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Effect of transglutaminase on mechanical and barrier properties of edible films made from soybeen and why protein isolate

Alsaadi. R. A
Researcher, Dep. of Food Sci., Coll. Agric. Engin. Sci., University of Baghdad, Baghdad, Iraq
Corresponding author email: rowaid.ahmed1202a@coagri.uobaghdad.edu.iq

Sedrah. Z. T
Assist. Prof. Dep. of Food Sci., Coll. Agric. Engin. Sci., University of Baghdad, Baghdad, Iraq
Email: ziad.t@coagri.uobaghdad.edu.iq

***Abstract*---**This study was aimed to study the effect of adding transglutaminase (TGase) on the mechanical and reservation properties of the edible films manufactured from soybean meal protein isolate (SPI) and whey protein isolate(WPI). The results showed an improvement in the properties with increase in the WPI ratios. Thickness of the SPI films amounted 0.097 mm decreased to 0.096 mm for the WPI: SPI films at a ratio of 2:1, when TGase was added decreased to 0.075 mm. While the tensile strength increased from 7.64 MPa for SPI films to eight MPa for the WPI: SPI films at a ratio of 2:1, when TGase was added increased to 11.04 MPa. Also, the elongation of the WPI: SPI films at a ratio of 2:1 presence of the TGase decreased to 40.6% compared without TG ase amounted 47.75% after being in the SPI film 90%. TGase also reduced the water vapor permeability of the WPI: SPI films at a ratio of 2:1, reaching 5.608 (g. mm/cm². Pa. day), while without TGase reached 7.6548 (g. mm /cm². Pa. day) after it was 8.3082 (g. mm /cm². Pa. day) in SPI films. The solubility decreased from 79.2% for the SPI films to 57.12% for the WPI: SPI films at a ratio of 2:1 and also decreased to 9.69% in the presence of the TGase.

***Keywords*---**cross-linking, elongation, water vapor permeability, edible protein film.

Introduction

Cross-linking is defined as the process of linking polymer chains through non-covalent or covalent bonds to form three-dimensional networks .TGase enzyme works on the formation of crosslinking links by incentivize the interactions between the chains of the amino acid glutamine and between it and the residues of the amino acid lysine. it is used to modify the structure of the protein. Through the cross-linking provided by this enzyme by linking the polymer chains by the covalent bonds that it forms, and through the studies conducted by the researchers, they found its high potential to improve the properties of the protein membranes, where the cross-linking is one of the most accepted methods of modifying the properties of the protein (1). Most of the studies focused on whey proteins, soybeans, collagen, gelatin, casein, corn zein and wheat gluten as one of the most protein sources used in preparing edible films and coatings (2). Edible films prepared a suitable alternative to Plastic food containers and bags classified under (Polyethylene Terephthalate) . which is used in preserving some foods because of its negative effect on foods. It was observed that when used in heating foods in microwaves or heating to boiling point, the concentrations of all heavy metals rose above the permissible limits set by the Food and Drug Administration (FDA) and European Commission. EU (3). Edible films and coatings are thin layers of edible materials, which have a high ability to provide a barrier against moisture and gases in addition to controlling the movement of dissolved substances within foods. It helps maintain the freshness of foods and facilitates transportation, storage and display, in addition to improving mechanical properties, moisture and gas barriers, and protection against microbes, as well as increasing the shelf life of food products. (4). Most of the recent research focused on discovering the Complementary advantages that are obtained through composite films or multi-component films. Where it was observed through the study conducted by the researcher (5) an improvement in the mechanical, barrier and antioxidant properties of the modified whey protein membranes by adding tea extract. In this regard, the current study aimed to use the enzyme transglutaminase in the manufacture of edible films from SPI and WPI in addition to a mixture of the two in the ratio (1:2 and 2:1, w/w).

Materials and Methods

Extraction and partial purification of transglutaminase

Transglutaminase was extracted from the chard and partially purified using ammonium sulfate and dialysis bags, after cleaning the plant, washing with distilled water, and then extracting and partial purification according to the method mentioned by (6**).**

Preparation of protein isolate

Protein isolate was prepared from soybean meal. Argentine soybean meal was used in the extraction. It was cleaned of impurities and then ground by a laboratory mill, and then the sieving process was carried out using a laboratory sieve (60 mash). Then the process of defatting and alkaline extraction of the protein isolate was carried out according to the method mentioned (7).

