ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Chemical Modification of Cold Water Fish Gelatin using Natural Phenolic Cross-linker Caffeic acid.

M Araghi*, and Z Moslehi.

Food Biopolymer Research Group, Food Science and Technology Department, Damghan Branch, Islamic Azad University, Damghan, Semanan, Iran.

ABSTRACT

Nowadays use of edible films and coatings is increasing due to their biodegradability properties. Fish gelatin obtained from fish skin wastes can be used as an appropriate protein compound for preparing the edible films. It can be also replaced pork gelatin. In this study, films were prepared from fish gelatin and phenolic compound caffeic in four levels of 0%, 1%, 3% and 5% at pH>10, temperature of $60^{O}C$ under continuous injection of O_2 and addition of the sorbitol /glycerol plasticizers. Some quality requires tests were then done for prepared films. The test results show that solubility and water vapor permeability were decreased: and the highest effect was observed at concentration of 5%. FTIR results showed that a new peak was appeared on 2800-2900 cm^{-1}. In conclusion, caffeic is a phenolic compound that can increase safety of biodegradable packaging materials for pharmaceutical and food products by improving barrier and physico-chemical properties.

Keywords: Caffiec acid; Solubility; Water vapor permeability.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

In recent years use of natural polymers to prepare the edible films for food packaging and pharmaceutical applications has been developed due to their biodegradability properties [1-3]. Proteins, Polysaccharides, lipids and their derivatives are some examples of these polymer substances, used for production of edible films [1, 4-6]. Although these compounds are not completely replacing synthetic films, they reduce their consumption [1, 2]. Proteins are able to produce edible films with appropriate mechanical properties through formation of side chains via cross-linking [7]. Gelatin is a protein which obtains from the hydrolysis of collagen. Skin, bone, cartilage and tendon of animals such as pork, fish, beef are some sources of gelatin extraction [1, 4, 8, 9]. For some reasons like biodegradability, renewability[10, 11], high productivity with low cost [12], and improving of elasticity, consistency and stability [1, 13, 14], gelatin has found wide applications in the food and pharmaceutical industries [12].Fish gelatin is an inexpensive compound which obtains from residual of fish skin and bones .Since consumption of pork and its derivatives is forbidden in Islam and Judaism, fish gelatin may be an appropriate alternative to pork gelatin [15]. Behavior and characteristics of fish gelatin is very different in compared to mammals' gelatin. Fish gelatin, especially cold water fish gelatin has much more hydrophobic amino acid content. It has also a little proline and hydroxy proline that has the lower gel time as well as lower melting point than mammals gelatin [6, 15-17].Gelatin has poor hydrophobic and mechanical properties when exposed to moisture that lead to a low water vapor barrier property [1,10,16,18]. Structure modification may improve mechanical and barrier properties of gelatin. Some methods of structure modification include physical (radiation, ultrasound, etc.); chemical treatments (e.g. aldehydes like glutaraldehyde, calcium salts); combination with the other proteins and polysaccharids such as chitosan and casein; using cross- linking compounds like genipin, formaldehyde, transglutaminase, and natural plant products such as phenolic compounds (e.g., tannic acid, ferulic acid) [18].Therefore, caffeic acid (CA) is used for modifying of cold water fish gelatin in this study. CA is resulted from secondary metabolism of plant polyphenols that contains biochemical, anti- bacterial and anti- viral properties [19]. There are many studies on using CA for bovine gelatin modification such as Zhang et al (2010). However, there is no study about the effects of this acid on cold water fish gelatin and its barrier and physico-chemical properties.

MATERIAL AND METHODS

Materials

Gelatin from cold water fish (G7041-100G) was purchased from Sigma–Aldrich Co. (Kuala Lumpur, Malaysia, Canada producing). Food grade glycerol and liquid sorbitol were prepared in the laboratory grade. Caffeic acid (CA) was obtained from Merk Co. agency in Tehran, Iran (Germany producing).

