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ORIGINAL ARTICLE

The Effects of Ribose on Mechanical and Physicochemical Properties of Cold Water Fish Gelatin Films

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KEYWORDS

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ABSTRACT: Native fish gelatin has some disadvantages such as high hydrophilic, and solubility in cold water. Mixing with other biopolymers and crosslinking by sugars may improve functional properties of fish gelatin. So in this research, the effects of ribose were investigated on moisture sorption isotherm, solubility in water, and mechanical properties of cold water fish gelatin (CWFG) films. Ribose sugar was incorporated into CWFG solutions at different concentrations (e.g. 0, 2, 4, and 6% w/w dried gelatin). Physicochemical properties such as water solubility, moisture sorption isotherm and mechanical properties of the films were measured according to ASTM standards. Results showed that incorporation of ribose sugar significantly improved functional properties of CWFG films. Solubility, moisture content and monolayer water content of the matrixes were decreased by increasing the ribose contents. Mechanical properties of biocomposites were improved more than 20% and moisture sorption isotherm curve significantly shifted to lower moisture contents. The results of this study could be explored for commercial use, depending on industrial needs for either production of edible films or for packaging purposes.

INTRODUCTION

The use of plastics is increasing dramatically in the world and it has an approximately 5% growth annually [1, 2]. Some synthetic packaging materials like Polyethylene terphthalate (PET), polyvinylchloride (PVC), have been very popular [3] because of their low cost, good mechanical properties, good barrier properties to gases, heat sealability and so on. In spite of good properties, their use should be restricted due to

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their non-biodegradability [4]. Increasing use of synthetic packaging films will contribute to environmental problems [5].

In recent decade the demand for environmentally friendly polymers is growing and has been focus of many researchers' efforts [6]. Attempts have been made to utilize natural polysaccharides, proteins, and lipid biopolymers to develop edible and non-edible packaging materials. Biopolymers have advantages over the synthetic polymers; biopolymers are biodegradable and renewable materials [7]. Permeability inhibition of gases by biopolymers increases shelf life of fresh products such as fruits and vegetables. Among the various types of films investigated and developed, biopolymer films based on proteins have shown best mechanical properties. Although protein-based films have been proclaimed to have wide applicability in the food industry, poor water vapor resistance and lower mechanical strength in comparison with synthetic polymers have limited their applications[8].

Gelatin, a heterogeneous mixture of water-soluble proteins (of high average molecular weight) is derived by hydrolytic action from collagen. Recently, gelatin has gained high attention in food industries due to its unique properties. Gelatin films are also being explored due to their easy availability and biodegradable nature. However, edible or packaging films produced from gelatin do not possess the desired mechanical and water vapor barrier properties, which limit their commercial use [9]. To overcome this problem, researchers have tested physical (e.g., radiation treatments, ultrasound) and chemical (e.g., aldehydes, especially glutaraldehyde, calcium salts) treatments along with the use of natural plant products like phenolic compounds (e.g., tannic acid, ferrulic acid) for their ability to improve the cross-linking properties of films. Some sugars, especially ribose, induce Maillard cross-linking after mild heat treatments and are able to cross-link proteins [10].

In recent years fish gelatin has gained importance, mainly because of religious sentiments (e.g., Judaism and Islam forbid the use of pig gelatin, whereas Hindus do not use gelatin produced from cows), the low production cost, and its easy availability worldwide [11].

The main objective of this study was to investigate the changes induced in fish gelatin films (via possible improvement in cross-linking) by the addition of the ribose (an aldo-pentose monosaccharide having five carbon atoms and an aldehyde functional group in a linear form). We evaluated physicochemical and mechanical properties of the films as well as moisture sorption isotherm of the films to provide fundamental data for their application in food and other industries.

MATERIALS AND METHODS

Materials

Gelatin from cold water fish (G7041-100G) was purchased from Sigma–Aldrich. Food grade glycerol and liquid sorbitol were prepared from R & M Marketing (Essex, UK). Ribose sugar was obtained from Merck (Germany).

