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Effect of pretreatment conditions and bleaching on physico-chemical and functional properties of gelatin prepared from cuttlefish skin

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ABSTRACT

Gelatin was extracted from the skin of cuttlefish *Sepia pharaonis* after pretreatment with sodium hydroxide (NaOH) and bleaching with hydrogen peroxide (H_2O_2) for varying durations. The extracted gelatin was lyophilised and quality characteristics were assessed. There was a significant increase (15.5-17.1%) in the yield of gelatin extracted from skin treated with 5% H_2O_2 for 24 h when compared to control samples. Viscosity of the gelatin samples were in the range of 3.76-6.54 cP which can be considered to be in the mid-range since viscosities for commercial gelatin have been reported to be in the range 2.0-7.0 cP. Bleaching the skin with H_2O_2 significantly improved the bloom strength of extracted gelatin. Maximum bloom strength was obtained for gelatin extracted from skin bleached for 48 h (133-140 g) whereas it was only in the range of 22-28 g for gelatin from unbleached skins. Gelatin from treated skin samples had significantly higher L* values in the range of 90.4-92.8 than control samples indicating superior white colour. Functional properties *viz.,* foaming and fat binding properties were also significantly improved by bleaching. Gelatin extracted from the ventral skin of cuttlefish after bleaching with H_2O_2 exhibited characterestics of Type B gelatin with desirable functional properties suitable for commercial utilisation. The skin of cuttlefish, which is considered to be an environmental hazard as a processing waste has the potential to yield good quality gelatin.

Keywords: Bleaching, Cuttlefish skin, Functional properties, Gelatin, Pretreatment

Introduction

Gelatin is derived by thermal degradation of collagen. It has multiple functional roles in food processing and formulations. Gelatin from marine sources (fish skins, bones and fins) is a possible alternative to bovine gelatin (Kim and Mendis, 2006; Rustad, 2003). One major advantage of marine sources of gelatin is that they are not associated with the risk of Bovine Spongiform Encephalopathy.

Processing of fish and shellfish leads to the generation of a large biomass of waste, and it is estimated that approximately 7.3 million t year is generally discarded (Kelleher, 2005). Fish processing generates solid wastes that can be as high as 50-80% of the original raw material (Wasswa *et al*., 2007). In India, industrial fish processing generates 302,750 t of waste (Anon., 2005). Among the maritime states, maximum fish processing waste generation is in Gujarat (30.51%) followed by Maharashtra (23%) and Kerala (17.5%). An important waste reduction strategy for the industry is the recovery of marketable byproducts from fish processing wastes. Utilisation of byproducts generates additional revenue and reduces disposal costs. Cuttlefish (*Sepia pharaonis*) is an important item in the Indian marine export trade. In 2010-11 India exported 59,159 t of frozen cuttlefish products valued at ₹1104.57 crores (Anon, 2012). The skin of cuttlefish form a significant component of waste generated during industrial fish processing in coastal regions of India. Thanonkaew *et al*. (2008) reported that soaking cuttlefish in a solution of 5% sodium chloride (NaCl) and 0.3% hydrogen peroxide (H_2O_2) together with 0.5% tripolyphosphate, improved the colour and decreased solubility and thaw drip in frozen cuttlefish. Soaking squid skin in 1% H_2O_2 in 0.01 M sodium hydroxide (NaOH) for 48 h improved colour of resulting collagen (Kolodziejska *et al.*, 1999). 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 *viz*., hydroperoxyl anion (HOO^-), and 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). Although cuttlefish skin can be a good source of gelatin, pigments in the skin may cause quality problems to the extracted gelatin.

The objective of the present work was to evaluate the physico-chemical and functional properties of the gelatin extracted from the skin of cuttlefish (*Sepia pharaonis*)

under different pre-treatment conditions and bleaching with hydrogen peroxide (H_2O_2) prior to extraction.