Preparation of films

Granules of cold water fish gelatin were dissolved in the deionized water to obtain a concentration of 5 g/50 ml in 55 °C for 1 hr. Caffiec acid was dispersed in 50 ml deionized water at concentrations of 1,3,5% (w/v) one by one. PH was adjusted in PH≥10 with sodium hydroxide 10 normal. Materials were then heated to 55°C with continuous stirring and injection of oxygen for 1 hr to produce a homogenized solution. Both cold water fish gelatin and acids solutions were mixed together while pH was adjusted in pH≥8 by sodium hydroxide 1 normal. Mixing of solution with continuous stirring and injection of oxygen for 30 min is stopped when the temperature reached to 55°C. The dispersion was cooled to room temperature once gelatinization was completed. Finally, the solution was cast on plates to form a 16 × 16 cm^2 film. The films were dried in an oven at 40 ºC for 20 hr. Dried films were stored at 23 ± 2 ºC, and 50 ± 5% relative humidity (RH) till tested time.

Thickness of film

The thickness of each film was measured at five different locations with a micrometer (Mitutoyo, Tokyo, Japan).

Water vapor permeability (WVP)

The modified gravimetric cup method based on ASTM E96-05 was used to determine the water vapor permeability (WVP) of films[20, 21]. The test cups were filled with 20 g of silica gel (desiccant) to produce a 0% RH below the film. The sample was placed between the cup and the ring cover of each cup coated with silicone sealant (high vacuum grease, Lithelin, Hannau, Germany). The air gap was at approximately 1.5 cm between the film surface and desiccant. The water vapor transmission rates (WVTR) of each film were measured at 55 ± 2% RH and 25 ± 2 ºC. The initial weight of the test cup was measured, and the cup was placed into an incubation chamber with an air velocity rate of 125 m/min. Weight gain measurements were taken by weighing the test cup to the nearest 0.0001 g with an electronic scale (Sartorious Corp.) every day for 7 days. A plot of weight gained versus time was used to determine the WVTR. The slope of the linear portion of this plot represented the steady state amount of water vapor diffusing through the film per unit time (g/h). The WVTR was expressed in grams per square meter per day. Six samples per treatment were tested. The slopes yielded regression coefficients of 0.99 or greater. The WVP of film was calculated by multiplying the steady WVTR by the film thickness and dividing that by the water vapor pressure difference across the film.

Solubility of films

The films solubility in water was determined according to the method of Maizura et al. [23] with some modifications. Pieces of each film in 2 × 3 cm^2 size were cut and placed in desiccators content P_2O_5 with 0% RH for 2 days. Samples were then weighed and placed in beakers with 80 ml deionized water. The samples were stirred with constant agitation for 1 hr at room temperature. The remaining pieces of film after soaking were filtered through filter paper (Whatman no.1), followed by oven drying at 60 °C to constant weight. Samples were measured in triplicates and the percentage of total soluble matter (% solubility) was calculated as follow:

$$Solubility\ (\%) = \frac{(Initial\ dried\ weight\ of\ film - Final\ dried\ weight\ of\ film)}{Initial\ dried\ weight\ of\ film} \times 100$$

FTIR investigation for chemical interaction

FTIR spectra of the films were recorded using an attenuated total reflection (ATR) method in Smart iTR (Thermo Scientific, Madison, USA). The thin films were applied directly onto the ZnSe ATR cell. For each spectrum, 64 consecutive scan at 4 cm^{-1} resolutions were averaged following methods described by Zhou et al [25].

Statistical analysis

ANOVA and Tukey tests were used to compare means of physical, mechanical, thermal, and antimicrobial properties of gelatin films at the 5% significance level. Statistical analy-sis was conducted using PASW 18.0 for windows (SPSS Inc. Chicago,IL) and GraphPad Prism 5 (GraphPad Software Inc., La Jolla, USA).

RESULTS AND DISCUSSION

Film color

Films prepared from Caffeic acids at different concentrations showed color change so that by increasing concentration, film color tend to be darker. In general interaction between natural phenolic compounds and proteins at O2 presence and alkaline conditions leads to oxidation of phenolic structure and quinon formation [10]. Quinon further reacts with amino acid groups in protein chains such as the sulfuhydryl group of cystine, the amine group of lysine and arginine. It may be supposed that hydroxyl and carboxy group of phenolic compounds reacted with amino acids of gelatin by hydrogen bond or esterifcation, thus produced the cross-linked network to improve the properties. Color change created in each concentration shown at figure1 indicates oxidation of phenolic compounds. Exaquing et al. (2010) found a color change in bovine gelatin- based film at Caffeic presence from pale yellow to dark brown. [8].

