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Antioxidative activity and emulsifying properties of cuttlefish skin gelatin–tannic acid complex as influenced by types of interaction

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ABSTRACT

The non-covalent interaction between cuttlefish skin gelatin and tannic acid was observed in gelatin modified with unoxidized tannic acid at pH 7, whereas covalent interaction was found in gelatin modified with oxidized tannic acid at pH 9. Degree of tannic acid incorporation into gelatin via non-covalent interaction was more pronounced than that found via covalent interaction as evidenced by lowered free amino group content and increased total phenolic content and hydroxyl group and aromatic ring determined by FTIR. Gelatin modified with oxidized tannic acid had the slight decrease in surface hydrophobicity, with no changes in particle size distribution of the emulsions. Modification of gelatin with tannic acid, especially via non-covalent interaction, increased *in vitro* antioxidative activity, compared with the control gelatin. Gelatin modified with tannic acid via covalent interaction rendered the emulsion with high stability and could inhibit lipid oxidation of menhaden oil-in-water emulsion effectively throughout the storage of 12 days.

Industrial relevance: Cuttlefish skin gelatin modified with tannic acid possessing both emulsifying activity and the improved antioxidative activity can be used as a natural and safe additive in food industry. Therefore, cuttlefish skin, a by-product from seafood processing industry, can be produced as the high value added product with wider applications.

1. Introduction

Many food products containing lipids are commonly found in emulsion forms either as water-in-oil, for example, butter and margarine, or oil-in-water, for example, mayonnaise, milk and cream. Lipid oxidation negatively affects the quality of foods, especially emulsion type products by altering appearance, odor, flavor, shelf-life and nutritional value. This results in unacceptability by consumers (Kellerby, Gu, McClements & Decker, 2006). To retard or prevent such changes, antioxidants are increasingly used to control lipid oxidation in emulsions. Due to the anxiety of possible toxicity of synthetic antioxidants, the natural antioxidants have gained increasing attention among consumers. Polyphenol compounds have received attention as antioxidant agents in food products. The antioxidant properties of phenolics are mainly because of their redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers (Rice-Evans, Miller & Paganga, 1997). Additionally, these phenolic compounds can bind metal ions and scavenge the radicals (Moure, Domínguez & Parajó, 2006). Nevertheless, phenolic compounds with higher antioxidative activity generally consist of the higher number of hydroxyl group in structure (Prasad, Soundar, Gyarahally & Somaradhya Mallikarjuna, 2005). These polar antioxidants are sparingly soluble in oil. In oil-in-water emulsions system, polar antioxidants readily partition into the aqueous phase, decreasing their concentration in the lipid phase and thus lowering their capability to prevent oxidation (Yuji, Weiss, Villeneuve, Giraldo, Figueroa-Espinoza & Decker, 2007). However, the effectiveness of polar antioxidant in oil-in-water emulsions can be improved by increasing their surface activity and ability to accumulate at the oil–water interface where oxidative reactions take place (Yuji et al., 2007).

Proteins are surface/interfacial active and are widely used as emulsifiers in foods to produce oil-in-water emulsions with desirable physicochemical properties and improved stability (McClements, 2004). They can adsorb on oil–water interfaces and various hydrophobic segments penetrate into the oil phase. To improve the amphiphilic property of protein, the appropriate modification by incorporating the phenolic compound could be a potential means to obtain the modified protein with the higher emulsifying property as well as antioxidant activity. A protein–phenolic complex is concentrated at the oil–water interface, yielding the surface-active nature of the protein and antioxidative activity of phenolic compounds (Almajano & Gordon, 2004). The interaction between proteins and phenolic compound may occur through non-covalent interaction (such as hydrogen bonding and

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hydrophobic interaction) or covalent interaction (Kroll, Rawel & Rohn, 2003).

Cuttlefish is one of the major seafood products of Thailand. During processing, the skin is removed and becomes the by-product with a low market value. In general, it has been used as animal feed. Recently, Aewsiri, Benjakul and Visessanguan (2009) extracted the gelatin from cuttlefish. However, it showed low bloom strength. To exploit the gelatin from cuttlefish skin, it has been used as an emulsifying agent after modification with some selected phenolic compounds (Aewsiri, Benjakul, Visessanguan, Eun, Wierenga & Gruppen, 2009). The incorporation of oxidized phenolic compounds especially tannic acid in gelatin from cuttlefish skin can increase antioxidative activity and emulsifying properties of resulting gelatin. Tannic acid is affirmed as Generally Recognized as Safe (GRAS) by the Food and Drug Administration (FDA) for the use as a direct additive in some food products such as baked goods and baking mixes, alcoholic and non-alcoholic beverages, frozen dairy desserts and mixes, hard candy and cough drop as well as meat products (21 CFR184. 1097, US Code of Federal Regulation, 2006). Tannin contains sufficient hydroxyls and other groups such as carboxyls to form strong complexes with proteins and other macromolecules (Kroll et al., 2003). Nevertheless, a little information regarding the type of interaction between gelatin and tannic acid and its impact on stability and lipid oxidation of emulsion system has been reported. Therefore, the objective of this study was to investigate the influence of the modification of cuttlefish skin gelatin with tannic acid via covalent and non-covalent interaction on antioxidative activity and emulsifying properties of resultant gelatin.