Materials and methods

Raw material

Ventral skin of cuttlefish (*Sepia pharaonis*) was collected from a preprocessing centre near Cochin and brought to the laboratory in iced condition. On reaching the laboratory, the skins were washed with cold tap water, drained, cut into small pieces (10-12 mm), packed in polythene bags and kept at −20°C until use, with frozen storage period not exceeding one month.

Extraction of gelatin

Gelatin was prepared as per the method of Gomez-Guillen *et al.* (2002) with some modifications on pretreatment conditions. Prior to gelatin extraction, the frozen cuttlefish skin was thawed and washed in running water. The cleaned cuttlefish skins were drained for 5 min, and squeezed manually to remove water. They were soaked in 0.05 N NaOH with a skin:solution ratio of 1:10 (w/v) for 4, 6 and 8 h at room temperature (26-28°C) with gentle stirring. The solution was changed every 1 h, to remove non-collagenous proteins. Alkali treated skin was washed with distilled water to neutralise pH. The pretreated skin was subjected to bleaching with 5% H_2O_2, at 1:10 (w/v) ratio for 24 and 48 h at 4°C. Bleached samples were washed three times with distilled water. The alkali-treated skin without bleaching was used as the control. Gelatin was extracted from the alkali treated skin with and without bleaching using distilled water at 60°C for 16 h, with sample:water ratio of 1:2 (w/v). During extraction, the mixture was stirred occasionally. The extracts were filtered and the filtrate was freeze dried in a lyophiliser (Gamma LSC 1-16, Martin Christ, GmBH, Osterode, Germany).

Proximate composition and pH

The moisture, protein, fat and ash contents of the raw skin and extracted gelatin were determined as per AOAC (2000) methods. For protein determination in gelatin sample, a nitrogen conversion factor of 5.4 was used as per Eastoe and Eastoe (1952). The pH of the gelatin solution was measured as per, British Standard 757: 1975 method (BSI, 1975).

Yield

The yield of gelatin was calculated as described by Muyonga *et al.* (2004). The following equation was used for gelatin yield calculation:

$$\text{Yield (\%)} = \frac{C \times V}{M} \times 100$$

where C = light liquor concentration (g ml^{-1}), V = liquor volume (ml) and M = weight of skin sample (g) used for extraction.

Viscosity

Viscosity was measured as per the method described by Cho *et al.* (2005). The viscosity (cP) of 10 ml of the gelatin solution of 6.67% (w/v) was determined using Brookfield digital viscometer (Model DVE Brookfield Engineering, USA) equipped with a No.1 spindle, at 30±0.5°C.

Colour

Colour analysis was performed with a Hunter lab Miniscan ®XE plus spectrocolorimeter (Hunter Associates Laboratory, Inc. Reston, Virginia, USA). Measurements were recorded using the L* a* *b** colour scale (CIE, 1986).

Melting point

Determination of melting point was based on the method by Wainewright (1977). Gelatin solutions 6.67% (w/w) were prepared and a 5 ml aliquot of each sample was transferred to a small culture test tube of 12 × 75 mm. The samples were degassed in a vacuum chamber (Heraeusvacutherm–Germany). The tubes were then covered with parafilm and heated in a water bath (Julabo TW 20, Germany) at 60°C for 15 min, cooled immediately in ice chilled water and matured at 10°C for 16-18 h. Five drops of a mixture of 75% chloroform and 25% red dye were placed on the surface of the gel. The gels were then put in a water bath (circulating bath – Haake D3 Germany) at 10°C and the water heated at the rate of 0.2°C per min. The temperature at which the drops began to move freely down the gel was taken as the melting point.

Bloom

The bloom (gel strength) was determined as per BSI (1975) using a texture analyser (Lloyd Instruments, Model LRX Plus, U.K.)

Foam expansion and foam stability

Foam expansion (FE) and foam stability (FS) of gelatin solutions were determined, as described by Aewsiri *et al.* (2009). Gelatin solution with 1% protein concentration was transferred to 100 ml cylinders and homogenised at 13,400 rpm for 1 min at room temperature. The sample was allowed to stand for 30 min. FE and FS were then calculated using the following equations:

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

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

where V_T is total volume after homogenising, V_0 is the original volume before whipping and V_t is the total volume after leaving at room temperature for 30 min.

