Manufacture of edible films using pea protein, buffalo milk casein and their mixtures and study their physical and mechanical properties

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Abstract:
Given the importance of packaging, especially bio-packaging for various food products, the current study was conducted and aimed to extract pea proteins and study the optimal conditions for extraction and then manufacture edible films using each of the pea proteins extracted in different proportions represented by treatment T_1 and buffalo casein protein represented by treatment T_2 and mixtures of pea protein and buffalo casein protein with three mixing ratios (25,50,75)% represented by treatments T_3, T_4,T_5 and study the physical and mechanical properties of these films. Various tests were conducted for the mentioned earlier films, which included tests of protein extraction conditions, best time, pH, and water addition rate, while physical tests included water vapor, while mechanical tests included both tensile strength and elongation limit tests. The results showed that the best conditions for extracting pea proteins were a time of 90 minutes, a mixing ratio of pea flour to water of 1: 20 w/v, the best pH for protein solubility was 10, the best pH for protein sedimentation was 4.5, and the protein yield was 18.22 g. As for the results of the mechanical tests, it was not clear that there were significant differences of $0.01\leq$ P between the pure Treatments (T_1 and T_2), with significant differences when comparing the pure Treatments and mixtures (T_3, T_4, T_5). The importance of the results of our study highlights the opening of the field for manufacturing composite protein coatings with improved mechanical properties made from pea protein and buffalo casein protein, which enhances sustainable packaging and maintains the food product properly

Keywords: buffalo casein, pea protein, water vapor permeability, tensile strength, elongation.

Introduction:

Packaging is a critical component of food processing operations, protecting food products during transportation and distribution while maintaining their quality [3]. In recent years the environmental impact of traditional food packaging materials has been the subject of increasing awareness and interest worldwide [4]. Due to the increase in the production and use of plastics resulting from petroleum polymers in the manufacture of covers and films [26], studies indicate that the daily use of harmful plastic waste reaches 35 million tons, but the recycling rate does not exceed 7% [42] ,it is also noted that these materials consist of harmful chemical components, which makes their irresponsible use and disposal a source of serious risks to ecosystems [41] .

The search for environmentally acceptable and sustainable alternatives has led to increased pursuit and innovation in the field of food packaging biomaterials [25].The possibility of reducing plastic waste has led to a great deal of interest in biodegradable

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polymers that decompose spontaneously over time [49]. Currently, edible and biodegradable wrappers and coatings are an integral part of food packaging, and these wrappers consist of proteins, polysaccharides, fats, or a combination of the above materials and may be supported by some antimicrobial components, and these wrappers are in the form of a thin layer that is safe for consumption with food [26]. Protein-based membranes are highly efficient at blocking oxygen even at low relative humidity (RH) and are effective at blocking hydrophobic substances such as oil, odor, and microbes. They can also be fortified with antimicrobial agents, antioxidants, and many food additives [41].

Protein-based films are widely used in a variety of foods such as fruits, cheeses, and meat [39], and plant-derived proteins (such as soy protein or wheat gluten) or proteins of animal origin (such as gelatin, shingles protein, or casein) can also be used as membrane-creating agents in edible coatings [16].

Peas are one of the sources that have gained great popularity at the present time as a source of plant protein, as they contain a high percentage of protein, reaching about 20-25%. The majority of these proteins consist of globulin 65-80% and albumin 10-20%. Studies have also indicated that Pea protein has its nutritional value and health benefits, as it contains a good percentage of amino acids It is also sufficiently available and low cost [48]. Pea protein can be used in baked goods, meat, and dairy products because of the functions it can provide such as emulsification, condensation, coagulation, and foaming [53]. (For concentrates or isolates) of pea protein can contribute to barrier properties and aid envelope stability [31].

As a Source of animal proteins used in edible coatings, casein is the main protein in milk (80% of milk proteins), which is available in several forms, including sodium caseinate and calcium caseinate [44]. Casein is consumed all over the world, especially in developing countries, due to its widespread availability and large quantities [28].

. Casein has been shown to have nutritional and health properties as well as antioxidant properties [28]. It also has properties such as enzymatic hydrolysis, emulsification and gel formation capabilities that contribute to improving the texture and texture of foods. Due to these properties, casein is used in the food industry [45] and [43]. Casein-based biopolymers have generally been prepared to improve the physical and chemical properties of coatings (e.g. mechanical properties) [35]. Caseins have the ability to create edible coatings from aqueous solutions without any additional processing [38]. Buffalo milk has a higher protein content than the milk of other animal species [27], so it was useful to use it in this study.

Buffalo casein peptides have also been shown to inhibit angiotensin-converting enzyme, which may protect blood vessels and the heart [47]. This study aimed to prepare and extract buffalo casein protein and pea protein, manufacture edible casings from each of the two proteins with 100% purity, manufacture composite casings by mixing pea protein with buffalo casein protein at mixing ratios of 25, 50 and 75%, and conduct membrane tests (mechanical tests: elongation, cutting limit and tensile strength test).

Materials and methods:

Materials:

Buffalo milk from the fields of the Qasim region in Babylon was used to prepare casein

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protein, and pea grains from local markets were used to extract pea proteins.

Factors affecting the production yield of pea protein extract:

Determine the best extraction time:

The best time period for extracting defatted pea meal protein was determined according to the method reported by Hayat *et al*. [27] and the mixing time periods were (30, 60, 90 and 120) minutes to determine the best time period to produce the highest percentage of the protein extract.

Determine the best water mixing ratio:

The best mixing ratio for pea flour removed from fat with water was determined according to the method mentioned by Gayol *et al*. [21] using mixing ratios (1:10), (1:15), and (1:20) weight/volume to achieve the best ratio for the protein extract quotient.

Determine the best pH for protein solubility:

Protein solubility was estimated according to Horax *et al*. [30] ،take 2 grams of defatted pea flour and add 20 ml of distilled water. Adjust the pH numbers to (2, 4, 6, 8, 10, and 12) using a standard sodium hydroxide solution (N)1 and a standard hydrochloric acid solution (1N) to observe the best pH for protein solubility, while stirring continuously using magnetic mixing at a temperature of 25°C for 30 minutes, then expose to cryogenic centrifugation At a speed of 10,000 rpm, the protein content was then measured after taking the filtrate using the Micro Kjeldahl method, and the percentage of protein solubility was calculated according to the following equation:

$$Solubility\ percentage\ \% = \frac{\text{filtering in protein ratio}}{\text{sample in protein ratio}} \times 100$$

Determine the best hydrogen number for protein deposition:

The best pH for the precipitation of defatted pea flour protein was determined according to the method mentioned by Karaça *et al*. [34], where the filtrate with pH 10 (previous paragraph) was taken and distributed into pre-weighted 20 ml test tubes after which the pH was reduced to (3, 3.5, 4, 4.5, 5 and 5.5) by means of HCL(1N) hydrochloric acid, The cryogenic centrifugation process was then performed at a speed of 10,000xg for 20 minutes, after which the filtrate was neglected and the precipitate was weighed to determine the most appropriate pH for protein deposition to produce the highest yield of protein isolate.

