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Extraction and characterisation of gelatin from the skin of striped catfish (*Pangasianodon hypophthalmus*) and studies on its colour improvement

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Functional properties of gelatin from skin of striped catfish with and without bleaching for 48 h by 5% H_2O_2 (w/v)) were studied. Gelatin from skin bleached with 5% H_2O_2 for 48 h showed the highest yield (16.18 g). Bleaching not only improved the colour of gelatin gel by increasing the L* (lightness)-value and decreasing a*(redness/greenness)-value, but also enhanced the bloom strength and the emulsifying and foaming properties of the resulting gelatin. 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, hence improved functional properties.

Key words: Gelatin, catfish, color, bloom strength.

INTRODUCTION

Gelatin, the denatured form of collagen, has been extensively applied in the food industry as an ingredient to improve the elasticity, consistency and stability of foods. Its parent form, collagen, constitutes approximately 30 g/100 g of total animal protein. Skin, bones, the vascular system, tendons and the connective tissue sheaths surrounding muscle are the major sources of collagen. In general, gelatin is manufactured from the waste generated during animal slaughter and processing, that is, skin and bone (Patil et al., 2000). Generally, pig and cow skin and bones are the main sources of gelatin. Recently, outbreaks of mad cow disease (bovine spongiform encephalopathy, BSE) have caused anxiety for customers. Additionally, the gelatin obtained from pig skin and bone cannot be used in kosher and halal foods due to religious constraints (Sadowska et al., 2003), while as Sikhs and Hindus, they do not use bovine gelatin due to religious constraints (Singh et al., 2011). Furthermore, an increasing attention to health issues of consumers has also gained momentum. As a consequence, increasing interest has been paid to alternative sources of gelatin, especially from the skins and bones from fish processing by-products (Kittiphattanabawon et al., 2005). In addition, the collagens extracted from bovine sources are prohibited for Sikhs and Hindus, whilst porcine collagen cannot be consumed by Muslims and Jews, both of whom require bovine to be religiously prepared. As a consequence, the alternative sources of collagen,

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especially from aquatic animals including freshwater and marine fish and mollusks have received increasing attention (Singh et al., 2011).

Pangasianodon hypophthalmus or Pla Sawai (in Thai), a large freshwater catfish, belongs to the order Siluriformes and is a member of Pangasidae family. It is one of the most important aquaculture species in Thailand (Froese and Pauly, 2007), especially in the northeast part of Thailand. This fish is also known as Siamese shark or sutchi catfish and is native to the Chao Phraya River in Thailand and the Mekong in Vietnam. It has become an important fish for many countries like Indonesia, Malaysia and China (Roberts and Vidthayanon, 1991). This freshwater fish normally lives in a tropical climate and prefers water with a pH of 6.5 to 7.5 and a temperature range of 22 to 26°C. Adults reach up to 130 cm (4 ft) in length and can weigh up to a maximum of 44.0 kg (97 lb) (Roberts and Vidthayanon, 1991). Its meat has been popular among the consumers worldwide. During processing and filleting, a huge amount of skin from this fish is generated as a byproduct, which can be used as a potential source for collagen extraction. The skin from this fish is thick and tough, which may be associated with the collagen cross-links, especially cross-linking caused by hydroxylysine. The information on composition and molecular properties of collagen from the skin of this species has been reported in our previous study (Singh et al., 2011). The mother substrate of the gelatin obtained from this species was a bit darker in color and hence it was apprehended that its gelatin may not draw the attention for industrial uses. 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 et al., 1999; Thanonkaew et al., 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 hemolytic cleavage of O–H or O–O bonds, with the formation of highly reactive products: hydroperoxyl anion (HOO^-), hydroperoxyl (HOO^-) and hydroxyl (OH^-) 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 et al., 2006). Hence, this study was undertaken to isolate and characterize gelatin, to study the effect of H_2O_2 on the bleaching of striped catfish skin and its impact on the functional properties of resulting gelatin.