Film Preparation

Ribose sugar was dispersed in water at different concentrations (0% (control), 2%, 4%, and 6%; w/w of total CWFG dried mater), and stirred for 0.5 h. The solution was used to prepare the aqueous CWFG dispersion at 8% (w/w). A mixture of sorbitol and glycerol (3:1) at 25% (w/w) of total solid was added as plasticizer in accordance with past works [12]. CWFGs were heated to 58 ± 2°C. The CWFG solutions were cooled to 45 °C and the bubbles were removed by vacuum pump. A portion (45g) of the dispersion was cast on Perspex plates fitted with rims around the edge to yield a 16×16 cm^2 film-forming area. Films were dried under controlled conditions in a humidity chamber (25°C and 50% RH). Dried films were peeled and stored

at 23 ± 2ºC and 50 ± 5% relative humidity (RH) until experimentation. The thickness of each film was measured at five different locations and to the nearest 0.01 mm with a hand-held micrometer (Mitutoyo, Tokyo, Japan). All films (including control) were prepared in triplicate.

Moisture sorption isotherm

The moisture sorption isotherm of the films at 25 °C was studied using the method described by Bertuzzi and colleagues[13]. Moisture content at equilibrium (g absorbed water/g dry film) was measured in triplicate for each relative humidity. Experimental sorption data were fitted using the GAB equation [14]:

$$W = \frac{w_m CKa_w}{(1-Ka_w)(1-Ka_w+CKa_w)}$$

Where w_m, K, and C are the GAB parameters, W is moisture content (dry basis), and a_w is water activity. To evaluate the accuracy of the GAB model for experimental sorption isotherm of the gelatin films, we calculated the percentage of mean relative deviation modulus (E) using the following formula:

$$E = \frac{100}{N}\sum_{i=1}^{N}\frac{|m_i - m_{pi}|}{m_i}$$

Where N is the number of experimental data and m_i and m_{pi} are the experimental predicted values, respectively. A modulus (E) value below 10% indicates a good fit [15].

The 3rd degree of polynomial model for moisture sorption isotherm also fitted to practical data.

W=Ba_w^3+Ca_w^2+Da_w

Where B, C, and D, are the constants, W is moisture content (dry basis), and a_w is water activity.

Solubility in water

Solubility of the composite CWFG films in water was determined following Torabi and Mohammadi Nafchi [16] with some modifications. Pieces of film (2 × 3 cm) were cut from each film type and were stored in a desiccator with P_2O_5 (0%RH) for 2 days. Samples were weighed to the nearest 0.0001 g and placed in beakers with 80 mL deionized water (18 MΩ). Then, samples were stirred with constant agitation for 1 h at room temperature. The remaining pieces of film were separated using a filter paper (Whatman no.1), followed by oven drying at 60 °C to constant weight. Samples were measured in five replicates and the percentage of total soluble matter (%solubility) was calculated as follows:

Solubility (%) = ((Initial dried weight of film-Final dried weight of film)) / (Initial dried weight of film) ×100

Mechanical properties

ASTM D882-10 [17] was used to determine the mechanical properties under standard conditions. Film strips were cut to 100 mm long and 20mm wide and conditioned at least for 48h in 25ºC and 55% RH. Texture analyzer (TA.XT2, Stable Micro System, and Surrey, UK) equipped with Texture Exponent 32 software V.4.0.5.0 was used for measuring mechanical properties of the films. The initial grip separation was 50 mm and crosshead speed was 0.5 mm/s. Elongation and tensile strength at break were calculated from the deformation and force data recorded by the software.

STATISTICAL ANALYSIS

ANOVA and Tukey's Post Hoc tests were used to compare the means of the physical and mechanical properties of CWFG films at 5% significance level. Statistical analyses were conducted using GraphPad Prism 6 (GraphPad Software Inc., La Jolla, USA). Curve fitting for polynomial sorption isotherm was evaluated by non-linear regression using the solver module in Microsoft Excel® 2010.

