CHARACTERIZATION OF NOVEL EDIBLE FILM FROM DEAMIDATED RICE *(Oryza sativa*) BRAN PROTEIN

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ABSTRACT

This study aimed to investigate the characteristics of new edible film prepared from deamidated rice bran protein (RBP). Deamidation was carried out at temperatures of 80, 100, and 120°C under varying pH conditions of 8, 10, and 12 for durations of 15 and 30 minutes to enhance protein solubility. The resulting film was then assessed for its physical, mechanical, and optical properties. The results revealed that protein solubility of deamidated RBP improved across all temperatures and pH levels compared to the control. A film made from deamidated protein under conditions of 120°C and pH levels of 8, 10, and 12 was evaluated using protein concentrations of 3, 4, and 5%. The solubility of the film increased as the pH was raised from 8 to 12 compared to the control. Swelling also increased with a higher protein percentage. However, films prepared through deamidation at pH levels of 10 and 12 at 120°C became fully soluble in water and completely dissolved. The puncture strength of deamidated, control, and SBP films increased as the RBP concentration rose from 3% to 5%. Among all RBP films, the deamidated (D) film exhibited the highest puncture strength, although it was still lower than that of the soybean protein (SBP) film. The elongation also improved with the increase in puncture strength. The opacity of RBP films increased with higher protein levels but remained lower than that of the control.

Keywords: , FTIR, physical, mechanical, optical properties.

توصيف فيلم غذائى جديد مجهز من بروتين رجيع الارز مزال منه مجموعة الاميد

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المستخلص

هدفت الدراسة الى دراسة خصائص تجهيز نوعية جديدة من أغلفة غذائية من بروتين رجيع الارز بعد اجراء نزع مجموعة الاميد من البروتين بهدف زيادة ذائبية البروتين فى الماء ثم دراسة خواصها. أجريت عملية نزع مجموعة الاميد على درجات حرارة 80 ، 100 ، 120م تحت ظروف حموضة (pH) مختلفة وهى 8 ، 10 ، 12 لمدد زمنية 15 ، 30 دقيقة. وتم تقييم بعض الصفات الطبيعية والميكانيكية والضوئية للافلام الناتجة. حيث اشارت النتائج الى ان ذائبية البروتين ازدادت نتيجة نزع مجموعة الاميد على كل ظروف الحرارة والحموضة مقارنه بالكنترول. تم تقييم الافلام الناتجة من البروتين بتركيزات 3 ، 4 ، 5% بعد عملية نزع الاميد على درجات حرارة 120م وحموضة 8 ، 10 ، 12حيث وجد أن: – ذوبان الفيلم فى الماء ازداد بزيادة درجة الحموضة من 8 إلى 12 مقارنة بمعاملة السيطرة. درجة الانتفاخ تزداد بزيادة تركيز البروتين فى الفيلم. على الرغم من ان الفيلم المجهز من البروتين منزوع الاميد على درجة حموضة 10 ، 12 على 120م ذوبان كاملا فى الماء. Puncture لكل من الفيلم المجهز من البروتين منزوع الاميد ومعاملة السيطرة وكذلك المجهز من بروتين الصويا ازداد بزيادة تركيز البروتين من 3 الى 5%. اختبار الثقب للفيلم المصنع من بروتين منزوع الاميد عند حموضة 12 وحرارة 120م لمدة 15 دقيقة (D) كانت الافضل بين العينات ولكنها اقل منها فى فيلم بروتين الصويا. تحسنت الاستطالة بزيادة الثقب. ضبابية الافلام المعاملة ازدادت (انخفاض الشفافية) بزيادة تركيز البروتين فى الفيلم ولكنها ما زالت اقل من معاملة السيطرة.

الكلمات المفتاحية: FTIR، الصفات الطبيعية، الميكانيكية، الضوئية.

