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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

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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).

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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)

SDS, 20% (v/v) glycerol and 10% (v/v) β-ME) at a ratio of 1:1 (v/v). Fifteen micrograms of protein, determined by the Biuret method (Robinson & Hodgen, 1940), were loaded onto the gel. Electrophoresis was conducted using the Protean II xi vertical cell and the 1000 Powerpac (Bio-Rad Laboratories, CA) at a constant current of 15 mA. Gels were stained using 0.05% Coomassie Brilliant Blue R250 dissolved in 15% (v/v) methanol and 5% (v/v) acetic acid, and de-stained with 30% (v/v) methanol and 10% (v/v) acetic acid. Protein markers, including myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase b (97 kDa), bovine serum albumin (66 kDa), glutamic dehydrogenase (55 kDa), ovalbumin (45 kDa), glyceraldehyde 3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), trypsin inhibitor (20 kDa), and α-lactalbumin (14 kDa), were used for estimation of molecular weight.

2.7. Fourier transform infrared (FTIR) spectroscopy

Spectra of gelatins from bleached and unbleached cuttlefish skin were obtained using a Bruker Model Equinox 55 FTIR spectrometer (Bruker, Ettlingen, Germany) equipped with a deuterated L-alanine triglycine sulphate (DLATGS) detector. The Horizontal Attenuated Total Reflectance Accessory (HATR) was mounted into the sample compartment. The internal reflection crystal (Pike Technologies, Madison, WI), which was made of zinc selenide, had a 45° angle of incidence to the IR beam. Spectra were acquired at a resolution of 4 cm^{-1} and the measurement range was 4000–600 cm^{-1} (mid-IR region) at room temperature. Automatic signals were collected in 16 scans at a resolution of 4 cm^{-1} and were ratioed against a background spectrum recorded from the clean, empty cell at 25 °C. Analysis of spectral data was carried out using Opus 3.0 data collection software.

2.8. Protein determination

Protein content was determined by the Biuret method, using bovine serum albumin as a standard.

2.9. Statistical analysis

All data were subjected to analysis of variance and differences between means were evaluated by Duncan's multiple range test. For pair comparison, *t*-test was used (Steel & Torrie, 1980). The SPSS statistical program (Version 10.0) (SPSS Inc., Chicago, IL) was used for data analysis.

3. Results and discussion

3.1. Yield

Yields of gelatins extracted from dorsal and ventral cuttlefish skin without bleaching were 36.82% and 59.69%, respectively (Table 1). Generally, dorsal skin showed a lower yield than ventral skin ($p < 0.05$). Dorsal and ventral skins might have different structural complexation, in which the gelatin extraction from both skins was different. Nevertheless, increases in yield of gelatin were observed as the skin was bleached with 5% H_2O_2 ($p < 0.05$). Longer bleaching time (48 h) resulted in higher yield in comparison with the shorter time (24 h). The highest yield was observed in gelatin extracted from skin bleached with 5% H_2O_2 for 48 h (49.65% and 72.88% for dorsal and ventral skin, respectively).

H_2O_2 was found to break the hydrogen bond of collagen (Courts, 1961). Donnelly and McGinnis (1977) reported that tissue containing collagen was liquefied through agitation with H_2O_2 for 4–24 h. In the presence of sufficient H_2O_2 (5%), hydrogen bonds of collagen molecules in cuttlefish skin might be broken, resulting in an increased efficiency in gelatin extraction, as evidenced by the increased yield. However, no increases in yield were observed in gelatin from skin bleached with 2% H_2O_2 at both 24 and 48 h, compared with that of the control (without bleaching).

Table 1
Yield and carbonyl content of gelatins extracted from dorsal and ventral cuttlefish skin with and without bleaching by H_2O_2 at different concentrations for various times.