Estimating the yield of protein extract:

The percentage of protein extract from defatted pea flour was calculated according to the method mentioned by Gao *et al*. [20] as in the following equation:

$$\text{Protein isolate yield \%} = \frac{\text{(g) isolated protein concentrate weight}}{\text{(g) pea−free fat flour weight}} \times 100$$

Pea protein preparation:

Pea flour was treated in a 1:4 hexane/acetone mixture to remove color and oils from pea flour by leaving the mixture of pea flour with the hexane and acetone mixture with magnetic mixing for 60 minutes, then centrifuging to separate the mixture from the pea flour, repeating the process to ensure the removal of all oils. After that, the method mentioned by Acquah *et al* [2] was followedwith modification to extract pea protein isolate, where pea flour was dispersed in water at a mixing ratio of pea flour to water of 20:1) weight/volume) at 25 degrees Celsius, after which the pH was modified to (10 PH =) using Sodium hydroxide solution (NaOH) titrated (2 N) for 90 minutes Followed by centrifugation

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at 18,000 g and 4 degrees Celsius for 30 minutes. The supernatant was then acidified to a pH of 4.5 using (2 N) hydrochloric acid for 90 min to precipitate the proteins, followed by centrifugation at 18,000 g and 4 C for 30 min.

Preparation of acidic buffalo casein:

Acid casein was prepared according to the method reported by [9] by sorting whole buffalo milk imported into the laboratory of the College of Food Sciences – Al-Qasim Green University. The milk was pasteurized at a temperature of 63 m° for half an hour, then cooled to 40 m°, after which the pH was reduced to (4.6), which is the electrical neutral point of casein, for the purpose of precipitating caseins, using an amount of a solution of hydrochloric acid at a concentration of 1 molar (1M HCL). When the white precipitate appeared, indicating the deposition of caseins, they were separated from the whey using a piece of malmal cloth, then washed with cold water to cool them and get rid of the remains of the whey and other materials. The clot was pressed under a pressure of 1 kg / cm2 for half an hour to reduce humidity as much as possible, then spread on a stainless steel table to dry under fan air and room temperature ≈ 23 m° with stirring and left until the next day until the drying process was complete. Then the dry clot was ground with an electric grinder and stored in the refrigerator using polyethylene bags until use at a temperature of 11 ± 1 m°.

Edible films preparing:

The method mentioned by (2009), Majid, was adopted with some modifications to form the protein films by taking 10 grams each of (pea protein powder and buffalo casein protein powder), then dissolving them in distilled water separately, then doing the following on both solutions: Mixing on a magnetic mixer for two hours After that, the solution was heated to

Protein precipitates were collected and then frozen at -18 for 24 h followed by freeze-drying to obtain Pea Protein Isolate (PPI) isolate

50°C for half an hour, then the solution was cooled to room temperature and the pH was adjusted to 7 using (N 1) sodium hydroxide NaOH. After that, glycerol was added at a concentration of 3% and mixed for five minutes. After that, 3 mixtures of the two solutions were prepared in mixing ratios of 25:75, 50:50 and 75:25, in addition to two samples with a purity of 100% From the two solutions, then transfer 6 ml of the mixtures to a Petri dish with a diameter of 9.1 cm. The dishes were then left to cool and dry on a flat surface at room temperature 2±25° degrees Celsius for 12-18 hours (a leveling device (known as qaban) was used to achieve the desired flatness of the surface) and then transferred to a dryer where a saturated sodium bromide solution was placed in the base to maintain relative humidity at 23 percent 1± In addition to a temperature of 1±55° degrees Celsius. They were allowed to dry for an additional day, after which the dry wrappers were separated and placed in polyethylene bags for storage in a refrigerated environment for later examinations**.**

Estimation of membrane permeability to water vapor:

The permeability of membranes to water vapor (WVP) was estimated by weight according to the American Society for Testing and Materials (ASTM) modified standard method numbered 80-96 E, known as the cup method as described by Gontard *et al*. (1994) and Al-Bayati (2005). The cups used in this test are made locally from Teflon with an outer diameter of 4.5 cm, an inner diameter of 3.4 cm, and a depth of 3.4 cm. Fill the cup with a desiccant, calcium

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chloride (zero relative humidity), up to 0.6 cm from the top edge of the cup mouth. The top surface of the cup is then coated with a thin layer of silicone grease. The membrane sample, which has been cut into a circular shape with a diameter equal to the outer diameter of the cup, is then placed over silicone grease to cover the cup opening. The membrane sample is fixed by placing a hollow stainless steel circular ring on top of it, which has an outer and inner diameter equal to the outer and inner diameter of the cup to ensure the sample is stable and tightly adheres to the surface of the mug. Weigh the cup and its contents with a sensitive scale to the nearest 0.1 mg, then transfer it to a glass dryer containing distilled water to obtain 100% relative humidity, then leave it for 24 hours to reach a stable state, after which seven weight readings were taken over the next seven days and regularly to monitor the increase in the weight of the cup. The permeability to water vapor was calculated after drawing the relationship, which represents the increase in the weight of the mug over time, on graph paper, and a straight line was obtained, which indicates reaching a stable state.

$$WVP = W.X / A.T (P2-P1)$$

Where:

WVP = permeability to water vapor (g mm / m2. Day.mm Hg)and W = weight gained (g) by the mug in time T(day).X = thickness of membrane sample (mm), (average of at least five readings per sample). A= The area of the membrane exposed to the permeability (m^2) covering the mug nozzle.P1 = water vapor pressure (zero mm Hg) inside the mug.P2 = water vapor pressure (32.23 mm Hg) outside the mug.

Estimate of tensile strength and elongation to the cutting limit:

The tensile strength and elongation to cut were estimated for all edible film samples under study using a Universal-Test device supplied by China and according to ASTM D-882.91 of 1996 [50] This was done by cutting two rectangular strips of pre-conditioned membranes, length (80 mm) and width (25 mm), for examination, and the model's drag speed was 50 mm/second.

Statistical analysis:

The statistical program Statistical Packages of Social Sciences-SPSS [51] was used in data analysis to study the effect of the envelope/sample on the studied characteristics (thickness, tensile pressure, maximum elongation, elongation ratio, and water vapor permeability), and the significant differences between the averages were compared with the test of the lowest significant difference (Least Significant Difference -LSD) and with the significance of ($p \leq 0.01$).

Results and discussion:

Protein extraction of pea grains:

The extraction results of pea flour showed that every 100 grams of pea flour yields 18.22 grams of protein extract, the protein content of which was 80%. This result is consistent with what was found by Berghout *et al*. [4] and Gao *et al*. [20] who indicated that the protein content in pea extract was 80%, and is also consistent with what was mentioned by Boukid *et al*., [7] who indicated that the percentage of protein in pea protein extract was 80%.

It is clear from the above results that pea grains had a high protein content, and this is consistent with what was stated by Lu *et al*. [37] and Windsor *et al*. [52] , who stated that the average protein content in pea grains was between 20-25%.

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Factors affecting the yield of pea protein extract:

Determine the best protein extraction time:

The results shown in Figure (1) show the results of the effect of ascending time periods on the protein content of defatted pea flour, where the protein content percentages were 10.24, 13.41, 16.22, and 16.26%, with an effect of 30, 60, 90, and 120 minutes, respectively.