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INTRODUCTION

Rice (*Oryza sativa L.*) is a crucial cereal crop and serves as a primary food source for over half of the global population (6, 28). Rice bran is a by-product produced from the rice milling industry. It was obtained from the outer layer of brown (husked) rice kernels during the milling process. It represents about 10% of the total weight of rough rice (9). It is an excellent source of protein, minerals, lipids, and dietary fiber (13). Annually, 90% of the world's rice bran is utilized at a low cost as livestock and poultry feed, while the remaining portion is utilized for extracting rice bran oil (20, 33). Rice bran contains approximately 10–15% premium proteins. A protein formulation derived from rice bran can assist in addressing protein-related nutritional deficiencies. Furthermore, its distinct hypoallergenic and anticancer qualities make rice bran proteins more advantageous than the cereal proteins (7). Edible films and coatings made from biodegradable materials have seen a growing interest in research over the past twenty years because of their potential applications in food packaging (15). Protein films are anticipated to serve as effective oxygen barriers under conditions of low relative humidity. Various protein sources have been used to create edible films, including casein, gelatin, whey protein, corn zein, soy protein, wheat gluten, peanut protein, and mung bean protein (3 , 19). The limited solubility of RBP in aqueous solutions, due to its elevated levels of glutamine, asparagine remnants, and non-polar amino acids, hinders the protein in food applications. So, enhancing its solubility and optimizing its functional properties in water-based solutions through suitable modification techniques is an important. Deamidation, a chemical alteration technique, significantly influences on the protein's charge and I.e.p (14). Deamidation is a modification technique used to enhance the solubility and other functional characteristics of proteins by removing amide groups from originally uncharged amino acids, converting them into acidic residues (32). This process can separate protein polymers, enhancing electrostatic repulsion between protein molecular chains, while elevating the surface hydrophobicity and elasticity of the protein molecules (18). Heating causes the dissociation of protein quaternary structures and denaturation of protein subunits. Furthermore, increases in protein cluster development through the formation of disulfide bonds, as well as hydrophobic and electrostatic interactions (2). Wang and Johnson (25) found that the direct use of steam infusion significantly enhanced the SBP concentrate solubility, foaming, and emulsifying characteristics. After 30 seconds of steam jet cooking, the concentrate's solubility rose to 56%, and its emulsifying properties increased approximately fourfold. At lower pressures (e.g., 200 MPa), protein solubility remains low because there were creation of higher molecular weight, insoluble clusters of protein isolate. However, at higher pressure (e.g., 600 MPa), these insoluble clusters transform into soluble clusters with reduced mean molecular weight, leading to enhance of solubility (23, 26). This finding is supported by the results of protein electrophoresis conducted both before and after high pressure treatment (31). Protein solubility is highly influenced by pH, with a significant reduction in droplet size occurring under high-pressure conditions in an alkaline pH (17, 26). So, this research focused on developing a novel edible film using deamidated rice bran protein and evaluating of its physical, mechanical and optical characteristics.

MATERIALS AND METHODS

Rice bran (Hassawey) was obtained from a local farm in Al-Ahsaa, Saudi Arabia. Glycerol, hexane, sodium hydroxide, hydrochloric acid, and sodium bicarbonate were sourced from Sigma Company. All chemicals were of analytical grade. Soybean protein (SBP) concentrate, Carboxymethyl cellulose (CMC) and Teflon sheets were procured from Mifad Co., 6th of October City, Egypt.

Sample preparation

Rice bran oil was extracted using three volumes of hexane through cold extraction. The resulting defatted rice bran (DRB) was stored at 5°C for future use.

Rice bran protein extraction

Rice bran protein was extracted using the methods outlined by **Adebiyi *et al.* (1)** with some modification as follows: The defatted

bran was suspended in distilled water at ratio (1:10, w/v). The pH of the slurry was adjusted to 9 using NaOH solution (4M), continuously stirred for 1h. The mixture was filtered throw cheesecloth and cotton and centrifuged at 12,600g for 15 minutes. The pH of the supernatant protein solution was adjusted to 4.5 using 4M HCl, stirred for 30 minutes, and then left undisturbed overnight at 4°C to allow for cold precipitation. The supernatant was carefully removed, and the precipitated protein was washed 3–4 times with distilled water. The pH of the slurry was adjusted to 7, dialyzed against water for 24 hours, and then freeze-dried using a tray lyophilizer (Rikakikai, Tokyo, Japan).

Determination of RBP solubility

Protein samples (100mg) were immersed in 2mL of deionized water and mixed for 30 minutes at room temperature. Samples were centrifuged at 15,000 xg for 20 minutes. The supernatants were moved to separate tubes and dried at 40°C. The residual protein was weighed and protein solubility was calculated as follows:

$$\textit{Protein solubility} = \left(1 - \frac{\textit{Residual protein weight}}{\textit{Total protein weight}}\right) \times 100$$

Deamidiation of RBP

Approximately 0.25 g of rice bran protein was dispersed in 25mL of a 0.1 molar sodium bicarbonate solution. The pH was adjusted to 8, 10, and 12 using 1 molar sodium hydroxide or 1 molar hydrochloric acid. The solutions were heated at 80°C and 100°C for either 30 or 60 minutes. Additionally, samples were heated at 120°C for 15 and 30 minutes. Following the heating process, the protein solution was cooled, neutralized, and dialyzed against water at 4°C for 48 hours before being freeze-dried.

Film preparation

Protein samples of 3, 4, or 5 grams were dissolved in 100 mL of distilled water. Carboxymethyl cellulose (CMC) was added to enhance the film's strength at a protein-to-CMC ratio of (1:0.1, w/w), and the pH was adjusted to 7. Protein solution samples were stirred at 80 ^{0}C for 30 min. After complete dispersing of protein and CMC, 50% of glycerol (on protein weight) was added as plasticizer. The mixture was cooled to room temperature. The solution was degassed at room temperature using a vacuum pump for 20 min and casted on plastic plates coated with Teflon sheet (15 × 20 cm^2). The plates were placed on levelled surfaces to obtain films of homogenous thickness. The films were dried in air oven at 25 ^{0}C for 12 h. The dried films were peeled-off and always conditioned again at 30±1% RH and 25±1 ^{0}C for 48 h prior to testing**.**

Fourier transform infrared (FT-IR) spectroscopy: FT-IR spectra of RBP were recorded using an FT-IR spectrometer (Model, FT-IR670/IRT-30, Japan Spectroscopic Co.). The measurements were conducted over a spectral range of 400–7000 cm^{-1} with a resolution of 1 cm^{-1}, utilizing 254 scans against a background spectrum. Composite bands of the amide 1 region (1700 – 1600 cm^{-1}) were used to determine the secondary structure of rice bran protein.

