Properties of Biodegradable Films Produced from Rice Bran and Roasted Sesame Meal through Chemical Modifications

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Biodegradable films were prepared from roasted sesame meal and rice bran. Acetic anhydride, succinic anhydride, and formaldehyde were added to the film-forming solutions, and their effects on tensile strength, percent elongation, water vapor permeability, and water solubility of the films were studied. Roasted sesame meal did not form film without acylation or addition of formaldehyde. Acylated roasted sesame films had higher tensile strength and water-solubility, and lower % elongation than rice bran films. Acylation with acetic and succinic anhydrides increased tensile strength, percent elongation, and water solubility of rice bran films, but decreased water vapor permeability. Treatment with formaldehyde increased tensile strength of roasted sesame and rice bran films and % elongation of rice bran films, while reducing water-solubility of roasted sesame and rice bran films and water vapor permeability of rice bran films.

Key words: *biodegradable film, rice bran, roasted sesame meal.*

Proteinaceous materials have the ability to form films, which are applied in food packaging. Biodegradable and edible protein films or coatings function as moisture and oxygen barriers to retain crispness and inhibit rancidity. Protein-based films have been made from collagen,[1] casein,[2] whey proteins,[3] soy protein,[4] and wheat gluten.[5,6] Rice bran and roasted sesame cake are the low cost, underutilized by-products of rice milling and sesame oil production. A number of papers have been published on the extraction of proteins from both full-fat and defatted rice bran[7-9] and the functional properties of rice bran protein concentrates.[10] Though rice bran films have been studied for food purpose, commercialization of rice bran films requires improvements in mechanical properties.[11] On the other hand, studies on the functional properties of sesame proteins have been limited to studies on fresh sesame flour and proteins,[12,13] and those on roasted sesame protein very rare. Defatted sesame meal, the by-product of sesame oil, contains about 30% good quality proteins, but direct use in the food industry is limited due to its high content of oxalate and fiber.[14] Moreover, during commercial processing for sesame oil, sesame seeds were roasted at 210°C for 5 min to provide the favorable flavors to the resulting sesame oil,[15] and, thus, the protein in the residual oil meal cake was denatured to varying degrees depending upon the roasting temperature and used primarily for animal feeding. Functional properties of proteins are often chemically modified through acylation.[16] Acetic and succinic anhydrides are the commonly used protein acylating agents. Acylation increases the net negative charges of protein, introduces bulky side groups, and changes the protein conformation. Hence, it affects not only protein functionalities but also protein-mineral-phytate interactions.[17] Treatment with aldehydes is another means of protein modification. The ability of mono- and bifunctional aldehydes to promote covalent intermolecular and intramolecular cross-linking of protein is well documented in the literature.[18] Aldehydes have been utilized for tanning of collagen in the production of sausage casing.[19] Keratin is hardened with aldehydes in wool fabric production.[20] The cross-linking effect of aldehydes on wheat gluten proteins was also verified.[21,22] Thus, the objectives of this study were to produce biodegradable films utilizing rice bran and roasted sesame meal and to determine the effects of acetic anhydride, succinic anhydride and formaldehyde on the properties of the films produced.

Materials and Methods

Materials. Samples of sesame cake and rice brans used in this study were purchased locally on an as-needed basis. Cake refers to the residue from the expeller extracting process after roasting dehulled sesame seeds at 210°C for 5 min. All chemicals used were of reagent grade.

Preparation of biodegradable films. Biodegradable films were prepared using the modified methods of Gnanasambandam *et al.*[11] and Ghorpade *et al.*[23] Sesame cake and rice bran were dispersed in distilled water and 20% ethanol solution [sample to solvent ratio of 1 : 5 (w/v)], and

