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Science and Technology
Meri-Rastilantie 3 B, FI-00980
Helsinki, Finland
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Journal of Food, Agriculture & Environment Vol.11 (1): 146-150. 2013 *www.world-food.net*

Gelling strength improvement and characterization of a gelatin from scales of bighead carp (*Aristichthys nobilis*)

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Received 8 November 2012, accepted 28 January 2013.

Abstract

Response surface method (RSM) was used to optimize enzymatic extraction conditions aiming at improving gelling strength of gelatin from scales of bighead carp (*Aristichthys nobilis*). The optimal extraction conditions were derived as follows: extracting temperature 46.98°C, extracting time1.27 h, pH 4.00 and pepsin concentration 547 U/g, respectively. The gel strength of the gelatin derived with the optimized process was 318±7 g, which was significantly higher than the gelatin extracted with conventional non enzymatic process (216±3 g, $p<0.01$). The yield of optimized extraction process (17.46±0.61%) was comparable with that of conventional extraction process (19.10±0.60%). The composition and functional properties of the gelatin from the scales of bighead carp (BSG) were compared with that of porcine skin gelatin (PSG). There were very significant differences in content of threonine, methionine, isoleucine, phenylalanine and lysine, significant differences in content of hydroxyproline, glycine and alanine between BSG and PSG. BSG showed lower imino acid (proline and hydroxyproline) content (20.75±0.82%) than PSG (24.01±0.70%). The gelling temperatures of BSG and PSG were 21°C and 24°C, and melting temperatures were 26°C and 29°C, respectivly. Assessment on dynamic viscoelastic properties indicated that BSG had better gelling ability and lower thermo-stability than PSG. Bighead carp scale is a good source of high quality fish gelatin for potential application in food industry. Extracting gelatin from bighead carp scales might be a promising solution in terms of both reducing environment pollution and increasing the economic efficiency of the industry.

Key words: Fish gelatin, gelling strength, enzymatic extraction, response surface methodology, viscoelastic properties.

Introduction

Gelatin is mostly extracted from porcine or bovine skins and bones by using acid or alkaline. The physical, chemical and rheological properties of gelatin or collagen depend upon their sources as well as the extraction conditions. The gelling and melting temperatures of fish gelatin are lower than those of mammals [1, 2], which make fish gelatin not so widely used as porcine or bovine gelatin in commerce. However, compared with mammalian gelatin, fish gelatin does not bear risks of Bovine Spongiform Encephalopathy or Swine Influenza Virus. Moreover, fish gelatins are the major alternative to mammalian gelatins in Judaism and Islam food cultures. Gel strength of warm-water fish gelatin was higher than that of cold-water fish gelatin [3], suggesting that warm-water fish may be a better resource for fish gelatin. Fish gelatin was usually extracted from fish skin [4-8] though a few studies on gelatins from fish scales were reported on silver carp [9], lizardfish[10], Nile tilapia [11] and grass carp [12].

China has the world's richest freshwater fish resources with a total over 700 species, including 40 to 50 species of major cash fish. Bighead carp (*Aristichthys nobilis*), distributed in South and East China, is one of the most commonly commercially produced fresh water fish in China. The fish meat and skin are used for human food, leaving almost 50-70% wastes such as bone, scale and viscera [13]. With the rapid expansion of the fish food process industry in Southeast China, the wastes disposal poses a big environmental challenge. The extraction of gelatin or other bio-materials from these production wastes might be a promising solution in terms of both reducing environment pollution and increasing the economic efficiency of the industry.

Gelatin was generally extracted by hot water for 5-12 h according to existing literatures [2, 8, 14]. Pepsin has been frequently used for collagen extraction [15-17] to improve extracting efficiency. High temperature may improve the yield of gelatin [18], but long time of hot water extraction may reduce gel strength because high temperature may lead to gel dehydration [19] and breakage of gel network structure.