Physical properties

Film thickness: Film Thickness was measured with a Caliper Micrometer (No. 7326, Mitutoyo Manufacturing Co. Ltd., Japan) at 8 random positions of the film.

Film solubility in water

The films were cut into 4×4 cm pieces and dried at 105°C for 24 hours. Following the drying process, the films were precisely weighed to an accuracy of ±0.0001 g to determine their dry matter. Film pieces were put separately into 100 mL beakers filled with 40mL of distilled water. The beakers were covered and stored at 25 ^{o}C for 24 hours. Afterward, the film pieces were removed and dried at 105◦C for 24 hours to determine their final dry weight. This process was repeated three times. The total soluble matter loss was calculated based on the difference between the initial and final dry weights of the films **(8).**

Swelling index

The samples were obtained from five separate films of the same kind. Each sample was cut into 2×2 cm pieces and weighed. The pieces were submerged in distilled water at 25°C for 2 minutes. After immersion, the wet samples were gently wiped with filter paper to remove excess liquid before being weighed again. The percentage of water absorbed was calculated. This measurement was repeated three times for each type of film, and the arithmetic mean was taken as the final result **(4).** The swelling index

(SI) measures the difference in the film's weight before (W1) and after (W2) soaking in deionized water for 2 minutes, relative to its original weight, as follows: SI (%) = (W2-W1)/ W1 × 100

Mechanical properties

Puncture strength (PS) and Elongation (%)

The puncture strength of the films was measured using a Texture Analyser (Yamaden Ltd, Japan) fitted with computer software. Each film specimen was cut into a round shape and securing it between two metal rings, each with an 18mm diameter and a central hole measuring 10mm in diameter. A cylindrical probe with a diameter of 1 mm was attached to a load cell and positioned perpendicularly at the hole centre of where the film was placed. The probe moved orthogonally toward the film surface with a steady speed of 5mm/s till the film ruptured. The puncture strength was calculated by dividing the peak force by the cross-sectional area of the probe, with the data captured by a computer (**1).** Elongation at break was measured according to the following equation:

$$Elongation = \left(\frac{L0 - Lc}{Lc}\right) \times 100$$

Note: L_0 = initial length, L_C= final maximum length

Optical properties==Opacity

A spectrophotometer (UV-1800, Shimadzu, Japan) was used to measure film opacity as outlined by **Cho and Rhee, (4).** Three pieces, each measuring 1.5×0.7cm, were cut from each film and adhered to the interior surface of plastic cuvette. The absorbance (A) was recorded across a wavelength range of 400–800 nm. Opacity was quantified as the region under curve, calculated using an integration method, and expressed accordingly: **O = Abs nm/x** Where: O = Film opacity, A = Absorbance of the film at (400-800) nm, x = Film thickness (mm).

Statistical analysis

Analysis of variance and Bonferroni multiple comparisons were conducted using Systat (SPSS Inc., Chicago, IL) to identify significant differences in film properties at a 5% confidence level.

RESULTS AND DISCUSSION

The yields and solubility of deamidated rice bran protein (RBP): The data in Table (1) shows that protein solubility improved after deamidation process at different temperatures and pH values compared to control. Solubility increased to 85.42% when deamidation was performed at 120 ^{0}C and pH 8 for 30 min compared to 18.02% for control. On the other hand, protein solubility increases as a function of increase pH to 12 at the same temperature and time which recorded 89.92%. Deamidation can enhance the functional characteristics of proteins by converting glutamine and asparagine residues into glutamate and aspartate, which improves protein solubility **(14).**

Table 1. The yields and solubility of deamidated rice bran protein (RBP).

pH	Temperature (°C)	Time (min)	Yield (%)	Solubility (%)
Untreated RBP				$18.02^{l} \pm 0.11$
8	80	30	$70.8^{g} \pm 0.12$	$55.27^{k} \pm 0.16$
		60	$70.4^{g} \pm 0.11$	$58.52^{i} \pm 0.13$
	100	30	$67.4^{h} \pm 0.14$	$62.85^{h} \pm 0.09$
		60	$68.8^{gh} \pm 0.09$	$64.41^{g} \pm 0.12$
	120	15	$67.6^{h} \pm 0.12$	$83.55^{d} \pm 0.14$
		30	$70.0^{g} \pm 0.31$	$85.42^{c} \pm 0.22$
10	80	30	$86.0^{a} \pm 0.11$	$54.95^{k} \pm 0.08$
		60	$80.0^{b} \pm 0.23$	$62.35^{h} \pm 0.11$
	100	30	$76.0^{e} \pm 0.21$	$66.86^{f} \pm 0.13$
		60	$76.6^{de} \pm 0.12$	$67.51^{f} \pm 0.20$
	120	15	$80.4^{b} \pm 0.15$	$84.71^{cd} \pm 0.22$
		30	$79.6^{bc} \pm 0.13$	$87.49^{b} \pm 0.17$
12	80	30	$79.2^{bc} \pm 0.15$	$58.73^{i} \pm 0.19$
		60	$74.8^{ef} \pm 0.13$	$57.13^{j} \pm 0.16$
	100	30	$75.2^{e} \pm 0.23$	$85.22^{c} \pm 0.11$
		60	$73.0^{f} \pm 0.12$	$87.07^{b} \pm 0.13$
	120	15	$78.2^{cd} \pm 0.21$	$75.50^{e} \pm 0.17$
		30	$76.4^{e} \pm 0.13$	$89.92^{a} \pm 0.14$