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the pHs of the dispersions were adjusted to 9 by the addition of 2 N NaOH solution to improve the protein extraction. Chemical modifications were conducted according to the modified method of Bera and Mukherjee.[10] Acetic and succinic anhydrides were added to the dispersions at levels of 0.25 g per g protein, maintaining the pH at 9 by adding 2 N NaOH, with constant stirring for 60 min. The mixtures were then centrifuged at 10,000 × g (Sorvall RC2-B Refrigerated Centrifuge) for 10 min to remove insoluble materials. The supernatants were separated and filtered through filter paper (Whatman No. 1). The protein content and the extent of modifications of each supernatant were analyzed. Glycerol was added to the protein solutions prepared from the roasted sesame cake and the rice bran at 2% (w/v) as plasticizer, and the solutions were stirred for about 2 min to facilitate uniform dispersion of glycerol. Film-forming solutions were heated to 80°C on a hot plate. One hundred milliliter of solutions were evenly spread on glass plates and dried for 24 h at 60°C in a convection oven. The films were peeled off manually from the plates and stored in a closed container at ambient temperature for further evaluation. Some films were also prepared by adding 2% formaldehyde to the film-forming solutions as a cross-linking agent.

Chemical analysis. The extents of protein modification, acetylation, and succinylation were quantified from the free lysine content of the modified and unmodified protein samples through the modified ninhydrin assay.[24-26] Ninhydrin solution (1 m*l*) was added to a 1% protein solution (1 m*l*), and the mixture was heated in a boiling water bath for 5 min and cooled immediately to 25°C. Distilled water (5 m*l*) was added, and the absorbance was determined at 580 nm against a distilled water-ninhydrin solution blank. The absorbance indicates the number of free amino acid groups available for reaction with ninhydrin reagent, and the difference in absorbances between unmodified and modified proteins reflects the extent of acetylation and succinylation.

$$Modification(\%) = \frac{No.\ of\ amino\ groups_{unmodified} - No.\ of\ amino\ groups_{modified}}{No.\ of\ amino\ groups_{unmodified}} \times 100$$

Protein contents were determined according to the method of AOAC[27] using a nitrogen to protein conversion factor of 6.25.

Film thickness. Film thickness was measured using a micrometer with a sensitivity of 1×10^{-3} mm (Model 293-561-30, Mitutoyo, Japan). Film strips were placed between the jaws of the micrometer, and the gap was reduced until the first indication of contact. Mean thickness from measurements at five different locations was recognized as the film thickness.

Tensile strength and percent elongation. Tensile strength and percent elongation were evaluated with a Texture Analyzer ($TAXT_2$, Stable Microsystem, England) according to ASTM Standard Method D 882.[28] After removal of films from glass plates, they were cut into 10 mm wide by 20 mm long strips. Film strips were conditioned at ambient temperature and 50% RH for 48 h prior to textural analyses. The film strips were pulled 20 mm apart at a speed of 120 mm/min in a tension mode. Tensile strength was determined at maximum load and expressed as maximum load per unit cross-sectional area of a film strip, and percent elongation was calculated at break.

Water vapor permeability. Water vapor permeability was evaluated using a modified ASTM Standard Method E 96[29] with a 50% RH gradient. Sample films were conditioned in a chamber at 25°C and 50% RH for 48 h. Films were then placed over the open mouth of cup (area = 12.56 cm^2), secured with rubber band, and returned to the chamber. Distilled water was placed in the cup with an air gap of 2 cm above the water surface. The high humidity side was maintained by distilled water and the low humidity side by use of the chamber set at 50% RH and 25°C. Weight of the cup with water was measured every 30 min for a minimum of 10 h. Water vapor permeability was calculated as described by McHugh *et al.*[3]

$$Water\ vapor\ transmission\ rate = \frac{Weight\ loss\ of\ water\ in\ the\ cup/sec}{Film\ area}$$

$$Water\ vapor\ permeability = Water\ vapor\ transmission\ rate \times \left(\frac{Film\ thickness}{\Delta p}\right)$$

where, Δp is the difference of partial pressures between inside and outside of cup.

Water solubility. Water solubility of films was measured as described by Stuchell and Krochta[30] at temperature range of 40~100°C. The percentage of initial dry matter in each type of film was determined by drying in an oven at 100°C for 24 h. Approximately 1.0 g of film sample was cut, weighed, and immersed in 30 m*l* distilled water for 24 h at the given temperature with periodic agitation when necessary. The film pieces were removed from the water and dried to determine the weight of the insoluble film matter. Water solubility was defined as the percentage of soluble matter in the sample on initial dry weight basis.