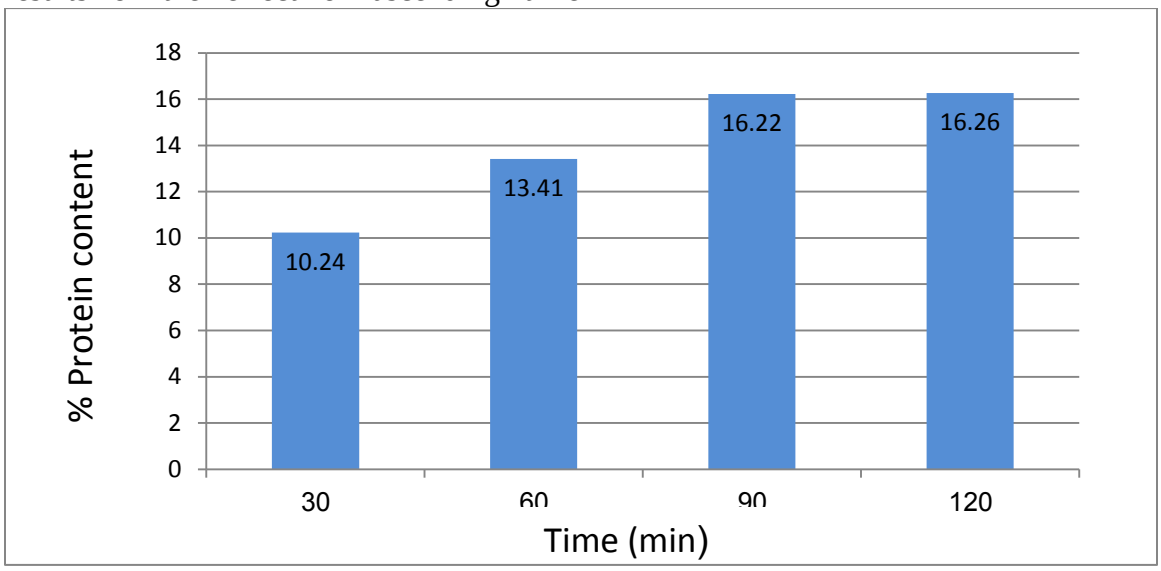

Figure 1: Effect of extraction time on the protein content of defatted pea flour. **LSD =2.507**

The lowest protein yield was 10.24% at 30 minutes, while the highest protein yield was 16.26% at 120 minutes. The results of the statistical analysis of the protein yield ratios due to the effect of time showed that there were significant differences (P≤0.01) in the different extraction periods on the protein yield ratios, with no significant differences in the extraction ratios in a period of 90 and 120 minutes, respectively. Therefore, a period of 90 minutes was adopted in producing the protein extract of pea grains.

. Ghasemi *et al*. [22] found that the highest extraction rate was at 90 minutes when different times of 30, 60 and 90 minutes were used to extract black lentil protein.

2- Determine the best mixing ratio for water:

The results shown in Figure 2 illustrate the results of the effect of adding different proportions of water on the protein content of defatted pea flour, where the resulting protein content ratios were 11.24%, 14.41%, and 17.25% for mixing pea flour and water in mixing ratios of 1:10, 1:15, and 1:20, respectively.

The results of the statistical analysis showed that there were significant differences (P≤0.01) between the mixing ratios of flour and water and the protein yield ratio, as the lowest protein yield ratio at the mixing ratio was 1:10 and was 11.24%, while the highest protein yield ratio at the mixing ratio of 1:20 was 17.25%.

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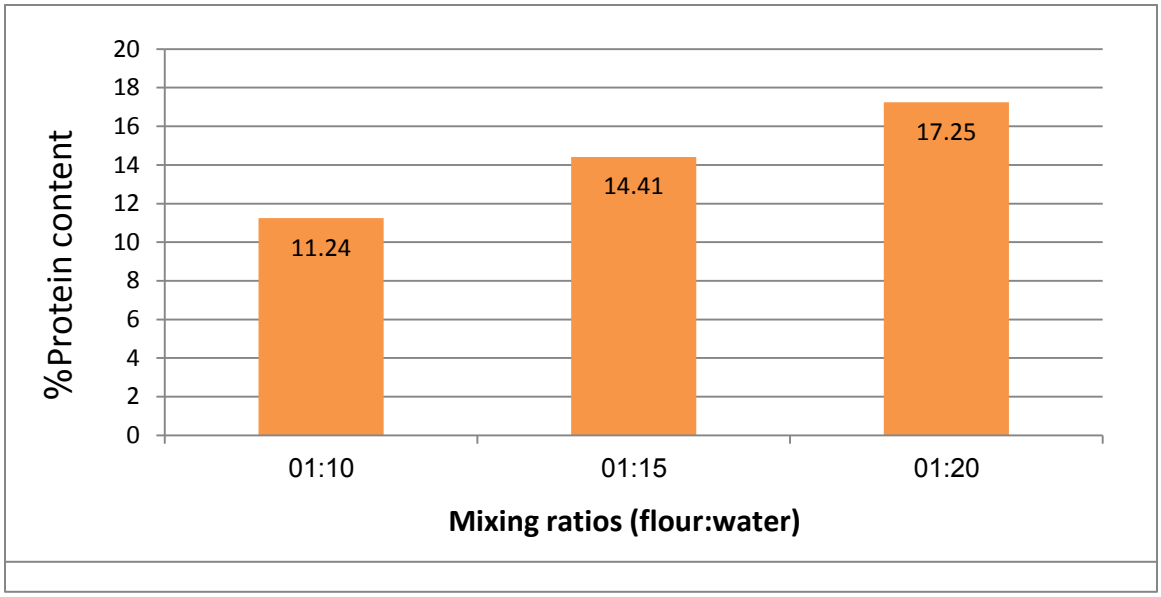

Figure 2: Effect of mixing ratios of defatted pea flour with water on protein yield**. LSD=2. 094**

Thus, a mixing ratio of 1:20 was adopted to produce the protein extract due to it giving the highest significant protein yield ratio.

Caldeira *et al*. [10] found The optimal water mixing ratio for producing chickpea powder protein extract was 1:12 out of several different ratios and was from 5:1 to 16:1. Gayol *et al*. [21] also found that the mixing ratios used were 10:1, 20:1, and 30:1. 1 does not significantly affect the extraction ratios of peanut powder.

Determine the best pH for protein solubility:

The results shown in Figure 3 showed the effect of using ascending pH values (pH) on the protein content ratios of defatted pea flour, as the highest protein solubility was at pH 10, which amounted to 67.33%, while for values 2, 4, 6, 8, and 12, it was 27, 9.4, 22.5, 36, and 59.81%, respectively. It is also noted from the results that the lowest solubility of the protein was at pH 4, and the reason for this may be due to the protein reaching the point of electrical neutralization.

The statistically analyzed results showed significant differences ($P \leq 0.01$) in the effect of pH values on protein solubility ratios, which confirms that pH has a clear effect on protein yield ratios, while there were no significant differences in pH values 2 and 6, and the protein yield ratios were close.

Jarpa-Parra *et al*. [32] found that pH 9 was optimal for the solubility of lentil flour protein, while raising pH to 10 led to partial degradation of the protein, which led to an increase in protein solubility but with a change in properties. Butseekhot *et al*. [8] also found that the optimal extraction and highest solubility of red bean proteins was at pH 9, which recorded the highest protein extraction rate, which was about 71-74%,

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while the extraction rate and solubility decreased when the pH rose, making the lowest extraction rate at pH 11.