RESULTS AND DISCUSSIONS

Mechanical Properties

Figure 1 shows the effect of ribose sugar on tensile strength (TS) of CWFG films with 25% plasticizer. Addition of ribose significantly ($p < 0.05$) increased the tensile strength of CWFG films from 30 MPa to 40 MPa. A possible reason for this difference might be that ribose is a good cross-linking agent and might have induced Maillard cross-linking after initial heat treatments. However, the marked increase in TS might also be due to enhanced protein-protein interactions via non-covalent forces (e.g., Van de Walls, electrostatic, hydrogen bond, hydrophobic, etc.) [18]. Figure 2 and 3 show elongation at break (EB) and Young's modulus of CWFG films. Elongation at break has a reverse relation with tensile strength in most cases and Young's modulus was directly related to tensile strength. Young's modulus is a measure of the rigidity of the material and improves as ribose sugar was introduced. The elasticity of the films is related to interactions of the macromolecules and can be reduced by addition of plasticizer. TS plays an important role in determining the mechanical properties of edible or packaging films developed for use in many food applications. TS are an indication of film strength, whereas EB is an indicator of stretchability of films prior to breakage. Water that has a plasticizing role [19, 20] on the gelatin films and addition of ribose, decrease the water content and consequently reduced the elasticity (elongation at break) of the films.

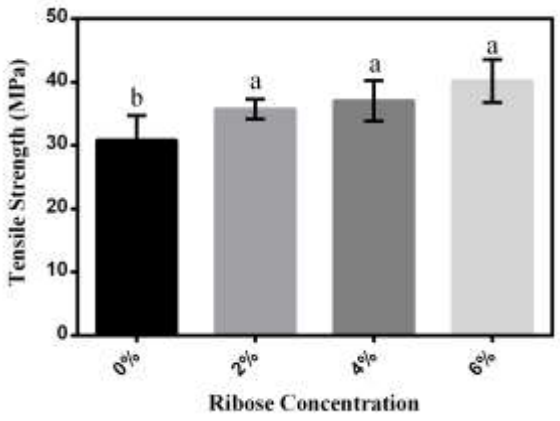

Figure 1. Effects of ribose sugar on tensile strength of CWFG films. The bars show mean (n=8) ± SD. Different letters on the bars represent the significant difference at 5% level of probability.

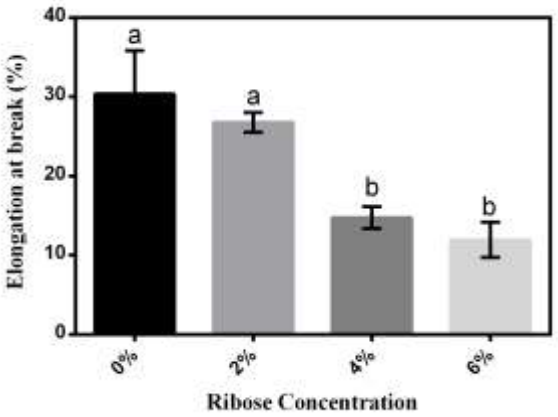

Figure 2. Effects of ribose on elongation at break of CWFG films. The barsshow mean (n=8) ± SD. Different letters on the bars represent the significantdifference at 5% level of probability.

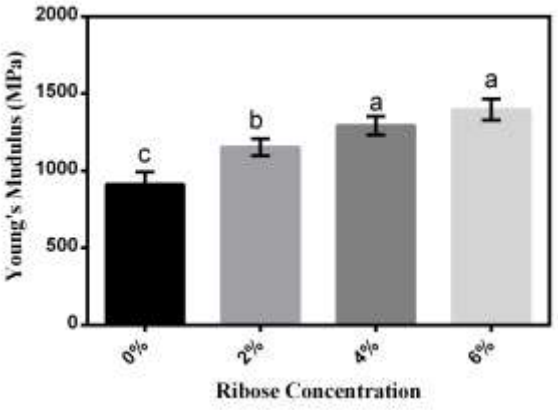

Figure 3. Effects of ribose on Young's modulus of CWFG films. The bars show mean (n=8) ± SD. Different letters on the bars represent the significantdifference at 5% level of probability.