2. Materials and methods

2.1. Chemicals

Tannic acid, 2,4,6-trinitrobenzenesulfonic acid (TNBS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,4,6-tripyridyl-triazine (TPTZ), Trolox and menhaden oil were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Hydrogen peroxide (H_2O_2), trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA), Folin–Ciocalteu's phenol reagent, sodium sulfite and ferric chloride ($FeCl_3$) were obtained from Merck (Darmstadt, Germany). Sodium dodecyl sulfate (SDS) was procured from Bio-Rad Laboratories (Hercules, CA, USA). All chemicals were of analytical grade.

2.2. Preparation of cuttlefish skin

Ventral skin of cuttlefish (*Sepia pharaonis*) was obtained from the 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.

2.3. Extraction of gelatin from cuttlefish skin

Cuttlefish skin gelatin was prepared according to the method of Aewsiri et al. (2009b). Skin was treated with 10 volumes of 0.05 N NaOH for 6 h with a gentle stirring at room temperature (26–28 °C). The alkaline solution was changed every 1 h for up to 6 h. Alkali treated skin was then washed with distilled water until the neutral pH of wash water was obtained. The prepared skin was subjected to bleaching with 10 volumes of 5% H_2O_2 at 4 °C for 48 h at room temperature and then washed with 10 volumes of water for 3 times. Gelatin was extracted from bleached skin using distilled water (60 °C) for 12 h with a sample/water ratio of 1:2 (w/v). During extraction, the mixture was stirred continuously. The extract was centrifuged at 8000 ×*g* for 30 min using a refrigerated centrifuge (Beckman Coulter, Avanti J-E Centrifuge, Fullerton, CA, USA) to remove insoluble material. The supernatant was collected and freeze-dried using a freeze dryer (ScanVac Model CoolSafe 55-4, Lynge, Denmark). Gelatin extracted from cuttlefish skin consisted of 87.97% protein, 11.17% moisture, 0.94% fat and 0.53% ash contents as determined by AOAC (2000). The molecular weight of major proteins in cuttlefish skin gelatin was estimated to be 97 kDa as analyzed by SDS-PAGE using 4% stacking gel and 10% separating gel (Laemmli, 1970). pI of gelatin was estimated to be 4.5 using a zeta potential analysis following the method of Kittiphattanabawon, Benjakul, Visessanguan, and Shahidi (2010).

2.4. Modification of cuttlefish skin gelatin by tannic acid

Cuttlefish skin gelatin was modified with tannic acid using different conditions to obtain gelatin–tannic acid complexes via covalent or non-covalent interactions. For covalent interaction, the gelatin was modified by oxidized tannic acid (OTA) at pH 9. The gelatin was dissolved in distilled water containing 0.02% sodium azide (NaN_3) to obtain a final concentration of 1.2% protein (w/v). The pH of gelatin solution was adjusted to 9 using 1 M NaOH. To prepare OTA, tannic acid was dissolved in distilled water to obtain the concentration of 2% (w/v), followed by pH adjustment to 9 with 1 M NaOH. Solution was then bubbled with oxygen at 40 °C for 1 h to convert tannic acid into OTA, an oxidized form. To 75 ml of gelatin solution, the solution of OTA (2.25 mL) was added to obtain the final concentration of 5% (based on protein content). Final volume was raised to 90 ml using distilled water (pH 9), in which a final protein concentration of 1% (w/v) was obtained. The mixtures were stirred continuously using a magnetic stirrer (IKAMAG® model RO 10 power, IKA LABORTECHNIK, Selangor, Malaysia) at a speed of 200 rpm at room temperature for 12 h. Thereafter, the samples were dialyzed for 24 h against 20 volumes of water to remove free phenolic compounds (unbound to proteins).

For non-covalent interaction, the gelatin was modified by tannic acid at different pH (pH 7 and 9). The reaction solutions were prepared as previously described but tannic acid solution was not converted into OTA. Both gelatin and unoxidized tannic acid (TA) solutions were adjusted to pH 7 or 9 prior to mixing together thoroughly as previously described. The control gelatin was prepared in the same manner except that either TA or OTA was excluded. Modified gelatin and the control gelatin were subjected to analyses.

2.5. Determination of incorporation of tannic acid into gelatin

Modified samples (1% of protein, 18 ml) either via covalent or non-covalent interaction were added with 2 ml of 10% sodium dodecyl sulfate (SDS) solution to obtain the final SDS concentration of 1%. The mixture was stirred gently for 1 h at room temperature to disrupt the non-covalent interaction of gelatin–tannic acid complexes. The untreated sample was prepared in the same manner except the distilled water was added instead of 10% SDS solution. Thereafter, the samples were dialyzed for 24 h against 20 volumes of water to remove unbound TA or OTA and SDS. Subsequently, gelatin attached with tannic acid via covalent bond was retained in the dialysate after treatment with SDS and dialysis. All samples treated without and with SDS were subjected to analyses.

2.5.1. Determination of free amino group content

Free amino group content of samples treated without and with SDS was determined according to the method of Benjakul and Morrissey (1997). To diluted samples (125 μl), 2.0 ml of 0.2125 M phosphate buffer, pH 8.2 and 1.0 ml of 0.01% TNBS solution were added. The solution was mixed thoroughly and placed in a temperature-controlled water bath (Model W350, Memmert, Schwabach, Germany) at 50 °C for 30 min in the dark. The reaction was terminated by adding 2.0 ml of 0.1 M sodium sulfite. The mixtures were cooled at room temperature for 15 min. The absorbance was read at 420 nm using a UV–vis