MATERIALS AND METHODS

Chemicals

Sodium dodecyl sulfate (SDS), acetic acid, hydrogen peroxide (H_2O_2), and sodium hydroxide were obtained from Merck (Darmstadt, Germany). Methane sulfonic acid, citrate buffer, calf skin gelatin (bloom strength 120 to 150 g) and calf skin acid-soluble type I collagen were from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). All chemicals for electrophoresis were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Fish skin preparation

Whole fresh farmed striped catfish (*P. hypophthalmus*) (approximately 2 years old) weighing 1 ± 0.5 kg/fish stored on ice were procured from the local fish market of Hatyai, Thailand. Fish were stored in ice with a fish/ice ratio of 1:2 (w/w) and transported within 1 h to the laboratory. Upon arrival, fish were washed using tap water and deskinned. The skin was washed with cold water (5 to 8°C) and cut into small pieces (0.5 to 0.5 cm^2). The prepared skin samples were packed in polyethylene bags and kept at 20°C until used. The storage time was not longer than 1 month.

Extraction of fish skin gelatin

Gelatin was extracted from washed giant catfish skin as described by Jongjareonrak et al. (2006). To remove non-collagenous proteins and pigments, washed skin was soaked in 0.2 mol/L NaOH with a skin to solution ratio of 1:10 (w/v) at 4 ±1°C with a continuous gentle stirring. The solution was changed every 40 min for three times. Alkaline-treated skin was then washed with tap water until neutral or faintly basic pH (pH<7.5) of wash water was obtained. To remove the fat content of the skin, it was treated with butanol with a skin to solution ratio of 1:10 with a continuous stirring at 4°C. The solution of the skin was changed every 8 h and six defatting changes were done. Butanol treated skin was washed with ten volumes of water till the water pH become basic. The prepared skin was subjected to bleaching in 2% H_2O_2 using a sample:solution ratio of 1:10 (w/v) for 48 h. Bleached samples were washed three times with 10 volumes of water. The alkali-treated skin without bleaching was used as the control. To swell, the collagenous material in the fish skin matrix, the alkaline-treated skin was soaked in 0.05 mol/L acetic acid with a skin to solution ratio of 1:10 (w/v) for 3 h at room temperature (25±1°C) with a continuous gentle stirring with change of the solution at hourly intervals. Acid-treated skin was washed as previously described. The swollen fish skin was soaked in distilled water with a skin/water ratio of 1:10 (w/v) at 45±1°C for 12 h with continuous stirring to extract the gelatin. The mixture was then filtered using two layers of cheese cloth. The resultant filtrate was freeze dried and the dry matter from freeze-dried process was ground and referred to as ‘‘gelatin powder’’.

SDS-Polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed using the method of Laemmli (1970). Gelatin samples were dissolved in 5% SDS solution. The mixtures were then heated at 85°C for 1 h, followed by centrifugation at 8500 ×g for 5 min using a microcentrifuge (MIKRO20, Hettich Zentrifugan, Germany) to remove undissolved debris. Solubilized samples were mixed at 1:1 (v/v) ratio with the sample buffer (0.5 M Tris-HCl, pH 6.8 containing 4% SDS and 20% glycerol). Samples were loaded onto polyacrylamide gel made of 7.5% separating gel and 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA/gel. After electrophoresis, gels were stained for 1 h with a mixture of 0.05% (w/v) Coomassie blue R-250 in 15% (v/v) methanol and 5% (v/v) acetic acid and destained for 1 h with a mixture of 30% (v/v) methanol and 10% (v/v) acetic acid and destained again with the same mixture for 30 min. High molecular weight markers (GE Healthcare UK Limited, Buckinghamshire, UK) were used to estimate the molecular weight of proteins.

Fourier transform infrared (FTIR) spectroscopy

FTIR analysis of freeze-dried gelatins of the bleached and non bleached skins was conducted and compared. A Bruker Model EQUINOX 55 FTIR spectrometer (Bruker, Ettlingen, Germany) equipped with a deuterated L-alanine triglycine sulfate (DLATGS) detector was used. The horizontal attenuated total reflectance (HATR) accessory was mounted into the sample compartment. The internal reflection crystal (Pike Technologies, Madison, WI, USA), 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 to 650 cm^{-1} (mid-IR region) at room temperature. Automatic signals were collected in 32 scans at a resolution of 4 cm^{-1} and were ratioed against a background spectrum recorded from the clean, empty cell at 25°C.

