As. J. Food Ag-Ind. **2012**, 5(06), 512-520

OPEN ACCESS

Asian Journal of Food and Agro-Industry

ISSN 1906-3040
Available online at www.ajofai.info

Research Article

Qualities of gelatin from Thai *panga* fish skin as affected by skin pretreatment

Trakul Prommajak and Patcharin Raviyan*

Division of Food Science and Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, 50100, Thailand.

*Author to whom correspondence should be addressed, email: patraviyan@gmail.com

Abstract

Gelatin was extracted from the skin of the Thai *panga* fish using different pretreatment methods, namely, shaking in sodium chloride, in sodium hydroxide, in hydrogen peroxide solution or in a mixture of these solutions. Skin pretreatment in 1% hydrogen peroxide with 0.1 M sodium hydroxide resulted in the highest degree of gelatin yield and gel lightness, but the lowest degree of gel strength. Pretreatment using 0.8 M sodium chloride with 0.1 M sodium hydroxide resulted in a high degree of gelatin yield, gel lightness and gel strength and demonstrated it is a suitable method for extracting gelatin from fish skin.

Keywords: *Pangasius bocourti* Sauvage, extraction, freshwater fish, additives, Thailand.

Introduction

Gelatin is a proteinaceous substance derived from collagen by a process involving the destruction of cross-links between polypeptide chains of collagen. Gelatin has been used in production of desserts, candies, bakery products, jellied meat, ice-cream and dairy products [1]. Currently, gelatin is mainly produced from bovine and porcine skin and bone. However, outbreaks of bovine spongiform encephalopathy and foot-and-mouth disease have caused consumer concern about food additives made from terrestrial animals. Consequently, focus has been shifted onto marine animals as an alternative source of gelatin production [2].

The Thai *panga* fish (*Pangasius bocourti* Sauvage) is an economic freshwater fish also known as *basa* (a member of the catfish family) that is being promoted for culture in areas along the Mae Khong River in Thailand. The flesh of this fish is normally frozen and exported to Europe and the USA as fish fillets. The freezing process leaves the remaining part of the fish as an industrial waste [3]. About 30% of the wastes are skin and bone, both of which have high collagen content and can be used for gelatin production [4].

In the gelatin production process, fish skin needs to be pretreated with alkaline, salt or hydrogen peroxide solution prior to gelatin extraction. Alkaline solutions, e.g., sodium hydroxide or calcium hydroxide, are mainly used to pretreat the raw materials in commercial gelatin production. The alkaline solution will remove non-protein materials such as mucopolysaccharide, sulphurcontaining compounds, sugars, lipids and non-collagenous proteins. It has been found that pretreatment of Alaska Pollock skin using alkaline solution at the concentration of hydroxyl ion lower than 0.5 M could eliminate non-collagenous protein without solubilization of collagen into the pretreatment solution [5].

Figure 1. ***Pangasius bocourti*** **Sauvage.**

Pretreatment of fish skin using salt solutions, such as sodium chloride or potassium chloride, could increase gelatin yield, gel strength and viscoelastic properties of the derived gelatin effectively when compared with pretreatment using magnesium chloride, magnesium sulphate or without using the pretreatment. Chloride ion from salt assists solubilization, and thus helps elimination of the myofibrillar protein from fish skin [6].

Hydrogen peroxide is an oxidant commonly used for bleaching marine animals for seafood processing. Hydroperoxyl anion, hydroperoxyl radical and hydroxyl radical formed during dissociation of hydrogen peroxide in water can react with many substances, including chromophore, which causes loss of pigments. In addition to increasing lightness of gelatin, pretreatment of cuttlefish skin using 5% hydrogen peroxide solution for 48 h also increases gelatin yield and gel strength [7].

However, comparative studies of these methods have not been elucidated. The objective of this study is to compare the effects that different skin pretreatments, namely, the use of sodium hydroxide, sodium chloride and hydrogen peroxide solutions, have on gelatin yield, gel strength and gel colour of the gelatin extracted from the skin of the Thai *panga* fish.

Materials and Methods

Materials

The skin of Thai *panga* fish was obtained from a processing plant in Nakhonphatom province of Thailand. The skin samples were kept at -20°C and used within 6 months.