Samples	Treatment	Yield (%)	Carbonyl content (nmol/mg protein)
Dorsal skin gelatin	Control	36.82 ± 1.53a*	3.91 ± 0.59a
	2% H_2O_2 24 h	37.14 ± 2.01a	5.74 ± 0.48b
	2% H_2O_2 48 h	36.83 ± 1.44a	5.91 ± 0.47b
	5% H_2O_2 24 h	45.00 ± 2.67b	8.33 ± 0.50c
	5% H_2O_2 48 h	49.65 ± 0.84c	8.53 ± 0.36c
Ventral skin gelatin	Control	59.69 ± 3.74a	2.49 ± 0.28a
	2% H_2O_2 24 h	58.91 ± 1.47a	6.11 ± 0.62b
	2% H_2O_2 48 h	59.78 ± 1.95a	6.23 ± 0.14b
	5% H_2O_2 24 h	68.44 ± 2.59b	6.45 ± 0.15b
	5% H_2O_2 48 h	72.88 ± 1.20c	6.57 ± 0.08b

Mean ± SD from triplicate determinations.
* Different letters in the same column within the same gelatin indicate significant differences ($p < 0.05$).

3.2. Carbonyl content

Carbonyl content of gelatin from cuttlefish skin is shown in Table 1. Carbonyl content is one of the most reliable measures of protein oxidation (Levine et al., 1990). Gelatins from both dorsal and ventral skin bleached with 5% H_2O_2 showed marked increases in the carbonyl content, compared with those from their unbleached counterparts ($p < 0.05$). However, bleaching with 2% H_2O_2 showed no impact on the carbonyl content of the resulting gelatin from both dorsal and ventral skins. For the control gelatin, that from dorsal skin contained a higher carbonyl content than that from ventral skin ($p < 0.05$). Hawkins and Davies (1997) found that hydroxyl radical, generated from a Fe(II)–H_2O_2 redox couple, attacked collagen, resulting in the generation of carbonyl compounds. The peroxide decomposition products, such as the hydroxyl radicals and superoxide anion radicals (O_2^-), are thought to cause the oxidation of protein and are responsible for the conversion of some amino acid residues to carbonyl derivatives (Butterfield & Stadtman, 1997).

Carbonyl content in gelatin increased with increasing concentration of H_2O_2 used for bleaching. However, bleaching time showed no effect on the carbonyl content in resulting gelatin, regardless of skin portion and H_2O_2 concentration. The most sensitive amino acids toward oxidation are heterocyclic amino acids. In addition, amino and phenolic groups of amino acids are susceptible to oxidation. Not only tryptophan, histidine and proline, but also lysine, cysteine, methionine and tyrosine, are prone to oxidation, where a hydrogen atom is abstracted from OH-, S- or N-containing groups (Doorn & Petersen, 2002). Oxidation of protein is associated with the alteration of protein structure, peptide chain scission, formation of amino acid derivatives and polymers, decreases in solubility, and changes in the functional properties (Decker, Xiong, Calvert, Crum, & Glanchard, 1993). Susceptibility of proteins to oxidation induced by H_2O_2 in cuttlefish dorsal skin was greater than that in ventral skin. This suggested a different composition of proteins in both portions.

3.3. Bloom strength of gelatin gel

The effect of bleaching of cuttlefish skin with H_2O_2 on bloom strength of gelatin gels is shown in Fig. 1. The lowest bloom strength was observed in gels of the control gelatin from dorsal and ventral skin (35.2 and 30.0 g, respectively). This result was in agreement with Gómez-Guillén et al. (2002), who found that gelatin gel extracted from squid skin was extremely soft and showed little cohesive force (~10 g). According to Holzer (1996), the gel strength of commercial gelatin, expressed as bloom value, ranges from 100 to 300 g but gelatin with bloom values of 250–260 g are most desired.