Preparation of film solution

Film forming solution was prepared according to the method described by (8), with some modifications for adding the enzyme as follows: 6 g of the protein isolate was dissolved in 100 ml of deionized water under stirring; glycerol was added at 40% of protein as a plasticizer. The pH was adjusted to 10 by 2N NaOH, and then the mixture was mixed for one hour at 70°C. After that, the solution was left to cool at room temperature and filtered using a piece of gauze to avoid any lumps or insoluble residues. Then enzyme was added in the amount of 4 units per gram of protein (9), 15 minutes were added to the time of preparation of membranes in the presence of the enzyme (10).table1 shows the number of concentrations used in preparing samples of membrane solutions.

Film Formation

According to (11), eight grams of the film solution transferred to Petri dishes with a diameter of 9.1 cm and spreading quietly while moving the dish left and right on a flat surface. The dishes were stayed at laboratory temperature for 20-24 hours, and then films were removed from the dishes using a spatula, stored in polyethylene bags, and kept in the refrigerator until tests were conducted on them.

Films symbol	Percentage of protein used from SPI %	Percentage of protein used from WPI %	Glycerol %	TGase Units
T1	6 %	0	2.4 %	0
T2	4 %	2 %	2.4 %	0
T3	2 %	4 %	2.4 %	0
T4	0	6 %	2.4 %	0
T1A	6 %	0	2.4 %	24U
T2A	4 %	2 %	2.4 %	24U
T3A	2 %	4 %	2.4 %	24U
T4A	0	6 %	2.4 %	24U

Table 1. Shows the proportions of the edible film components

Determination of the film thickness

Seven parts were chosen randomly from the periphery to the center of the film then the average of these readings was calculated according to (13).

Determination of tensile strength and elongation at break

Tensile Strength Tester was used to measure the tensile strength and the percentage of elongation at break according to the method mentioned (14). The sample was cut into strips of 100 mm in length and 20 mm in width. and the speed of withdrawal of the model was is 5 mm/s.

Water vapor permeability

The water vapor permeability (WVP) was estimated by weight and according to the standard method (ASTM) numbered E-96-95 mentioned by (15).

Determination of the solubility of films in water

The percentage of solubility was determined according to the method described by (16).The film was cut into small pieces weighed 2 grams from piece were dried in a vacuum oven at 70 °C for 48 h, weighed, and assigned an initial dry weight ،then immersed into 50 ml of distilled water and left for 24 h at room temperature (35±2) °C, then the solution was filtered by whatman filter paper No.1(pre-weighted) to separate the insoluble fractions, then the filter papers and their residues of insoluble biofilms were dried in an vacuum oven at a temperature of (105°C) until the weight was stable, following equation was used to computed the percentage of solubility:

$$\%\text{solubility} = \frac{\text{Initial dry weight} - \text{final dry weight}}{\text{Initial dry weight}} \times 100$$

Determination of the water holding capacity of films

The percentage of holding water of the edible films was determined according to the method mentioned by (17), by weighing one gram of the film, and immersing it in distilled water for ten seconds for two successive times, then taken out of the water and left in the oven for a little while until the weight of the film is stable. Following equation was used to computed the percentage of water holding capacity(WHC):

$$\%\text{WHC} = \frac{F_w - I_w}{I_w} \times 100$$

Where F w = Final weight after immersion. I w = Initial weight before immersion.

Results and Discussion

Mechanical and reservation tests

Films thickness

The thickness test is one of the important tests when performing the rest of the tests. Prepared edible films were homogeneous in addition to not containing bubbles. Thickness changes according to the materials included in the films composition, where we note through Figure1. Results of thickness films decreased by simple differences for the different types of films according to the type of protein isolate used in preparing the film, where the results of the treatments T1, T2, T3, T4 reached 0.097, 0.096, 0.096, 0.086 mm, respectively. In addition to the decrease in the thickness of the films modified by TGase to 0.08, 0.075, 0.07,

0.065 mm, respectively. Decrease in thickness may be due to the cross-linking provided by the enzyme, which reduced the moisture content of the film, and led to a reduction in thickness. These results were in agreement with the results obtained by (18), where the thickness of the films prepared from olive leaf extract decreased from (0.301 mm) to (0.138 mm) in the presence of the TGase, attributing the reason for the cross-linking provided by the enzyme causing a decrease in films swelling, thus reducing the Moisture content causes low thickness.

