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Properties of film from cuttlefish (*Sepia pharaonis*) skin gelatin incorporated with cinnamon, clove and star anise extracts

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ABSTRACT

Properties of film from cuttlefish (*Sepia pharaonis*) ventral skin gelatin without and with partial hydrolysis (1.2% degree of hydrolysis) incorporated with 1% ethanolic extract of cinnamon (CME), clove (CLE) and star anise (SAE) were determined. Films with different herb extracts (without and with oxidation) had higher tensile strength (TS) but lower elongation at break (EAB), compared with the control film (without addition of herb extracts) ($p < 0.05$). Lower water vapor permeability (WVP) and L^*-value but higher b^*- and ΔE^*-values were observed when the extracts were incorporated ($p < 0.05$). Electrophoretic study revealed that cross-linking was pronounced in films containing different herb extracts. Oxidized extracts yielded films with higher TS and WVP than those without oxidized herb extracts ($p < 0.05$). Generally, similar properties were noticeable for films from gelatin with and without partial hydrolysis. Nevertheless, higher mechanical properties were obtained for the latter. FTIR spectra indicated that protein–polyphenol interactions were involved in the film. Thermo-gravimetric analysis revealed that films incorporated with SAE or SAE with oxidation (OSAE) exhibited lower heat susceptibility and weight loss in the temperature range of 50–600 °C, compared with control film. Films with SAE and OSAE had smoother surface for gelatin without hydrolysis; however, coarser surface was observed in film from gelatin with partial hydrolysis. Thus, the incorporation of different herb extracts directly affected the properties of film from cuttlefish skin gelatin with and without hydrolysis.

1. Introduction

Biodegradable films made from renewable biopolymers have become an important environmental friendly packaging, thereby reducing plastic wastes (Hoque, Benjakul, & Prodpran, 2010a; Prodpran & Benjakul, 2005; Tharanathan, 2003). Most synthetic films are non-biodegradable, leading to environmental pollution and serious ecological problems (Tharanathan, 2003). Gelatin is a natural biopolymer obtained by thermal degradation of collagen (Arvanitoyannis, 2002). Gelatin has been used as a material for preparing biodegradable films with an excellent gas barrier property (Jongiareonrak, Benjakul, Vesessanguan, Prodpran, & Tanaka, 2006; Tharanathan, 2003). Protein-based films have good barrier characteristics against gas, organic vapor and oil as compared to synthetic films, which can maintain the quality of food products (Jiang, Tang, Wen, Li, & Yang, 2007). However, the poor mechanical properties and the high water vapor permeability are the main drawbacks of protein films for their application as a packaging material (McHugh & Krochta, 1994).

To modify the properties of protein film, different chemical cross-linking agents and enzyme including gossypol, formaldehyde, glutaraldehyde (de Carvalho & Grosso, 2004), glyoxal (Nuthong, Benjakul, & Prodpran, 2009a) and transglutaminase (Mariniello et al., 2003) have been used. However, the toxicity and high cost of some cross-linking agents have led to their limitation for the application in films (Cao, Fu, & He, 2007). Polyphenols are known to react with side chain amino groups of peptides, leading to the formation of protein cross-links (Strauss & Gibson, 2004).

Different plant or herb extracts have been incorporated in gelatin films to enhance antioxidant and/or antimicrobial properties such as murta ecotypes leave extracts incorporated in tuna-skin gelatin film (Gómez-Guillén, Ihl, Bifani, Silva, & Montero, 2007), oregano or rosemary aqueous extracts in bovine-hide and tuna-skin gelatins film (Gómez-Estaca, Bravo, Gómez-Guillén, Alemán, & Montero, 2009) and borage extract in sole skin or commercial fish skin gelatin (Gómez-Estaca, Giménez, Montero, & Gómez-Guillén, 2009). Additionally, tuna-skin gelatin film incorporated with oregano and rosemary extracts had altered properties including higher glass

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transition temperature, decreased deformability and increased water solubility (Gómez-Estaca, Montero, Fernández-Martín, Alemán, & Gómez-Guillén, 2009) and improved light barrier properties (Gómez-Estaca, Bravo, et al., 2009). Rattaya, Benjakul, and Prodpran (2009) found that fish skin gelatin films incorporated with oxygenated seaweed (*Turbinaria ornata*) extract exhibited higher EAB, and lower WVP and film solubility than the control film ($p < 0.05$). Recently, Hoque, Benjakul, and Prodpran (2010b) found that gelatin with shorter chain length produces film with poorer properties. Incorporation of selected herb extracts may improve the properties of those films. However, there is no information about the effect of incorporation of herb (cinnamon, clove and star anise) extracts on the properties of gelatin-based film. Thus, the objectives of the present study were to study the effects of three different herb (cinnamon, clove and star anise) extracts with and without oxidation on the properties of films from cuttlefish skin gelatin with and without partial hydrolysis.

2. Materials and methods

2.1. Chemicals

L-Leucine, 2,4,6-trinitrobenzenesulphonic acid (TNBS), bovine serum albumin and wide range molecular weight protein markers were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Hydrogen peroxide (H_2O_2), glycerol, *p*-dimethylaminobenzaldehyde and tris(hydroxymethyl) aminomethane were obtained from Merck (Darmstadt, Germany). Sodium dodecyl sulfate (SDS), Coomassie Blue R-250 and *N*, *N*, *N*′, *N*′-tetramethylethylenediamine (TEMED) were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Folin–Ciocalteu's reagent and gallic acid were obtained from Fluka (Buchs, Switzerland). Alcalase 2.4 L was provided by Novozymes (Bagsvaerd, Denmark). All chemicals were of analytical grade.

