Food Chemistry 115 (2009) 243–249

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Functional properties of gelatin from cuttlefish (*Sepia pharaonis*) skin as affected by bleaching using hydrogen peroxide

Tanong Aewsiri [a], Soottawat Benjakul [a,*], Wonnop Visessanguan [b]

[a] Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand
[b] National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 113 Paholyothin Rd., Klong1, Klong Luang, Pathumthani 12120, Thailand

ARTICLE INFO

Article history:
Received 30 July 2008
Received in revised form 8 October 2008
Accepted 2 December 2008

Keywords:
Gelatin extraction
Cuttlefish
Bleaching
Hydrogen peroxide
Functional properties

ABSTRACT

Functional properties of gelatin from dorsal and ventral skin of cuttlefish with and without bleaching by H_2O_2 at different concentrations (2% and 5% (w/v)) for 24 and 48 h were studied. Gelatin from skin bleached with 5% H_2O_2 for 48 h showed the highest yield (49.65% and 72.88% for dorsal and ventral skin, respectively). Bleaching not only improved the colour of gelatin gel by increasing the L^*-value and decreasing a^*-value but also enhanced the bloom strength, and the emulsifying and foaming properties of the resulting gelatin. Gelatin from bleached skin contained protein with a molecular weight of 97 kDa and had an increased carbonyl content. Fourier transform infrared spectroscopic study showed higher intermolecular interactions and denaturation of gelatin from bleached skin than that of the control. These results indicated that hydrogen peroxide most likely induced the oxidation of gelatin, resulting in the formation of gelatin cross-links, giving improved functional properties.

1. Introduction

Gelatin is a denatured form of collagen and can be used widely in the food and pharmaceutical industries (Cho, Gu, & Kim, 2005). Gelatin is commercially made from skins and skeletons of cattle and pigs by alkaline or acidic extraction (Gilsenan & Ross-Murphy, 2000). However, the occurrence of bovine spongiform encephalopathy (BSE) and foot and mouth diseases have caused major concerns for human health, and thus byproducts of mammals are now less popular for the production of collagen and gelatin as functional foods, cosmetics and pharmaceutical products (Cho et al., 2005). Additionally, porcine gelatins can cause objections from some religions. As a consequence, increasing interest has been paid to other gelatin sources, especially fish skin and bone from seafood processing waste. So far, gelatin from skin of different fish species has been intensively studied (Gómez-Guillén et al., 2002; Muyonga, Cole, & Duodu, 2004). However, gelatin from marine resources has poorer bloom strength, compared with mammalian gelatin, due to its lower imino acid content. Therefore, bloom strength of fish gelatin has been improved by chemical modification (e.g. $MgSO_4$, glycerol) or enzyme modification (e.g. transglutaminase) (Fernández-Díaz, Montero, & Gómez-Guillén, 2001).

Cuttlefish has become an important fishery product in Thailand, and is mainly exported worldwide. During processing, skin is generated as a byproduct. Skin has a low market value and is used as animal feed. The extraction of gelatin from cuttlefish skin could increase its profitability. Nevertheless, the pigments in skin may pose a colour problem and bleaching could be performed prior to gelatin extraction. Hydrogen peroxide is a potent oxidant that is widely used as bleaching agent in seafood processing (Kolodziejska, Sikorski, & Niecikowska, 1999; Thanonkaew, Benjakul, Visessanguan, & Decker, 2008). Kolodziejska et al. (1999) reported that soaking squid skin in 1% H_2O_2 in 0.01 M NaOH for 48 h could improve the colour of the resulting collagen. The decomposition of H_2O_2 in aqueous solution occurs by dissociation and homolytic cleavage of O–H or O–O bonds, with the formation of highly reactive products: hydroperoxyl anion (HOO^-), and hydroperoxyl ($HOO^{\cdot}$) and hydroxyl ($OH^{\cdot}$) radicals, which can react with many substances, including chromatophores (Perkins, 1996). Wash water containing H_2O_2 also showed a gel-enhancing effect in surimi, *via* induced protein oxidation (Phatcharat, Benjakul, & Visessanguan, 2006). Currently, no information regarding the use of H_2O_2 as a bleaching agent in cuttlefish skin prior to gelatin extraction and its effect on the functional properties and yield of gelatin has been reported. The objectives of this work were to study the effect of H_2O_2 pretreatment on bleaching of cuttlefish skin and to investigate its impact on the functional properties of the resulting gelatin.

* Corresponding author. Tel.: +66 7428 6334; fax: +66 7421 2889.
E-mail address: soottawat.b@psu.ac.th (S. Benjakul).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.12.012

2. Materials and methods

2.1. Chemicals

β-Mercaptoethanol (β-ME), bovine serum albumin and protein markers were purchased from Sigma Chemical Co. (St. Louis, MO). Hydrogen peroxide (H_2O_2), *p*-dimethylaminobenzaldehyde and tris(hydroxymethyl)aminomethane were obtained from Merck (Darmstadt, Germany). Sodium dodecyl sulphate (SDS), Coomassie Blue R-250 and *N,N,N′,N′*-tetramethylethylenediamine (TEMED) were purchased from Bio-Rad Laboratories (Hercules, CA). Type I collagen from calf skin was purchased from Elastin Products Co., Inc. (Owensville, MO). Food grade bovine bone gelatin was obtained from Halagel (Thailand) Co., Ltd. (Bangkok, Thailand).

2.2. Collection and preparation of cuttlefish skin

Dorsal and 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), placed in polyethylene bags and stored at −20 °C until use. Storage time was not greater than 2 months.