Fat binding capacity

Fat binding capacity of gelatin was determined as described by Cho *et al.* (2004). One gram of gelatin was placed in a centrifuge tube and 10 ml refined sunflower oil was added and held at room temperature for 1 h. It was mixed using vortex mixer for 5 seconds in every 15 min. The gelatin solutions were then centrifuged at 450 g for 20 min in a REMI cooling centrifuge (Model CPR 24, REMI Instruments, India). The supernatant was filtered with Whatman No. 1 filter paper and the volume recovered was measured. The difference between the initial volume of sunflower oil added to the gelatin sample and the volume of the supernatant was determined, and the results were reported as ml of oil absorbed per gram of gelatin sample.

Statistical analysis

All data were analysed using analysis of variance (ANOVA) and Duncan's multiple range test was carried out to determine the significant difference between the means. Statistical package used in the study was SAS, Version 9.3. All the data represented are mean ± SD of triplicate analyses.

Results and discussion

Proximate composition and pH

The proximate composition of cuttlefish skin gelatin is shown in Table 1. Cuttlefish ventral skin contained 80.16% moisture, 16.11% protein, 1.20% fat, and 3.36% ash. Maximum protein content (91.26%) was observed for cuttlefish skin pretreated with NaOH for 4 h. Protein content decreased for samples with a longer duration of pretreatment with NaOH followed by bleaching with H_2O_2. The peroxide decomposition radicals such as hydroxyl radicals causes oxidation of proteins, resulting in the generation of carbonyl derivatives. Gelatin obtained from cuttlefish skin subjected to pretreatment with NaOH for 8 h had significantly less protein content. The protein content of cuttlefish skin gelatin was higher than that reported for fish skin gelatin from different sources such as brown stripe red snapper - 88.6% (Jongjareonrak *et al*., 2006); sin croaker - 69.2% and short fin scad (Cheow *et al.,* 2007); yellowfintuna - 78.1% (Rahman, *et al.*, 2008) and Niletilapia - 89.4% (Songchotikunpan *et al.,* 2008).

The pH of the gelatin samples were in the range 5.51-6.12 (Table 1) and can be categorised as Type B gelatin. Type B gelatin is produced when the raw material is subjected to alkali pretreatment whereas Type A gelatin is produced by acid pretreatment of the raw material. The pH of Type A gelatin ranges from 3.8-5.5 and that of Type B gelatin from 5.0-7.5. Cole (2000) reported that for Type B gelatin, viscosity is minimal and gel strength is maximum at a pH of 5. Some functional properties of gelatin *viz.*, gel strength and turbidity are influenced by the pH. The gel strength can be controlled by adjusting pH close to the isoelectric point of the gelatin (Gudmundsson and Hafsteinsson, 1997). In this study there was significant increase of gel strength in samples treated with H_2O_2 (Fig.1). However, this cannot be attributed to the pH of the samples and the reasons are described elsewhere.

Yield

The yield of gelatin extracted from cuttlefish skin with and without H_2O_2 treatment is shown in Table 2. The yield of gelatin from whole skin without H_2O_2 bleaching was in the range of 5.70-8.89%. A significant increase was observed in the yield of gelatin from skin treated with 5% H_2O_2. The maximum yield (17.12%) was observed in sample A_1 obtained from pretreatment with NaOH for 4 h and 5% H_2O_2 for 24 h. In general, there was a near threefold increase in the yield of gelatin extracted from skin treated with 5% H_2O_2 for 24 h when compared to control samples except in sample C_1,

Table 1. Proximate composition and pH of gelatin extracted from ventral skin of cuttlefish under different pretreatment conditions