ISSN: 0975-8585

Table 1: Effect of concentration of Caffeic acid on the color of cold water fish gelatin.

Color of films	
Concentration of Acid	**Caffiec Acid**
0%	Pale yellow
1%	Yellow
3%	Pale Brown
5%	Brown

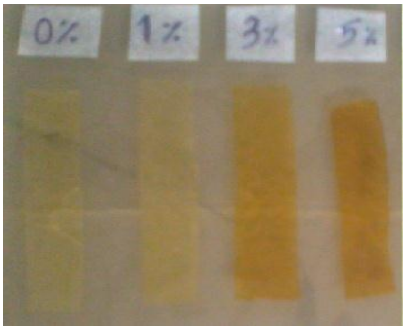

Figure 1: Effect of different concentrations of caffeic acid on color change of cold water fish gelatin films.

Solubility

Formation of cross-links between polymers can also reduce significantly solubility of edible film constituents [23]. Solubility of gelatin film of cold water fish modified by CA is indicated at Figure2. Results based on concentration of each acid showed that an increase in concentration led to decrease in solubility, due to formation of cross- linking between polymers. Modification of fish gelatin by phenolic compound Caffiec Acid led to reduced solubility which can be attributed to interaction of polymers by hydroxyl or carbonyl leading to formation of hydrogen or covalent bond (involving C–O–H and N–C). This in turn resulted in formation of cross- linkings thus reduction in solubility [10]. The moisture barrier properties of a film can be improved by decreasing the solubility of the protein in water [23]. Also reduction solubility in fish gelatin film using ribose has been reported by Bhat & Karim (2012).

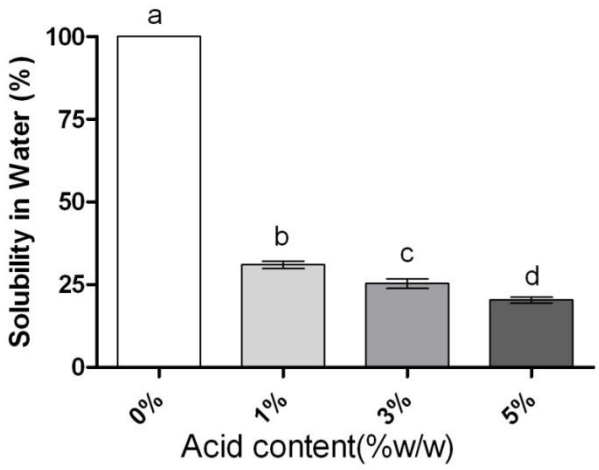

Figure 2: Solubility of fish gelatin films with caffiec acid.

*Different letters in each column indicate, significant differences at the level of Statistically 95% ($p<0.05$).

Water vapor permeability

Film diameter is a factor influencing water vapour permeability. According to Fick' law, water vapour permeability is decreased in thin layers of <60µm diameter [7]. In fact, water vapor transfer rate and permeability decreased when thickness of material coating increased from 0 to 60 µm (table2) [23]. In this study no significant difference in film diameter was found. In CA films increase in concentration led to

decrease in water vapor permeability ($p<0.05$). However in film Generally protein films have a weak water vapor permeability leading to limited use of them in packaging [25]. Regarding that water vapor permeability is depended on hydrophobic and hydrophilic components of film [7] and that water vapor transfer is performed by hydrophilic component [6], cross- linking created by phenolic compound CA in cold water fish gelatin has led to reduction in water vapor permeability. Reduced water vapor permeability is of highly importance because substances used for pharmaceutical and food products with appropriate barrier properties can improve packaging condition through lowering moisture transfer between food product and the environment [4]. Bhat & Karim (2012) reported a reduced water vapour permeability of fish gelatin film in which cross-linking has been created by ribose [18].