2.2. Preparation of herb extracts

2.2.1. Collection and preparation of herb

Three different herbs namely cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*) and star anise (*Illicium verum*) were obtained from local market in Hat Yai, Thailand. Herbs were dried using a hot-air oven at 35 °C until the final moisture content of 10–12% was obtained. Dried herbs were then ground and sieved to obtain uniformity using a screen with mesh 35 with an aperture size of 500 μm, ASTM E11, serial number 5666533 (FRITSCH GMBH, Laborgerätebau, Industriestrasse 8, D-55743 Idar-Oberstein, Germany).

2.2.2. Preparation of herb extracts

Herb extracts were prepared according to the method of Santoso, Yoshie-Stark, and Suzuki (2004) with some modifications. To prepare herb extracts, the herb powder (25 g) was mixed with 80% ethanol using a powder/solvent ratio of 1:20 (w/v). The mixtures were then stirred continuously at room temperature for 3 h. The mixtures were centrifuged at 5000×g for 10 min at room temperature using a refrigerated centrifuge (Beckman Coulter, Avanti J-E Centrifuge, Beckman Coulter, Inc., Palo Alto, CA, USA) to remove undissolved debris. The supernatant was collected and filtered through a Whatman filter paper No. 1 (Schleicher & Schuell, Maidstone, England). The solvent in the extract was removed by a rotary evaporator (EYELA, Rotary Vacuum Evaporator, N-1000 series, Tokyo Rikakikai Co., Ltd., Chuo-ku, Tokyo, Japan) at 40 °C to obtain the concentrated herb extract. To remove the residual ethanol, the extract was purged with nitrogen gas. The extract was further dried using a freeze dryer to obtain the dry extract. Dried extract was ground into a powder using a mortar and pestle. Extract powder was referred to as cinnamon extract (CME), clove extract (CLE) and star anise extract (SAE). CME, CLE and SAE powder was placed in amber bottles, closed tightly and stored in a dessicator until use.

2.2.3. Determination of total phenolic content of herb extracts

Total phenolic content of herb extracts was determined with Folin–Ciocalteu reagent according to the method of Singleton, Orthofer, and Lamuela-Raventos (1998) using gallic acid as a standard. Extract was dissolved in 25% ethanol to obtain the concentration of 0.5% (w/v). The solution (0.5 mL) was added to 100 μL of Folin–Ciocalteu reagent (two-fold diluted with de-ionized water) and mixed thoroughly. After 3 min, 1.5 mL of 2% Na_2CO_3 was added. The mixture was allowed to stand at room temperature for 30 min. The absorbance was measured at 760 nm using a spectrophotometer (UV-160, Shimadzu, Kyoto, Japan). The concentration of total phenolic compounds in the extract was calculated from a standard curve generated with gallic acid (0.01–0.1 mg/mL) and expressed as mg of gallic acid equivalent (GAE)/g powder.

2.2.4. Preparation of oxidized herb extracts

Oxidized herb extracts were prepared as per the method of Strauss and Gibson (2004) with some modifications. Herb extracts (CME, CLE and SAE) were dissolved using 25% ethanol to obtain a concentration of 0.5% (w/v). The solutions were then adjusted to pH 12 by adding 6 M NaOH. The prepared solutions were placed in a temperature controlled water bath (Memmert, GmbH+Co. KG, D-91126, Schwabach, Germany) at 40 °C and subjected to oxygenation by bubbling the solution with oxygen gas with a purity of 99.8% (TTS Gas Agency, Hat Yai, Songkhla, Thailand) for 30 min to convert the phenolic compounds to quinone. After being oxygenated for 30 min, the solution was then neutralized using 2 M HCl and the final volume of the solution was adjusted to 25 mL by using distilled water and was referred to as "oxidized herb extract" named oxidized cinnamon extract (OCME), oxidized clove extract (OCLE) and oxidized star anise extract (OSAE). All oxidized extracts were analyzed for quinone formation using Folin–Ciocalteu reagent according to the methods of Balange and Benjakul (2010) with a slight modification. The conversion of phenolic compounds in different herb extracts to quinone was monitored indirectly by determining total phenolic content in different herb extracts before and after oxidation. The differences in the total phenolic content before and after oxidation indicated the formation of quinone and were expressed in percentages.

2.3. Collection and preparation of cuttlefish skin

Ventral skin of cuttlefish (*Sepia pharaonis*) was obtained from a dock in Songkhla, Thailand. Cuttlefish skin was stored in ice with a skin/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University within 1 h. Upon arrival, cuttlefish skin was washed with tap water and cut into small pieces (1×1 cm^2), placed in polyethylene bags and stored at −20 °C until use. Storage time was not longer than 2 months. Prior to gelatin extraction, the frozen skin was thawed using running water (25–26 °C) until the core temperature reached 0–2 °C.

2.4. Preparation of gelatin and partially hydrolyzed gelatin from cuttlefish skin

Gelatin was extracted from cuttlefish skin according to the method of Hoque et al. (2010a). Skin was soaked in 0.05 M NaOH with a skin/solution ratio of 1:10 (w/v) with gentle stirring at room