Reagents

Extraction chemicals included sodium hydroxide (Merck, Germany), sodium chloride (Union Science, Thailand), hydrogen peroxide (Carlo Erba Reagenti, USA) and glacial acetic acid (Labscan, Thailand). Analytical reagents included cupric sulphate 5-hydrate (J.T. Baker, USA), potassium sodium tartrate (Univar, Australia) and bovine serum albumin (Sigma-Aldrich, Canada).

Proximate compositions of fish skin
Proximate analysis of the skin of Thai *panga* fish was performed using AOAC (2000) methods. Moisture content was analyzed by drying the fish skin in a hot-air oven at 100°C until constant weight was obtained. Crude protein was measured by Dumas combustion method using FP-528 nitrogen/protein analyzer (Leco, Michigan). Nitrogen conversion factor was 5.4 [8]. Crude lipid was analyzed by Rose-Gottlieb method. Crude ash was determined by incineration in muffle furnace at 550°C until the sample weight was constant. Proximate analysis was performed at least in triplicate.

Fish skin pretreatments and gelatin extraction
Fish skin was manually scraped off the flesh and cut into pieces of 1-2 cm in size. The skin was soaked in different pretreatment solutions as shown in Table 1, with a skin-per-solution ratio of 1:20 (w/v) [9]. The mixture was continuously shaken by using a orbital shaker (Gallenkamp, England) at 120 rpm. The pretreated skin was washed 3 times with tap water.

Table 1. Order of treatments to study the effects of different pretreatment methods.

<table>
<tr><th rowspan="2">Treatment order</th><th colspan="8">Extraction time</th></tr>
<tr><th>A</th><th>B</th><th>C</th><th>D</th><th>E</th><th>F</th><th>G</th><th>H</th></tr>
<tr><td>0.8 M NaCl</td><td></td><td></td><td>10 min</td><td rowspan="2">4 h</td><td></td><td></td><td></td><td rowspan="2">4 h</td></tr>
<tr><td>0.1 M NaOH</td><td></td><td>4 h</td><td>4 h</td><td>4 h</td><td rowspan="2">4 h</td><td>4 h</td></tr>
<tr><td>1% (v/v) H_2O_2</td><td></td><td></td><td></td><td></td><td>30 min</td><td></td><td></td></tr>
<tr><td>Gelatin extraction</td><td colspan="6">Water 3 h</td><td colspan="2">0.05 M acetic acid 3 h</td></tr>
</table>

Fish skins pretreated with treatments A to F were then extracted with deionised water. Because gelatin is well soluble in acid solution, fish skin pretreated with the same solution as B and D was extracted in 0.05 M acetic acid solution to compare the effect. The acid extraction methods were assigned as G and H. The ratio of initial skin weight per water was 1:6 (w/v). Extraction was conducted in water bath (Memmert, Germany) at 50°C for 3 hours.

Gelatin solution was filtered through a double-layer cheese cloth and then centrifuged at 2000g (Rotina 46R, Hettich, Germany) for 30 min to obtain the supernatant of gelatin solution. The protein content in the supernatant was analyzed by the Biuret method. The gelatin solution was dried out overnight using forced-air oven at 70°C (Termaks, Norway) to obtain gelatin sheets with 12-13% moisture content. The dried gelatin sheets were analyzed for gel strength and colour.

Gelatin yield determination
The protein content of the gelatin solution was determined by the Biuret method [10]. A Biuret reagent was prepared by dissolving 9 g of sodium potassium tartrate and 3 g of copper sulphate pentahydrate in 0.2 M sodium hydroxide solution and the final volume was then adjusted to 1,000 ml. For protein analysis, 100 µl of the sample was mixed with 300 µl water and 1.6 ml Biuret reagent. The solution was then kept for 30 minutes at room temperature before measuring the optical density at 550 nm using bovine serum albumin (Sigma-Aldrich, St. Louise, MO) as the standard. The gelatin yield was calculated as follows:

$$\text{Yield (\%)} = \text{protein content in supernatant (g)} \times 100 / \text{weight of fish skin used (g)}$$

Gel strength determination
Gelatin solution of 6.67% (w/w) was prepared by dissolving dried gelatin with distilled water and heated at 60±1°C for 30 minutes in a water bath. The gelatin solution was then put in a cup (30 mm diameter × 15 mm height) and kept at 2±0.4°C for 16-18 h [11]. The gel strength was measured by the texture analyzer (TA.XT Plus, Stable Micro System, England), using a 12.7 mm

diameter plunger (P/0.5R probe), 0.5 mm/s compression rate and 4 mm penetration depth. The gel strength is the maximum force required in penetration.

