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Original paper

Assessing the structure and interaction of sustainable Bio – Composite Films Prepared form deamidated Rice Bran Protein

MOHAMMED HAMED HASSAN[1]*, RAGAA ZEIN[1], OSMAN M. F. [1]

[1]Food Technology Department, Faculty of Agriculture, Kafrelsheikh University, Kafrelshiekh, Egypt

Abstract

Rice bran protein (RBP) was utilized to prepare bio-composite films after deamidation modification process with deferent percentages. The physical, mechanical properties, exposure to light properties, FT-IR analysis, surface shape by SEM, thermal stability by DSC and X-ray diffraction were applied to study the potential interaction, structure and stabilizing the thermal property of the prepared films. The deamidation process has proven successfully, depending on the cleared changes of shapes of FT-IR curves resulting from RBP compared with deamidated rice bran protein (DRBP). The deamidation process enhanced tensile strength, elongation at break, haze, transparency, gloss and opacity properties of produced films from DRBP. Additionally, the produced films from DRBP presented smooth and homogenous surface with increasement of thickness, solubility and decrement of water vapor permeability and crystallinity compared with films from RBP.

Keywords Deamidation, edible films, deamidated rice bran protein, film characteristics

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*Corresponding author: MOHAMMED HAMED HASSAN, Food Technology Department, Faculty of Agriculture, Kafrelsheikh University, Kafrelshiekh, Egypt
E-mail: Mohammed_Yousef@agr. kfs. edu. eg

Introduction

In recent years, the development for newly degradable films from protein isolates has become a must [1]. Therefore, protein films offer the potential for reducing non-renewable resources and may lead to the use of agricultural raw materials for film preparation. Coatings and edible films define as, thin layers of edible substances wrapped on the food products that have an important for distributing, storage and marketing. Some of their functions are to product from mechanical damage, physical, chemical and microbiological activities. The bio-composite films are first molded as solid sheets, which are then applied as a wrapping on the food products [2]. The bio-composite films are analyzed for different applications because of their ability to provide a barrier to mass transfer, carry food ingredients, and improve mechanical integrity of foods. Additionally, it can also enable reduction and simplification of the wrapping material required for a food product. Furthermore, coating material has been utilized in reducing the loss of water, delay of the ripening, reducing the chilling and mechanical damage, reduce decay and added shine, or gloss to the coated commodity [1, 3]. films act films act as a carrier for nutrients and other bioactive compounds that affect the health on a positive way, due to the techniques of microencapsulation or nano-encapsulation. during that, the film substance acts for transporting the bioactive materials to the target sides without losing its activity [4]. The bio-composite films could sustain a modified packaging? passive atmosphere, which encourage some changes in minimally and fresh processed food staff such as : firmness, inhibition of microbial growth, antioxidant properties, color, ethylene production, sensory quality, and volatile compounds as a resulting of anaerobic processes [5].

Polysaccharides, proteins, and lipids are the main polymeric materials to produce edible coatings and films. In many instances two or all of these ingredients could be blended to produce composite coating films [6]. Proteins from plant are more often used than animal proteins for films production because of the availability and lower costs. The by-product from the cereal agro-industrial processing may be a source of protein that can be recovered for formation of protein – based films. Rice bran (RB) is a by-product which derives from the processing of the grain, which proteins were extracted to produce bio – based [7, 8]. The reason why its excellent property as barrier for oxygen, its tightly packed hydrogen bonded structure. Edible coating from protein has good O2 barrier property at low RH. It consists of good organoleptic and physomechanical properties [9]. However, the utilization of rice bran protein (RBP) is still limited because of its low solubility in water. To enhance the food protein solubility various modification approaches, such as physical and chemical modification were made [10, 11]. One method that is commonly used to enhance the functional properties of food proteins is deamidation. Deamidated forms residues of Glutamine and Asparagine to their increase the protein solubility [12]. In this study, RBP was deamidated under various alkaline conditions of temperature, pH, and reaction time. Changes in solubility of protein were examined to determine the degree of deamidation. The objective of the present study was to fabricate new bio-composite films with enhanced manners by utilizing modified RBP

Materials and Methods

Materials

Rice bran (Giza 178) was obtained from Rice Research Center at Sakha, Agricultural Research Center, Egypt. Glycerol, Ascorbic acid, Citric acid, Sodium hydroxide (NaOH), Hydrochloric acid (HCl), N-hexane, Sodium bicarbonate were purchased from El- Gomhoria Co. for Chemicals and Drugs. Teflon plates (20×30) used to cast all the films. All reagents were of analytical grade.