In this experiment, we explored the application of pepsin to shorten the extracting time and thus to improve gel strength of the gelatin from scales of bighead carp (BSG). Response surface method was used to optimize conditions of the extraction with the objectives of improving gelling strength while maintaining comparable yield. The chemical composition and functional properties of BSG were characterized in comparison with commercial mammalian gelatin. These research results would offer a better understanding of BSG, which would in term provide scientific and technological basis for possible industrial production and utilization of the gelatin in the future.

Materials and Methods

Materials and pretreatment: Fresh bighead carps (around 1.5-2.0 kg bodyweight) were collected from a local fish market in Hangzhou, Zhejiang province, China. The scales were scratched after the fish was killed in 15 min. The scales were washed by water, air-dried with a modest temperature of 40°C for 10 h, and stored at 4°C for one month. Before extraction, the scales were treated with 10 volumes (v/w) of 0.1 M NaOH for 5 h to remove non-collagen protein and pigment. The scales were demineralized with 0.4 M EDTA disodium salt solution (in pH 7.5 Tris-HCl buffer) at ratio of 1:30 (w/v) for 8 h. After this treatment, the scales were neutralized by washing under running water until they had a pH of about 7.0. Then the scales were washed in distilled water and drained.

Gelatin extraction: In the preliminary experiments, pH (2.0-6.0), temperature (20-60°C), time (1-8 h) and pepsin concentration (179-893 U/g) were chosen as the factors of gelatin extraction. The pretreated scales were mixed with distilled water, in a ratio of dried material to water of 1:10 (w/v). The pH was adjusted to a range from 2.00 to 4.00 before the pepsin was added. Then the mixtures were put in a water bath at designated temperature from 30°C to 50°C for 1-3 h. When the extraction was finished, the mixture was put in a boiling water bath for 5 min to inactivate the enzyme. The extracted solution was filtered firstly through double layer gauze to remove scale residue and then through double layer filter paper by suction filtration. The filtrate was then lyophilized into powder which was referred to as bighead carp scale gelatin (BSG).

The conventional non enzymatic process was conducted according to the method of Cho *et al.* [2] with slight modification. The pretreated scales were mixed with 10 volumes (v/w) of distilled water and extracted in the water bath at 60°C for 5 h. The extracted solution was filtered and lyophilized like BSG.

Determination of gel strength: Gel strength was determined by the method described by Liu *et al.* [20]. A 6.67 % (w/v) gelatin solution was prepared by mixing the lyophilized gelatin and chilled distilled water together. The mixture was kept at 10°C for 60 min and transferred to a water bath of 45°C for 30 min with the solution stirred intermittently. The solution was kept at 10°C for 17-18 h. After maturation, the gel strength tests were carried out by using a texture analyzer (Stable Microsystems, UK) with a 5 kN load cell, equipped with a 1.27 cm diameter probe. Gel strength is the maximum force (in grams) required for the probe to press the gel by 4 mm depression at a rate of 0.5 mm/s.

Amino acid analysis: Gelatin samples were mixed with 6 M HCl and hydrolyzed in vacuum-sealed tubes at 110°C for 22 h. After hydrolysis, the samples were dissolved in buffer and analyzed by amino acid analyzer (Sykam, Germany) after being dried by vacuum drier. Hydroxyproline content was measured by the method of Codex [21].

Dynamic viscoelastic properties: Dynamic viscoelastic properties were measured by an AR-G2 rheometer (TA Instruments-Waters LLC, USA). As Liu *et al.* [20] described, dry gelatin was mixed with distilled water (6.67 %, w/v) for 60 min and then the solution was kept in water bath at 45°C for 20 min. The measurement was performed at a scan rate of 1°C/min, frequency 1 Hz, oscillate applied stress of 3.0 Pa. The gelatin solution was cooled from 40°C to 5°C, kept at 5°C for 10 min, and then heated from 5°C to 40°C, at the rate of 1°C/min. The elastic modulus (G2; Pa), the viscosity modulus (G3; Pa) and the phase angle (rad) were plotted as a function of temperature.