Gelatin	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	pH
A	6.48 ±0.2^a	91.26±1.67^a	0.51±0.01^a	0.75±0.07^a	5.73±0.12^a
A_1	6.20±0.15^a	90.15±2.01^a	0.48±0.08^a	0.68±0.03^a	5.94±0.12^a
A_2	5.90±0.11^a	89.88±1.50^a	0.45±0.03^b	0.78±0.02^a	5.54±0.11^b
B	6.11±0.16^a	88.22±1.67^a	0.40±0.01^c	0.43±0.03^b	5.80±0.12^a
B_1	6.26±0.21^a	88.40±0.91^a	0.49±0.01^a	0.45±0.02^b	5.51±0.05^b
B_2	5.80±0.13^a	88.17±2.29^a	0.47±0.02^b	0.46±0.02^b	5.64±0.02^b
C	5.87±0.13^a	86.93±0.60^b	0.45±0.01^b	0.77±0.04^a	6.11±0.07^a
C_1	6.15±0.18^a	87.10±1.56^b	0.40±0.02^c	0.76±0.01^a	6.10±0.04^a
C_2	6.23±0.12^a	86.39±1.01^b	0.42±0.02^c	0.79±0.03^a	6.12±0.05^a

A - Control - NaOH 4 h, A_1 - 5% H_2O_2 24 h, A_2 - 5% H_2O_2 48 h, B - Control - NaOH 6 h, B_1 - 5% H_2O_2 24 h, B_2 - 5% H_2O_2 48 h,
C - Control - NaOH 8 h, C_1 - 5% H_2O_2 24 h, C_2 - 5% H_2O_2 48 h.
*Mean ± SD of triplicate analyses. Values being different superscripts in the same column indicate significant difference ($p< 0.05$)

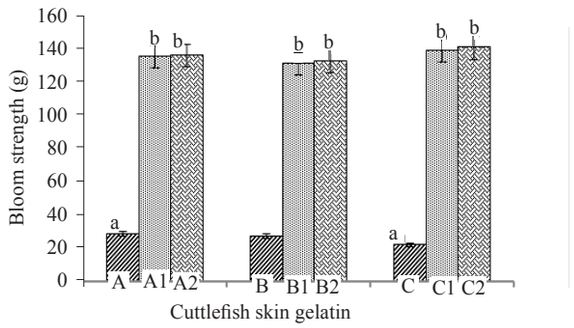

Fig. 1. Bloom strength of cuttlefish skin gelatin extracted under different pretreatment conditions*

A- Control - NaOH 4 h, A_1 - 5% H_2O_2 24 h, A_2 - 5% H_2O_2 48 h
B - Control - NaOH 6 h, B_1 - 5% H_2O_2 24 h, B_2 - 5% H_2O_2 48 h,
C - Control - NaOH 8 h, C_1 - 5% H_2O_2 24 h, C_2 - 5% H_2O_2 48 h,
*Bars represent standard deviation from average of triplicate analyses

where it was only 88.1% increase when compared to the control. The yield decreased when H_2O_2 treatment was prolonged for 48 h in all cases. Hydrogen peroxide 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, hydrogen bonds of collagen molecules in cuttlefish skin might have broken down, resulting in an increased efficiency in gelatin extraction. Aewsiri *et al*. (2009) reported a marked increase in the yield of gelatin extracted with H_2O_2 compared to the yield of the gelatin extracted without H_2O_2 bleaching. On an average the extraction yield of fish gelatin is between 6% and 19% (Karim and Bhat, 2009). The yield of gelatin from cuttlefish skin reported in the present study is higher than that reported from many fish sources (Binsi *et al.,* 2009; Sai-Ut *et al.,* 2010; George *et al*., 2011; Mehdi *et.al.,* 2011).