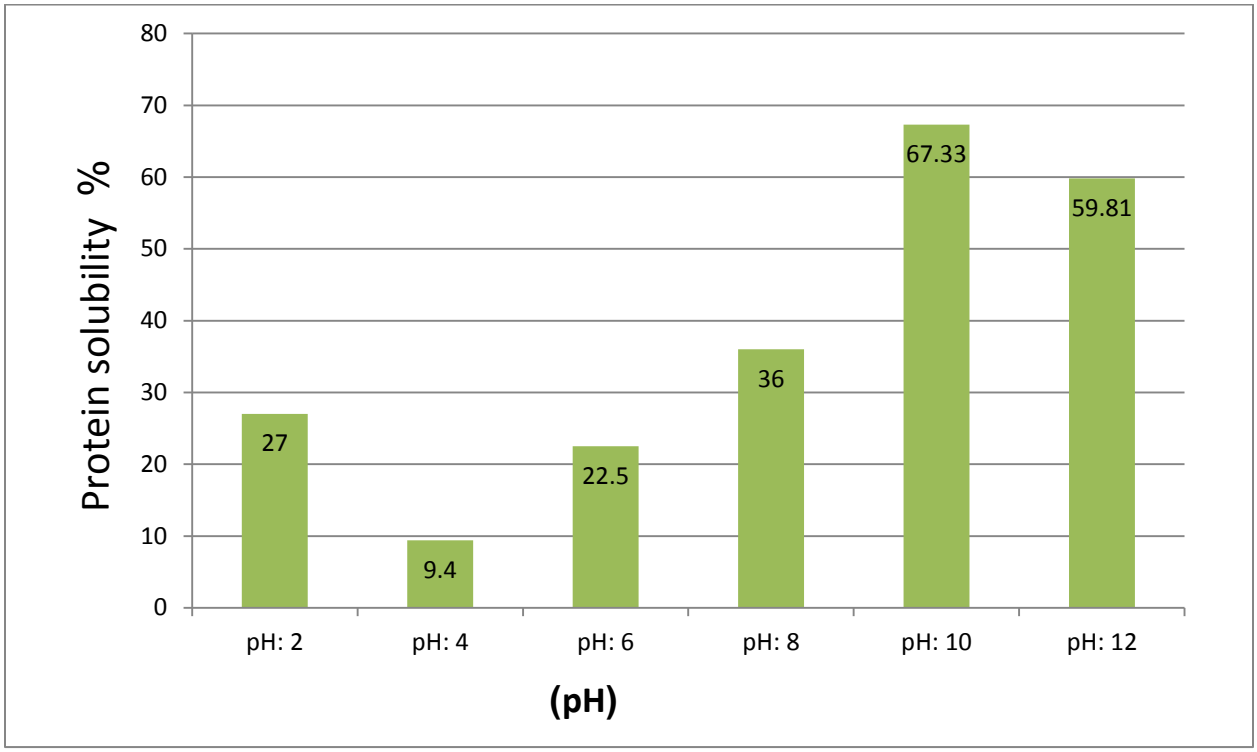

Figure 3: Effect of pH on the solubility of defatted pea flour protein. **LSD= 6.419**

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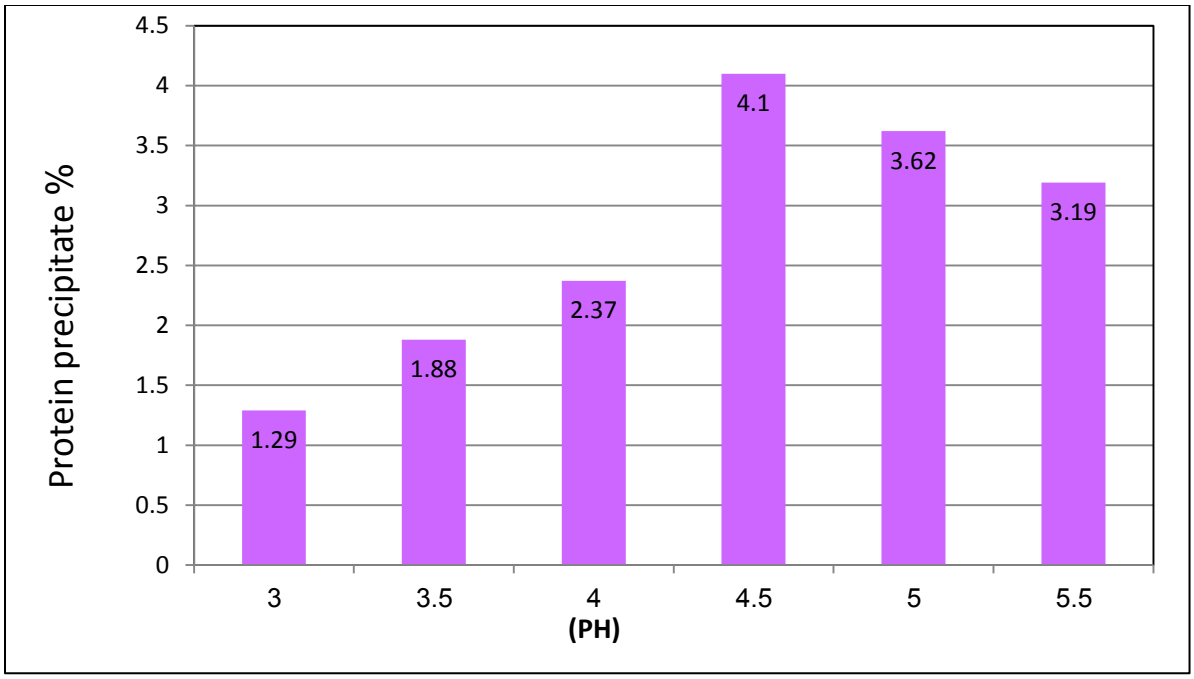

Figure 4: Effect values of pH on defatted pea flour protein precipitation. **LSD= 1.061**

Determine the best pH for protein deposition:

The results shown in Figure 4 showed the effect of pH on the sedimentation rate of defatted pea flour protein, as the highest sedimentation rate was at pH 4.5 and reached 4.1%, which represents the electrical neutral point of pea protein. It is also noted from the results that the sedimentation rates decrease when the pH falls or rises below 4.5, and the lowest sedimentation rate was at pH 3. The results of the statistical analysis indicate that there are significant differences in the effect of pH on protein precipitation rates ($P \leq 0.01$). Ghasemi *et al*. [22]found that the best sedimentation rate for black lentil proteins was 3.23% and was at pH 4.5, which represented the optimal pH among the hydrogen numbers used.

Water vapor permeability:

The results shown in Figure 5 showed that the water vapor permeability values of the edible films under study were 0.172 gm mm / m^2. day.mm Hg for the film made from pea protein treatment T_1,this value is close to that reported by Jiang *et al*. [33] who indicated that the water vapor permeability of the coating made of pea protein isolate/sodium carboxy methyl cellulose (PPI/CMC) was 2.6 gm mm / m^2. day.mm Hg Our results also contradict what was stated by Hopkins *et al*. [29] who indicated that the permeability values of leguminous proteins range between 1.6–8.0 gm mm / m^2. day.mm Hg.The water vapor permeability values of the membrane made from buffalo casein proteins treated 2T were 0.135 g mm / m2. day.mm Hg. These results contradict what was stated by Chen *et al*. [12] who indicated that the water

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vapor permeability of the coatings made of casein protein was approximately 22.4 g mm / m2. day.mm Hg. It is also noted from the results that the water vapor permeability value of the casings made from pea grains is higher compared to the water vapor permeability of the casings prepared from buffalo casein proteins. This is consistent with what was mentioned by Zioga *et al*. [54] who indicated that pea proteins (PPI) form a less complex, less tight and homogeneous network compared to casein networks, which provides more and easier paths for the passage of water vapor molecules [18] and this may be attributed to the hydrophilic nature of pea proteins, and this is consistent with what was stated by Acquah *et al*. [1] Who pointed out that the permeability of water vapor is significantly affected by the affinity of pea proteins for water

.