Table 2: Effect of concentration of phenolic compound CA on the thickness and water vapour permeability of cold water fish gelatin

Acid Level	Caffiec Acid	
	Average Thickness(µm)	WVP(ng.m/(m^2*Pas)
0%	21.16±0.94	7.52±0.1a
1%	19.93±0.81	6.84±0.1b
3%	21.81±0.61	6.55±0.2c
5%	21.68±0.62	5.94±0.2d

In general, solvent-cast protein films, for which the solvent is water, are typically formed at room temperature and stabilized through electrostatic interactions, hydrogen bonding, and van der Waals forces among the protein chains. The protein film network may be improved through heat-denaturation, which improves the tensile and barrier properties of solvent casted films by induction of cross-linking between the protein chains. Disulfide bond formation, which occurs with heat-denaturation in protein based films, is often used to modify film properties [7].

Fourier Transform Infrared (FTIR)

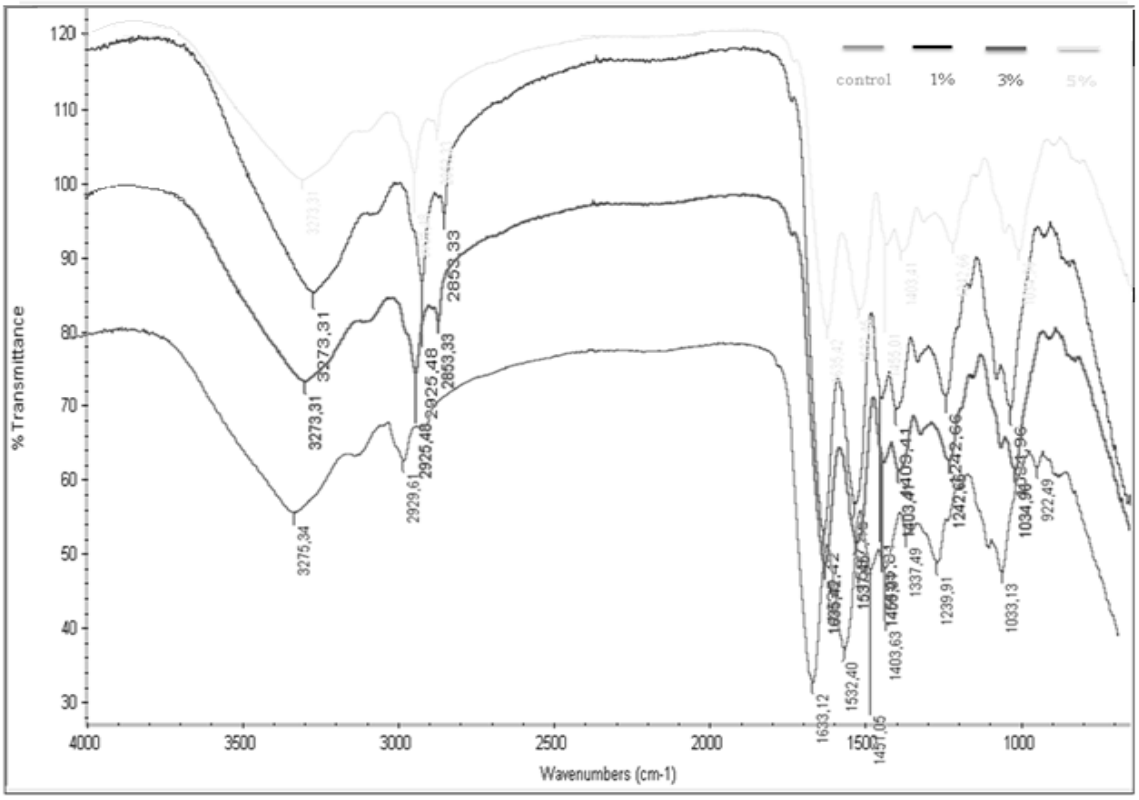

Figure 3: FTIR spectra of caffeic acid in cold water gelatin film

Fourier transform infrared spectroscopy (FTIR) is a useful technique to supplement micro structural characterization of composite films, since it may be used to evaluate interactions between film components. The light source of transmittance was 650–4000 cm^{-1}. FTIR spectral analyses of the preparations showed network formations of cross-linked gelatin and caffeic acid by hydroxyl and carboxy group of caffeic acid reacted with amino acids of gelatin by hydrogen bond or esterification, thus produced the cross-linked network to improve the properties. According to Figure 4, and comparison of different concentrations of caffeic acid can be concluded, existence new peak in 2800 to 3000 show created cross-linking in the basement is fish gelatin.