Methods

RBP extraction

RB samples were treated by microwave to inhibit the enzymes action for five minutes. Oil was elicited from RB using n- hexane based on the method outlined by Kahlon [13]. The defatted rice bran was stored at -18oC until used. After that, defatted rice bran was suspended in distilled water (1:10 w/w). slurry's pH was set at 9. 0 using 4 M NaOH solution under continuously stirring for 1 h, then centrifuged for 15 minutes at 12600 g. supernatant protein solution was set to pH 4. 5 using 4 M HCl, stirred for 30 min then left overnight at 4 °C for cold precipitation. The supernatant has been siphoned off then the resulted precipitated protein was washed 3 to 4 times with distilled water. The protein slurry has been set to pH 7 and lyophilized [14].

Production deamidated rice bran protein

Protein deamidation was performed as following; briefly, 0. 25 g of RBP was suspended in 25 mL of 0. 1 M NaHCO3 and pH was set to 8, 10, and 12 with 1 M NaOH or 1 M HCl. The solutions were then heated at 80 or 100°C for 30 or 60 min, or 120°C for 15 or 30 min. Then, samples have been neutralized, dialyzed against water at 4˚C for 48 hrs, and lyophilized.

Determination of protein solubility

Fifty mg of protein sample has been suspended in 5 mL of deionized water, mixed for 30 min at room temperature, and centrifuged at 15000 g for 20 min. The supernatant has been transported to another tube, after dried at 40 °C, weight of residual protein was determined based on the method outlined by Bradford [15].

Formation of edible film

RBP 1% or DRBP with different concentrations (1, 2 and 3%) were dissolved in distilled water, stirred at 80˚C for 30 min. Glycerol was added and pH was adjusted. Ascorbic and citric acid were added as anti-browning agent and antimicrobial (1. 5% (w/v). Film solution was left in the room temperature for 1h to remove air bubbles and cast into Teflon plates (20×30 cm) then dried at 40˚C in oven. Films have been stored at plastic bags until the measurement were performed.

Mechanical properties

Tensile strength and Elongation

Tensile strength (TS, Mpa) and elongation percentage (%E) at break of film were determined at 22 ± 10C and RH=31% using an Instron Universal Testing Machine (Model 1011, UK), according to Ferreira et al. [16].

Characterization of physical properties.

Film thickness

Thickness of film has been calculated with an accuracy of 0. 01 mm using a digital micrometer (Mitutoyo digimatic indicator corporation, model: pk-1012 E, Tokyo, Japan). The mean thickness have been used to measure the water vapor permeability and tensile strength [17].

Determination of films solubility and swelling power

Solubility and swelling degree of the formed films were measured by using the method of Riaz et al. [18] with a slight modification. The film pieces were cut into 2x 2 cm then dried at 105°C until constant weight to calculate the initial dry mass (M1). Then, those were took place in 100 mL beakers with 50 mL distilled water, covered with plastic wraps and stored at 25°C for 24 h. Later, the films have been dried using filter paper and dried at 105°C until fixed weight to determine final dry mass (M2). The following equation was used to measure film solubility:

$$\text{Film solubility}=((M1–M2)/M1)*100$$

films were placed in beakers (50 mL) that contain distilled water (30 ml) for 24 h at 25°C after weighing (M1). Then, wet film was dried using filter paper then weighed (M2). The swelling degree calculated with this equation:

$$\text{Film swelling degree (\%)}=((M1-M2)/M1)*100$$

Moisture content

Moisture content was determined based on the way of Araujo-Farro et al. [19]. Moisture content was measured by Eqs. 1. film samples have been cut into squares of 2 ×2 and weighted (W1). The samples have been dried at 105°C until constant weighted (W2) obtained. Triplicates of each film samples were tested.

$$\text{Moisture content (\%)}=((W1–W2)/W1)*100$$

Water vapor permeability (WVP)

Modification of the ASTM E96-92 gravimetric method to measure the relative humidity (RH) at the film underside was used for measuring WVP [20]. Distinct glass cups have been used with 4 cm diameter then filled with anhydrous $CaCl_2$ then film samples (5×5 cm) have been sealed above each glass cup. The cups have been placed in a desiccator at 75% RH which was maintained by a saturated sodium chloride solution. The glass cups have been then weighted change against time was determined by linear regression.