The water vapor permeability of the composite casings from mixing certain proportions of pea protein and buffalo casein protein with a mixing ratio of (P 75:25 C) represented by treatment T_3, a mixing ratio of (50 p: 50 C) represented by treatment 4T, and a mixing ratio of (75 P:25C) represented by treatment 5T (0.153, 0.147, and 0.142) g.mm / m2. day. mm Hg, respectively. The results show a decrease in the water vapor permeability of the coatings manufactured from protein mixtures, directly proportional to the increase in the percentage of buffalo casein protein used in the preparation. The water vapor permeability decreased with the increase in the percentage of buffalo casein in the manufactured coating, which reached 0.153, 0.147, and 0.142) g.mm / m2. day. mm Hg for the coatings in the treatments T_3, T_4 and T_5. respectively, and this is consistent with what was stated by Ghosh *et al*. [23] who indicated the efficiency of casein protein in controlling the transfer of water vapor molecules and protecting food from water loss or gain. Thus, it can be said that introducing buffalo casein protein into the treatment composition in increasing proportions led to an improvement in the permeability of the treatment, and this is shown by the permeability values for the mixing treatments T_3, T_4 and T_5. The reason for this improvement in permeability values may be due to the content of buffalo casein protein from the functional groups of casein protein that enhance the condensation of the protein network and thus hinder the spread of water vapor molecules. This is consistent with what was stated by Fematt-Flores *et al*. [18] and Chaudhary *et al*. [11] who indicated the superiority and ability of casein protein in hydrophobic properties and the formation of a cohesive protein network.

The results of the statistical analysis show that there are significant differences of $p \leq 0.01$ between the pure pea protein coat and the pure buffalo casein protein coat, with no of these differences between the treatments of the T_3, T_4, and T_5 mixture film .

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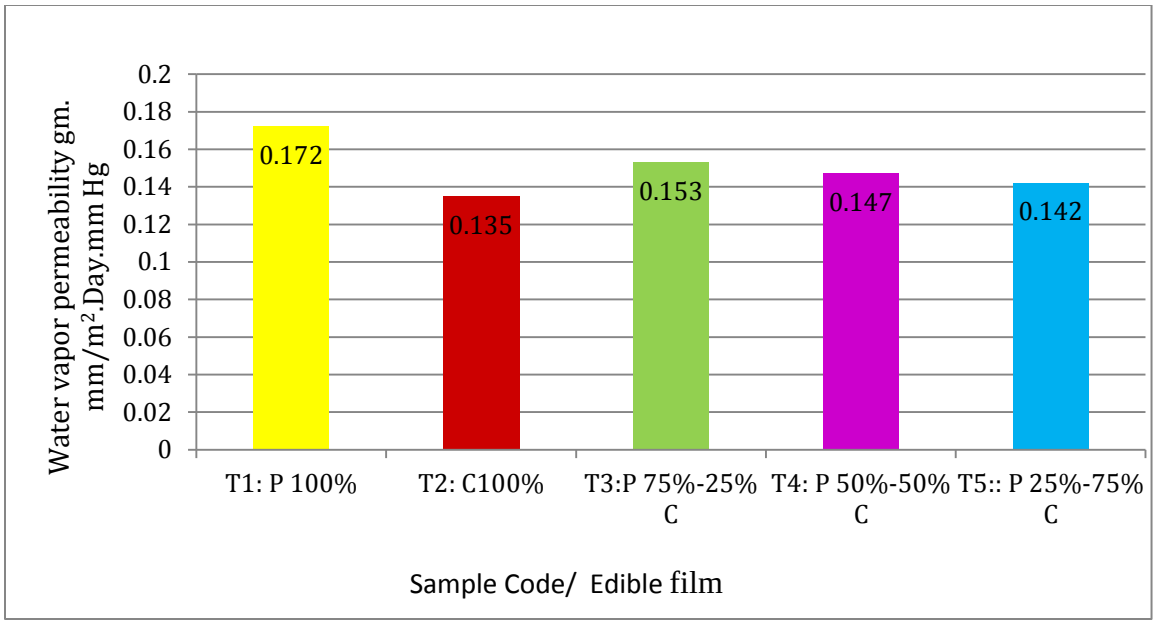

Figure 5: Water vapor permeability values of coatings prepared from pea bean protein, buffalo casein protein and their mixtures. **LSD =0.0319**

Mechanical properties:

The mechanical properties of polymeric materials, which include tensile strength and elongation limit, are basic criteria. They express the durability and flexibility of membranes and their suitability for use in various applications, especially food, and in transportation and handling conditions, especially biological membranes [46].

Tensile strength of coating:

The results shown in Figure 6 show the tensile values of pure edible protein films and protein films made from both pea bean protein and buffalo casein protein, where the tensile strength of pure films T_1 and T_2 was 0.63 and 3.22 MPa, respectively, while the tensile strength of the films was mixtures (P 75:25 C) represented by treatment T_3 and mixing ratio (50 p: C 50) Represented by treatment T_4 and mixing ratio (25 P:C 75) represented by treatment T_5 1.85, 2.32 and 3.42 MPa respectively .As is evident from the results shown in Figure 6, the tensile strength value of treatment T_1 decreased by 0.63 MPa compared to the tensile strength value of treatment T_2, which amounted to 3.22 MPa. These results for the tensile value of pea protein are consistent with what was found by Cheng *et al*. [13], who indicated that the tensile strength value of the pea protein isolate was about 0.7 MPa, which indicates that the pea protein without modification gives coatings with relatively weak tensile strength. Our results for the tensile value of the T_2 buffalo casein protein coat are close to what was stated by Chen *et al*. [12] who indicated that the tensile strength of the casein coats was approximately 2.5 MPa using glycerol as a plasticizer at a rate of 50%.

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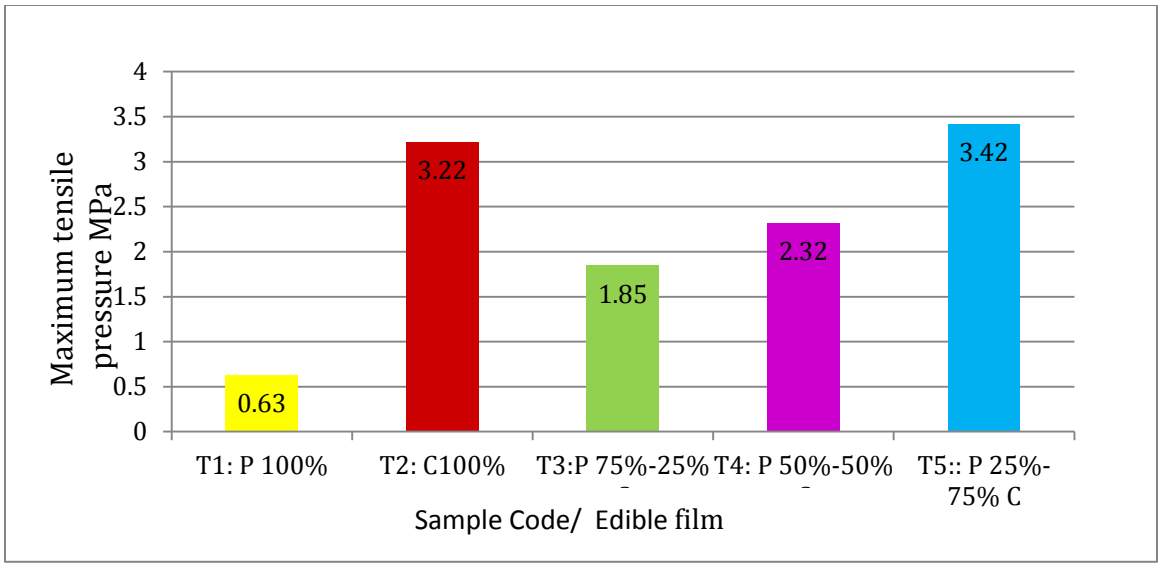