Values are means ± SD

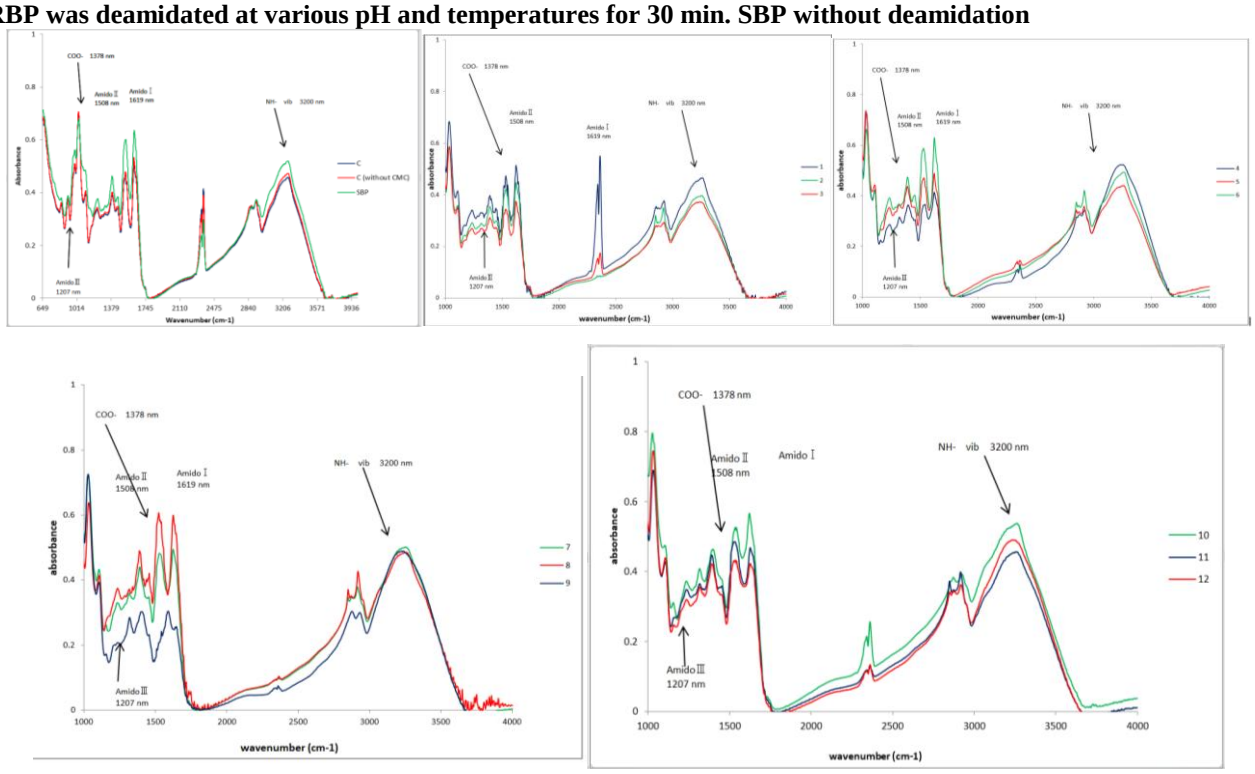
FT-IR spectroscopy

FT-IR spectroscopy is an effective method for analyzing the secondary structure of insoluble proteins by examining the band of amide I. Key structural features include α-helices (1653 cm^{-1}), β-sheets (1620, 1635, and 1683 cm^{-1}), β-turns (1669 and 1675 cm^{-1}), and random coils (1645 cm^{-1}). Additionally, the band at 1660 cm^{-1} is predominantly attributed to the carbonyl stretching of glutamine side chains (Fig. 1). The bands at 1683 and 1920 cm^{-1} are thought to be linked to the aggregation process. The analysis was conducted to evaluate the alterations in the secondary structures of rice bran protein caused by deamidation (Table 2). Before deamidation, RBP exhibited 15% α-helix, 34% β-sheet, 25% β-turn, and 26% other structures. FTIR infrared measurements revealed that, the dense structure of rice bran protein underwent noticeable changes during deamidation. Tan et al. (22) observed a strong correlation between protein water solubility and secondary structure, with correlation factor of -0.964 ($p < 0.01$) for α-helix and 0.743 for β-sheet. Additionally, it was reported that Tilapia-soybean protein co-precipitates exhibited lower α-helix content and higher β-sheet content compared to Tilapia protein isolate, leading to a notable improvement in their water solubility.