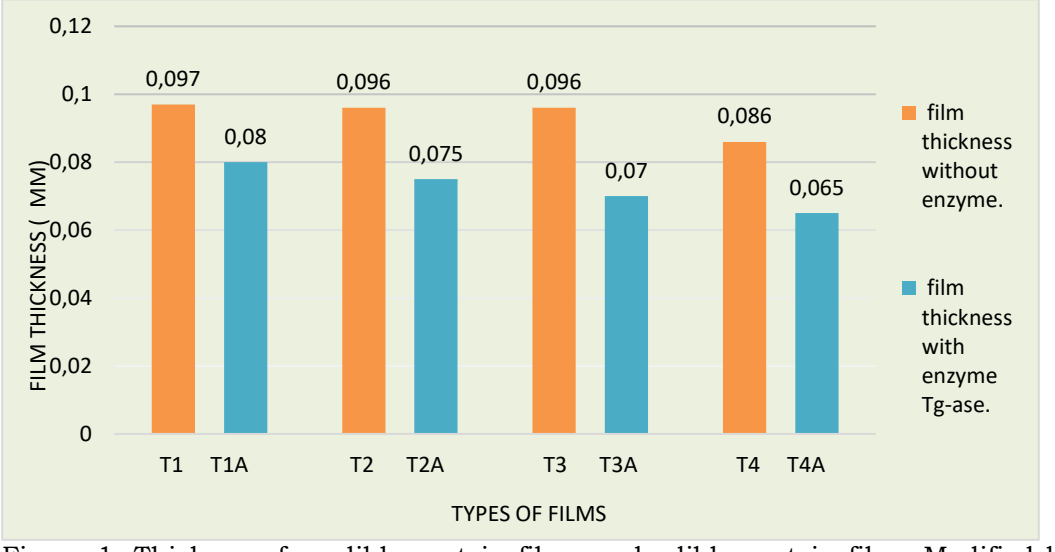

Figure 1. Thickness for edible protein films and edible protein films Modified by transglutaminase. T1, T2, T3, and T4 refer to the protein films in different concentrations, T1A, T2A, T3A, and T4A refer to the protein films Modified by TGase

Tensile strength (TS)

Tensile strength refers to maximum tension the film can withstand during handling, storage and transportation, figure 2. Showed results for edible protein films with different mixing ratios, TS for treatment T1 was 7.64 Mpa this result was close to what was found by (19) where the TS was reached 8.4 Mpa. While, the treatment T4 formed 8.14 MPaWhere this result came approach of what was found by (20) where TS formed 9.32 MPa. Results of the treatments T2 and T3was reached 7.91 and 8 MPa, respectively. This discrepancy in ratios may be due to several reasons, including the difference in the concentration of the protein isolate, as the higher the percentage of the protein isolate, the greater the tensile strength of the films as a result of the interconnections that occur between the protein chains. It provides an opportunity to increase the interactions between protein molecules as a result of releasing the hydrophobic and sulfhydryl groups. Which leads to an increase in the bonding between the protein chains and an increase in the tensile strength of the films. In addition to the basic conditions of the solution forming the films, increased TS with an increase in the percentage of whey proteins due to the increase in the protein-protein bonds in the most alkaline conditions, which led to an increase in the bonding between protein

molecules and the formation of strong bonds between the protein chains, which led to an increase in the tensile strength (21). Also noticed from Figure 2. TS ratio of all films modified with TGase enzyme increased to 8.3, 10.5, 11.04, 12.7 Mpa, respectively. This increase is consistent with the result reached by (22) where the TS of the films increased from 2.21 MPa to 2.58 in the presence of TGase. These results also agreed with the findings of (18), where the TS in the presence of the enzyme increased from 3.97 MPa to 4.02. This increase is likely due to the cross-linking achieved by the enzyme forming a highly elastic gel. It is possible that the gel caused by the enzyme is due to the difference in the nature of the free amino acids present in the protein, especially those able to interact with the TGase (glutamine and lysine), amount and distance between available amino acid residues and the number of enzyme inhibitors that may be present in the reaction system plays a major role in the variability in the rate of gel formation and the increase in tensile strength. (23)