Statistical analysis. Each replication included individual preparation of film from film forming solution. Three replications were performed in a completely randomized design. A minimum of three observations were collected. Data were analyzed using general linear model procedures, a package program of the Statistical Analysis System.[31] Specific differences were determined by least significant differences. All comparisons were made at a 5% level of significance.

Results and Discussion

The protein contents of starting materials, commercial sesame meal roasted at 210°C and rice bran were 33.7 and 11.2% (wet basis), respectively. Recovering these proteins from the starting materials into film-forming solutions was the main yield-determining factor for their utilization. According to our previous research (data not shown), the maximum extraction of rice bran protein was achieved by extraction at pH 9~11 with 20% ethanol solution. Therefore, these extraction conditions were applied in the preparation of film-forming solutions to maximize the protein recovery. The protein contents and modification extent in film forming solutions are described in Table 1. The protein contents of acetylated or succinylated film forming solutions were lower than those of the unmodified samples due to the acetic and succinic acid residues bound to the proteins and the NaCl produced during neutralization. Slightly lower protein contents in sesame samples might be caused by the higher contents of soluble matters, such as phytic acids and sugar, in sesame samples.

Film formation. Films were produced for all experimental points except roasted sesame film prepared with no modification, regardless of the presence of formaldehyde. Film forming ability of proteins may be influenced by the composition, distribution, and polarity of amino acid, conditions necessary for formation of ionic cross-links between amino and carboxyl groups of amino acid side chains, presence of hydrogen bonding groups, and intramolecular and intermolecular disulfide bonds.[32] Additionally, alkali treatment has been suggested as a method of improving three dimensional network formation by unfolding the soy proteins.[33] However, the proteins in this study differ considerably in that they were partially heat-denatured and/or unfolded by succinylation or acetylation and, therefore, would respond differently to alkali treatment. It is conceivable that the combination of alkali and heating causes partial hydrolysis and other rearrangements detrimental to film formation. Alkali treatment has been shown to affect the disulfide linkages of proteins,[34] which would probably affect the film formation of roasted sesame. Films prepared with roasted sesame were darker and had a reddish yellow tan color, as a result of maillard reaction during the roasting procedure. Rice bran films, especially unmodified, were less transparent than roasted sesame films. Decreased solubility of rice bran proteins caused by evaporation of ethanol during heat treatment of film forming solution at 80°C, might have resulted in a tighter film structure and/or precipitation of proteins causing a higher reflection of light and lower transparency. The thicknesses of biodegradable films produced in this study are described in Table 2. Mean thickness of the unmodified rice bran film prepared without the addition of formaldehyde was 195 μm, which was very close to 190 μm reported by Gnanasambandam *et al.*[11] Acetylation and succinylation reduced the thickness of rice bran film.

Tensile strength. Tensile strengths of roasted sesame and rice bran films modified through acetylation and succinylation with absence (a) and presence (b) of formaldehyde are higher than that of isolated soy protein film (2.119~2.881 MPa)[4] and described in Fig. 1. Acetylated and succinylated sesame films, regardless of the addition of formaldehyde, had much higher tensile strengths than rice bran films, although unmodified sesame film was not

Table 1. Protein contents (dry basis) and extent of acetylation or succinylation in film-forming solutions prepared from rice bran and roasted sesame cake.

	Rice bran			Roasted sesame cake		
	Unmodified	Acetylated	Succinylated	Unmodified	Acetylated	Succinylated
Protein content	63.6%	62.5%	58.7%	55.6%	51.9%	53.5%
Extent of modification	-	73.8%	72.8%	-	71.4%	69.7%

[a]Values are means of three determinations.

Table 2. Thicknesses of films prepared from modified rice bran and roasted sesame cake.

Thickness(μm)	Rice bran			Roasted sesame cake		
	Unmodified	Acetylated	Succinylated	Unmodified	Acetylated	Succinylated
Absence of formaldehyde	195 ± 16[a]	122 ± 10[c]	128 ± 7[c]	-	146 ± 13[a]	172 ± 18[b]
Presence of formaldehyde	158 ± 11[b]	113 ± 11[c]	116 ± 9[c]	-	132 ± 12[a]	141 ± 13[a]

[a,b,c]The same letters in the same starting material indicate no significant difference at $p<0.05$ level by Duncan's multiple range test.
[d]Values are means of five determinations.