CONCLUSION

Gelatin has been used as a coating by the food and pharmaceutical industry for years [24].In this study cold water fish gelatin was prepared from a combination of phenolic compound caffiec acid. Results showed that solubility, O2 permeability and vapour permeability have been reduced especially at concentration of 5%. With regard to importance of barrier and physico-chemical properties for packaging products, CA is appropriate phenolic compound for packaging pharmaceutical and food products.

REFERENCES

[1] Cao, Na, Yuhua, Fu, Junhui, He. Food Hydrocoll 2007;21(4):575-584.
[2] Bourtoom T. Int Food Res J 2009;16:1-9.
[3] Zeppa C, Gouanve F, Espuche E. J App Poly Sci 2009;112:2044–2056.
[4] Voon HC, Bhat R, Easa A, Liong MT, Karim AA. Food Bioproc Technol 2010; DOI:10.1007/s11947-010-0461-y.
[5] Detduangchan N, Wittaya T. World Acad Sci Eng Technol 2011;57:1058-1063.
[6] Gómez-Guillén MC, Pérez-Mateos M, Gómez-Estaca J, López-Caballero E, Giménez B, and Montero P. Trends Food Sci Technol 2009;20(1):3-16.
[7] Dangaran K, Tomasula PM, and Qi P. 2009. Structure and function of protein-based edible films and coatings. In *Edible Films and Coatings for Food Application*s (Huber, K. C. and Embuscado, M. E., Ed.), 25-56, Springer.
[8] Kosaraju SL, Puvanenthiran A, Lillford P. Food Res Int 2010;43(10):2385–2389.
[9] Bigia A, Cojazzib G, Panzavoltaa S, Roveria N, Rubini K. Biomater 2002;23:4827–4832.
[10] Zhang X, et al. Biomacromol 2010;11(4):1125-1132.
[11] Zhang X, et al. J Agr Food Chem 2010;58(11):6809-6815.
[12] Carvalho RA and Grosso CRF. Brazilian J Chem Eng 2006;23(01):45 - 53.
[13] See SF, Hong PK, Ng KL, Wan Aida WM and Babji AS. Int Food Res J 2010;17: 809-816.
[14] Irwandi J, et al. Int Food Res J 2009;16:381-389.
[15] Sothornvit R and Krochta JM. J Food Sci 2000;65(4):700-703.
[16] Yi JB, et al. J Food Sci 2006;71(9).
[17] Sabato SF, et a. J Agr Food Chem 2001;49:1397-1403.
[18] Bhat R and Karim AA. J Food Sci Technol 2012.
[19] Hagiwara A, et al. Cancer Res 1991;51:5655-5660.
[20] ASTM. 2005. Standard Test Methods for Water Vapor Transmission of Materials E96/E96M-05, in Annual Book of ASTM Standards, Philadelphia, PA.
[21] McHugh TH, Avena-Bustillos R and Krochta JM. J Food Sci 1993;58(4):899-903.
[22] Maizura, M, Fazilah A, Norziah MH and Karim AA. J Food Sci 2007;72(6):C324-C330.
[23] Olives GI, and Barbosa-Canovas. 2009. Edible Films and Coatings for Fruits and Vegetables. In *Edible Films and Coatings for Food Application*s (Huber, K. C. and Embuscado, M. E., Ed.), 211-244, Springer.
[24] Hamaguchp PY, Shiku Y and Tanaka M. J Packaging Sci Technol 2003;12(5).
[25] Zhou J, L Ren, et al. Carbohydr Poly 2009;78:888-893.