Viscosity

Viscosity of 6.67% (w/v) gelatin samples was in the range of 3.76 -6.54 cP (Table 2). Viscosity is the second most important commercial physical property of gelatin (Wards and Courts, 1977), which is partially controlled by molecular weight and polydispersity of the peptides. Significantly higher viscosity was obtained for the gelatin which was bleached with H_2O_2 for 48 h. Viscosity of the gelatin from cuttlefish skin can be considered to be in the mid-range since viscosities for commercial gelatin have been reported to be in the range from 2.0 to 7.0 cP for most gelatins and up to 13.0 cP for specialised ones (Johnston-Banks, 1990). The viscosity of gelatin solutions varies with source in terms of molecular weight and molecular size distribution of proteins, concentration, pH as well as temperature of the gelatin solutions (Sperling, 1985; Cho *et al.,* 2006; Arnesen and Gildberg, 2007).

Colour

Instrumental colour measurements of vacuum dried gelatin powders with and without bleaching under different conditions are shown in Table 2. Gelatin from skin without bleaching was more dark in colour, as indicated by a lower lightness (L*) value but a higher a* value, when compared with gelatin from bleached skin. Lightness (L*) value was highest for 48 h bleaching with 4 h NaOH treatment. The a* values was high for the control whereas it was negative for 24 h and 48 h bleaching, indicating a shift of colour towards green. The b* values were positive indicating the degree of yellowness. Significantly low b* value was shown by 48 h bleached and 4 h NaOH treated gelatin. Thus, soaking cuttlefish skin in 5% H_2O_2 solution improved the colour of gelatin gel by increasing L* value and decreasing a* value and b* value. In general, the control had higher colour intensity than the gel from bleached skins, most likely due to the higher content of chromatophores in the skin. The L* values reported by Aewsiri *et al.* (2009) for gelatin extracted from the dorsal and ventral skin of cuttlefish was lower than that reported in this study, indicating a better colour for gelatin prepared from the ventral skin of cuttlefish. Oxidising agents

Table 2. Physical properties of gelatin extracted from ventral skin of cuttlefish under different pretreatment conditions*

Gelatin	Yield (%)	Viscosity (cP)	Melting point (^{0}C)	Colour		
				L*	a*	b*
A	5.97± 0.90^a	5.73± 0.24^a	30.1 ± 0.8^a	73.28± 1.21^a	8.05± 1.10^a	9.32± 0.40^a
A_1	17.12± 2.11^b	5.94± 0.19^a	22.2 ± 0.5^b	90.62± 2.30^b	-0.72 ± 0.21^b	7.94± 0.90^b
A_2	12.56± 1.86^c	6.54± 0.11^b	22.5± 0.3^b	92.82± 2.22^c	-0.81 ± 0.34^b	7.71± 0.87^b
B	5.70 ± 0.55^a	3.76± 0.48^c	32.9 ± 0.8^a	75.89± 1.77^a	6.53± 0.19^a	8.69± 1.11^a
B_1	15.49± 3.18^b	4.77 ±0.21^d	19.5± 0.5^b	90.51± 1.03^b	-0.87± 0.29^b	9.43± 0.36^a
B_2	14.45± 3.20^b	6.01 ± 0.13^b	19.1 ± 0.5^b	90.44± 2.30^b	-0.91± 0.15^b	9.35± 0.25^a
C	8.89 ± 0.60^a	5.56± 0.10^a	30.9 ± 0.8^a	79.07± 2.77^a	5.42± 0.69^a	8.12± 0.72^a
C_1	16.72 ± 3.91^b	5.64 ± 0.25^a	18.9 ± 0.4^b	90.62± 2.41^b	-0.69± 0.27^b	7.94± 0.40^b
C_2	15.81± 2.83^c	6.12± 0.22^b	19.9 ± 0.5^b	90.88±0.81^b	-0.53± 0.50^c	7.63± 0.44^b

*Mean ± SD of triplicate analyses. Values being different superscripts in the same column within the same pretreatment and different bleaching conditions indicate significant differences ($p < 0.05$)

viz., hydroperoxyl anion, derived from the decomposition of H_2O_2 was 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). Also, 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 which was responsible for white color of the gelatin powder.