The WVP was measured by the following equation

$$WVP = \left(\frac{\text{Slope} \times L}{A \times \Delta P}\right)$$

Where L is the average film thickness (m), A is the transfer area (m2) and ΔP the partial water vapor pressure difference.

Exposure to light properties

Haze and Transparency

Haze and transparency of samples were determined by Hazemeter (BYK- Gardner Gmbh model haze-grad plus, Germany, according to ASTM., (2001).

Film gloss

Gloss is a way to measure of the ability of the film to reflect incident light at angle 450. The sample's gloss has been measured by glossmeter (BYK- Gardner Gmbh model micro- gloss 45, Germany, according to ASTM., (1997). Standard measure for tensile properties of thin plastic sheeting. D882- 97, Annual book of American Standard Testing Methods, ASTM, Philadelphia, PA. Gloss values measurement based on 6 random positions per sample from double faces and three samples per film.

Films opacity

Spectrophotometer (Model PU 8800 UV/VIS, Pye Unicam Ltd., Cambridge, UK) has been used to determine film opacity as described by Sun et al. [21]. Three strips (1 x 4 cm) were cut from the soaked films and placed on the inner side of a transparent plastic cuvette. The adsorption spectrum was determined with a wavelength domain of 400–800 nm, and opacity was taken as the area under the curve, as determined through an integration procedure, and termed as Absorbance Units in nanometers (A nm).

Characterization of RBP films

Analysis of SEM

Scanning electron microscopy (SEM, SU8010, Hitachi, Japan) at 10 K V has been used to study film surface morphology. The films were cut in pieces (10 x 10 mm) then, dried and mounted on aluminum stubs using a double – sided carbon tape and sputter- coated with gold [21].

Analysis of FT- IR

Fourier Transform – Infrared (FT- IR) spectrometry (Nicolet IR200, Thermo, USA) was used to study prepared films structure through KBr module. FT- IR spectra were recorded in the frequency range of 4000 to 400 cm-1.

Differential scanning calorimetry (DSC)

DSC analysis was performed using a Q1000 DSC system (TA instruments, USA) following the way of Akhtar et al. [22]. Film pieces (10 mg) were sealed in a standard aluminum pan and heated at a constant rate of 10 °C / min from 0 to 450 °C at a nitrogen atmosphere.

X-ray diffraction

The crystal structures of films was explored following the way of Ali et al. [17] using x-ray diffractometer (D8 Advance, Bruker. USA) at a voltage of 40kV and 100 Ma. The scattered radiation was detected in the angular range 2ϴ= 10- 40º with scanning speed of 5 º/ min.

Statistical analysis

Data were analyzed statistically by SPSS 20. 0. Analysis of the data (ANOVA) assessed the difference between factors and levels. To identify the significance of differences among mean values. Tukey's multiple range tests were executed. Differences were considered significant when $p< 0. 05$.

Results and discussions

Effect of deamidation process on RBP solubility

Different alkaline pH values were utilized with various temperature for different times to modify protein structure by removing amides to characterize RBP solubility. as shown in Table (1).

The results showed that, protein solubility increased from 18 to 93% when deamidation was achieved at pH 12 for 15 min at 120˚C. RBP was deamidated by altering the heating, temperature, pH and period. By raising these parameters, the grade of deamidation has been increased. As expected, solubility with increasing the deamidation degree. That is, Asparagine and Glutamine residues in RBP converted to Aspartate and Glutamate residues upon deamidation, so that the surface polarity of the protein increased to improve its solubility. RBP has high thermo-stability because of its compact structure, with also cause poor solubility. Temperature needed for denaturing of RBP decreased with the deamidation process, which result in unfolding of the proteins. However, deamidation conditions such as high temperature (<120˚C) and high pH are likely to affect the denaturation more strongly. With increasing pH more denaturation and unfolding the protein happened, so deamidation of RBP under alkaline pH improves the solubility of rice bran protein. While, severe alkaline conditions ((pH<12) result in the racemization of amino acids [12].

Table1. The solubility of deamidated RBP under different pH, temperature and time conditions

pH	Temperature (°C)	Time (min)	Solubility (%)
Untreated RBP			18.54
8	80	30	36.53
		60	36.52
	100	30	44.84
		60	41.09
	120	15	54.75
		30	52.10
10	80	30	34.97
		60	30.41
	100	30	39.31
		60	49.83
	120	15	46.09
		30	71.63
12	80	30	51.21
		60	39.52
	100	30	36.71
		60	72.52
	120	15	93.20
		30	92.42

Mechanical properties

Elongation at break (EB) and Tensile strength (TS) are main parameters which indicate film stretchability upon breakage and the strength of the film. Improvement of the mechanical properties of films explains the increase in its endurance versus stress during storage and transportation hence inhibits tearing of composites and perforation [23]. TS and EB values from the films are shown in Figure 1. DRBP with different concentrations films showed significant differences for TS and EB values compared with RBP. The highest TS and EB were found 57. 22 Mpa and 66. 73 %, respectively in DRBP (3%) samples.