Figure 6: Tensile strength values of coatings prepared from pea bean protein, buffalo casein protein and their mixtures . **LSD = 0.841**

The reason for the difference in tensile strength values between pea protein treatment and buffalo casein protein may be due to the nature of casein protein, as it is a protein of animal origin that is characterized by the ability to form dense and cohesive polymeric networks, which is reflected in the tensile value [15].

improvement in the tensile strength of the mixture coatings compared to pure pea protein coatings, as the composite coating T_3 with a mixing ratio of (P 75:C 25) showed a tensile strength of 1.85 MPa ، This value is consistent with what was found by Kowalczyk ,[36] who stated that the tensile strength was 1.70 MPa for pea protein isolate coatings. As for the tensile strength of the T_5 and T_4 treatments coatings, which amounted to 2.32 and 3.42 MPa, respectively, it is close to what was found by Kowalczyk ,[36] who indicated that the tensile strength was 3.99 MPa for the coatings of pea protein isolate with the addition of sorbitol as a plasticizer and 0.025% calcium chloride $CaCl_2$.As is clear

The tensile strength values of the films prepared by mixing certain proportions of pea protein and buffalo casein protein with a mixing ratio (P 75:25 C) represented by treatment T_3, a mixing ratio (50 p :C 50)represented by treatment T_4, and a mixing ratio (25 P: C 75) represented by treatment T_5 1.85, 2.32 and 3.42 MPa, respectively. The results also show an from the results, the mixture coatings witnessed an improvement in the tensile strength values with the increase in the percentage of buffalo casein used. Therefore, it is noted that the T_3 composite coating with a mixing ratio of (P 75:25 C) had the weakest tensile strength among the mixture treatments, but it was better when compared to the pea protein coating with a purity of 100% represented by the T_1 treatment This may be due to the effect of introducing buffalo casein protein into the treatment structure, as casein protein partially enhances the treatment structure by forming a more cohesive network by increasing the density of bonds in the

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protein treatment network. This is consistent with Bhatia *et al*. [5].

The statistically analyzed results showed that there were no significant differences of 0.01 P≤ between the treatments of the mixture coatings compared to the treatments of the pure coatings and that these differences existed between the treatments of the pure coatings.

Percentage elongation of films:

The results shown in Figure 7 show the total elongation percentage values of pure protein films made from pea protein and buffalo casein protein and films made from mixtures of both proteins, where the total elongation percentage of pure films made from pea protein treated T_1 was 33%, and this result is close to what was mentioned by Kowalczyk, [36] who indicated that the elongation rate of the cutting limit for casings made from pea protein isolate reached 37.01 and 32.67%, using sorbitol as a plasticizer and using calcium chloride at a rate of 0 and 0.01%.

The total elongation rate of the envelope made from pure buffalo casein protein T_2 was 51.5% and this result is close to what was found by Fematt-Flores *et al*. [18] who indicated that the elongation rate of the cut-off limit of the casein protein envelope was 49.6% , but it contradicts what was stated by Chen *et al*. [12] who stated that the elongation rate of the casein envelopes was 65%. Buffalo casein protein films are structurally similar to those of bovine casein proteins, as hydrophobic bonds and calcium bonds make the treatment cohesive and at the same time flexible, and the presence of a plasticizer such as Glycerol reduces the strength of the bonds and increases the distance between the chains, allowing good elongation [6]. It is also noted from the results that the elongation rate of pea seed protein films is lower compared to that of buffalo casein protein films. This result is consistent with what was mentioned by Cheng *et al*. [13] who indicated that films made from pea proteins of plant origin tend to have low elongation and little flexibility compared to proteins of animal origin and are improved by modification or addition of plasticizers this is due to the fact that pea proteins, during the production stages of alkaline and thermal preparations, produce a network of strong hydrophobicity and disulfide bonds between the treatment network, resulting in a cohesive but inelastic treatment with an almost low elongation ratio [17].

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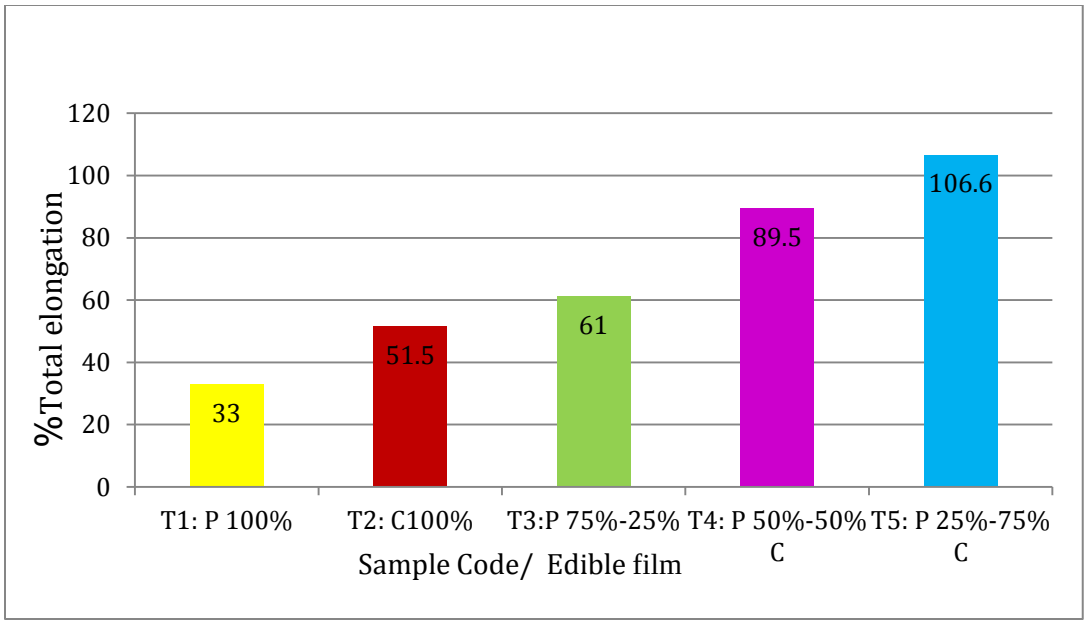

Figure 7: Total elongation ratio values of protein films prepared from pea bean protein, buffalo casein protein and their mixtures**.** **LSD =9.863**

The total elongation ratio of the casings of mixtures consisting of pea grain protein and buffalo casein protein was (P 75:25 C) represented by treatment T3, a mixing ratio of (C50: 50 p)represented by treatment T4, and a mixing ratio of (25 P: C 75)represented by treatment T5 61, 89.5 and 106.6, respectively. It is also noted from the results that there is a clear improvement in the total elongation ratio of the composite films, and this is consistent with what was mentioned by Dutta *et al*. [14] who indicated that the casein protein improves the fine structure of the treatment in terms of thickness and homogeneity, which is reflected in its effect on the mechanical properties of the films, including the elongation ratio.