Melting point

Significant differences were observed in the melting temperatures of gelatins obtained by different treatments and bleaching (Table 2). Melting point was found to be high for gelatin from control samples (30.1-32.9°C) whereas it was found to be significantly low for gelatin from bleached skin (18.5–22.5°C). The melting temperature of gelatin has been found to correlate with the proportion of the imino acids proline and hydroxyproline in the original collagen (Piez and Gross, 1960; Veis, 1964; Ledward, 1986). The melting points of cuttlefish skin gelatins were lower compared with Alaska pollack skin and commercial bovine skin (Kim *et al*., 1994), but were higher compared with cod skin (Gudmundsson and Hafsteinsson, 1997). One of the major applications of fish gelatin with low melting point is in the microencapsulation of vitamins and other pharmaceutical additives and cuttlefish skin gelatin can be a potential material for this application. Another area of application can be in the manufacture of gel desserts which are to be stored at low room temperature.

Bloom

Bloom strengths of gelatin from cuttlefish skin subjected to different pretreatments are shown in Fig. 1. Bleaching with H_2O_2 significantly improved the bloom strength. Bloom strength was maximum for gelatin from 48 h bleached skin (133-140 g). Bloom strength of gelatin from unbleached skin was only 22-28 g. Bleaching time had no significant effect on the bloom strength. Hydrogen peroxide might induce oxidation of protein with 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. Moreover, OH^- 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 improved bloom strength. Oxidation of protein is associated with the alteration of protein structure, peptide chain scission, formation of amino acid derivatives and polymers, decrease in solubility and changes in the functional properties (Decker *et al.,* 1993). Although bleaching significantly improved the bloom strength of cuttlefish skin gelatin samples, these yielded only gelatins with low bloom strength when compared to mammalian and warm water fish species gelatin. For commercial products, gelatin is commonly divided into three categories based on the bloom *viz.*, low bloom gelatin (<125 Bloom), medium bloom gelatin (150-200 Bloom) and high bloom gelatin (> 220 Bloom). Mammalian gelatins of bovine and porcine origin have high bloom strength in the range of 250-350 g which renders them versatile with many applications. However there is a wide range of bloom strength for fish gelatins (0-250 g) with reported values above 400 g for tuna skin gelatin. Cuttlefish skin gelatin with low bloom values can be used as an ingredient at 0.5-1% level in wafers, juices and desserts.

Foam expansion (FE) and foam stability (FS)

Foam expansion (FE) and foam stability (FS) of cuttlefish skin gelatin are shown in Fig. 2a and b respectively. Minimum FE was observed in control samples *i.e.,* gelatin extracted without bleaching. The FE was maximum for gelatin samples extracted after pretreatment with 6 h NaOH followed by bleaching with 5% H_2O_2 and the FS was highest for gelatin samples extracted after bleaching with 5% H_2O_2 for 48 h. Foam formation