SDS-PAGE: Gelatin samples were dissolved at 5 mg/ml in distilled water at 60°C for 20 min. Buffer containing 20 % β-mercaptoethanol was added. After denatured at 95°C for 5 min, the gelatin samples (10 µl) were analyzed by SDS-PAGE according to Laemmli [22] using 7.5% resolving gels and 5% stacking gels. Protein bands were stained with Coomassie Brilliant Blue R250.

Experiment design: Box-Behnken design was adopted in the optimization of gelatin extraction from bighead carp scales. Based on preliminary experiments, the extracting temperature (X_1, °C), extracting time (X_2, h), pH (X_3) and pepsin concentration(X_4, U/g) were selected as variables with significant effects on extracted gelatin. Table 1 shows the independent variables and their range of three levels in this study. Gel strength (Y, g) was selected as the dependent variable. Regression analysis was performed to fit the response function with the experimental data by design-expert software (Version 8.0.6, Stat-Ease, Inc. USA).

Table 1. Range and values of the independent variables in design.

Independent variables	Symbol	Coded levels		
		-1	0	1
The extracting temperature (°C)	X_1	30	40	50
The extracting time (h)	X_2	1	2	3
pH	X_3	2	3	4
Pepsin (U/g)	X_4	357	536	714

Results and Discussion

Optimization of gelatin extraction by response surface method: The two-dimentional effects of the experimental variables on the gel strength of extracted gelatin are shown in Fig 1. In general, when temperature or pH variables were fixed, the gel strength increased with extracting time until 1.5~2 h, and decreased thereafter. There were significant interactions among extracting temperature, time and pH. The pH had no substantial effect at low temperature and a positive linear effect at high temperature on the gel strength. The gel strength increased with the increase of the quantity of pepsin until 459 U/g and further increase of pepsin has no effect on gel strength. High quantity of pepsin will hydrolyze gelatin extracted from scales, and break gel network structure.

The final response surface regression equation obtained by RSM is as follows:

$$Y= -1837.34+60.12X_1+348.95X_2+245.97X_3-0.3X_4-3.29X_1X_2+5.98X_1X_3-27.20X_2X_3+0.04X_2X_4+0.24X_3X_4-0.84X_1^2-42.01X_2^2-70.29X_3^2.$$

The regression model had statistical evalution values of "Prob>F" ≤ 0.0500 , p-value for lack-of-fit analysis 0.7683, R^2 = 0.9715, "Adj R^2" = 0.9430" and Pred R^2" = 0.8838, indicating that the model terms are statistically significant.

The optimal extraction conditions for maximum gel strength deduced from the RSM model were temperature 46.98°C, time 1.27 h, pH 4.00, and pepsin concentration 547 U/g, respectively. The