Colour measurement

The 6.67% gelatin solution was prepared as described in gel strength determination and measured for colour in the L*C*h* scale using Minolta Chroma Meter (Minolta, Japan).

Statistical analysis

All experiments were conducted in triplicate. Differences between means were tested by analysis of variance and Duncan's multiple range test using SPSS software (SPSS Inc., Chicago, Illinois).

Results and Discussion

Proximate composition

Fresh Thai *panga* fish skin contained 60.86±0.65% moisture, 35.83±2.61% crude protein, 2.19±0.64% lipid and 0.18±0.08% ash.

Gelatin yield

Gelatin extracted using treatment H showed the highest yield of 18.36±0.94%, which was not significantly different (P>0.05) from the yield obtained from treatment G (17.61±1.11) as shown in Figure 1. The reason for the high yield resulting from acid treatment was mainly due to the good solubility of collagen in acid solution and also the breakdown of hydrogen bond and hydrophobic bond between alpha chains of collagen triple helix [12, 13].

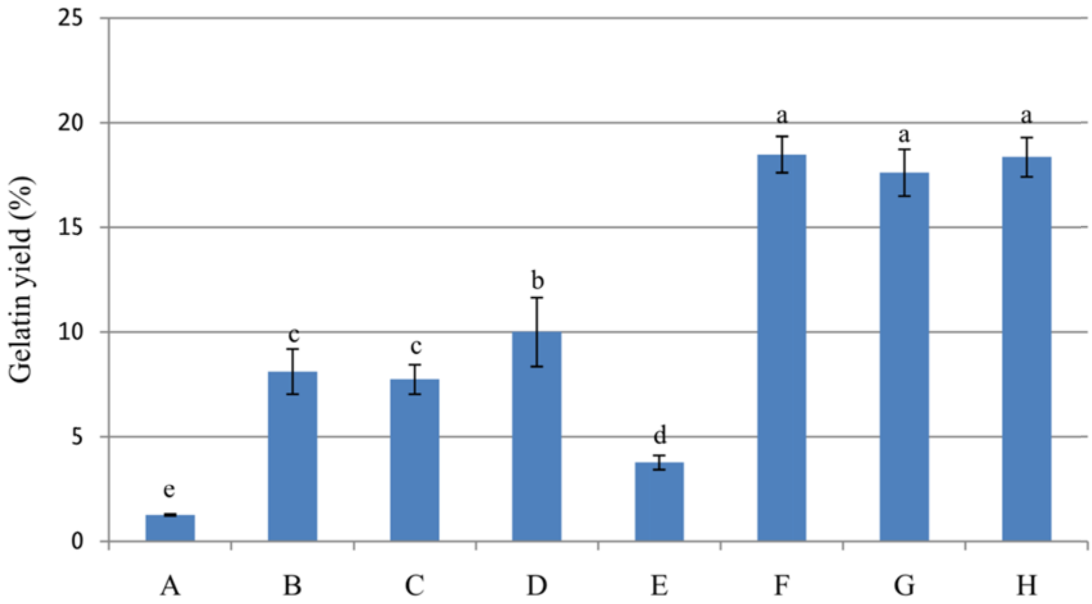

Figure 2. Gelatin yield (%) from fish skin pretreated with different pretreatment solutions.

Among the methods of gelatin extraction using water as the solving agent, pretreatment using hydrogen peroxide (treatment F) resulted in the highest gelatin yield (18.48±0.87%) and the yield obtained was not significantly different (P>0.05) from those extracted by acetic acid solution (treatment G and H). However, pretreatment using 0.1 M NaOH followed by hydrogen peroxide solution for 30 min (treatment E) provided a low amount of gelatin yield (3.78±0.34%), but the yield was still higher than that extracted without pretreatment (treatment A). The difference between gelatin yield from treatment E and F may be because of the difference in pH of the extracting solution. This result conformed with the study on cuttlefish by Aewsiri *et al* [7]. The hydrogen peroxide pretreatment of cuttlefish skin caused higher gelatin yield compared with that obtained from the control. Hydrogen peroxide increased gelatin yield by disruption of hydrogen bond between collagen molecules which resulted in easier extraction of gelatin.