Bleaching with 2% and 5% H_2O_2 for 24 and 48 h resulted in marked increases in bloom strength ($p < 0.05$). Bloom strength of gelatin gel from ventral skin increased with increasing H_2O_2 concentration and bleaching time ($p < 0.05$). However, no pronounced effect of both H_2O_2 concentration and bleaching time on bloom strength of gelatin from dorsal skin was noticeable. The highest bloom strength of gelatin was obtained from dorsal and ventral skins bleached in 5% H_2O_2 for 48 h (126 and 137 g for dorsal and ventral skin gelatin, respectively). Bloom strength of resulting gelatins was about four times higher than that of the control. This result suggested that H_2O_2 might induce the oxidation of protein with the concomitant formation of carbonyl groups. Those carbonyl groups might undergo Schiff base formation with the amino groups, in which the protein cross-links were most likely formed (Stadtman, 1997). Moreover, $OH^{\cdot}$ can abstract H atoms from amino acid residues to form carbon-centred radical derivatives, which can react with one another, to form C–C protein cross-linked products (Stadtman, 1997). The larger protein aggregates were mostly associated with the improved bloom strength. However, the bloom strength of cuttlefish skin gelatin gel from all treatments was lower than that of bovine bone gelatin gel, most likely due to the lower hydroxyproline content of the former.

3.4. Colour of gelatin gel

L^*, a^* and b^*-values of gelatin gels from cuttlefish skin with and without bleaching under different conditions are presented in Table 2. Gelatin gel from skin without bleaching was more pink-purple in colour, as indicated by a lower L^*-value but a higher a^*-value, when compared with gelatin gel from bleached skin. Thus, soaking cuttlefish skin in 2% or 5% H_2O_2 solution could improve the colour of gelatin gel by increasing L^*-value and decreasing a^*-value. For dorsal skin, increases in H_2O_2 concentration and bleaching time resulted in increases in L^* and lower a^*-value of the resulting gelatin ($p < 0.05$). For ventral skin, a^*-value of gelatin gel decreased with increasing H_2O_2 concentration and bleaching time ($p < 0.05$), but no changes in L^*-value were found. In general, the control gel from dorsal skins had a higher colour intensity than the gel from ventral skins, most likely due to the higher content of chromatophore in the former. Thus, higher H_2O_2 concentration was necessary for improvement of colours of gelatin from dorsal skin, in comparison with ventral skin.

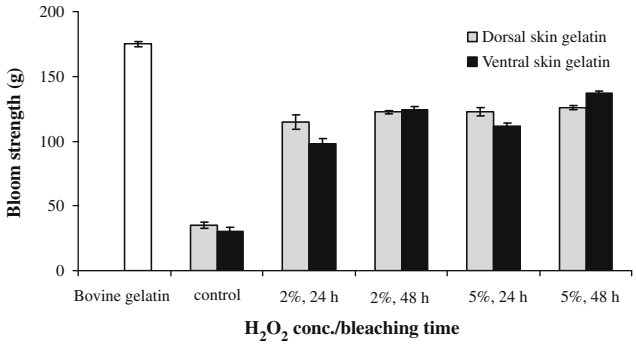

Fig. 1. Bloom strength of gelatin extracted from dorsal and ventral cuttlefish skin with and without bleaching by H_2O_2 at different concentrations and various times. Bars represent the standard deviation from triplicate determinations.

H_2O_2 is widely used in the cephalopod industry as the bleaching agent. The cephalopod needs to be bleached because the flesh could be stained by ink, viscera and colour pigments during handling and processing (Thanonkaew et al., 2008). Oxidising agents, derived from the decomposition of hydrogen peroxide, were able to destroy the chromophore. Hydroperoxyl anion is a strong nucleophile which, during bleaching, is able to break the chemical bonds that make up the chromophore. This changes the molecule into a different substance that either does not contain a chromophore, or contains a chromophore that does not absorb visible light (Perkins, 1996). On the other hand, hydroperoxyl and hydroxyl radical ($OH^{\cdot}$) generated by the decomposition of hydrogen peroxide may induce free radicals, causing the oxidation of protein, changes in protein structure and functional properties of gelatin. As a result, bleached skin contained a low content of chromophore, or still had the chromophore, which was colourless.