2.3. Extraction of gelatin from cuttlefish skin without and with bleaching

Gelatin was prepared according to the method of Gómez-Guillén et al. (2002) with some modifications. Skin was soaked in 0.05 N NaOH with a skin:solution ratio of 1:10 (w/v) for 6 h with a gentle stirring at room temperature (26–28 °C). The solution was changed every 1 h, to remove non-collagenous proteins, for up to 6 h. Alkali-treated skins were then washed with distilled water until the neutral pH of wash water was obtained. The prepared skin was subjected to bleaching in 2% and 5% H_2O_2, using a sample:solution ratio of 1:10 (w/v) for 24 and 48 h at 4 °C. Bleached samples were washed three times with 10 volumes of water. The alkali-treated skin without bleaching was used as the control. Gelatin was extracted from skin with and without bleaching using distilled water at 60 °C for 12 h, with a sample:water ratio of 1:2 (w/v). During extraction, the mixture was stirred continuously. The extracts were centrifuged at 8,000 g for 30 min using a refrigerated centrifuge (Sorvall Model RC-B Plus, Newtown, CT) to remove insoluble material. The supernatant was collected and freeze-dried (Model Dura-Top™ μP/Dura Dry™ μP, FTS® System, Inc., Stone Ridge, New York). The yield of gelatin obtained was calculated and expressed as the percentage of dry matter of gelatin relative to dry matter of cuttlefish skin. Gelatins were subjected to analyses.

2.4. Determination of carbonyl content

Carbonyl content of gelatin was determined according to the method of Liu, Xiong, and Butterfield (2000). Gelatin solution (0.5 ml, 4 mg protein/ml) was added to 2.0 ml of 10 mM 2,4-dinitrophenylhydrazine (DNPH) in 2 N HCl. The mixture was allowed to stand for 1 h at room temperature. Thereafter, 2 ml of 20% (w/v) TCA were added to precipitate protein. The pellet was washed twice with 4 ml of ethanol:ethyl acetate (1:1, v/v) mixture, to remove unreacted DNPH, blow-dried, and dissolved in 1.5 ml of 0.6 M guanidine hydrochloride in 20 mM potassium phosphate (pH 2.3). The absorbance of solution was measured at 370 nm using a spectrophotometer (UV-160, Shimadzu, Kyoto, Japan). A molar absorptivity of 22,400 M^{-1} cm^{-1} was used to calculate carbonyl content (Levine et al., 1990).

2.5. Determination of functional properties

2.5.1. Determination of bloom strength

Bloom strength of gelatin was determined, according to the method of Gómez-Guillén et al. (2002), with a slight modification. Gelatins were dissolved with 30 ml of distilled water (60 °C) in a 50 ml beaker with an inner diameter of 3.8 cm for 30 min to obtain a final concentration of 6.67% (w/v). Gelatin solution was kept at 5 °C for 18 h prior to measurement. Bloom strength of sample (2.7 cm height) was measured at 8–10 °C using a texture analyser with a load cell of 5 kN, cross-head speed of 1 mm/sec, equipped with a 1.27 cm diameter flat-faced cylindrical Teflon plunger. Maximum force (in grams), taken when the plunger had penetrated 4 mm into the gelatin gels, was recorded.

2.5.2. Determination of colour

Gelatin gel (6.67%, w/v) was prepared as described previously. Colour of gel samples was determined using a colourimeter (ColourFlex, HunterLab Reston, VA). CIE L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) values were measured.

2.5.3. Determination of emulsifying properties

Emulsion activity index (EAI) and emulsion stability index (ESI) of gelatin were determined according to the method of Pearce and Kinsella (1978), with a slight modification. Soybean oil (2 ml) and gelatin solution (1% protein, 6 ml) were homogenised (Model T25 basic; IKA Labortecnik, Selangor, Malaysia) at a speed of 20,000 rpm for 1 min. Emulsions were pipetted out at 0 and 10 min and 100-fold diluted with 0.1% SDS. The mixture was mixed thoroughly for 10 s using a vortex mixer. A_{500} of the resulting dispersion was measured using a spectrophotometer (UV-160, Shimadzu). EAI and ESI were calculated by the following formulae:

$$EAI(\mathrm{m^2/g}) = (2 \times 2.303 \times A \times \mathrm{DF})/l\phi C$$

where $A = A_{500}$, DF=dilution factor (100), l = path length of cuvette (m), ϕ = oil volume fraction and C = protein concentration in aqueous phase ($\mathrm{g/m^3}$);

$$ESI(min) = A_0/\Delta A \times \Delta t$$

where A_{500} = absorbance at 500 nm, $\Delta A = A_0 - A_{10}$ and Δt = 10 min.

2.5.4. Determination of foaming properties

Foam expansion (*FE*) and foam stability (*FS*) of gelatin solutions were determined, as described by Shahidi, Xiao-Qing, and Synowiecki (1995), with a slight modification. Gelatin solution with 1% protein concentration was transferred into 100 ml cylinders. The mixtures were homogenised for 1 min at 13,400 rpm for 1 min at room temperature. The sample was allowed to stand for 0, 30 and 60 min. *FE* and *FS* were then calculated using the following equations:

$$FE\ (\%) = (V_T/V_o) \times 100$$
$$FS\ (\%) = (V_t/V_0) \times 100$$

where V_T is total volume after whipping; V_0 is the original volume before whipping and V_t is total volume after leaving at room temperature for different times (30 and 60 min).

2.6. SDS–polyacrylamide gel electrophoresis (SDS–PAGE)

Protein patterns of gelatin samples extracted from both dorsal and ventral skins with and without bleaching were analysed using sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE), according to the method of Laemmli (1970), using 10% separating gel and 4% stacking gel. Gelatin solution was mixed with sample buffer (0.5 M Tris–HCl, pH 6.8 containing 4% (w/v)