Pretreatment with 0.1 M NaOH (treatment B) resulted in higher gelatin yield compared with that obtained without the pretreatment (treatment A) or pretreatment using sodium hydroxide followed by hydrogen peroxide solution (treatment E). Sodium hydroxide caused collagen molecules to swell and removed non-collaganous proteins, thereby making gelatin extraction easier [14]. The gelatin yield through pretreatment using 0.8 M NaCl for 10 min followed by 0.1 M NaOH (treatment C) was not different from the yield through treatment B ($P>0.05$). Meanwhile, pretreatment using 0.8 M NaCl together with 0.1 M NaOH provided higher yield as compared with the yields obtained from pretreatments using sodium hydroxide alone (treatment B) and from using NaCl followed by NaOH solution (treatment C), respectively. The difference in gelatin yield might be caused by using different NaCl contact times of 0, 10 or 240 min in treatment A, B, and C, respectively. Similar to the extraction of gelatin from the Dover sole (*Solea vulgaris*) skin, pretreatment using NaCl solution resulted in higher gelatin yield as compared with the control (no salts were used). Saline ion caused lyotropic hydration of collagen molecules by altering water structure around the collagen molecules, interrupting hydrogen bonds or interacting with internal hydrophobic bond [6]. Therefore, the triple helix of collagen became unstable and easier to be extracted. As treatment A and E resulted in low gelatin yield, they were then excluded from the following physical properties analyses.

Gel strength

The important property of gelatin used by the food processor is its gelling effect. Gel strength is the primary factor to determine quality and price of gelatin [15]. The lowest gel strength was obtained from pretreatment using 1% hydrogen peroxide with 0.1 M NaOH solution (treatment F) as shown in Figure 2. Aewsiri *et al* reported that treatment of fish skin with 2% hydrogen peroxide solution for 24 h could increase gelatin extracted from cuttlefish skin [7]. The controversial results could be caused by the difference in raw materials or by the dissociation of hydrogen peroxide at different pH. In alkaline condition, hydrogen peroxide decomposed to perhydroxyl anion (HOO^-) which is a strong nucleophile. This nucleophile could attack the peptide bonds causing cleavage of the protein backbone [16].

Among the treatments using water as the extracting solution, the maximum gel strength could be obtained from the pretreatment using 0.8 M NaCl and 0.1 M NaOH (treatment D). The gel strength of the gelatin obtained using the mixture of NaCl and NaOH was higher than that obtained from the pretreatment using only NaOH (treatment B) ($P<0.05$). However, the addition of NaCl in NaOH solution had no significant effect on the gel strength ($P>0.05$) when extraction was performed in acid solution (treatment G and H). This result was in accordance with the study of Giménez *et al*, which reported that pretreatment of the Dover sole skin in NaCl solution resulted in gelatin with high gel strength as compared with those obtained from the control (no salts were used) [6]. This indicated that NaCl solution could maintain the α-chain of collagen, which was a main contributor to gel strength [17]. Gelatin extracted by acid solution (treatment G and H) exhibited higher gel strength than those obtained from water solution (treatments B and D).

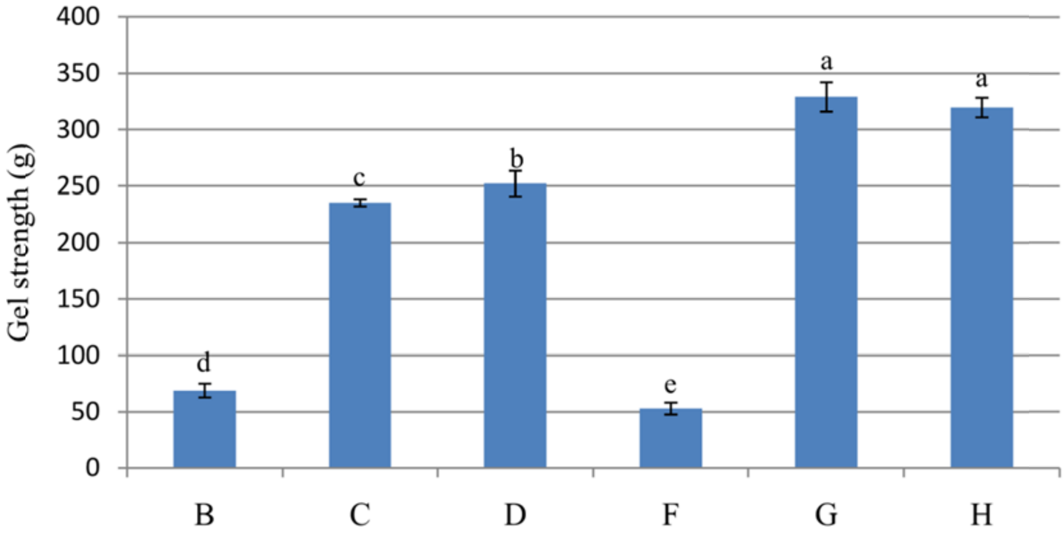

Figure 3. Gel strength (g) of gelatin obtained from fish skin pretreated with different pretreatment solutions.

