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Effect of Formaldehyde Treatment on the Barrier and Mechanical Properties of Oat Protein Films

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Abstract

Oat protein 5% with 40% glycerol as a plasticizer was used to prepare edible films, and formaldehyde was chemically added to the film's solution at four concentrations. The films' barrier and mechanical properties were then investigated. The films were characterized by being medium transparency, homogeneous, odorless, and with a thickness ranging between 0.12 and 0.15 mm. It was also observed that the water vapor permeability decreased whenever the concentration of formaldehyde increased, with 1.963 at 4% of formaldehyde compared to 2.559 in untreated simple films. Tensile strength increased from 3.251 MPa for untreated films to 4.014 MPa for treated films when the formaldehyde concentration rose. The untreated films' elongation decreased from 27.25% to 19.79% at a concentration of 3%. The solubility also increased in value to 14.63% whenever the concentration of the chemical treatment increased, compared to 12.28% in the untreated simple films. The results show an improvement in the mechanical and barrier properties of the oat protein films after chemical treatment with formaldehyde.

Keywords: Formaldehyde treatment, barrier and mechanical properties, oat protein films, edible film

INTRODUCTION

To increase the shelf life of meat and other food goods, coating procedures must be used. Coating techniques are important for extending the shelf life of meat and other food products. However, most coatings processors use non-biodegradable synthetic materials like plastic, nylon, and polyester [1] exacerbating the environmental damage caused by the accumulation and failure of plastic waste containers to decompose. Aside from the toxic risks, it contains substances that, when transferred and interacted with food, pose a risk to the consumer [2, 3].

Because of increased consumer awareness of food safety and the negative environmental impacts of non-biodegradable coatings, consumer demand for safe and stable foods has increased [4]. Recent research has concentrated on the development of coating materials made from natural polymers such as proteins, polysaccharides, or fats, particularly edible ones. These biodegradable polymers are a great replacement for traditional plastics. Furthermore, it works to extend the storage period of the food while maintaining its quality elements for as long as possible; it preserves the physical and chemical properties of the coated food by fortifying it or making chemical modifications [5].

Proteins differ from other biological compounds in that they have a distinct structure. They are composed of 20 different monomers, which allow them to bind with other molecules. They also have superior mechanical and barrier properties for

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gases when compared to fats and polysaccharides, making them suitable for the production of edible films [6].

Improving protein film's barrier and mechanical properties compared to synthetic polymers necessitates modifying it to improve its fibrous structure formation, such as using enzymes or thermal treatments [7]. Alternatively, modification may be done using formaldehyde [8]. The current study aims to determine the effect of formaldehyde treatment on the mechanical and barrier properties of oat protein films.

MATERIALS AND METHODS

Preparing the Simple Coats

The protein film formation solution was prepared according to the method conducted by Habibi Zarabadi et al. [9], with a slight modification to it. Five grams of oat protein concentrate were dissolved with distilled water to obtain (w/v), and then the pH value was adjusted to 10 using a 0.5 M solution of NaOH. Next, the solution was heated at 90°C for 30 minutes using a water bath, and then the film solution was left to cool at a laboratory temperature of 24°C. Glycerol was added to the solution at 40% (w/w of protein). An additional solution mixing step was followed by this step with a magnetic homogenizer for 10 minutes. After conducting several preliminary experiments to obtain a suitable film thickness, 10 mL of the film-forming solution was poured into plastic dishes with a diameter of 9 cm on a flat surface. Finally, the films were dried at a temperature of 45°C for 6 hours inside the hot-air oven.

Preparation of Coating Treated with Chemicals (Formaldehyde)

Protein coating solutions were prepared at a concentration of 5% of oat protein, and then the coating solutions were treated with formaldehyde according to the method mentioned by Al-Rikabi e tal. [8] and De Carvalho and Grosso [10] with some minor modifications, where the previously prepared formaldehyde was added with four percentage additions of 0.01%, 0.02%, 0.03%, and 0.04% (formaldehyde: film solution), so that the concentration of the chemical added to the film solution is healthy and acceptable as follows 0.0001%, 0.0002%, 0.0003%, and 0.0004%. This quantity was taken after doing several preliminary experiments to obtain the appropriate film. Glycerol was added to the solution at 40% (w/w of protein). An additional solution mixing step was followed by this step with a magnetic homogenizer for 10 minutes. After conducting several preliminary experiments to obtain a suitable film thickness, 10 mL of the film-forming solution was poured into plastic dishes with a diameter of 9 cm on a flat surface. Finally, the films were dried at a temperature of 45°C for 6 hours inside the hot-air oven.

Dried films were removed from the templates and conditioned to 52% relative humidity within a desiccator at 23°C.