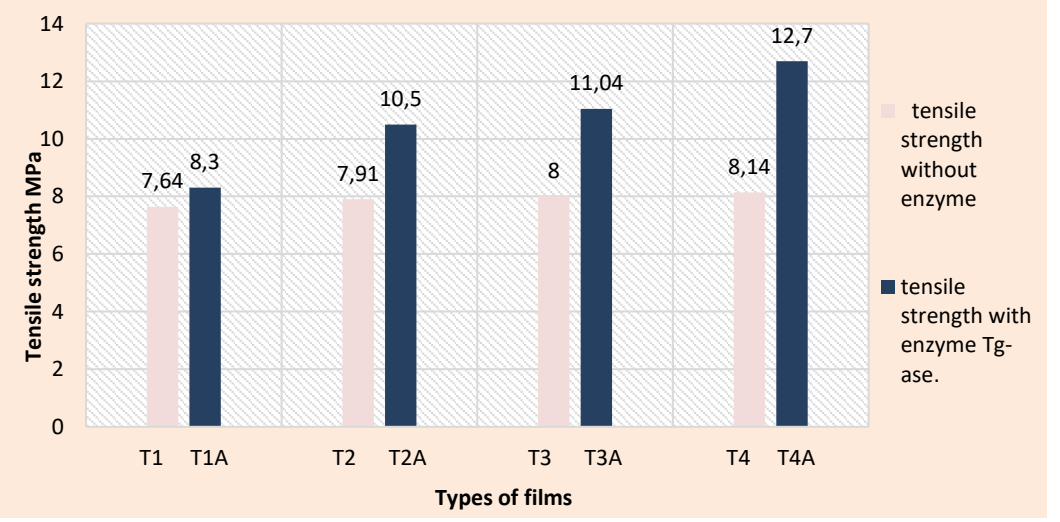

Figure 2. Tensile strength for edible protein films and edible protein films Modified by TGase.T1, T2, T3, and T4 refer to the protein films in different concentrations, T1A, T2A, T3A, and T4A refer to the protein films and Modified by TGase.

Elongation at break (EL)

Elongation at break a measure of the amount of stretching in the films, which expresses the percentage change in the original length of the films before discontinuation (24). Figure 3. Showed results for protein films with different mixing ratios. We note a gradual decrease in the percentage of elongation with an increase in the percentage of WPI, corresponding to a decrease in the percentage of SPI, where the results of the treatments T1, T2, T3, T4 decreased 90.0, 87.13, 47.75, 31.1 %, respectively. The result of the percentage of EL of SPI films is close to what was found by (25), where EL was 81.7%. In addition to a decrease in the elongation rate of films modified by TGase for treatments T1A, T2A, T3A, T4A to 40.3, 21, 40.6, 29.1 %, respectively. This decrease in the percentage of elongation may be due to the higher water carrying capacity of the WPI compared with SPI

and an explanation for this, the higher the percentage of WPI and the lower the percentage of SPI in the film preparation solutions led to a decrease in the percentage of elongation due to the increase in the water carrying capacity of the protein mixture in the film solution Which caused an increase in the structural cohesion of the films due to the transverse bonds formed between the polymer chains, which led to an increase in the cohesion strength and a decrease in the movement of molecules between the polymer chains, which resulted in a decrease in the stretch ability. In addition to the role of the transglutaminase enzyme when added to protein membranes, a Homopolymer has a high water-retaining capacity. This polymer was formed when the subject was one type of protein, which resulted in the formation of a molecular cross-linking. And if the subject material consists of two types of proteins or more, the enzyme works to form cross-links forming a heteropolymer characterized by the formation of a feast of functional properties (23). This cross-linking reduced the movement between the polymer chains, which led to This decrease in the percentage of elongation also agreed with the result reached by (22), where the percentage of elongation decreased from 159.87% to 105.88 in the presence of TGase enzyme with soybean protein isolate membranes, and this is due to the difference in the nature of the constituent protein of the membrane and the percentage of enzyme addition as well as the reaction conditions.