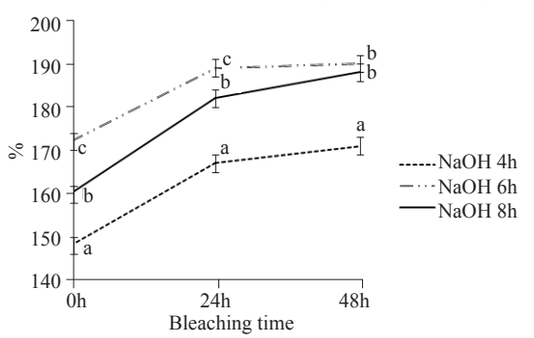

Fig. 2a. Foam expansion (%) of cuttlefish skin gelatin

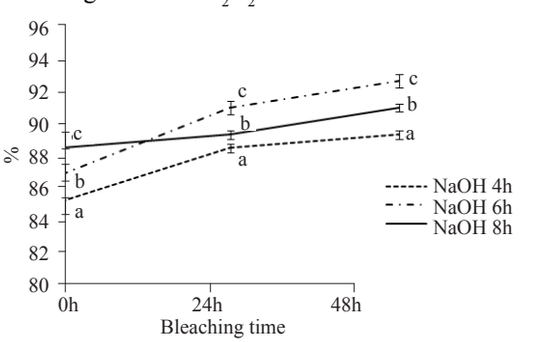

Fig. 2b. Foam stability (%) of cuttlefish skin gelatin

is generally controlled by transportation, penetration and reorganisation of protein molecules at the air–water interface. A protein must be capable of migrating rapidly to the air–water interface, unfolding and rearranging at the interface to express good foaming ability (Halling, 1981). Positive correlation between hydrophobicity of unfolded proteins and foaming characteristics has been reported by Townsend and Nakai (1983). The foaming capacity of protein was improved by making it more flexible, by exposing more hydrophobic residues and by increasing its capacity to decrease surface tension (Mutilangi *et al.*, 1996).

Foam stability of protein solutions is generally positively correlated with molecular weight of peptides (Van der Ven *et al.,* 2002). In addition, foam stability depends on the nature of the film and indicates the extent of protein–protein interaction within the matrix (Mutilangi *et al*., 1996). The decrease in FS might be due to gravitational force, causing water to drain. Air bubbles will come closer together during foam ageing (Zayas, 1997). 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). The results showed that foam expansion and foam stability were significantly higher ($p < 0.05$) for samples bleached with H_2O_2. Thus, foaming properties of gelatin from cuttlefish skin could be improved by bleaching the skin with H_2O_2 under appropriate conditions.

Versatility of the emulsifying and foaming properties of gelatin is particularly valued in emulsified powders and cream forming products (Klaui *et al.,* 1970). Cuttlefish skin gelatin with its foaming properties can find application as a whipping agent in different products like marshmallows, nougats, mousses, souffles, chiffon cakes and whipped cream.

Fat binding capacity

Fat binding capacity of cuttlefish skin gelatins are shown in Fig. 3. Fat binding capacity is a functional property that is closely related to texture by the interaction between components such as water, oil and other components. Gelatin extracted after 8 h pretreatment in NaOH followed by bleaching with H_2O_2 had high fat binding capacity (2.62 ml g^{-1}). The fat binding capacity was low for gelatin extracted without bleaching. Fat binding capacity depends on the degree of exposure of the hydrophobic residues inside gelatin. The hydrophobic amino acids *viz.*, tyrosine, leucine and isoleucine, were

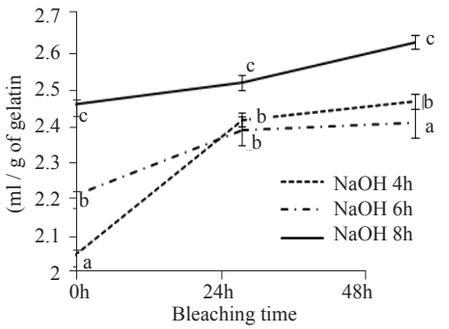

Fig. 3. Fat binding capacity of cuttlefish skin gelatin

present in cuttlefish as 5, 29 and 22 residues per 1000 (Balti *et al.,* 2010). It may be because of these hydrophobic residues that cuttlefish skin exhibited high fat binding capacity. High amount of tyrosine is probably responsible for the high fat binding capacity (Cho *et al.*, 2004). Fat binding capacity is a desirable property in ground meat formulations because it helps to retain flavour, improve palatability, and extend shelf life of baked goods, soups and meat products.

Gelatin extracted from the ventral skin of cuttlefish after bleaching with H_2O_2 was found to have characteristics of Type B gelatin with desirable physico-chemical properties suitable for commercial utilisation. Bleaching with 5% H_2O_2 significantly improved the yield, colour, viscosity and bloom strength of cuttlefish skin gelatin. Functional properties *viz*., foaming and fat binding properties were also significantly improved by bleaching. Cuttlefish skin gelatin produced by bleaching has wide potential application in food industry as whipping agent and as a component in wafers, juices and desserts. Considering the volume of skin waste generated during commercial processing of cuttlefish, a concerted effort is required to convert this processing waste as a source of gelatin with versatile applications.

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