3.5. Emulsifying properties of gelatin

Emulsion activity index (*EAI*) and emulsion stability index (*ESI*) of gelatin from cuttlefish skin with and without bleaching are presented in Table 3. No differences in *EAI* of gelatin from dorsal skin were observed, irrespective of bleaching. For gelatin from ventral skin, bleaching using 2% and 5% H_2O_2 for 48 h resulted in lower *EAI* ($p < 0.05$), compared with 24 h bleaching and no bleaching. It was presumed that bleaching of ventral skin for a long time caused aggregation of protein to a large extent. Aggregated proteins might be rigid and could not unfold rapidly at the interface and form a film around an oil droplet effectively.

Emulsions containing gelatin from bleached dorsal and ventral skin was more stable than that of the control ($p < 0.05$). Larger and longer peptides could stabilise the protein film at the interface more effectively. However, proteins oxidised to a higher degree might possess a lower ability for stabilising emulsions. It was noted that a longer bleaching time and higher H_2O_2 concentration led to a lower *ESI* of gelatin for all samples, except for gelatin from dorsal skin, in which the highest *ESI* was obtained when the skin was bleached with 5% H_2O_2 for 48 h ($p < 0.05$). Surh, Decker, and McClements (2006) found that the oil-in-water emulsion prepared with high molecular weight fish gelatin (~120 kDa) was more stable than that prepared with low molecular weight fish gelatin (~50 kDa). Thickness of an adsorbed gelatin membrane increased with increasing molecular weight. This was associated with the increased stability of emulsions to coalescence during homogenisation (Lobo & Svereika, 2003). *EAI* and *ESI* of bovine bone gelatin were higher than those of all cuttlefish skin gelatin samples. This possibly resulted from the differences in the intrinsic properties of proteins, composition and conformation of protein between gelatins from both sources (Damodaran, 1997).

3.6. Foaming properties of gelatin

Foam expansion (*FE*) and foam stability (*FS*) of gelatin extracted from cuttlefish skin with and without bleaching are shown in Table 3. Gelatin from unbleached skin, both dorsal and ventral, had a slightly lower *FE* than gelatin extracted from bleached skin ($p < 0.05$). However, bleaching condition had no marked impact on the *FE* of the resulting gelatin.

The foaming ability of proteins is related to their film-forming ability at the air–water interface. In general, proteins, which rap-

Table 2
Colour (L^*, a^* and b^*-values) of gel from gelatin extracted from dorsal and ventral cuttlefish skin with and without bleaching by H_2O_2 at different concentrations for various times.

Samples	Treatment	Colour[A]		
		L^*	a^*	b^*
Dorsal skin gelatin	Control	14.12 ± 0.30a[B]	18.08 ± 0.71d	8.86 ± 0.38a
	2% H_2O_2 24 h	41.66 ± 0.15b	12.61 ± 0.46c	19.81 ± 0.12e
	2% H_2O_2 48 h	41.43 ± 0.09b	6.80 ± 0.09b	19.17 ± 0.02d
	5% H_2O_2 24 h	48.68 ± 0.07c	6.34 ± 0.16b	13.68 ± 0.04c
	5% H_2O_2 48 h	48.89 ± 0.04c	5.87 ± 0.13a	12.77 ± 0.01b
Ventral skin gelatin	Control	47.78 ± 0.12a	11.20 ± 0.11d	15.56 ± 0.09e
	2% H_2O_2 24 h	63.65 ± 0.13c	−0.11 ± 0.36c	10.08 ± 0.06d
	2% H_2O_2 48 h	63.66 ± 0.05c	−1.02 ± 0.06a	5.77 ± 0.02a
	5% H_2O_2 24	63.16 ± 0.05b	−0.54 ± 0.03b	8.69 ± 0.03c
	5% H_2O_2 48	63.86 ± 0.04d	−1.06 ± 0.09a	6.35 ± 0.04b
Bovine bone gelatin		80.21 ± 0.19	−2.56 ± 0.18	14.39 ± 0.08

Mean ± SD from four determinations.
[A] 6.67% (w/v) gelatin.
[B] Different letters in the same column within the same gelatin indicate significant differences ($p < 0.05$).