Bloom strength

Gelatin gel was prepared as per the British Standard 757: 1975 (BSI, 1975) with a slight modification. Gelatin was dissolved in distilled water (60°C) to obtain a final concentration of 6.67% (w/v). The solution was stirred until gelatin was solubilized completely and transferred to a cylindrical mold with 3 cm diameter and 2.5 cm height. The solution was incubated at the refrigerated temperature (4°C) for 18 h prior to the analysis. The bloom strength was determined according to the British Standard 757: 1975 method (BSI, 1975). The bloom strength was determined at 8 to 10°C using a texture analyzer (Stable Micro System, Surrey, UK) with a load cell of 5 kg, cross-head speed of 1 mm/s, equipped with a 1.27 cm diameter flat-faced cylindrical Teflon® plunger. The maximum force (in grams), taken when the plunger had penetrated 4 mm into the gelatin gels, was recorded. The measurement was performed in triplicate.

Determination of colour

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

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:

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

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 (g/m^3);

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

where A_{500} = absorbance at 500 nm, $\Delta A = A_0 - A1_0$, and Δt = 10 min.

Determination of foaming properties

Foam expansion (FE) and foam stability (FS) of gelatin solutions were determined, as described by Shahidi et al. (1995) with a slight modification. Gelatin solution with 1% protein concentration was transferred into 100 ml cylinders. The mixtures were homogenized 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:

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

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

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

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 and Torrie, 1980). SPSS statistical program (Version 10.0) (SPSS Inc., Chicago, IL) was used for the data analysis.

RESULTS AND DISCUSSION

Yield

Gelatin was extracted from the skin of striped catfish with yields as shown in Table 1 on a dry weight basis. The gelatin yield recoveries have been reported to vary among fish species, mainly due to the differences in collagen content, the compositions of the skin as well as the skin matrix. The yields of gelatin obtained from fish skin have been reported for sin croaker (14.3 g/100 g), shortfin scad (7.25 g/100 g) (Cheow et al., 2007), big eye snapper (6.5 g/100 g) and brown stripe red snapper (9.4 g/100 g) (Jongjareonrak et al., 2006), Atlantic salmon (15.3 g/100 g) and cod (11.8 g/100 g) by a 2 step extraction (Arnesen and Gildberg, 2007). As shown in Table 1, it was found that the gelatin yield was higher in bleached samples as compared to non bleached samples and gelatin yield increased with increase in defatting changes. 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 to 24 h. In the presence of sufficient H_2O_2 (5%), hydrogen bonds of collagen molecules in striped catfish skin might be broken, resulting in an increased efficiency in gelatin extraction, as evidenced by the increased yield.

SDS page pattern of gelatin

Protein patterns of gelatin from the skin of striped catfish treated with and without H_2O_2 are as shown in Figure 1a and b, respectively. In general, no differences were seen

Table 1. Yield of gelatin isolated from skin of striped cat fish *(Pangasius hypothalamus*).

Sample	Treatment	Yield (g)
Skin gelatin without bleaching	Control	5.38 ± 0.01
	2 Defatting changes	4.15 ± 0.01
	4 Defatting changes	5.98 ± 0.01
	6 Defatting changes	7.54 ± 0.01
Skin gelatin with bleaching	Control	6.66 ± 0.03
	2 Defatting changes	12.68 ± 0.04
	4 Defatting changes	14.02 ± 0.02
	6 Defatting changes	16.18 ± 0.01