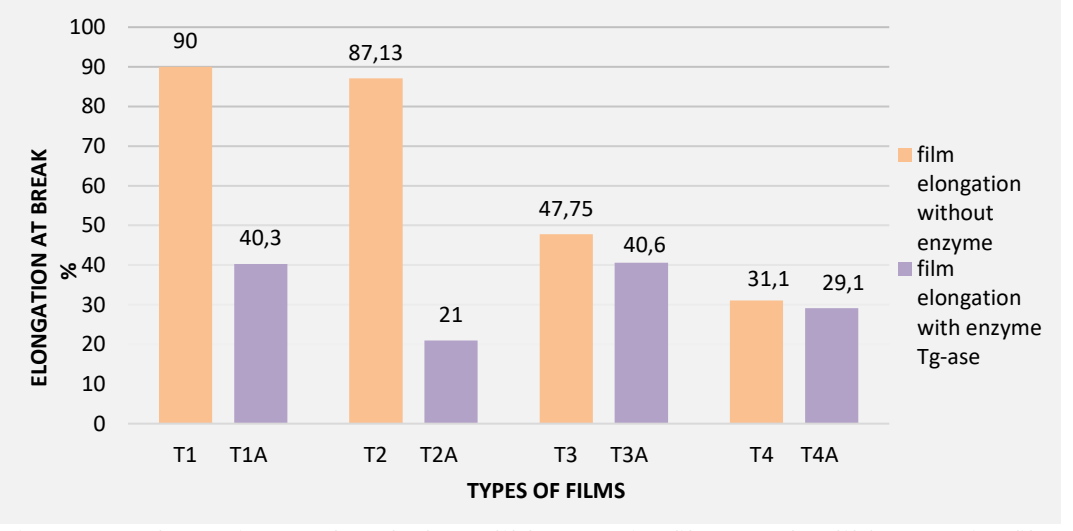

Figure 3. Elongation at break for edible protein films and edible protein films Modified by TGase .T1, T2, T3, and T4 refer to the protein films in different concentrations, T1A, T2A, T3A, and T4A refer to the protein films and Modified by TGase

Water Vapor Permeability (WVP)

One of the most important barrier tests for edible films has been WVP, because it is closely related to the moisture factor and its effect in providing appropriate microbial conditions for the growth of microorganisms in foods in the presence of a high moisture content (26). Figure 4. Showed results for protein films with different mixing ratios. In general, decrease in the WVP of the edible films with an

increase in the percentages of WPI for treatments T1, T2, T3, T4 were reached 8.3082, 8.225, 7.6548, 6.9892 (gm. mm./cm². Pa. day) respectively. The reason for this may be attributed to the higher ability of WPI to retain water compared to SPI, which leads to a reduction in the distance between protein molecules by increasing the concentrations of whey proteins, in addition to the heat treatment of 70 M and pH 10 that increased the solubility of the proteins, which led to reducing the distance between protein molecules with the formation of bonds between the protein molecules included in the formation of the film, which reduces the WVP of the films (26). In addition, the presence of enzyme TGase improved the permeability of the edible protein films, Where the results are decreased to 6.1828, 5.9610, 5.6080, 5.5673 (g. mm./cm². pas. day), respectively. These results are in agreement with the results found by (12), in the study that she conducted on whey proteins, where she noticed a decrease in WVP in the presence of the enzyme TGase. The reason may be attributed to the fact that the addition of the enzyme increases the content of the dry matter in the films through the cross-linking provided by the enzyme directly affects the reduction of the ability of proteins to bind and retain water through sharing and linking between the amino groups, especially when the amino acid lysine is present, and this linkage makes the opportunity impossible Available to link amino acids with water through hydrogen bonding (27), which leads to a decrease in the water-carrying capacity of the films, thus reducing the thickness of the film and improving the permeability of the edible films.