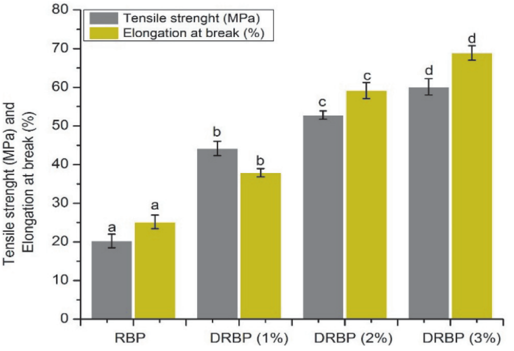

Fig. 1. Mechanical properties of RBP films and films with different concentration from DRBP.

Deamidation process created complex of protein-protein interaction which might make many types of cohesiveness due to changes in electrostatics and hydrophobicity of protein. Additionally, concentration of protein in the film forming solutions affect the self-adhesion and the average of matrix forming on film preparations. On increasing protein concentration, protein – protein interactions increased and resulted high cohesiveness medium [24]. Considering the EB of films depends on the TS [25]., the denaturation case of RBP to produce DRPB in alkaline environment and heating caused ruining of protein structure, breaking existing disulfide intermolecular bonds and exposing sulfhydryl hydrophobic groups, making them available for bonding. The cleavage of di-sulfide bonds results in polypeptide chains with lower molecular weights, destroying elasticity and cohesiveness of RBP. Upon casting and drying, sulfhydryl groups reform disulfide bonds by air oxidation, which leads to elongation of the film structure [26]. On the other hand, RBP was deamidated under various alkaline conditions of temperature, pH and reaction time. These might increase the surface electronic charge and polarity which improve structural flexibility and TS. Deamidation was adopted to modify the structure of RBP with denaturation without causing severe hydrolysis which improve elongation properties [27].

Physical properties

Thickness

The film's thickness significantly increased with increasing concentration of DRBP as show in Table 1 compared with RBP. The deamidation of films by using alkaline medium generated interaction between protein chains and this might lead to denaturation of DRBP which cause tighter binding of protein film [28]. The thicker films are favorable to retard penetrating moisture from surrounded environment to wrapped food.

Moisture content and swelling ratio

Data for moisture content ratio of film samples are shown in Table 2. It provides information about the water affinity, whereas swelling ratio may help to predict quality and stability changes in food product storage [29].

For moisture content and swelling ratio, significant differences were observed in all the films. The results showed that moisture content decreased due to increase protein content, It may be result of hydrophobicity of DRBP [30]. Swelling ratio decreased with increasing DRBP content and this could be because of formation of strong hydrogen bond between the unfolded protein which result from the denaturation process which happen during deamidation. This result improves the swelling property of the film, which need to be resistant to water to make it suitable for use as food packaging material.

Water solubility

Water solubility of the film is an essential property of biodegradable. It showed significant increase for the RBPD films in comparison to control RBP film (Table 2). The higher values of water solubility for RBPD films as for film solubility, it increases due to increase protein concentration from 1 to 3%. Also, alkaline medium led to increase film solubility in water. The higher values of water solubility for DRBP films because of the hydrophilic groups of DRBP that could easily interact with water molecules [31, 32].

Water vapor permeability

The most essential function of the film is to delay the deterioration of food products from the surrounding atmo-

Table 2. Physical properties of RBP and DRBP films

Samples	Thickness	Swelling degree (%)	Water solubility (%)	Moisture Content (%)	Water Vapor permeability (%)
RBP	38.50 ±0.03a	36.56± 0.36d	14.80± 0.45a	17.35±0.44d	5.75±0.77c
DRBP 1%	40.10±0.01b	32.85± 0.25c	19.64± 0.56b	15.90±0.19c	4.40±0.29b
DRBP 2%	42.30 ± 0.15c	28.71± 0.05b	25.82±0.81c	14.30±0.56b	2.80±0.22a
DRBP 3%	44.50± 0.17d	24.95± 0.06a	31.50± 0.51d	12.40±0.26a	2.75±0.08a