Table 2. Secondary structures of protein film prepared by deamidated RBP

pH	Temp. (°C)	α-helix (%)	β-sheet (%)	β-turn (%)	Other (%)
SBP		14	43	21	22
Untreated RBP		15	34	25	26
	80	17	32	26	25
8	100	17	37	24	22
	120	15	32	28	25
	80	17	39	22	22
10	100	21	32	23	24
	120	19	34	23	24
	80	16	38	23	23
12	100	16	39	22	23
	120	14	36	26	24

RBP was deamidated at various pH and temperatures for 30 min. SBP without deamidation



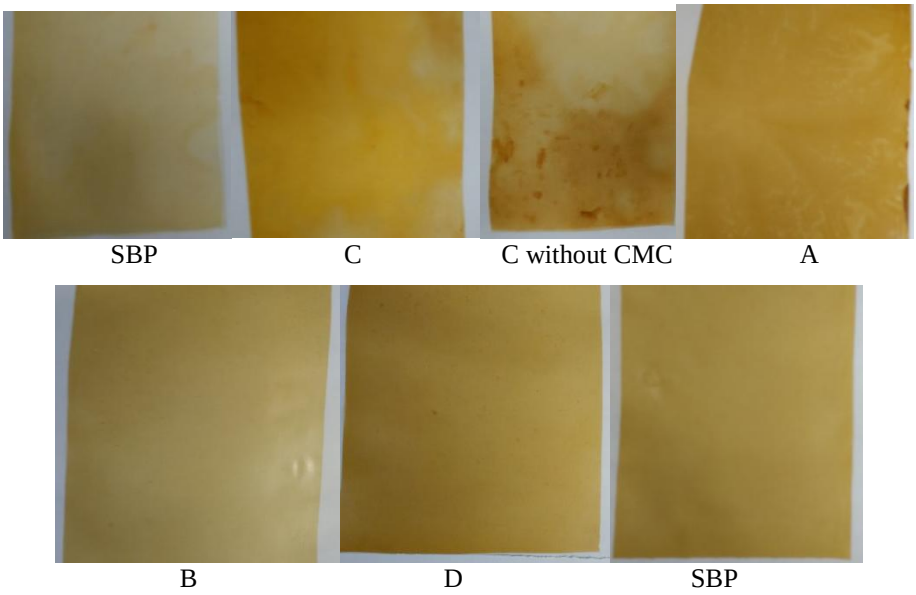


Fig 1. FT-IR chromatogram showing RBP film as affected by deamidation compared to SBP film.

Physical properties of film

Some physical properties of RBP film prepared by different levels of RBP as affected by deamidation process such as film thickness, solubility of water and % swelling were presented in Figures 2, 3 and 4.

Film thickness

The data in Fig (2) shows that film thickness ranged between 0.17 to 0.19mm when protein was used at 3% level; while it increased to 0.24mm and 27mm as a function of increase protein concentration to 4 and 5%; respectively. Film thickness may be varies depending on protein concentration and the surface area used to prepare the film. The increase in film thickness could be attributed to a higher concentration of total solids in the film composition.

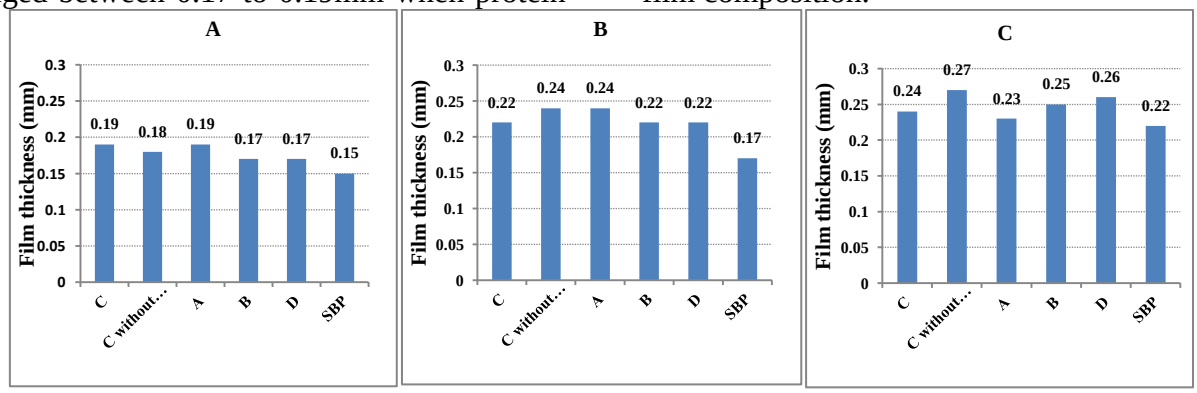


Fig. 2. Film thickness (mm) as affected by protein concentration and deamidation conditions. C = Control, Control without CMC, A = pH8, 120 ^{o}C, B = pH10, 120 ^{o}C, D = pH12, 120 ^{o}C, SBP = Soybean protein.