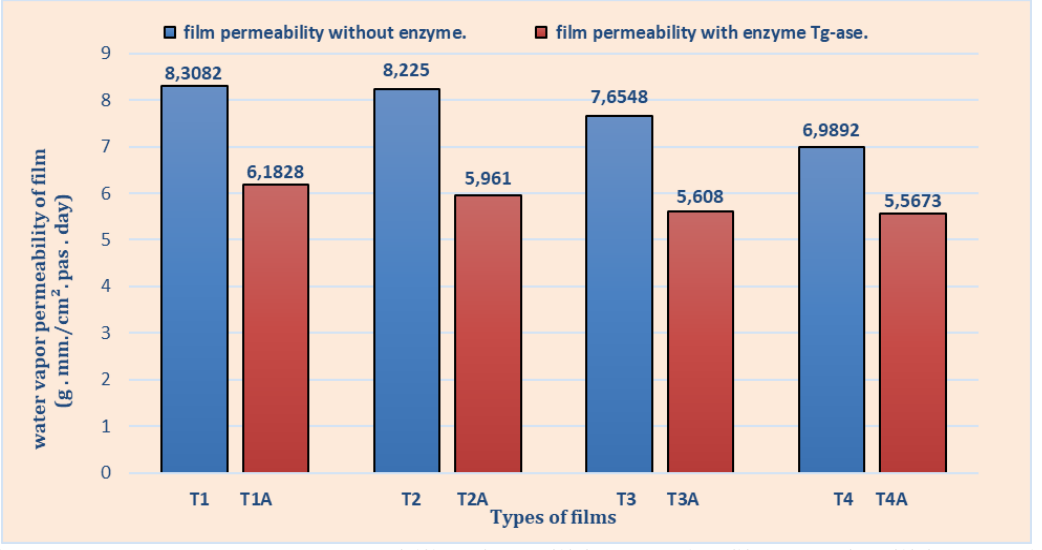

Figure 4. Water Vapor Permeability for edible protein films and edible protein films Modified by TGase T1, T2, T3, and T4 refer to the protein films in different concentrations, T1A, T2A, T3A, and T4A refer to the protein films and Modified by TGase

Water solubility of films

The results in the figure 5. Shows a decrease in the percentage of films solubility with an increase in WPI and a decrease in SPI concentrations. Where the results for treatmentsT1, T2, T3, T4 without the enzyme were reached 79.2, 65.15, 57.21,

45, 68 %, respectively, the percentages of T4 treatment were similar to what was found by (28), where the results of the solubility of WPI films in water were 42.4%. The reason may be attributed to the difference in the ability of proteins to dissolve in water with different hydrophilic and hydrophobic protein molecules present on the surface of the protein when in contact with the surrounding water. Where the fewer hydrophobic parts on the surface Increased solubility of the protein. In addition to the interaction between the external properties of the molecule with the internal properties of the solution such as pH, temperature, ionic strength as well as the heating time during the preparation of the coating, this depends critically on the interactions that proteins establish with other macromolecules present in the food (23). The figure 5. Also showed a decrease in the solubility of all films modified with the enzyme, where the results were 36.88, 17, 9.69, 5.36 %, respectively. These percentages also varied with the results found by (22) in the study he conducted on the protein isolate of soybeans without and with the presence TGase by adding 4 U/g of protein, the solubility results decreased from 40.12% to 31.44% in the presence of TGase. The reason may be attributed to the cross-linking caused by TGase, which reduced the moisture content of the protein molecules due to the lack of opportunity for the residues of amino groups, especially containing lysyl residues. The enzyme crosslinked is not available to bind with water via the hydrogen bond, which reduces its solubility (22).