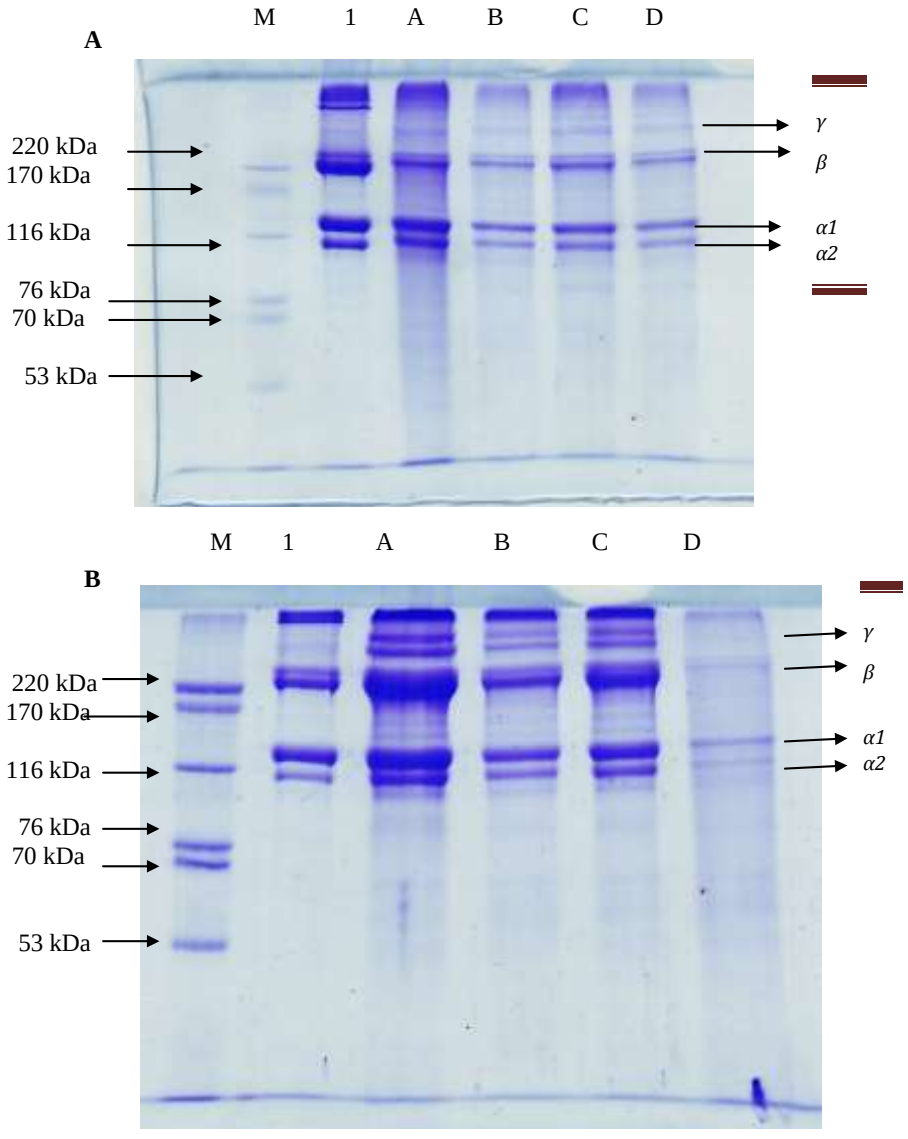

Figure 1. Protein patterns of the skin gelatin from striped catfish (A) with and (B) without H_2O_2 treatment (*Pangasius hypothalamus*). 1: type 1 calf skin collagen, A: gelatin with no defatting, B: gelatin with two defatting changes, C: gelatin with four defatting changes, D: gelatin with six defatting changes. M: Molecular weight markers.

in protein patterns of gelatin under bleached and non bleached conditions. α1- and α2-chains were found as the major components and similar to that of standard collagen type I. 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 obtained. Without bleaching, skin matrix was denser and the conversion of collagen to gelatin was less effective. 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. 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 composed mainly of α-chains and low content of dimmer (β-components) and higher molecular weight aggregates (γ-components) (Gomez-Guillen et al., 2002; Nagai et al., 2001).

FTIR spectra of gelatin

FTIR spectra of gelatin extracted from skin of striped cat fish with and without bleaching in 5% H_2O_2 for 48 h are as shown in Figure 2a and b, respectively. FTIR spectroscopy has been used to study changes in the secondary structure of gelatin. Spectra of both bleached and non-bleached 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 striped catfish skin gelatin were similar to those found in other gelatins (Muyonga et al., 2004). Bleaching skin with H_2O_2 resulted in decreases in the intensity of amide A, I, II and III bands of gelatin in case of both bleached and non-bleached skin. These changes are indicative of greater disorder (Friess and Lee, 1996) in gelatin and are associated with loss of triple helix state (Muyonga et al., 2004). 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 and Veis, 1988). Amide I is the most useful peak for infrared analysis of the secondary structure of protein including gelatin (Surewicz and Mantsch, 1988).