Film solubility in water

The water solubility of protein films plays a crucial role in determining their applications in the food industry. Films with high water solubility are well-suited for making hot-water-soluble pouches, whereas those with low solubility are ideal for coating or packaging high-moisture foods, such as meat products (21). A high solubility can be advantageous for certain applications. The solubility of films is also a key factor influencing their biodegradability when used as packaging material. As shown in Fig. 3, film solubility increased with a rise in pH from 8 to 12 compared to the control sample. The control sample without CMC exhibited lower solubility than the sample with CMC. This could be because CMC tends to form clusters, which may disrupt the polymer structure and increase moisture permeability at higher concentrations (11). The solubility of RBP films decreased with increasing protein levels.

Differences in water solubility were attributed to variations in protein characteristics such as composition and surface charge, which influenced their solubility. Our findings align with Tan *et al.* (22), who observed that Tilapia-SBP complexes, characterized by lower α-helix and higher β-sheet contents compared to Tilapia protein isolates, exhibited increased water solubility. Specifically, film D demonstrated the highest solubility, correlated with higher β-sheet and lower α-helix contents compared to other films.

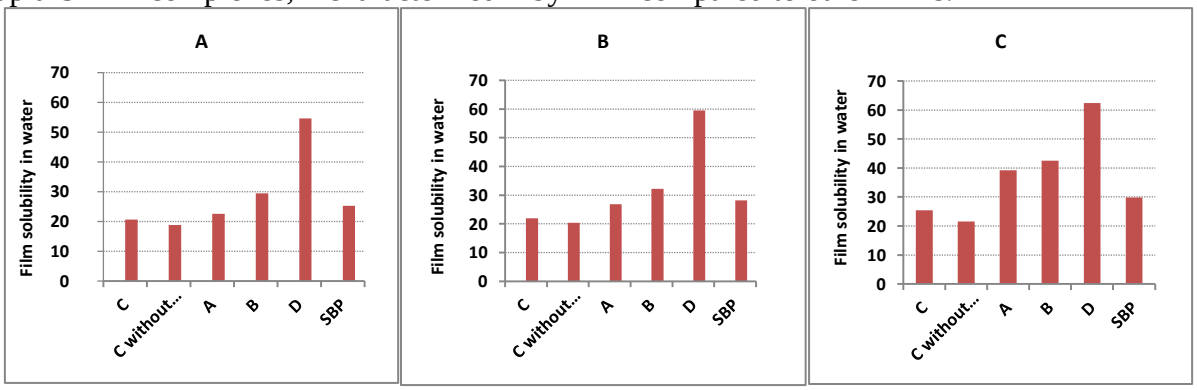


Fig 3. Film solubility in water as affected by protein concentration and deamidation conditions. C = Control, Control without CMC, A = pH8, 120 ^{0}C, B = pH10, 120 ^{0}C, D = pH12, 120 ^{0}C, SBP = Soybean protein. A = 3%, B = 4% and C = 5%.

Film swelling (%)======The swelling rate is a crucial parameter in film samples, as a high swelling rate adversely impacts the film's shelf life (10). Swelling degree values ranged from 67.71% for control RBP film at 3% level to 150.62% for sample A (Fig. 4). For the 3% SBP film, the value was 162.89%, likely due to the abundance of –OH groups, which enhance its interactions with water and subsequently reduce the water available for binding with soy protein. Samples B and D had solubilized and disappeared when immerged in water to determine swelling at all used concentrations. Generally, swelling index of RBP film increases as a result of increase RBP concentration. Mathew *et al.* (12) observed that the swelling properties of a film are directly influenced by water diffusion, functional groups, and ionic bonds. These properties also depend on the film's thickness, molecular interactions, and microstructure. Consequently, a decrease in hydrophilicity results in a reduced swelling rate.

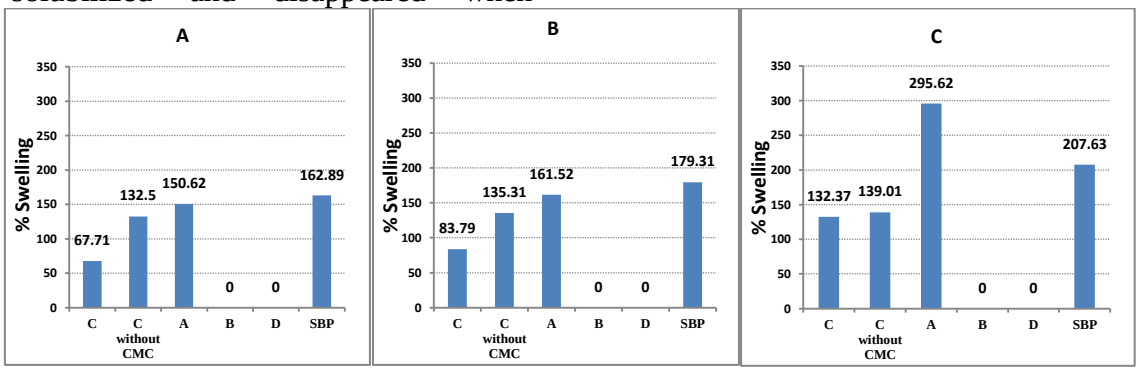


Fig. 4. Swelling (%) of RBP film as affected by protein concentration and deamidation conditions. C = Control, Control without CMC, A = pH8, 120 ^{0}C, B = pH10, 120 ^{0}C, D = pH12, 120 ^{0}C, SBP = Soybean protein. A = 3%, B = 4% and C = 5%.