Table 3
Emulsifying and foaming properties of gelatin extracted from dorsal and ventral cuttlefish skin with and without bleaching in H_2O_2 at different concentrations for various times.

Samples	Treatment	Emulsion[A] activity index (m^2/g)	Emulsion[A] stability index (min)	Foam expansion[A] (%)	Foam stability[A] (%)	
					30 min	60 min
Dorsal skin gelatin	Control	24.30 ± 0.51a[*]	15.14 ± 1.31a	170 ± 12.25a	89 ± 4.18aB[**]	76 ± 2.24aA
	2% H_2O_2 24 h	23.58 ± 1.20a	20.96 ± 2.97b	184 ± 8.94b	90 ± 0.00abB	81 ± 2.24abA
	2% H_2O_2 48 h	23.07 ± 2.56a	19.24 ± 2.81b	192 ± 4.47b	94 ± 2.24abB	82 ± 8.37abA
	5% H_2O_2 24 h	23.47 ± 1.23a	26.40 ± 1.71c	196 ± 5.48b	96 ± 2.24bcB	81 ± 2.24abA
	5% H_2O_2 48 h	22.05 ± 1.31a	17.84 ± 0.64ab	198 ± 8.94b	101 ± 8.22cB	86 ± 4.18bA
Ventral skin gelatin	Control	23.04 ± 0.21a	17.15 ± 0.29a	174 ± 4.18a	89 ± 5.48aA	82 ± 2.74aA
	2% H_2O_2 24 h	23.50 ± 0.99a	23.95 ± 0.86d	182 ± 10.95ab	91 ± 5.48aB	82 ± 2.74aA
	2% H_2O_2 48 h	18.91 ± 1.06b	21.10 ± 0.04c	190 ± 10.00b	93 ± 2.74aB	85 ± 4.18aA
	5% H_2O_2 24 h	22.72 ± 1.10a	21.20 ± 1.28c	192 ± 4.47bc	92 ± 2.74aB	78 ± 6.71aA
	5% H_2O_2 48 h	18.17 ± 1.00b	19.03 ± 1.19b	202 ± 8.37c	94 ± 5.48aB	80 ± 7.07aA
Bovine bone gelatin		28.27 ± 0.71	31.23 ± 0.90	190 ± 12.25	98 ± 7.58B	90 ± 0.00A

Mean ± SD from four determinations.
[A] Protein at a level of 1% (w/v) was used for determinations.
[*] Different letters in the same column within the same gelatin indicate significant differences ($p < 0.05$).
[**] Different capital letters in the same row of foam stability indicated significant differences ($p < 0.05$).

idly adsorb at the newly-created air–liquid interface during bubbling and undergo unfolding and molecular rearrangement at the interface, exhibit better foaming ability than proteins that adsorb slowly and resist unfolding at the interface (Damodaran, 1997).

At both 30 and 60 min, bleaching had no effect on *FS* of gelatin from ventral skin. Gelatin from dorsal skin bleached with 5% H_2O_2 for 48 h exhibited the highest *FS* at both 30 and 60 min ($p < 0.05$). No differences in *FS* were noticeable when 2% H_2O_2 was used, regardless of bleaching time.

Gravitational drainage of liquid from the lamella and disproportionation of gas bubbles *via* interbubble gas diffusion contribute to instability of foams (Yu & Damodaran, 1991). Coalescence of bubbles occurs because of liquid drainage from the lamella film as two gas bubbles approach each other, leading to film thinning and rupture (Damodaran, 2005). Thus, foam stability could be improved by bleaching the skin of cuttlefish with H_2O_2 under the appropriate conditions.