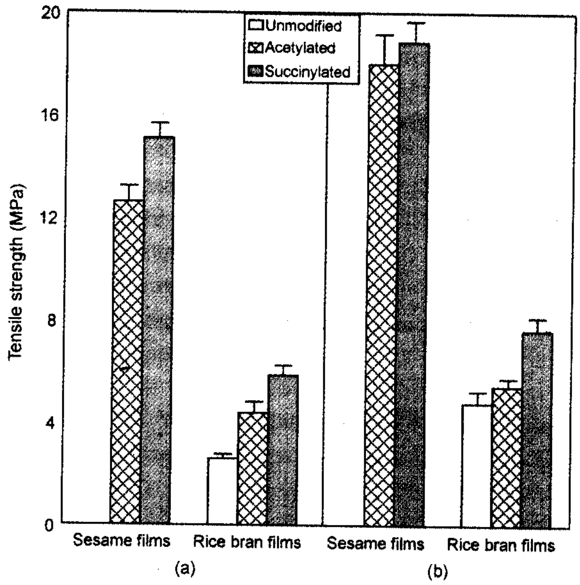

Fig. 1. Tensile strengths of roasted sesame and rice bran films modified through acetylation and succinylation with absence (a) or presence (b) of formaldehyde.

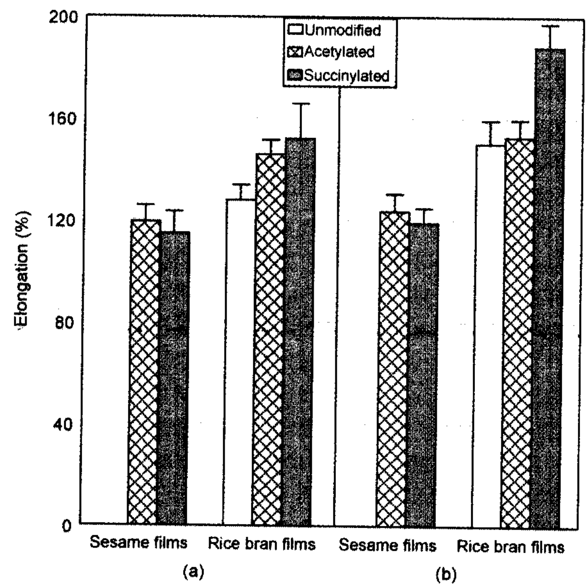

Fig. 2. Percent elongations of roasted sesame and rice bran films modified through acetylation and succinylation with absence (a) and presence (b) of formaldehyde.

formed. Acetylation and succinylation increased the tensile strengths of rice bran films. Succinylation was more effective in improving tensile strengths. The increased solubility of the proteins by the increased electrostatic repulsions resulting from acetylation and succinylation might contribute to the formation of strong films. Treatment with formaldehyde appeared to have a cross-linking effect on the roasted sesame and unmodified rice bran films since the tensile strength of the aldehyde-treated protein films [Fig. 1 (b)] were higher than untreated ones [Fig. 1 (a)]. Increases in mechanical strength also have been reported for formaldehyde-treated films and coatings from proteins such as corn zein[35] and collagen.[1] However, the cross-linking effect of formaldehyde on the acetylated rice bran film was not significant.

Percent elongation. Percent elongations of roasted sesame and rice bran films modified through acetylation and succinylation with absence (a) and presence (b) of formaldehyde are similar with that of isolated soy protein film (103.1~150.8%)[4] and described in Fig. 2. Acetylated and succinylated rice bran films prepared without the addition of formaldehyde and succinylated rice bran films prepared with the addition of formaldehyde showed slightly but significantly higher % elongations than roasted sesame films. Higher % elongation indicates that the film was more flexible when subjected to tension, impling that the film may resist mechanical damage when subjected to machinery or rough handling. In protein-based films, protein-protein interactions during film formation determine the characteristics of the films produced. Therefore, the differences in % elongations of roasted sesame and rice bran films might mainly be attributed to the differences in amino acid composition, distribution, and polarity of roasted sesame and rice bran. However, the presence of residual ethanol in rice bran film-forming solution could be another reason for higher % elongation of rice bran films. Rice bran proteins have a high content of hydrophobic amino acids which contribute to stability of proteins via hydrophobic interaction.[36] Transfer of free energy of most non-polar side chains of amino acids decreases as the solvent changes from water to alcohol. This shift in solvent system generally induces weakening of protein tertiary structure by reducing hydrophobic interactions and concomitantly promote helix formation by strenthening hydrogen bonding.[37] Although glycerol was added to every film forming solution prepared, additional ethanol was added to the rice bran samples to improve the protein extraction in the form of film-forming solution. Succinylation slightly but significantly increased the % elongations of rice bran films, regardless of the addition of formaldehyde. However, the effect of acetylation on the % elongation of rice bran film was insignificant, especially in the presence of formaldehyde. The effect of formaldehyde on the % elongation of roasted sesame films and acetylated rice bran film was not significant.