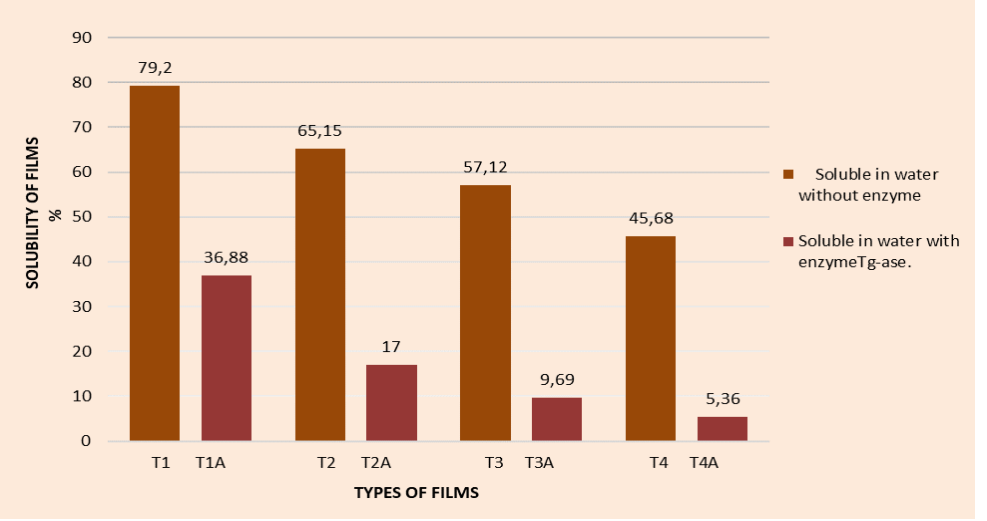

Figure 5. Water solubility for edible protein films and edible protein films Modified by TGase .T1, T2, T3, and T4 refer to the protein films in different concentrations, T1A, T2A, T3A, and T4A refer to the protein films and Modified by TGase

Water Holding Capacity of films (WHC)

Figure 6. Shows increase in the WHC of the container films high percentages of WPI and low percentages of SPI, results of treatments T1, T2, T3, and T4 without TGase were reached 37.28, 68.8, 87.7, and 108.3 %, respectively. Reason may be due to the abundance of hydrophilic groups in the structure of the film with the increase in the percentage of whey proteins, which helped to increase the binding

with water molecules, figure 6. Also showed the high WHC of the film modified at TGase for treatments T1A, T2A, T3A, T4A where reached 61.58, 109.55, 118.78, 140.41 %, respectively. Reason for the increase in the presence of enzymatic modification may be attributed this to the deamination of glutamine residues that increase the protein's hydrophobicity associated with the gel produced by the polymerization of protein chains, forming a strong gel structure It has a High porosity is able to immobilize water more efficiently. However, both deamination interactions and TGase-catalyzed cross-linker formation directly affect WHC in various protein sources (23).

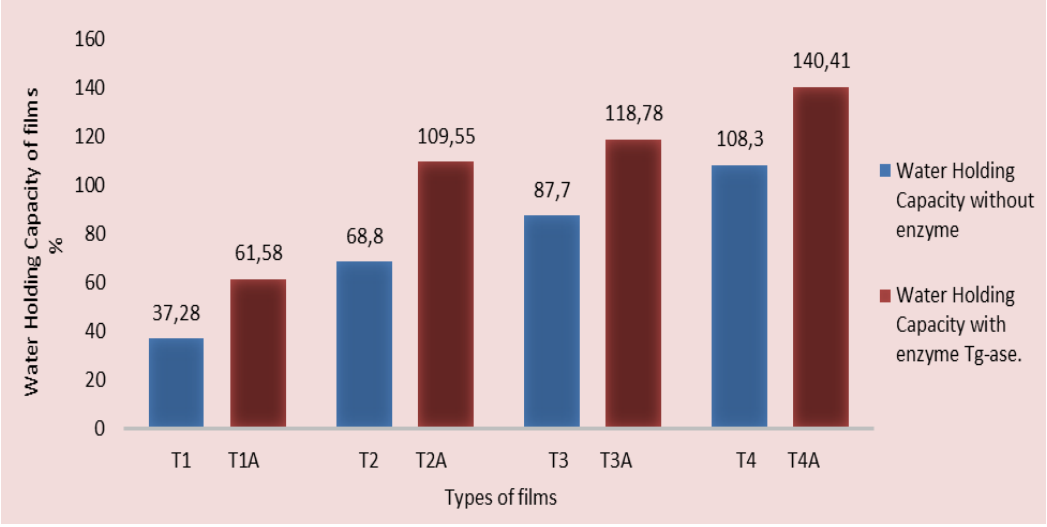

Figure 6. Water Holding Capacity for edible protein films and edible protein films Modified by TGase . T1, T2, T3, and T4 refer to the protein films in different concentrations, T1A, T2A, T3A, and T4A refer to the protein films and Modified by TGase

Conclusions

In our study in general use more than one protein source in addition to Enzymatic modification of edible protein films by (4 U / g protein) of TGase reveals an improvement in mechanical and reservation properties of edible films prepared from different protein sources and different concentrations.

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