The environmental temperature of fish influences the rheological properties of fish gelatin. Gel strength of the cold-water fish gelatin was generally lower than that obtained from the warm-water fish gelatin [2]. The maximum gel strength of gelatin from the skin of the Thai *panga* fish, a warm-water fish, was 329.0±13.08 g (treatment G) which was significantly higher than the gelatins obtained from such cold-water fish species as Alaskan pollock (186g) or Alaskan pink salmon (216 g) [18]. When compared with gelatins from other warm-water fish, gel strength of the Thai *panga* fish skin gelatin was lower than that obtained from certain types of fish such as yellowfin tuna skin gelatin (426 g), while higher than that obtained from the grass carp skin (267g) or the channel catfish head bone (282 g) [19, 20, 21]. However, species of fish was not the only factor that determined the gel strength of gelatin. Gel maturation temperature and time, amount of sample or container dimension were different between the above studies. These factors could affect the values of gel strength [2].

The difference in gel strength of gelatin depends mainly on the composition of amino acid and molecular weight distribution. The amino acid compositions of gelatins were influenced by fish species. Warm-water fish generally has higher content of imino acid (proline+hydroxyproline) which stabilizes the gelatin gel network by the formation of hydrogen bonds and electrostatic bridging [22-23].

Molecular weight distribution of gelatin was affected by processing conditions. Extraction using severe treatment caused degradation of gelatin molecules into smaller peptides which formed a weak junction zone, leading to lowered gel strength [2]. Sequential extraction of channel fish head bone also resulted in lowered gel strength of gelatin derived from the second and third extractions compared to the first extraction due to the low molecular weight peptides derived from prolonged heat treatment [21]. In this study, gelatin was extracted at 50°C for 3 h, while the extracting conditions of gelatin from yellowfin tuna, grass carp and channel catfish were between 52-75°C for 4-5 h [19, 20, 21]. The conditions used in these studies were not much different from that used in this study, so the differences in gel strength should be mainly due to the fish species. The molecular weight distribution of gelatin extracted from the Thai *panga* fish need to be explored further to confirm the effect of the molecular weight on gel strength.