The statistically analyzed results showed that there were no significant differences of 0.01 P≤ in the results of the total elongation ratio between pure films (T_1 and T_2), with significant differences when comparing pure films and mixtures (T_3, T_4 and T_5).

Conclusion

The results of this study indicate the possibility of manufacturing edible films by mixing two types of proteins of different origins: proteins of plant origin (pea) and proteins of animal origin (buffalo casein), and the possibility of improving the water vapor permeability and mechanical properties of films made from pea protein using buffalo casein protein

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References:

[1] **Acquah, C., Zhang, Y., Dub´ e, M. A., & Udenigwe, C. C. (2020).** Formation and characterization of protein-based films from yellow pea (Pisum sativum) protein isolate and concentrate for edible applications. *Current Research in Food Science*, 2, 61–69.

[2] **Al-Jubouri, N. R. A. A., Al-Badrani, D. I. J. (2024).** Study of the quality characteristics of Monterrey cheese coated with edible coating films made from whey proteins and black seed proteins. *Euphrates Journal of Agricultural Science*, *16*(3), 31–46.

[3] **Arunan, I., & Crawford, R. H. (2021).** Greenhouse gas emissions associated with food packaging for online food delivery services in Australia. *Resources, Conservation and Recycling*, 168, 105299.

[4] **Berghout, J. A. M., Pelgrom, P. J. M., Schutyser, M. A. I., Boom, R. M., & Van Der Goot, A. J. (2015)**. Sustainability assessment of oilseed fractionation processes: A case study on lupin seeds. *Journal of Food Engineering*, 150, 117–124.

[5] **Bhatia, S., Al- Harrasi, A., Al-Azri, M., Ullah, S., Makeen, H., Meraya, A., Albratty, M., Najmi, A., & Anwer, M. (2022).** Gallic Acid Crosslinked Gelatin and Casein Based Composite Films for Food Packaging Applications. *Polymers*, 14.

[6] **Bonnaillie, L. M., Zhang, H., Akkurt, S., Yam, K. L., & Tomasula, P. M. (2014).** Casein films: The effects of formulation, environmental conditions and the addition of citric pectin on the structure and mechanical properties. *Polymers*, *6*(7), 2018-2036.

[7] **Boukid, F., Rosell, C. M., & Castellari, M. (2021).** Pea protein pngredi ents: A mainstream ingredient to (re)formulate innovative foods and beverages. *Trends in Food Science & Technology*, 110, 729– 742.

[8] **Butseekhot, S., Sorndech, W., Ployphetchara, T., Auranwiwat, C., Sumsakul, W., Siricoon, S., ... & Aluko, R. E. (2024).** Effects of alkaline extraction on the physicochemical and functional properties of protein concentrates obtained from Vigna mungo and Phaseolus vulgaris L. legumes. *Food Research*, 8(4), 128-134.

[9] **Bylund, G. (1995).**CaScinate,In Tatra Pak Dairy processing Handbook ; Tetra Pak processing system AB. Teknotext ; Lund , Sweeden , pp 399 – 402

[10] **Caldeira, R., De Paiva Gouvêa, L., De Lima Azevedo, T., Wilhelm, A., De Grandi Castro Freitas De Sá, D., Galdeano, M., Felberg, I., Antoniassi, R., Mellinger, C., & Lima, J. (2025)**. Chickpea processing to obtain a protein ingredient for the plant-based market. *Frontiers in Food Science and Technology*.

[11] **Chaudhary, V., Kajla, P., Kumari, P., Bangar, S. P., Rusu, A., Trif, M., & Lorenzo, J. M. (2022).** Milk protein-based active edible packaging for food applications: An eco-friendly approach. Frontiers in Nutrition, 9, 942524.

[12] **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.

ISSN 2072-3857

[13] **Cheng, J., Li, Z., Wang, J., Zhu, Z., Yi, J., Chen, B., & Cui, L. (2022).** Structural characteristics of pea protein isolate (PPI) modified by high-pressure homogenization and its relation to the packaging properties of PPI edible film. *Food chemistry*, 388, 132974.

[14] **Dutta, D., & Sit, N. (2024).** Casein-based films reinforced with bamboo shoot fibers modified by ultrasound and cellulase. *Industrial Crops and Products*.

[15] **El-Sayed, S. M., & Youssef, A. M. (2024).** Emergence of cheese packaging by edible coatings for enhancing its shelf-life. *Journal of Food Measurement and Characterization*, 18(7), 5265-5280.

[16] **Essa, Y. R., Abd Elhady, R. S., Kassab, H. and Ghazi, A. (2015).** Isolation and Charcteristizaten of protein isolated from sesame seeds (sesamum indicum) meal. *Weber Agricultural Research & Management*.

[17] **Farshi, P., Mirmohammadali, S. N., Rajpurohit, B., Smith, J. S., & Li, Y. (2024).** Pea protein and starch: Functional properties and applications in edible films. *Journal of Agriculture and Food Research*, *15*, 100927.

[18] **Fematt-Flores, G. E., Aguiló-Aguayo, I., Marcos, B., Camargo-Olivas, B. A., Sánchez-Vega, R., Soto-Caballero, M. C., ... & Rodríguez-Roque, M. J. (2022).** Milk protein-based edible films: Influence on mechanical, hydrodynamic, optical and antioxidant properties. *Coatings*, 12(2), 196.

[19] **Gao, D., Helikh, A. O., & Duan, Z. (2021).** Functional properties of four kinds of oilseed protein isolates. *Journal of Chemistry and Technologies*, *29*(1), 155-163.

[20] **Gao, Z., Shen, P., Lan, Y., Cui, L., Ohm, J. B., Chen, B., et al. (2020).** Effect of alkaline extraction pH on structure properties, solubility, and beany flavor of yellow pea protein isolate. *Food Research International*, 131, 109045.

[21] **Gayol, M. F., Pramparo, M. D. C., Nepote, V., Fernandez, H., and Grosso, N. R. (2013).** Optimization of the protein concentration process from residual peanut oil-cake. *Grasas y aceites*, *64*(5), 489-496.

[22] **Ghasemi, P., Noshad, M., Behbahani, B. A., Nikfarjam, Z., & Vasiee, A. (2025).** Black Lentil Protein Isolate: Extraction optimization, Structural, thermal, and physicochemical properties. *Lwt*, 118160.

[23] **Ghosh, A., Ali, A., & Dias, G. (2009).** Effect of cross-linking on microstructure and physical performance of casein protein.. *Biomacromolecules*, 10 7, 1681-8 .

[24] **Gregory, D. A., Tripathi, L., Fricker, A. T., Asare, E., Orlando, I., Raghavendran, V., & Roy, I. (2021).** Bacterial cellulose: A smart biomaterial with diverse applications. Materials Science and Engineering: R*: Reports*, 145, 100623.

[25] **Gupta, V., Biswas, D., & Roy, S. (2022**). A comprehensive review of biodegradable polymer-based films and coatings and their food packaging applications. *Materials*, *15*(17), 5899.

[26] **Habeeb, N. D., & Al-Bedrani, D. I. J. (2022).** The effect of partial replacement of cow's milk prepared for the manufacture of low-fat Monterrey cheese with skim buffalo milk on the traits of product quality. *Natural*

ISSN 2072-3857

Volatiles & Essential Oils, *9*(2), 553–571.