3.7. Protein patterns of gelatin with and without bleaching

Protein patterns of gelatins extracted from dorsal and ventral cuttlefish skin with and without bleaching using 5% H_2O_2 for 48 h are shown in Fig. 2. Gelatins extracted from both bleached and unbleached skin had smear protein bands with molecular weight equivalent to γ-chain, α-chain and less than α-chain. Proteins with smear bands were generated during extraction. Muyonga et al. (2004) reported that during conversion of collagen to gelatin, the inter- and intra-molecular bonds linking collagen chains as well as some peptide bonds are broken. The more severe the extraction process, the greater the extent of hydrolysis of peptide bonds was obtained. Without bleaching, skin matrix was den-

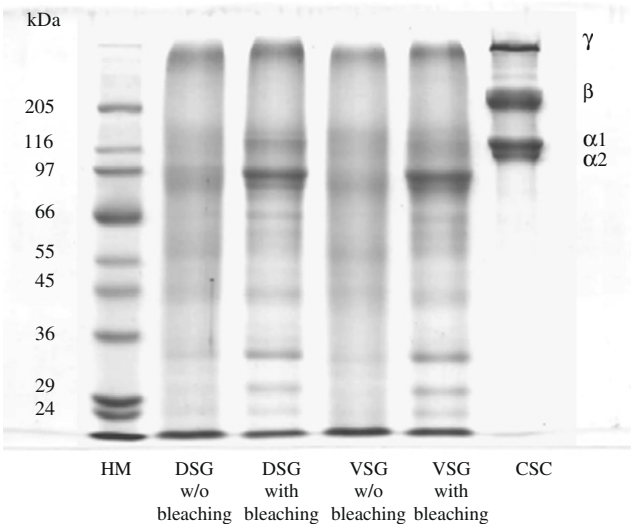

Fig. 2. SDS–PAGE pattern of gelatin extracted from dorsal (DSG) and ventral (VSG) cuttlefish skin with and without bleaching using 5% H_2O_2 for 48 h. HM and CSC denote high MW protein markers and collagen type I, respectively.

ser and the conversion of collagen to gelatin was less effective. Gelatin from dorsal and ventral skin with prior bleaching contained proteins with molecular weight of about 97 kDa as the dominant component. This suggested that the peroxide decomposition products, such as the hydroxyl radicals and superoxide anion radicals (O_2^-), were presumed to destroy H-bond-stabilising α-chains, resulting in increased extractability. However, the MW of 97 kDa protein was slightly lower than that of α1 and α2. H_2O_2 might induce some fragmentation of α-chain, leading to a slightly lower MW (97 kDa). Collagen extracted from both cuttlefish and squid skin was composed mainly of α-chains and low content of dimer (β-components) and higher molecular weight aggregates (γ-components and other) (Gómez-Guillén et al., 2002; Kolodziejska et al., 1999; Nagai, Yamashita, Taniguchi, Kanamori, & Suzuki, 2001).

3.8. FTIR spectra of gelatin

FTIR spectra of gelatin extracted from dorsal and ventral skin with and without bleaching in 5% H_2O_2 for 48 h are shown in Fig. 3. FTIR spectroscopy has been used to study changes in the secondary structure of gelatin. Spectra of both dorsal and ventral skin gelatin displayed major bands at 3264 cm^{-1} (amide A, representative of NH-stretching, coupled with hydrogen bonding), 1628 cm^{-1} (amide I, representative of C=O stretching/hydrogen bonding coupled with COO^-), 1550 cm^{-1} (amide II, representative of NH bending, coupled with CN stretching) and 1240 cm^{-1} (amide III, representative of NH bending). FTIR spectra of cuttlefish skin gelatin were similar to those found in other gelatins (Muyonga et al., 2004).