Mechanical properties of film

The mechanical properties of protein films indicate their expected integrity when subjected to stress during processing, handling, and storage. The strength and flexibility of film is described by its puncture strength (PS) and elongation (E).

Puncture strength

Figure (5) illustrates the behavior of the films under puncture force. The puncture test was performed to evaluate maximum force and elongation of the films in its transversal direction. The puncture strength of control RBP films was significantly influenced by the presence of CMC compared to films without

CMC. An increase in RBP concentration from 3% to 5% led to higher puncture strength in deamidated, control, and SBP films at higher protein levels, the abundance of hydroxyl and carboxyl groups along protein molecules may facilitate the formation of many hydrogen bridges among protein molecular chains. These widespread interactions among chains enhance the film mechanical properties (11). These findings differ from those reported by Vachon *et al.* (24), who observed a significant decrease ($p < 0.05$) in the puncture strength of films with higher whey protein concentrate (WPI) concentrations, reaching a minimum value of 0.04 N/µm for films composed solely of WPI. In contrast, soybean protein films exhibited greater puncture strength than RBP films across all concentrations (Fig. 5). Our findings align with those reported by Adebiyi *et al.* (1). RBP film (D) had the highest puncture strength among all RBP films. This could be due to the deamidation process at pH 12 and 120°C, which likely caused protein-protein interactions to form a complex. These interactions might have led to various types of cohesiveness as a result of changes in the protein's electrostatic properties and hydrophobicity. Additionally, the protein concentration in film-forming solutions influences self-adhesion and the uniformity of matrix formation during film preparation. As protein concentration increases, protein–protein interactions are enhanced, leading to a highly cohesive medium.

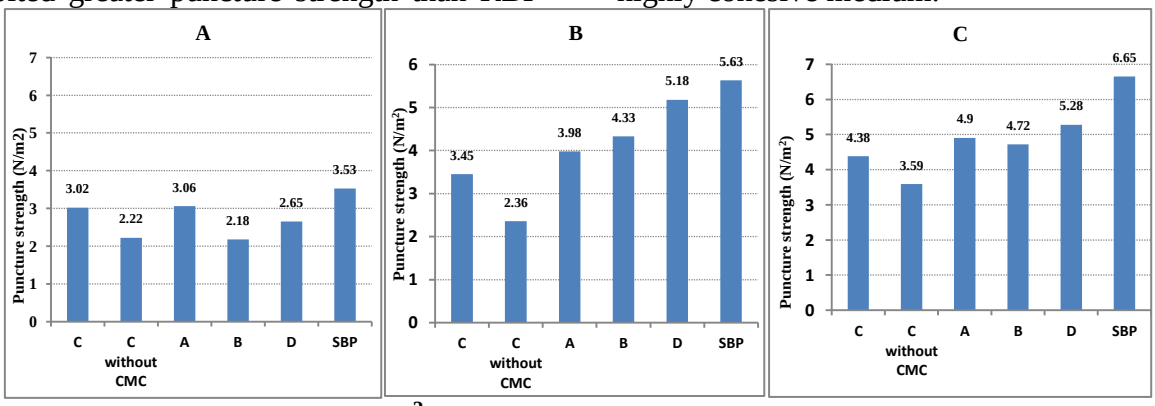


Fig. 5. Puncture strength (N/m^2) of RBP film as affected by protein concentration and deamidation conditions. C = Control, Control without CMC, A = pH8, 120 ^{0}C, B = pH10, 120 ^{0}C, D = pH12, 120 ^{0}C, SBP = Soybean protein. A= 3%, B = 4% and C = 5%.

Elongation: Elongation at break (E) and puncture strength (PS) are key parameters that reflect a film's stretchability at the point of breakage and its overall strength. Enhancing the mechanical properties of films increases their resistance to stress during storage and transportation, thereby reducing the risk of tearing or perforation of composites (29). A higher elongation signifies that the film is more flexible under tension or mechanical stress. The results showed that the percentage of puncture strength increased with increasing elongation for the majority of RBP and SBP films (Fig. 5 and 6). Elongation at break ratio of deamidated RBP films ranged between 3.13 to 3.82%, 4.68 to 4.97% and 5.49 to 9.1% at levels 3, 4 and 5% protein; respectively. The SBP and RBP (D) films at all protein concentrations exhibited the best mechanical properties with the highest values of puncture strength and elongation. Perez-Gago (16) established that the protein phase has a significantly greater impact on the strength of films compared to the lipid phase. Consequently, RBP films with higher protein concentrations were anticipated to exhibit greater strength.