[27] **Hassanin, A. A., Osman, A., Atallah, O. O., El-Saadony, M. T., Abdelnour, S. A., Taha, H. S., ... & Eldomiaty, A. S. (2022).** Phylogenetic comparative analysis: Chemical and biological features of caseins (alpha-S-1, alpha-S-2, beta-and kappa-) in domestic dairy animals. *Frontiers in Veterinary Science*, *9*, 952319.

[28] **Hayat, I., Ahmad, A., Rafiq, S., Gulzar, N., Khan, D., Amir, R., Murtaza, A., Tariq, H., & Ahmed, R. (2025).** Extraction Optimization of Red Kidney Bean (Phaseolus vulgaris L.) Protein using Response Surface Methodology. *Journal of Agriculture and Veterinary Science*.

[29] **Hopkins, E. J., Stone, A. K., Wang, J., Korber, D. R., & Nickerson, M. T. (2019).** Effect of glycerol on the physicochemical properties of films based on legume protein concentrates: A comparative study. *Journal of texture studies*, *50*(6), 539-546.

[30] **Horax, R., Hettiarachchy, N., Kannan, A., & Chen, P. (2011).** Protein extraction optimisation, characterisation, and functionalities of protein isolate from bitter melon (Momordica charantia) seed. *Food chemistry*, *124*(2), 545-550.

[31] **J. Cheng, L. Cui (2021).**Effects of high-intensity ultrasound on the structural, optical, mechanical and physicochemical properties of pea protein isolate-based edible film. *Ultrason. Sonochem*. 80, 105809

[32] **Jarpa-Parra, M., Bamdad, F., Wang, Y., Tian, Z., Temelli, F., Han, J., & Chen, L. (2014).** Optimization of lentil protein extraction and the influence of process pH on protein structure and functionality. *LWT-Food Science and Technology*, *57*(2), 461-469.

[33] **Jiang, P., Chen, G., Xu, S., Yu, J., Qu, Z., Li, S., & Chen, Y. (2024).** Quality Improvement of Pea Protein Isolate- Based Film: Effect of Sodium Carboxymethyl Cellulose on Film. *Starch - Stärke*.

[34] **Karaça, A., Low, N., & Nickerson, M. (2011).** Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food Research International*, 44, 2742-2750.

[35] **Khan, M. R., Volpe, S., Valentino, M., Miele, N. A., Cavella, S., & Torrieri, E. (2021).** Active casein coatings and films for perishable foods: structural properties and shelf-life extension. *Coatings*, *11*(8), 899.

[36] **Kowalczyk, D. (2024).** Comparative Study on the Effect of Calcium Chloride on the Physicochemical Properties of Glycerol-and Sorbi-tol-Plasticized Pea Protein Isolate Films.

[37] **Lu, Z.X.; He, J.F.; Zhang, Y.C.; Bing, D.J.(2020) .**Composition, physicochemical properties of pea protein and its application in functional foods. *Crit. Rev. Food Sci. Nutr*., 60, 2593–2605.

[38] **Mihalca, V.; Kerezsi, A.D.; Weber, A.; Gruber-Traub, C.; Schmucker, J.; Vodnar, D.C.; Dulf, F.V.; Socaci, S.A.; Fărcaș, A.; Mureșan, C.I.; et al. (2021) .**Protein-based films and coatings for food industry applications. *Polymers* , *13*, 769.

[39] **Mohammed, A. I., Aljabary, A. M. A., Marf, B. A., & Al-Saadi, J. M. (2023).** Effect of Bio-Based Protein

ISSN 2072-3857

Coating on the Quality and Shelf Life of Fig Fruit (Ficus Carica L.). *Passer Journal of Basic and Applied Sciences*, *5*(2), 356-361.

[40] **Ostad-Ali-Askari, K. (2022).** Management of risks substances and sustainable development. *Applied Water Science*, *12*(4), 65.

[41] **Pirsa, S., & Aghbolagh Sharifi, K. (2020).** A review of the applications of bioproteins in the preparation of biodegradable films and polymers. *Journal of Chemistry Letters*, 1(2), 47-58.

[42] **Porta, R. (2017).**Plastic Pollution and the Challenge of Bioplastics. *J. Appl. Biotechnol. Bioeng., 2, 111*.

[43] **Rahi, A. K., et al. (2023).** Effect of adding sodium caseinate, whey protein concentrate and milk protein concentrate on the physical, rheological and sensory properties of yogurt produced from goat milk. Journal of Applied and Natural Science, 15(4), 1434 – 1444.

[44] **Ríos-de-Benito, L. F., Escamilla-García, M., García-Almendárez, B., Amaro-Reyes, A., Di Pierro, P., & Regalado-González, C. (2021).** Design of an active edible coating based on sodium caseinate, chitosan and oregano essential oil reinforced with silica particles and its application on panela cheese. *Coatings*, 11(10), 1212.

[45] **Runthala, A., Mbye, M., Ayyash, M., Xu, Y., & Kamal-Eldin, A. (2023).** Caseins: versatility of their micellar organization in relation to the functional and nutritional properties of milk. *Molecules*, *28*(5), 2023.

[46] **Shah, Y., Bhatia, S., Al- Harrasi, A., Afzaal, M., Saeed, F., Anwer, M., Khan, M., Jawad, M., Akram, N., & Faisal, Z. (2023).** Mechanical Properties of Protein-Based Food Packaging Materials. *Polymers*, 15.

[47] **Shanmugam, V. P., Kapila, S., Kemgang, T. S., Reddi, S., Kapila, R., Muthukumar, S., & Rajesh, D. (2021).** Isolation and characterization of angiotensin converting enzyme inhibitory peptide from buffalo casein. *International Journal of Peptide Research and Therapeutics*, 27(2), 1481-1491.

[48] **Shanthakumar, P., Klepacka, J., Bains, A., Chawla, P., Dhull, S. B., & Najda, A. (2022).** The current situation of pea protein and its application in the food industry. Molecules, 27(16), 5354.

[49] **Shen, M., Song, B., Zeng, G., Zhang, Y., Huang, W., Wen, X., & Tang, W. (2020).** Are biodegradable plastics a promising solution to solve the global plastic pollution?. *Environmental pollution*, 263, 114469.

[50] **Sothornvit, R. and Krochta, J. M. (2000).** Oxygen permeability and mechanical properties of films from hydrolyzed whey protein. *Journal of Agricultural and Food Chemistry*, 48:(9) 3913-3916.

[51] **SPSS (2019).** Statistical Packages of Social Sciences- SPSS/ IBM Statistics 26 step by step. 16th Edition.

[52] **Windsor, N., Boatwright, L., Boyles, R., Bridges, W., Rubiales, D., & Thavarajah, D. (2024).** Characterizing Dry Pea (Pisum sativum L.) for Improved Nutritional Traits and the Potential for Biofortification. *Legume Science*, 6.

[53] **Wu, D. T., Li, W. X., Wan, J. J., Hu, Y. C., Gan, R. Y., & Zou, L. (2023).**

A comprehensive review of pea (Pisum sativum L.): chemical composition, processing, health benefits, and food applications. *Foods*, 12(13), 2527.

[54] **Zioga, M., Papantonopoulou, G., & Evageliou, V. (2023).** High internal phase emulsions and edible films with high methoxyl pectin and pea protein isolate or sodium caseinate. *Food Hydrocolloids.*

ISSN 2072-3857