Conditioning Films

The process of conditioning the edible coating was carried out before laboratory tests were carried out on it by transferring the dried films to a desiccator containing a saturated magnesium nitrate solution at the bottom of the desiccant to set the relative humidity at 52% ± 2% at a temperature of 25°C ± 1°C for at least 48 hours [11].

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Measurements for the Prepared Films

Film Thickness

The film thickness was estimated using an electronic micrometer (Total company). Then, the average was calculated by choosing several locations randomly [12]. The film thickness was estimated before any examination because its value is included in the calculations for estimating water vapor permeability and subsequent mechanical tests.

Determination of Solubility of Films in Water

The film solubility in water was estimated according to the method mentioned by Jancikova et al. [12] with some modifications. First, two square pieces (2 × 2 cm) were cut. Then the initial weight of each film was estimated using a sensitive balance. Next, the samples were immersed in a plastic cup containing 50 mL of distilled water. To this, sodium azide was added at a concentration of 0.02% (weight:volume) to prevent the growth of microorganisms. The cups were closed by their covers and then incubated at 25°C for 24 hours; the mugs were shaken gently and periodically during incubation. After the incubation period, rinse the sample gently with distilled water. Next, it was placed in a drying oven at 105°C for 2 hours. Finally, samples were weighed to obtain the final weight, representing the weight of the dry matter of the shell that was not dissolved in water. The solubility of the films in water was calculated according to the following mathematical equation:

$$Water\ solubility\ of\ the\ film\ (\%) = \frac{\text{Sample Initial Weight (gm)} - \text{Sample Final Weight (gm)}}{\text{Sample Initial Weight (gm)}} \times 100$$

Films Water Vapor Permeability determination:

The amount of water vapor permeable through the prepared and conditioned films was measured at a relative humidity of 52% ± 2% and at a temperature of 25°C ± 1°C for 48 hours, based on the standard method of the American Society for Testing and Materials (ASTM E96-96M-16) [13] with some minor modifications, which were mentioned by Mehdizadeh et al. [11]. Circular plastic cups with an outer diameter of 3.9 cm, an inner diameter of 3.75 cm, and a depth of 6.25 cm were used for this purpose. The cups were filled with up to 10 ml of distilled water, then covered with a circular piece of pre-cut film so that its diameter was larger than the outside diameter of the cup and sealed with a pair of circular rubber bands. The cup was weighed to the nearest 0.001 g using a sensitive scale and transferred to the desiccator, which had anhydrous copper sulfate to regulate the relative humidity at 0% at 25°C for 48 hours. The weight drop was recorded. The data was an average of two replicates per sample. The water vapor permeability calculations were carried out as follows:

First, the water vapor transmission rate is calculated according to the following equation, as follows:

$$\mathrm{WVT} = G/tA = (G/t)/A$$

G: Loss (change) in weight (g).

t: Amount of trial time (hours).

G/t: (Slope) The amount of slope from the straight line (g/hr) was calculated using the Excel program by drawing a relationship between weight loss (g) with time (hr) to the stability of the weight.

A: Cross-section area of the film (m^2), calculated according to the law of the area of the circle for the mouth of the inner cup and according to the equation.

The area of the film = the area of the circle = π x $(\text{radius})^2$; note that $\pi = 3.14$

WVT: Water vapor transfer rate (g/hr. m^2).

Second, the amount of water vapor permeability is calculated from the following equation:

$$\text{Permeance} = \mathrm{WVT}/\Delta p = \mathrm{WVT}/S\ (R1 - R2)$$

$$\mathrm{WVP} = \mathrm{WVT}/S \times (R1 - R2)$$

S: Saturated water vapor pressure at 25°C test temperature (3141.47 Pa).

R1: The amount of moisture to which the film was conditioned (52%)

R2: the amount of moisture in the desiccator (0%).

Determination of Tensile Strength and Elongation to Cut Ratio of Films

The film tensile strength and elongation to cut were estimated after the film had been conditioned at a relative humidity of 52% at 25°C. According to the standard method of the American Society for Testing Materials and numbered (D-882-10) [14] for the year 2010 and described by Gheorghita Puscaselu et al. [15], using a texture analyzer (Zwick Roell, Germany). The film was cut into lengths of 70 mm and widths of 10 mm. The test speed was 50 mm/min, while the initial grip between the two handles of the device was 50 mm. The following equation extracts the tensile strength of the sample:

$$Tensile\ Strength\ (MPa) = \frac{\text{Max Force (N)}}{\text{A}}$$

Max force: The maximum force needed to cut the tape (in Newtons)

A: cross-sectional area of the model (mm^2), and it is calculated as follows:

A cross section area (m^2) = Film width (m) × Film thickness (m)

As for the percentage of elongation at break, it was calculated by dividing the value of the elongation occurring at the moment of the tape break by the initial length of the tape, as in the following equation:

$$Elongation\ at\ break\ (\%) = \frac{\Delta L}{L} \times 100$$

ΔL: change in sample length when the strip is cut off (mm).