Solubility of CWFG films incorporated by ribose in Water

Water solubility is the measure of tolerance to water, and higher solubility of a film indicates lower water resistance. Solubility in water may be an important factor in defining applications for biopolymer composite films. Most of the biopolymers are sensitive to water. Crosslinking of the structure or incorporation of nanoparticles sensitivity to water could be decreased by

incorporating lipids [21, 22]. Figure 4 shows the solubility of CWFG films in deionized water after 1 hr. Ribose-containing films showed significantly decreased solubility compare to native films. This difference can be attributed to the possibility of higher cross-linking on incorporation of ribose. The presence of a high amount of cross-linkers in the film-forming solutions has been reported to lower the solubility. Galietta and colleagues (1998) reported decreased solubility of glycerol-plasticized films cross-linked with formaldehyde, and they suggested that the decrease was due to of the formation of covalent bonds [23]. In the present study, the presence of possible cross-linking agents might have initiated the formation of covalent bonds, thereby leading to decreased water solubility of the ribose-incorporated and pure gelatin films.

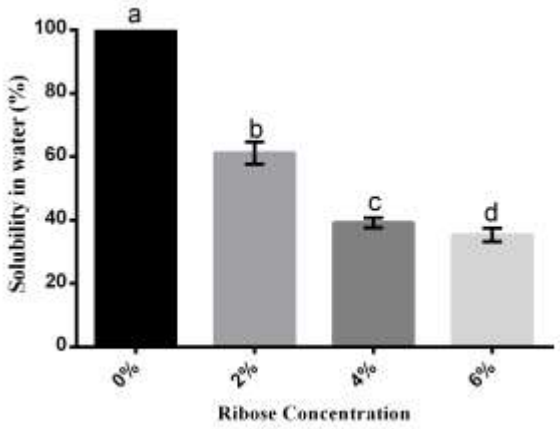

Figure 4. Effects of ribose on water solubility of CWFG films. The bars show mean (n=3) ± SD. Different letters on the bars represent the significantdifference at 5% level of probability.

Moisture sorption isotherm

The theoretical sorption isotherm curves fitted with the 3rd order polynomial equation and experimental data for CWFG films at 25 °C are presented in Figure 5. R2 > 0.99 for 3rd order polynomial equation represents excellent fitting of this model. Figure 3 shows that in all ranges of aw (0.1-0.9), the ribose incorporated CWFG films exhibited less equilibrium water content compared with control films. This observation may be attributed to the interaction between plasticizer, biopolymer matrix, and ribose, leading to reduced hydroxyl group availability to interact with water and, consequently, a less hygroscopic matrix.

Based on the Brunauer, Emmett, and Teller classification, films with 0≤K≤1 and CG>2 are type II and those with 0≤K≤1 and 0≤CG≤2 are type III (Blahovec, [24]; therefore, the CWFG films can be classified as type II. The monolayer factor (mo) for the films in this study is consistent with those of previous researches, since the addition of ribose decreased the hydrophilic behavior of the biopolymer films [6, 24 and 27].

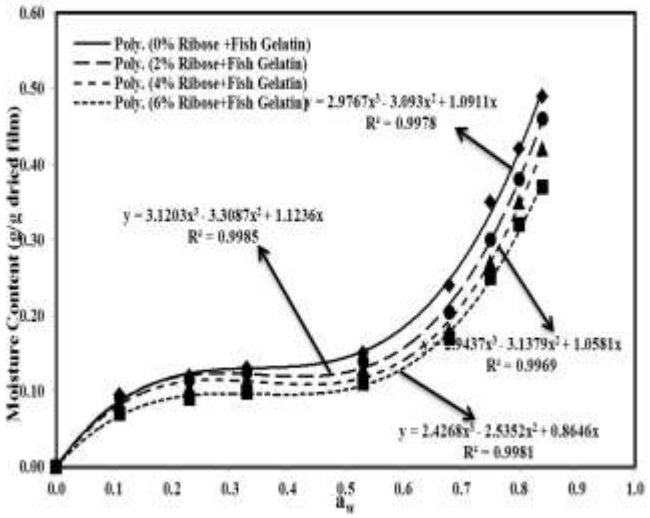

Figure 5. 3rd order polynomial moisture sorption isotherm for CWFG films and effects of ribose on moisture sorption isotherm.

CONCLUSION

In this study, ribose sugar introduced to the cold water fish gelatin matrix to fabricate a new biocomposites. Introduction of the ribose improved mechanical properties of the CWFG films. Water solubility and monolayer water content of the films significantly decreased. The results showed that biocomposites based on ribose and CWFG may have potential applications in food packaging as well as pharmaceutical industries.

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