Yakimets et al. (2005) reported that the absorption peak at 1633 cm^{-1} was the 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.

Bloom strength

The effect of bleaching of cuttlefish skin with H_2O_2 on bloom strength of gelatin gels is shown in Table 2. The lowest bloom strength was observed in gels of the control gelatin when compared with bleached skin gelatins. 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 to 260 g are the most desired. Bleaching 5% H_2O_2 for 48 h resulted in marked increases in bloom strength ($p < 0.05$). Bloom strength of gelatin gel from striped catfish skin increased with increasing defatting changes ($p < 0.05$). The highest bloom strength of gelatin was obtained from skins with six defatting changes followed by bleaching in 5% H_2O_2 for 48 h (316.63 ± 2.94 g). Bloom strength of the resulting gelatins was 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. These 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^- can abstract H atoms from amino acid residues to form carbon-centered 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.

Colour

L*, a* and b*-values of gelatin gels from striped cat fish skin with and without bleaching under different conditions are shown in Table 3. 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 5% H_2O_2 solution could improve the colour of gelatin gel by increasing L*-value and decreasing a*-value. For fish skin, H_2O_2 treatment resulted in increases in L* and lower a*-value of the resulting gelatin ($p < 0.05$). In general, the control gel from dorsal skins had higher colour intensity than the gel, most likely due to the higher content of chromatophore. Thus, higher H_2O_2 concentration was necessary for