Water vapor permeability. Water vapor permeability of film is an important property that greatly influences utility in food systems. Protein films, being hydrophilic by nature, are limited in their applications. Water vapor permeabilities of roasted sesame and rice bran films modified through acetylation and succinylation with absence (a) and presence (b) of formaldehyde are similar with that of isolated soy protein film ($1.90\sim3.04\times10^{-9}$ gm/m^{2}sPa)[4] and described in Fig. 3. No significant differences in water vapor permeability were observed among all types of roasted sesame films. The water vapor permeability of rice bran was significantly decreased by acetylation, succinylation, and/or

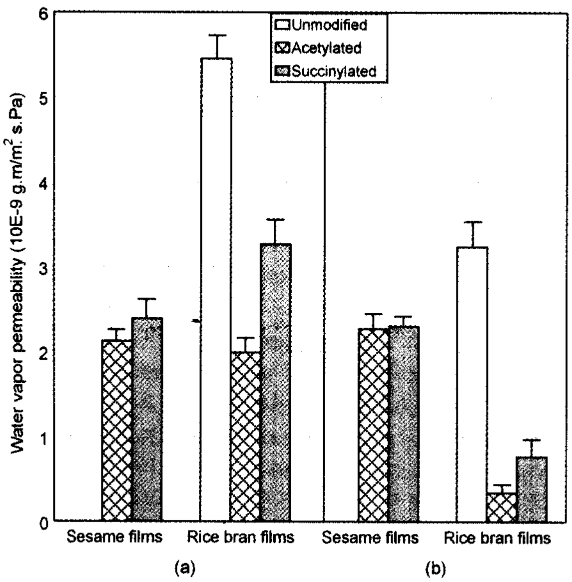

Fig. 3. Water vapor permeabilities of roasted sesame and rice bran films modified through acetylation and succinylation with absence (a) and presence (b) of formaldehyde.

addition of formaldehyde. However, water vapor permeability was considered to be influenced more by film thicknesses than by modifications. When calculating water vapor permeability of the films, the assumption was made that water vapor permeability was independent of film thickness. When film thickness increased, the water vapor transmission rate through the film decreased proportionally, thus the water vapor permeability calculated remained constant. However, there is a dependency of water vapor permeability on thickness for hydrophilic films, as shown for films from cellulose ether, sodium caseinate[39] and soy protein,[23] although the opposite is true for relatively hydrophobic films.[38] Similar result was observed in the present study (Table 2 and Fig. 3). Stuchell and Krochta[30] reported on an increase in water vapor permeability of soy protein-based films with increase in the level of glycerol added. The films in our study had similar amounts of glycerol (2%, w/v).

Water solubility. Water solubilities of films were determined to indicate their integrity in an aqueous environment. Generally, higher solubility signifies lower water resistance. However, a high solubility can be advantages for some applications. For an example, if a package of instant noodle soup-base is water-soluble, customers can add the whole package directly to the boiling water without bothersome process of tearing off. The solubility may also be an important factor that determines biodegradability of films used as packaging wraps. Changes in water solubilities of roasted sesame and rice bran films, modified through acetylation and succinylation without and with formaldehyde, upon water temperature changes from 40 to 100°C are shown in Figs. 4 and 5. The solubility of