Initial L: Initial length of the sample (mm)

Statistical Analysis

The data were statistically analyzed using a complete randomized (CRD) one-way analysis of variance (ANOVA). The data were analyzed by the statistical program (IBM SPSS Version 22), and the averages were compared using Duncan's test at a probability level of ($P < 0.05$) [16].

RESULTS AND DISCUSSION

General Description of Simple and Formaldehyde-treated Films

The simple films of oat protein (OP) were characterized by their transparent light yellow color. The films were also characterized by their soft texture, odorless and delightful taste. As for the coating treated with formaldehyde, its color was dark yellow, homogeneous, of acceptable transparency, odorless, and of good taste. They were glossy compared to the rest of the films.

Film Thickness Treated with Formaldehyde

The results of film thickness are presented in Table 1. The formaldehyde concentration of oat treated in preparing films significantly ($P \leq 0.05$) affected the thickness. Its thickness rose from 0.120 to 0.153 mm whenever the formaldehyde treatment was increased. The reason is that formaldehyde acts as a strong intermolecular bonding material [17], which leads to raising the thickness of the films and improving their mechanical properties. This agrees with previous studies [8, 18].

Table 1. Film thickness on treatment with formaldehyde (mm).

Formaldehyde treated (%)	0	0.01	0.02	0.03	0.04
Film thickness (mm)	0.120^{d}	0.137^{c}	0.142^{bc}	0.147^{ab}	0.153^{a}

*Different letters within the same horizon indicate significant differences ($P \leq 0.05$) between the treatments.

The Solubility of the Films in Water

The solubility of oat protein films at a concentration of 5% treated with four concentrations of formaldehyde is shown in Table 2. It increased significantly ($P < 0.05$) whenever the formaldehyde concentration in the film formation solution rose from 0.01% to 0.04%, so the solubility increased from 12.50% to 14.63%, respectively.

Table 2. Solubility of formaldehyde-treated films in water (%).

Formaldehyde treated (%)	0	0.01	0.02	0.03	0.04
Film thickness (mm)	12.284^{d}	12.509^{c}	13.275^{b}	14.591^{a}	14.633^{a}

*Different letters within the same horizon indicate significant differences ($P \leq 0.05$) between the treatments.

Oat protein films are poorly soluble in water due to their high bonding and high density of covalent and disulfur bonds between the chains. Therefore, the formaldehyde treatment will covalently bind the chains of small molecular weight in the protein network, with some small chains remaining free inside the polymer matrix, thus increasing the chances of its solubility [19].

The results agree with the findings of Al-Rikabi et al. [8]. It was observed from the results that the solubility of whey protein films increased, with an increase in the concentration of formaldehyde added to the film forming solution, with significant differences. The solubility ratio increased from 10.47% to 49.19% whenever the formaldehyde concentration was increased from 0.01% to 0.04% of the whey protein film at a concentration of 6%.

However, the findings of De Carvalho and Grosso [10] when gelatin films were treated with formaldehyde showed that the solubility decreased from 31.5% to 25.5%, and the decrease was directly proportionate to the formaldehyde concentration.

Water Vapor Permeability of Films

Water vapor permeability is one of the most important barrier tests for protein films, which helps in evaluating its performance and knowing its ability to protect foods from moisture loss during storage operations.

The values of the permeability of oat protein films to water vapor decreased significantly with the increase in formaldehyde concentration. The water vapor permeability decrease was from 2.281 to 1.963 (g.mm/m^2.h kPa) when the formaldehyde concentration increased from 0.01% to 0.04% (Table 3).

Table 3. Permeability of formaldehyde-treated films to water vapor (g.mm/m^2.h kPa).

Formaldehyde treated (%)	0	0.01	0.02	0.03	0.04
Film thickness (mm)	12.284^{d}	12.509^{c}	13.275^{b}	14.591^{a}	14.633^{a}

*Different letters within the same horizon indicate significant differences ($P \leq 0.05$) between the treatments.

This decrease was due to the fact that formaldehyde reacts with the protein in two stages: the first was the formation of the methylol compound, and the second corresponds to the formation of methylene bridges, which are cross-links between protein chains, giving strength and durability to protein films [20]. This agrees with the work of Hernández-Muñoz et al. [21], which indicated that the treatment of films based on the protein glutenin from wheat with aldehydes, especially formaldehyde, improves the functional properties of films. It also reduces the film permeability values to water vapor by 30%, the higher the formaldehyde content.