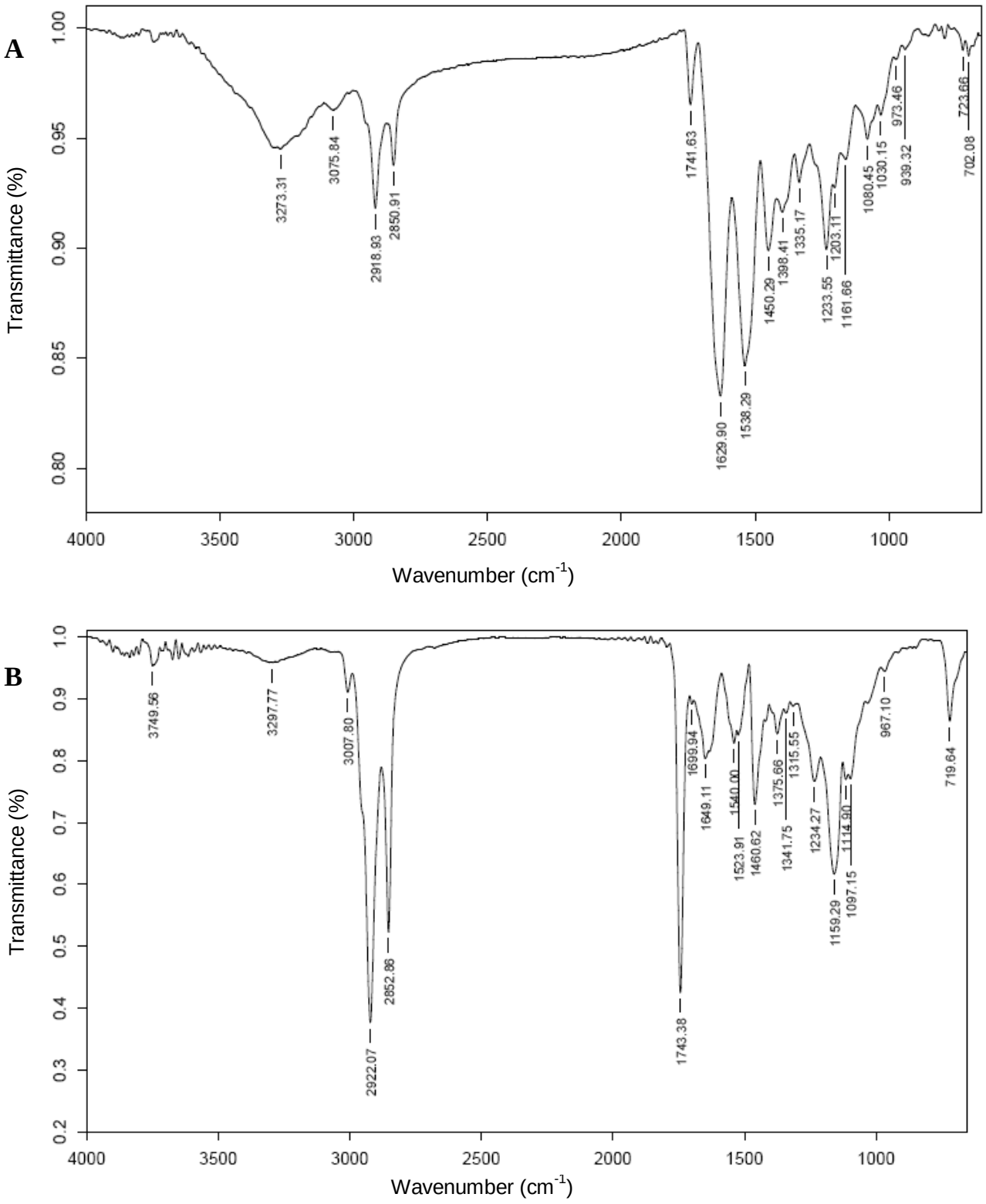

Figure 2. FTIR spectra of gelatin from skin of striped catfish (A) with no and (B) with bleaching.

improvement of colour of gelatin. The fish 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,

Table 2. Bloom strength of gelatin extracted from skin of striped cat fish *(Pangasius hypothalamus*) treated with and without H_2O_2.

Sample	Treatment	Bloom strength (g)
Skin w/o bleaching	Control	233.56 ± 6.51^{c}
	2 Defatting changes	237.33 ± 3.91^{c}
	4 Defatting changes	268.37 ± 7.1^{c}
	6 Defatting changes	292.08 ± 8.36^{c}
Skin with bleaching	Control	242.75 ± 4.3^{a}
	2 Defatting changes	294.87 ± 1.87^{a}
	4 Defatting changes	303.10 ± 2.02^{a}
	6 Defatting changes	316.63 ± 2.94^{a}

Data represent mean ± SD of four determinations. Letter in the same column with different superscripts differ significantly ($p<0.05$).

Table 3. Color of gelatin extracted from skin of striped catfish *(Pangasius hypothalamus*) with and without H_2O_2 treatment.

Sample	Treatment	L	A	B
Skin gelatin w/o bleaching	Control	62.69 ± 0.06^{a}	71 ± 0.56^{c}	5.56 ± 0.43^{d}
	2 Defatting changes	66.63 ± 0.02^{a}	-0.57 ± 0.58^{c}	6.48 ± 0.07^{d}
	4 Defatting changes	68.08 ± 0.08^{a}	-0.76 ± 0.72^{c}	7.08 ± 0.11^{d}
	6 Defatting changes	70.14 ± 0.45^{a}	0.24 ± 0.19^{c}	10.41 ± 0.05^{d}
Skin gelatin with bleaching	Control	47.25 ± 0.00^{b}	2.28 ± 0.02^{c}	17.13 ± 0.02^{d}
	2 Defatting changes	68.71 ± 0.01^{b}	-0.64 ± 0.58^{c}	14.11 ± 0.03^{d}
	4 Defatting changes	59.76 ± 0.14^{b}	-0.97 ± 0.58^{c}	14.69 ± 0.10^{d}
	6 Defatting changes	63.30 ± 0.02^{b}	-1.17 ± 0.58^{c}	11.90 ± 0.06^{d}

Data represent mean ± SD of four determinations. Letter in the same column with different superscripts differ significantly ($p<0.05$) while letter in the same row with differ superscripts differ significantly.

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^{-}) 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.