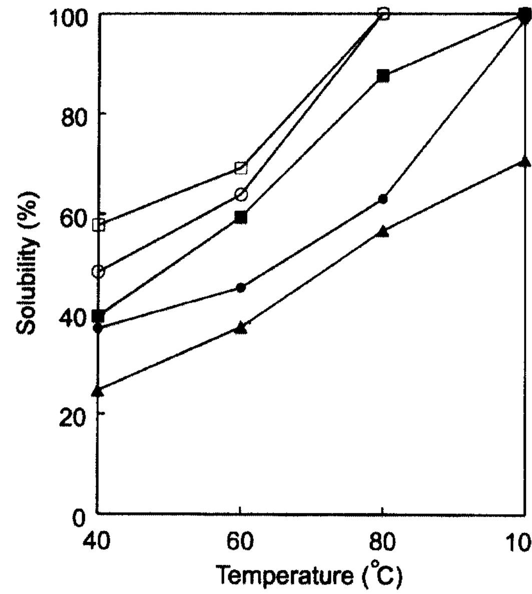

Fig. 4. Changes in water solubilities of roasted sesame and rice bran films, modified through acetylation and succinylation with absence of formaldehyde, upon water temperature changes from 40 to 100°C. (○, Acetylated sesame film; ❑, Succinylated sesame film; ▲, Unmodified rice bran film; ●, Acetylated rice bran film; ■, Succinylated rice bran film).

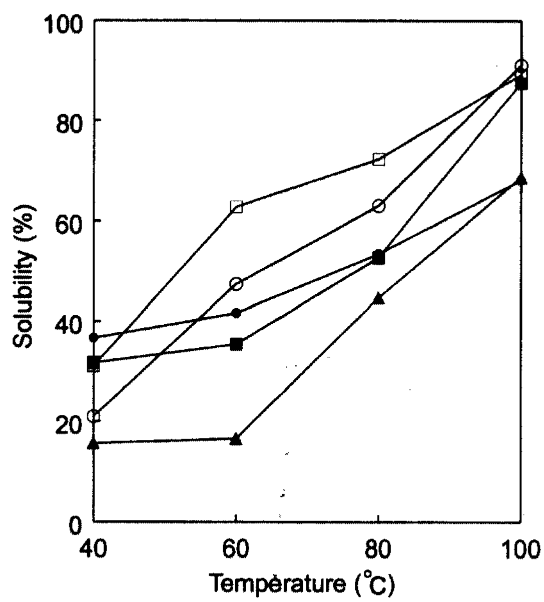

Fig. 5. Changes in water solubilities of roasted sesame and rice bran films, modified through acetylation and succinylation with presence of formaldehyde, upon water temperature changes from 40 to 100°C. (○, Acetylated sesame film; ❑, Succinylated sesame film; ▲, Unmodified rice bran film; ●, Acetylated rice bran film; ■, Succinylated rice bran film).

films increased with increase in temperature. Roasted sesame films had higher solubilities than rice bran films at all temperature ranges tested without formaldehyde (Fig. 4) and at temperatures over 60 when formaldehyde was added. Regardless of the presence of formaldehyde, both the acetylated and succinylated rice bran films had higher solubilities than the unmodified ones at all temperature ranges. This was expected since a decrease in protein hydrophobicity as a result of acetylation and succinylation had been documented.[16] Significant decreases in water solubilities of the films resulted from formaldehyde treatment (Figs. 4 and 5). This was most likely due to formaldehyde reacting with free amino groups of the basic amino acids which are among the primary water-bonding sites on the protein.

In conclusion, roasted sesame and rice bran films were successfully produced through acetylation, succinylation, and/or formaldehyde treatment. Roasted sesame produced strong films, while rice bran produced flexible ones. Reaction with formaldehyde improved the strength and % elongation but reduced water vapor permeability and water solubility. However, it is highly recommended not to apply formaldehyde to edible films as a cross-linking agent due to its toxicity. Acylation with acetic and succinic anhydrides led to formation of roasted sesame films and increased the strenth, % elongation, and water solubility of the films with reducing water vapor permeability. Reducing hot water solubility is still needed for practical uses of roasted sesame and rice bran films as biodegradable packaging material. However, considering their present low end uses, the economic merits of these products are favorable and attractive.

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