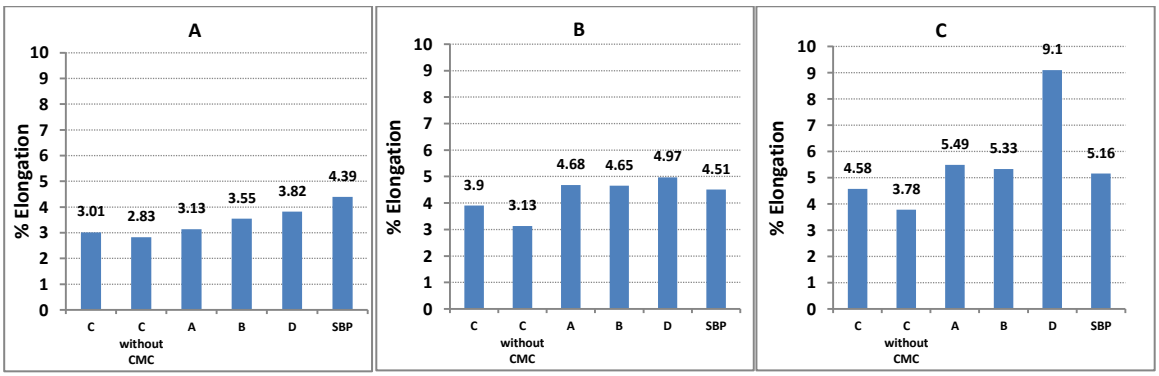


Fig. 6. %Elongation of RBP film as affected by protein concentration and deamidation conditions. C = Control, Control without CMC, A = pH8, 120 ^{0}C, B = pH10, 120 ^{0}C, D = pH12, 120 ^{0}C, SBP = Soybean protein. A = 3%, B = 4% and C = 5%.

Optical properties of film

Opacity: Film opacity measures a film's capacity to block light, which can trigger and encourage unwanted chemical reactions like lipid peroxidation in fatty foods. Additionally, it significantly affects the visual appeal of the packaged product (27). The opacity of films prepared from control and deamidated RBP at different concentrations are showed at Fig. (7), the results reflected that control sample had higher opacity (lower transparency) than control with CMC at all concentrations. It may be due to low solubility of protein which may aggregate and prevent light transmission. Also, opacity increased while transparency decreased with an increase in protein concentration from 3% to 5%. This could be attributed to enhanced protein–protein interactions, leading to the development of a tight network and an increase in insoluble matter. Also, opacity decrease as a result of increase deamidation pH from 8 to 12; it may be due to the increase of protein solubility led to increase of light transparency. Apparent also that opacity decrease (increase of transparency) with increase of wavelength from 400 to 800 nm for all samples. Our findings align with those of Zhang and Han (30), who observed that the light transmission rate increased with increasing wavelength. They also noted that establishing a clear correlation between the transparency and the components in the films was challenging.

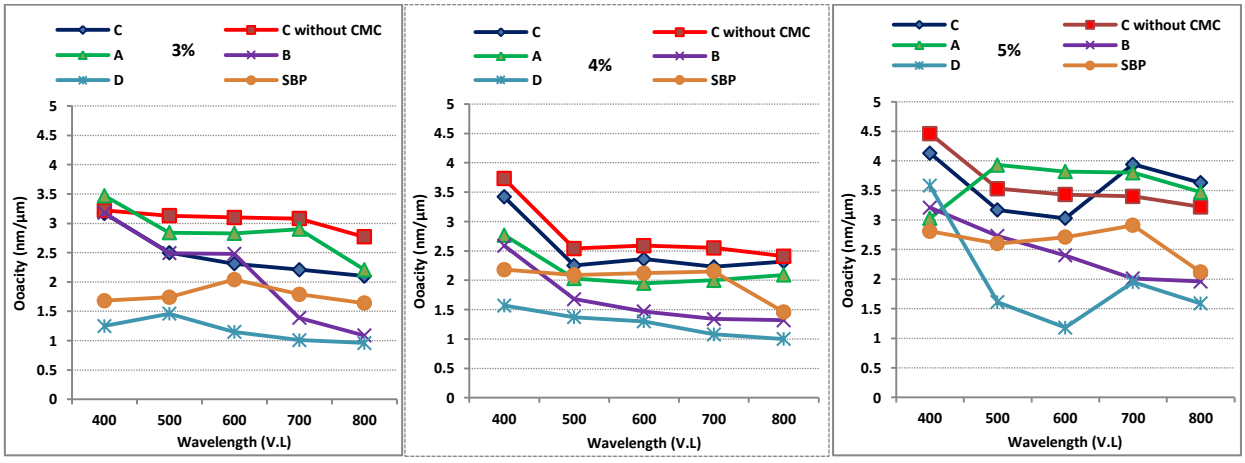


Fig 7. Opacity of RBP film as affected by protein concentration and deamidation conditions. C = Control, Control without CMC, A = pH8, 120 ^{0}C, B = pH10, 120 ^{0}C, D = pH12, 120 ^{0}C, SBP = Soybean protein.

It could be noted from the previous results that deamidation process can increase RBP solubility from 18 to 89.92%. Furthermore, the obtained protein can be used to prepare edible film which has good physical, mechanical and optical properties compared to soybean protein film.

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