Values are mentioned as mean ± standard deviation. Dissimilar letters in the same column show significant differences ($p<0.05$)

sphere. The Film is considered better if the moisture transmission between food staff and surrounding environment is as low as possible. Water vapor permeability is one of the main essential characters of bio-composite films for food packaging as it has direct contact with food products hence has high influence on its shelf life. The present results showed that DRBP had significant decreasing effect on WVP of film samples (Table 2). WVP of films decreased from 5. 75 to 2. 75% with increasing concentration of RBPD, because that at higher pH, protein denatures, unfolds and solubilizes, facilitating disulphide bonds by thiol-disulphide interchange and thiol oxidation reactions, resulted in stronger films with less permeability to gases and water [33, 34].

Exposure to light properties

Haze of film is defined as, the percentage (%) of light transferring through the film and refraction by angle more than 2. 5o from incident light. While, known as the percentage of light transfeerring through the film. Appearance of films as (Haze, Transparency and Gloss) are important properties of edible films because it could affect consumer acceptance in potential edible food applications. The consumer preference is greatly influenced by opacity and color of food packaging materials. Data in Table 3 explain result of RBP and rice bran protein deamidated concentration on exposure to light of RBP and DRBP film. Results show that, Haze, transparency and Gloss gradually increase as function of increasing DRPB from 1 to 3% in comparison with the control RBP film, it may be beacuse solubilisation of the protein under the alkaline pH and deamidation of RBP, which yielded in increasing solubility and exposure to light properties [28].

Additionally, Results in Table (3) show that film opacity affected by protein concentration and pH. It could be noted that film opacity increased due to increase protein concentration from 1 to 3 %. It may be because of increasing protein – protein interaction and forming a compact network and increasing insoluble matters [35].

Characterization of fabricated films

SEM

Characterization of fabricated films SEM morphology, homogeneity and Structure of the material have an essential role in the film's permeability [36]. SEM was applied to analyze the micro-structure of the film to resolve and test the film surface voids, smoothness, layer structure and homogeneity.

SEM images of the surface of rice bran protein film and DRBP films are shown in Fig 2. SEM image show that of rice bran protein film has bubbles, crakes and heterogeneous surface. The surface become more smooth, homogenous and has few bubbles with deamidation films. When, DRBP were 2 and 3% the SEM image have best result with smooth, no crake, uniform and homogenous surface. RBP has poor solubility, the solubility of rice bran protein improves with deamidation. Increasing the solubility affect and improve structure, homogeneity and morphology of the matrix, which result in quite smooth, uniform, ordered and homogeneous structure without bubbles or porous with no cracks in contrast to the film with native RBP.

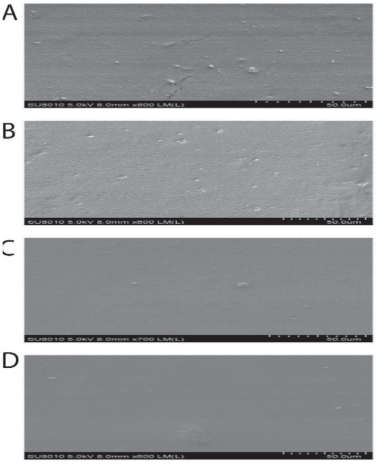

Fig. 2. SEM photographs of (A) RBP films, (B) DRBP 1%, DRBP 2% and DRBP 3% films.

FT-IR spectroscopy

Infrared spectroscopy is a rapid and a nondestructive technique that has been widely used to characterize different biomaterials. Moreover, FT-IR spectroscopy is a powerful

Table 3.Exposure to light properties of RBP and DRBP films

Samples	Haze	Gloss	Transparency	Opacity
RBP	56.07± 0.19a	16.87±0.03a	83.80± 0.25a	0.135 ± 0.01a
DRBP 1%	61.03± 0.23b	19.33± 0.06b	89.90± 0.01b	0.195±0.01b
DRBP 2%	63.46± 0.29c	25.18± 0.02c	91.23± 0.05c	0.325± 0.07c
DRBP 3%	63.46± 0.29c	27.70±0.05d	92.32± 0.15C	0.330± 0.04c

Values are mentioned as mean ± standard deviation. Dissimilar letters in the same column show significant differences ($p<0.05$)