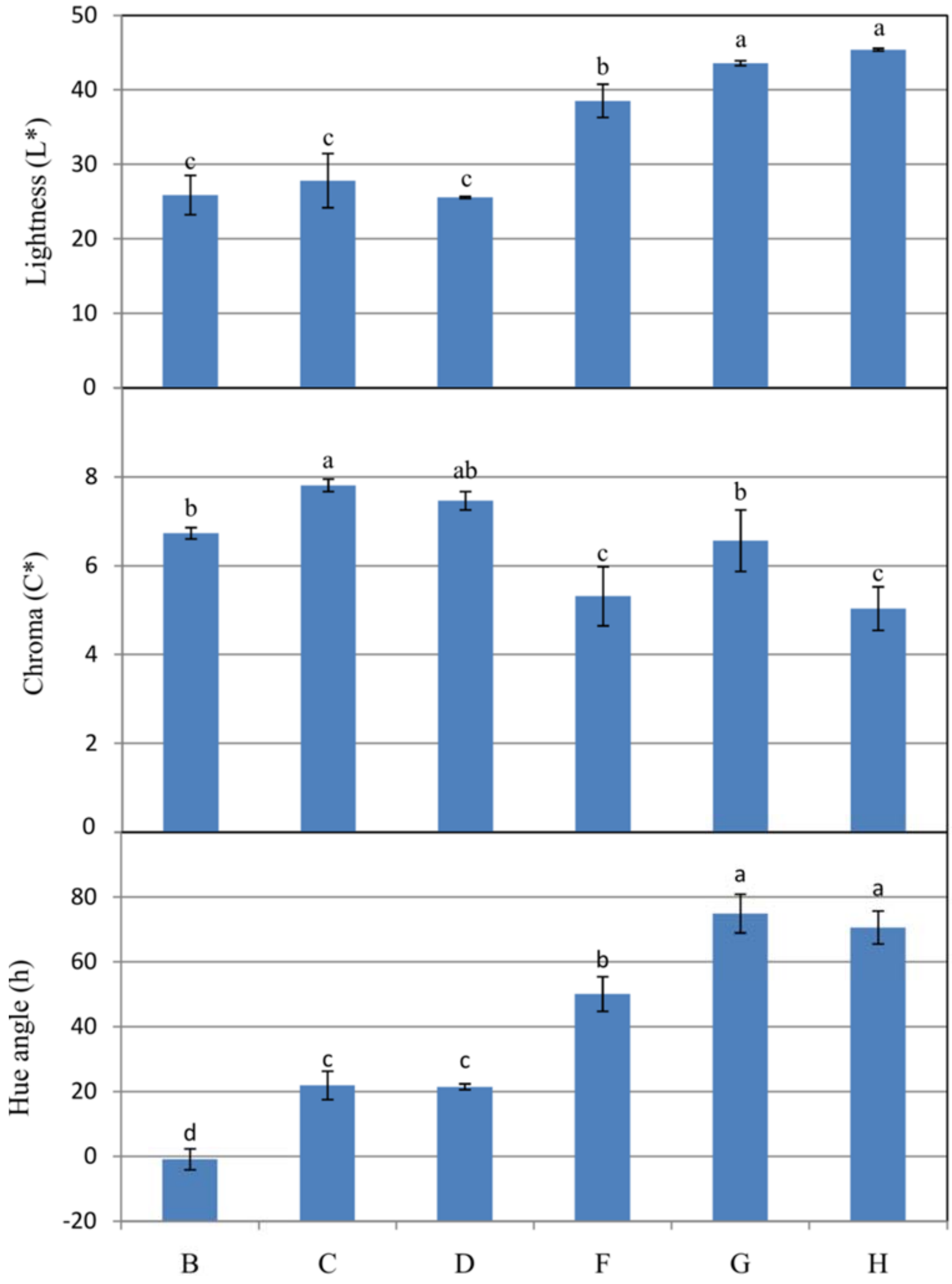

Figure 4. Colour in L*C*h scale of gelatins obtained from the Thai *panga* fish skin pretreated with different pretreatment solutions.

Gel colour

Although the hue (h) of gelatins varied from red (treatment B, C and D) to orange (treatment F) and yellow (treatment G and H), their colour shades could not be observed visibly because the chroma (C*) values were very low (ranging from 4.5 to 7.9). Consequently, lightness should be an appropriate indicator for the quality of gel colour (Figures 3).

Gelatin extracted by acid solution (treatment G and H) had the highest lightness ($P>0.05$). Among the treatments using water as the extracting solution, the pretreatment using hydrogen peroxide (treatment F) resulted in the highest lightness of gel, but still lower than those obtained by acid extraction. Hydrogen peroxide is a bleaching agent used in cephalopod industry. Oxidizing agents derived from the decomposition of hydrogen peroxide, such as hydroperoxyl anion, break the bond of a chromophore and change it to a new substance without a chromophore or a new chromophore that does not absorb visible light [7]. For treatments B, C and D, gel lightness was low and did not differ from each other (P>0.05).

Conclusions

Although pretreatment of fish skin using hydrogen peroxide together with sodium hydroxide solution resulted in the highest gelatin yield and gel lightness, its yielded lowest gel strength. Because gel strength is considered to be the most important property of gelatin, pretreatment using hydrogen peroxide together with sodium hydroxide solution may not be the most suitable pretreatment for the production of gelatin. As a result, pretreatment using sodium chloride together with sodium hydroxide solution, which provides high gelatin yield, gel strength and gel lightness, is considered to be the appropriate pretreatment method for the production of gelatin from the skin of the Thai *panga* fish.

References

1. Djagny, K.B., Wang, Z. and Xu, S. (2001). Gelatin: a valuable protein for food and pharmaceutical industries: review. ***Critical Reviews in Food Science and Nutrition***. 41: 481-492.

2. Regenstein, J.M. and Zhou, P. (2007). Collagen and gelatin from marine by-products. In: Shahidi, F. (Ed.). **Maximising the Value of Marine By-products**. Cambridge: Woodhead Publishing Limited.

3. National Food Institute. (2006). Economic marine animals development project. Available at: http://www.nfi.or.th/nfi/fish/ [Accessed 30 July 2009].