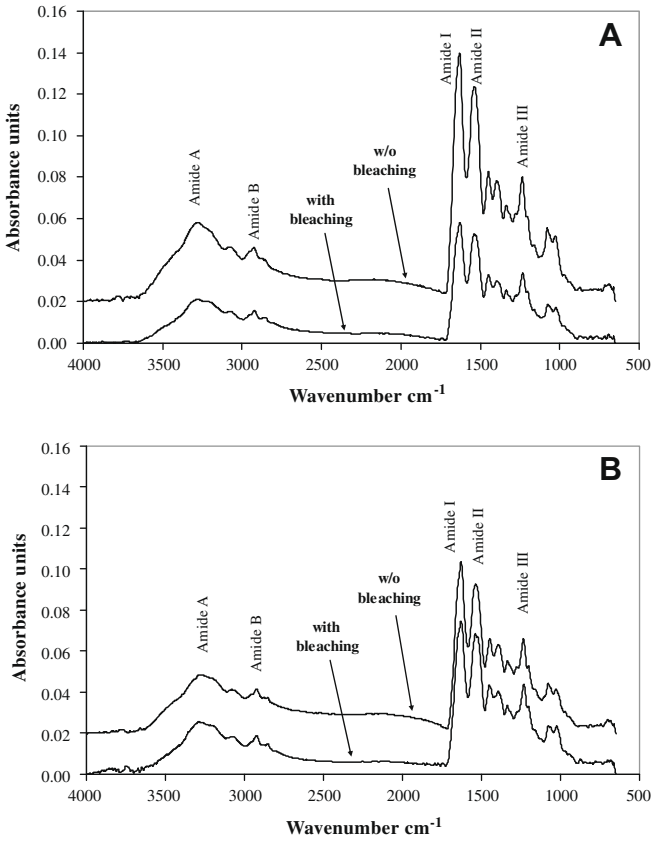

Fig. 3. Fourier transform infrared (FTIR) spectroscopic spectra of gelatin extracted from dorsal (A) and ventral (B) cuttlefish skin with and without bleaching using 5% H_2O_2 for 48 h.

Similar spectra were observed between gelatin from skin with and without bleaching for both dorsal and ventral skin (Fig. 3A and B). Bleaching skin with H_2O_2 resulted in decreases in the intensity of amide A, I, II and III bands of gelatin from both dorsal and ventral skin. These changes are indicative of greater disorder (Friess & Lee, 1996) in gelatin and are associated with loss of triple helix state (Muyonga et al., 2004). The shift to lower wave number was observed in gelatin from ventral skin bleached with 5% H_2O_2. However, no shift was noticeable in gelatin from dorsal skin. The amine A, I and II peaks of gelatin extracted from bleached ventral skin (3264, 1628 and 1535 cm^{-1}, respectively) had lower wave numbers than those of gelatin from unbleached ventral skin (3289, 1629 and 1538 cm^{-1}, respectively).

Muyonga et al. (2004) reported that the amide I and II peak of collagen extracted from adult Nile perch was at a higher frequency than the young fish skin collagen, due to more intermolecular cross-links in the adult fish collagen. A shift of peaks to lower wave numbers is associated with a lower molecular order (Payne & Veis, 1988). The amide I is the most useful peak for infrared analysis of the secondary structure of protein including gelatin (Surewicz & Mantsch, 1988). Yakimets et al. (2005) reported that the absorption peak at 1633 cm^{-1} was characteristic for the coil structure of gelatin. The change in amide I band of gelatin suggested that the use of H_2O_2 might affect the helix coil structure of gelatin. This result suggested that hydrogen peroxide might induce the changes in secondary structure and functional groups of resulting gelatin, associated with the increased intermolecular interactions and denaturation of gelatin.

4. Conclusion

Bleaching of cuttlefish skin with 5% H_2O_2 not only improved the colour of resulting gelatin but also enhanced the bloom strength effectively. Furthermore, bleaching could increase the yield of gelatin. Bleaching also improved emulsifying and foaming properties of the resulting gelatin, mostly *via* the oxidation of the gelatin molecule.

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