areas in the polymer network makes the gases easily spread through the film, thus causing increased permeability. When the extract is added to the films, the crystalline state of the films increase which gave it a greater potential in gas reserves (11). This decrease has an effective role in the effectiveness of antioxidants as its role is limited by the high interaction of film bonds so limited movement, which reduces the movement of antioxidant, but the indirect role in reducing the amount of oxygen passing, thus reducing of oxidation, The relative humidity has a significant role in the effectiveness of antioxidants because as low moisture reduces oxygen permeability through films, thus reducing the oxidation of fat, where The effective role of the film against oxidation reduces oxygen permeability (11).

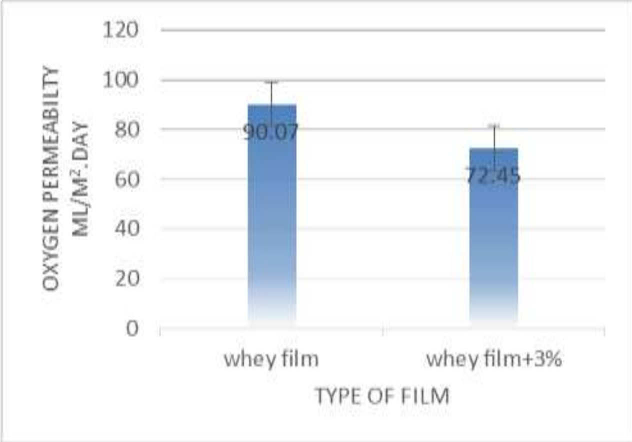

Figure 2. oxygen permeability of whey protein isolates film

Film permeability of water vapor

The process of measuring water vapor permeability of films is important for food packaging processes and its effect in determining the shelf life because it change the sensory properties of food products in addition to contributing to the creation of an environment suitable for the growth of microorganisms (15). Because of food-spoilage and enzymes work in a aqueous medium the process of film formation, temperature and relative humidity have an effect on film permeability (15). The water vapor permeability of the control film was 0.61 (gm. ml./hr.m^2.MPa) while the permeability value of the sample of the film supported by the extract was 0.22 (gm. ml./hr.m^2.MPa) as shown in (Fig.3). This result is lower than that reported by Erdoğan (10), where the value of permeability ranged between 3.1 - 15 (gm. ml./hr.m^2.MPa) . The addition of green tea extract to improve film properties towards water vapor, this reduction in the water vapor permeability is probably due to the complex internal structure of the whey proteins film after interfering with the green tea extract, which reduces the transmission of water vapor through the film (16).

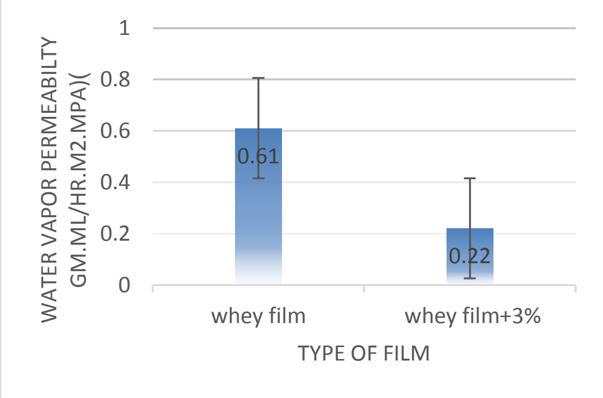

Figure 3. Water vapor permeability of whey protein isolates film

Water solubility of films

The results obtained indicated that the water solubility of the films increased when the extract was added to the film, whereas the water solubility of the control film was 41.2%. While the solubility of the films incorporated with 3% green tea extract reached 47.5%. These results are largely consistent with the results confirmed by Galus (12) Where the values of water solubility of whey protien film was 42.4, This value increases with the addition of the supporting substances. The increase in the water solubility when adding other material because whey proteins have the ability to bind with the chemical compounds and thus from soft structural structures increasing the solubility of films in water (12), (19).

Optical color characteristics

The optical properties of the film play a role in controlling the amount of light passing through the film ,therefore the amount of light passing through the film is effective for the processes of photo oxidation that get in food as the opacity of the film reflects the amount of light absorbed by the films and does not pass through. The chromatic properties determine the color of the film within the system expressed by the measurements of a *, b *, L * where each has a specific chromatic connotation, for example a *, b * reflect the extent of color consistency.The result of the examination of the film from whey protein isolates revealed that there was an increase in opacity when the green tea extract was added and the color changed to yellow, giving it better chromatic properties as compared to the control films so reduce the photo oxidation processes. 29.09% represents the degree of color difference in which color is determined, and L, a and b were respectively (28.3, -7.41, 51.01), the samples of the control films were ΔE 9.6% and (L, a, b), (6.6, -9.27.59.15) respectively. Table (5)

Table 5. Optical and color properties of film samples

Films	Bright.	Opa.	ΔE	a*	b*	*L
Control film	25	-18.7	9.6%	-9.27	6.6	59.15
Film+GTE%	8.49	-194.3	29.09%	-7.41	28.3	51.01

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