4. Wasswa, J., Tang, J. and Gu, X. (2007). Utilization of fish processing by-products in the gelatin industry. ***Food Reviews International***. 23: 159-174.

5. Zhou, P. and Regenstein, J.M. (2005). Effects of alkaline and acid pretreatments on Alaska pollock skin gelatin extraction. ***Journal of Food Science***. 70: C392-396.

6. Giménez, B., Gómez-Guillén, M.C. and Montero, P. (2005). The role of salt washing of fish skins in chemical and rheological properties of gelatin extracted. ***Food Hydrocolloids***. 19: 951-957.

7. Aewsiri, T., Benjakul, S. and Visessanguan, W. (2009). Functional properties of gelatin from cuttlefish (*Sepia pharaonis*) skin as affected by bleaching using hydrogen peroxide. ***Food Chemistry***. 115: 243-249.

8. Muyonga, J.H., Cole, C.G.B. and Duodu, K.G. (2004). Characterisation of acid soluble collagen from skins of young and adult Nile perch (*Lates niloticus*). ***Food Chemistry***. 85: 81-89.

9. Noitup, P. (2004). Collagen extraction from fish skin by-product in the frozen fish industry: study of some characteristics of extracted collagen. Ph. D. Dissertation, Kasetsart University.

10. Weaver, C. and Daniel, J. (2003). The Food Chemistry Laboratory: A Manual for Experimental Foods, Dietetics, and Food Scientists. Boca Raton: CRC Press.

11. Zhou, P. and Regenstein, J.M. (2004). Optimization of extraction conditions for pollock skin gelatin. ***Journal of Food Science***. 69: C393-C398.

12. O'neil, M.J., Smith, A., Heckelman, P.E. and Budavari, S. (2001). Merck Index, 13th ed. New Jersey: Merck.

13. Poppe, J. (1999). Gelatin. In: Imeson, A. (Ed.). **Thickening and Gelling Agents for Food**. Gaithersburg: Aspen Publishers.

14. Klaypradit, W. (1997). Production of gelatin from skins of red snapper (*Lutjanus sanguineus*). Master of Science Thesis, Kasetsart University.

15. Schrieber, R. and Gareis, H. (2007). Gelatine Handbook: Theory and Industrial Practice. Weinheim: Wiley-VCH Verlag GmbH & Co. KGaA.

16. Kimball, A.S., Lee, J., Jayaram, M. and Tullius, T.D. (1993). Sequence-specific cleavage of DNA via nucleophilic attack of hydrogen peroxide, assisted by flp recombinase. ***Biochemistry***. 32: 4698-4701.

17. Cole, C.G.B. (2000). Gelatin. In: Francis**,** F.J. (Ed.). **Encyclopedia of Food Science and Technology, 2nd edition**. New York: John Wiley & Sons.

18. Avena-Bustillos, R.J., Olsen, C.W., Olson, D.A., Chiou, B., Yee, E., Bechtel, P.J. and Mchugh, T.H. (2006). Water vapor permeability of mammalian and fish gelatin films. ***Journal of Food Science***. 71: E202-E207.

19. Cho, S.M., Gu, Y.S. and Kim, S.B. (2005). Extracting optimization and physical properties of yellowfin tuna (*Thunnus albacares*) skin gelatin compared to mammalian gelatins. ***Food Hydrocolloids***. 19: 221-229.

20. Kasankala, L.M., Xue, Y., Weilong, Y., Hong, S.D. and He, Q. (2007). Optimization of gelatine extraction from grass carp (*Catenopharyngodon idella*) fish skin by response surface methodology. ***Bioresource Technology***. 98: 3338-3343.

21. Liu, H.Y., Han, J. and Guo, S.D. (2009). Characteristics of the gelatin extracted from Channel Catfish (*Ictalurus Punctatus*) head bones. ***LWT - Food Science and Technology***. 42: 540-544.

22. Gómez-Guillén, M.C., Pérez-Mateos, M., Gómez-Estaca, J., López-Caballero, E., Giménez, B. and Montero, P. (2009). Fish gelatin: a renewable material for developing active biodegradable films. ***Trends in Food Science and Technology***. 20: 3-16.

23. Haug, I.J., Draget, K.I. and Smidsrød, O. (2004). Physical and rheological properties of fish gelatin compared to mammalian gelatin. ***Food Hydrocolloids***. 18: 203-213.