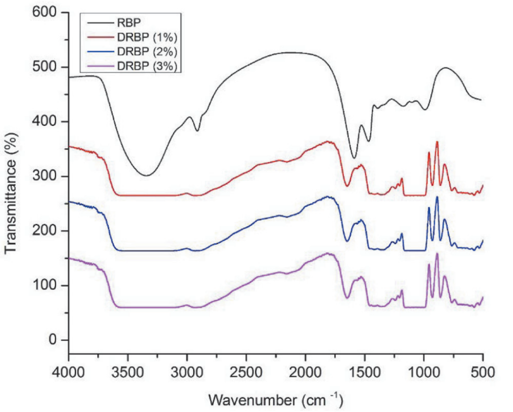

Fig. 3. FT-IR spectra of RBP films and films with different concentration from DRBP.

technique to evaluate polymer blend miscibility. Deamidation result in denaturation and unfolding of protein which facilitating chemical interaction at the molecular level, that increase the hydrogen bonds in protein matrix. These changes can be an indication of good miscibility of matrix. In this work, FT-IR showed that the absorption spectra of films from different formulations had similar absorption regions, differing only in the bands absorption intensity(Fig. 3). The absorption stands on the content of insoluble protein, which decrease with deamidation process [37].

DSC analysis of produced films

In the case of proteins, DSC can be utilized to investigate the thermodynamic stability, folding mechanism, thermal stability and denaturation of protein [38]. DSC thermograms of RBP and DRBP films were observed in Fig4. It was observed that the thermograms changed considerably by deamidation process. One transition with exo-shoulder corresponding to the unfolding of the proteins was observed in all samples. The apparent melting temperatures were 73. 5 °C for RBP films, and 78. 8 °C for DRPP; evidence that the deamidation process increased the thermal stability of proteins and delayed the unfolding process. The thermal property and secondary structure of RBPD determined by DSC and FT-IR was well preserved during the deamidation process [37].

XRD

To identify the crystalline structure of native RBP and study the result of deamidation on RBP. Deamidation process did not change the internal structure of RBP. The X-ray spectrum of the deamidated films showed only a decrease in the intensity of spectrum, and this may be because of decreasing the insoluble matter by deamidation (Fig. 5). The

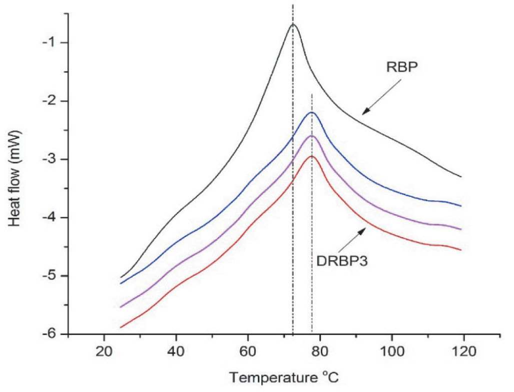

Fig. 4. DSC thermograms of RBP films and films with different concentration from DRBP.

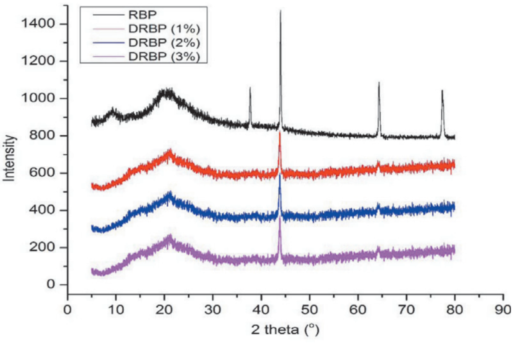

Fig. 5. XRD intensity of RBP films and films with different concentration from DRBP.

interactions that happen during deamidation in the crystalline structure may hinder the intra molecular hydrogen bonds and inter molecular hydrogen bonds in the denaturated unfolded protein, resulting in a low crystallinity. Hence, mechanical properties of the film strongly depend on the crystallites in its structure.

Conclusions

In the present study, solubility of RBP was improved by deamidation process by alkaline conditions. Overall, this study showed that, DRBP has the ability to develop protein films with good physical, mechanical and exposure to light properties. Deamidation process improves protein structure, solubility and increase the strength of hydrogen bonds between protein polymers, hence production of rice bran protein films with suitable physical, mechanical, Exposure to light properties and crystalline structure. That can be widely used as edible film for packaging food products. Hence, deamidation process can be applied on another protein material from food and agricultural manufacturing residues to increase their use as distinctive edible coating.

Conflict of Interest

The author has no conflict of interest to declare.

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