This was also consistent with what was proposed by De Carvalho and Grosso [10]. When treating gelatin films with formaldehyde, they noticed a decrease in water vapor permeability from 0.198 to 0.155 g.mm/m^2.h kPa when the formaldehyde concentration is raised from 0% to 8.8%. The authors attributed the low permeability values to the presence of lysine in the protein chains. Moreover, formaldehyde was more effective with lysine available than with other amino acids such as cysteine and histidine, which can lead to an increase in the degree of crosslinking between the polymer chains.

The results agree with the findings of earlier studies [8, 18], when treating whey proteins with formaldehyde, where the decrease in the permeability values was observed with an increase in the concentration of formaldehyde added to the films and to all concentrations of whey protein.

The results did not agree with those of Al-Abadi [22] when adding thyme essential oil to cellulosic films, where a rise in water vapor permeability was observed in the composite films with thyme essential oil, reaching 8.78 g.mm/m^2.h.kPa, compared with the simple films, which recorded 6.59 mg. mm/m^2.h.kPa when the oil concentration was raised from 0% to 3%. Al-Sadi [23] also indicated that the permeability of the films to water vapor increased when the lupine protein concentration increased from 6% to 8%, reaching 4.71 and 5.12 g.mm/m^2.h.kPa.

Tensile Strength of Formaldehyde-treated Films

Table 4 shows the results of the tensile strength of oat protein films with a concentration of 5% after treating them with different concentrations of formaldehyde. It was noticed that the tensile strength values significantly increased with the increase of the formaldehyde concentration, where the highest value of the tensile strength of the film was 4.014 MPa at a concentration of 0.03% formaldehyde, while the lowest value of tensile strength was recorded at 3.285 MPa at the low concentration of formaldehyde at 0.01%.

Table 4. Tensile strength of films treated with formaldehyde (MPa).

Formaldehyde treated (%)	0	0.01	0.02	0.03	0.04
Film thickness (mm)	3.251^e	3.285^d	3.590^c	4.014^a	3.905^b

*Different letters within the same horizon indicate significant differences ($P \leq 0.05$) between the treatments

Some chemicals, such as formaldehyde, were considered chemical binding agents that enhance and improve the mechanical properties of protein coats by their interaction between the network of polymer chains [24].

The best chemical bonding agent to increase the tensile strength and toughness of edible films used in food coating applications, according to Benbettaïeb et al. [17], is formaldehyde. These findings concur with those of Al-Garory [18], who found that the tensile strength of whey protein films increases as the film's formaldehyde concentration rises.

According to Wittaya [20], formaldehyde could be a cross-linking agent into protein chains; this gives strength and durability to protein films.

Elongation to Cut for Formaldehyde-treated Films Ratio

Table 5 shows the results of elongation of the oat protein film at 5% concentration, prepared by adding 40% glycerol and treated with four concentrations of formaldehyde. The maximum elongation was 25.10% at a 0.01% concentration, and the elongation was recorded at 20.27% and 20.03% for chemical concentrations of 0.02% and 0.04%, respectively, while the lowest value of the elongation ratio was 19.79% at a concentration of 0.03%.

Table 5. Elongation to cut of films treated with formaldehyde (%).

Formaldehyde treated (%)	0	0.01	0.02	0.03	0.04
Film thickness (mm)	3.251^e	3.285^d	3.590^c	4.014^a	3.905^b

*Different letters within the same horizon indicate significant differences ($P \leq 0.05$) between the treatments

From the results, it can be observed that the gradual decrease in the elongation ratio characteristic, the higher the proportion of formaldehyde-treated in the protein film, can be inferred that the increase in crosslinking in the polymer matrix is a result of the ability of formaldehyde to link the chains among themselves and the increase in the tensile strength in them, which was reflected in the ability of these films to elongate. This is in agreement with other studies [24, 25]. According to Benbettaïeb et al. [17], the interactions that occur within the chains between formaldehyde and amino acids at the terminal active groups tend to improve the strength and cohesion of edible films.

Our results agree with those of Wittaya [20] that formaldehyde is one of the simplest cross-linking agents because it has a unique mechanism that specializes in the binding between the amine group in the amino acid lysine and the side chains of amino acids in other chains containing cysteine, histidine, tryptophan, or arginine. Although formaldehyde has one functional group, it can interact bifunctionally and thus crosslink.

These results were in agreement with the findings of Al-Garory [18], who observed a decrease in the elongation rate of whey protein films as the formaldehyde concentration in the film increased.

CONCLUSIONS

The findings of this work revealed that edible oat films reticulated with formaldehyde had a higher thickness, solubility, elongation to cut, and tensile strength than untreated films. Also, the formaldehyde-treated film has significantly lower water vapor permeability than the untreated film. Those results showed that the barrier and mechanical properties of the edible oat films could be significantly improved by the cross-linking chemical agent (formaldehyde). Overall, this study suggests that the modified oat films could potentially be used as an edible coating for fresh beef burgers, thereby helping the food industry control the use of some non-biodegradable plastic films used in meat packaging.

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