Emulsifying properties of gelatin

EAI and ESI of gelatin from striped catfish skin with and without bleaching are shown in Table 4. For gelatin from bleached skin, bleaching using 5% H_2O_2 for 48 h resulted in lower EAI ($p < 0.05$), compared with skin without bleaching. It was presumed that bleaching of fish 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 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 the control ($p < 0.05$). Surh et al. (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 homogenization (Lobo and Svereika,

Table 4. Emulsifying properties. Emulsifying properties of the gelatin extracted from the skin of striped catfish *(Pangasius hypothalamus*) with and without H_2O_2 treatment.

Sample	Treatment	Emulsion activity index (m^2/g)	Emulsion stability index (min)
Skin gelatin w/o bleaching	Control	16.57 ± 0.06^{a}	15.14 ± 0.05^{c}
	2 Defatting changes	15.64 ± 0.21^{a}	18.57 ± 0.55^{c}
	4 Defatting changes	16.16 ± 0.07^{a}	18.28 ± 0.96^{c}
	6 Defatting changes	16.89 ± 0.05^{a}	35.92 ± 3.29^{c}
Skin Gelatin with bleaching (48 h)	Control	11.91 ± 0.06^{b}	16.96 ± 0.16^{d}
	2 Defatting changes	11.21 ± 0.72^{b}	22.18 ± 0.23^{d}
	4 Defatting changes	13.30 ± 0.26^{b}	34.89 ± 2.29^{d}
	6 Defatting changes	11.80 ± 0.27^{b}	31.17 ± 0.76^{d}

Data represent mean ± SD of four determinations. Letter in the same column with different superscripts differ significantly ($p<0.05$) while letter in the same row with different superscripts differ significantly.

Table 5. Foaming properties. Emulsifying properties of the gelatin extracted from the skin of striped catfish *(Pangasius hypothalamus*) with and without H_2O_2 treatment.

Sample	Treatment	Foam expansion (%)	Foam stability (%)	
			30 min	60 min
Skin gelatin w/o bleaching	Control	112.5 ± 10.61^{a}	127.50 ± 10.61^{c}	122.50 ± 10.61^{da}
	2 Defatting changes	127.50 ± 3.54^{a}	112.50 ± 3.54^{c}	107.50 ± 3.54^{da}
	4 Defatting changes	136.50 ± 0.71^{a}	130.50 ± 0.71^{c}	125.50 ± 0.71^{da}
	6 Defatting changes	127.50 ± 3.54^{a}	122.50 ± 3.54^{c}	117.50 ± 3.54^{da}
Skin gelatin with bleaching (48 h)	Control	119 ± 1.41^{b}	115.00 ± 0.00^{d}	110.00 ± 0.00^{ba}
	2 Defatting changes	127.50 ± 3.54^{b}	135.00 ± 0.00^{d}	118.00 ± 0.00^{ba}
	4 Defatting changes	131.50 ± 7.78^{b}	143.50 ± 3.54^{d}	128.00 ± 4.24^{ba}
	6 Defatting changes	142.00 ± 7.07^{b}	151.00 ± 7.07^{d}	138.00 ± 7.07^{ba}

Data represent mean ± SD of four determinations. Letter in the same column with different superscripts differ significantly ($p<0.05$) while letter in the same row with different superscripts differ significantly.

2003). This possibly results from the differences in the intrinsic properties of proteins, composition and conformation of protein between gelatins from different sources (Damodaran, 1997).

Foaming properties of gelatin

FE and FS of gelatin extracted from cuttlefish skin with and without bleaching are shown in Table 5. Gelatin from unbleached skin had a slightly lower FE than gelatin extracted from bleached skin ($p < 0.05$). The foaming ability of proteins is related to their film-forming ability at the air-water interface. In general, proteins, which rapidly 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 with 5% H_2O_2 for 48 h exhibited the highest FS ($p < 0.05$). Gravitational drainage of liquid from the lamella and disproportionation of gas bubbles via inter-bubble gas diffusion contribute to instability of foams (Yu and 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 catfish with H_2O_2 under the appropriate conditions.

Conclusion

Bleaching of striped catfish 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.

Conflict of interests

The authors have not declared any conflict of interests.

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