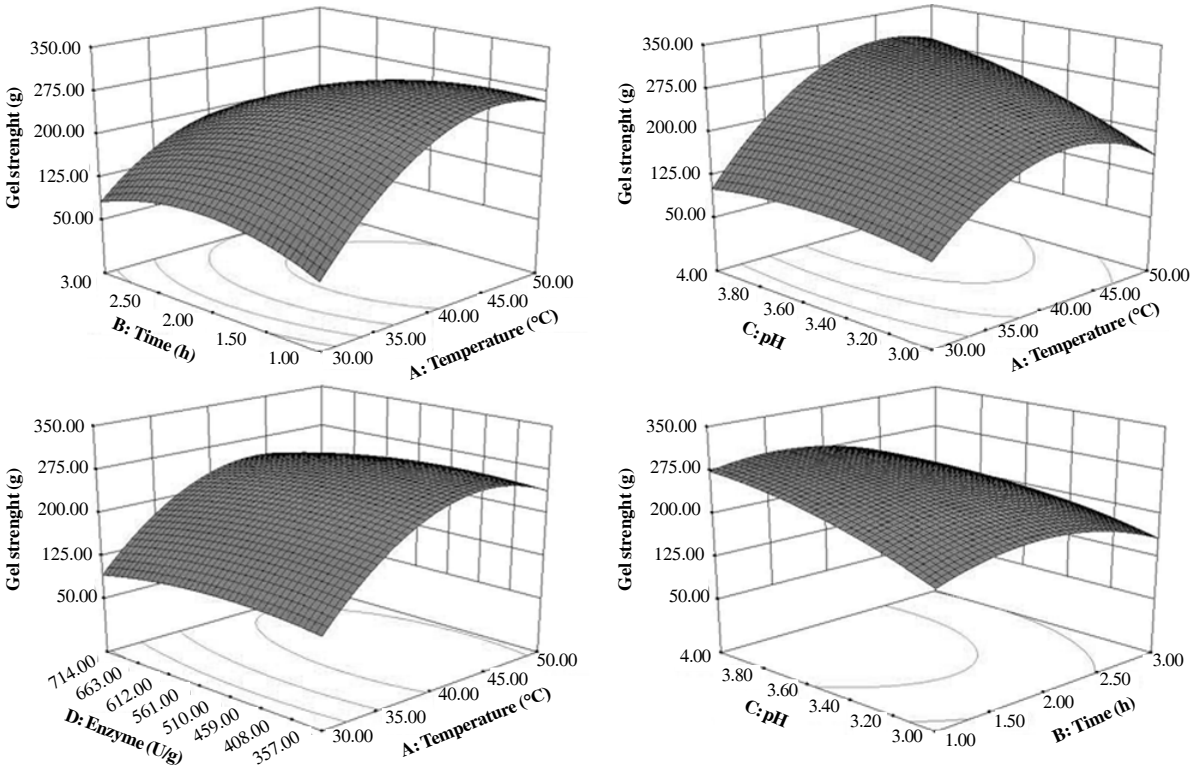


Figure 1. The response surface for optimization of BSG. The levels of the other two variables in each sub-figure were all fixed at central point except pH which was 3.5.

predicted gel strength value was 325 g which agreed quite well with the tested gel strength of 318±7 g ($p<0.01$). This gel strength is significantly higher then the gel strength of bighead fish scale gelatin extracted with conventional non enzymatic process (216±3 g, $p<0.01$). The yield of optimized extraction process (17.46±0.61%) was comparable with that of conventional extraction process (19.10 ± 0.60 %).

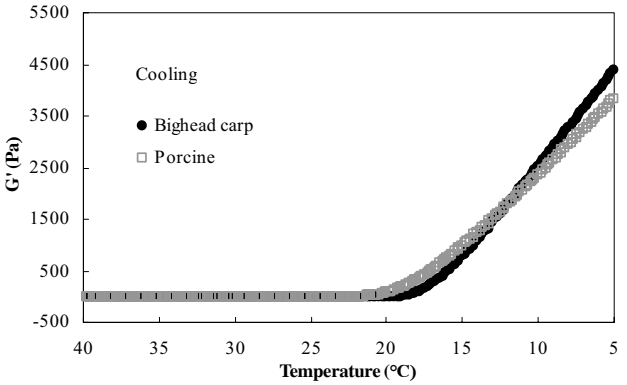
Amino acid composition: The amino acid composition of BSG and PSG is shown in Table 2. Both of them were rich in glycine, proline, and glutamic acid. By comparing each value between BSG and PSG, there were very significant diffrences in content of threonine, methionine, isoleucine, phenylalanine and lysine, significant diffrences in content of hydroxyproline, glycine and alanine. BSG showed a slightly lower imino acid (proline and hydroxyproline) content (20.75±0.82 %) than PSG (24.01± 0.70%), and the differences of degrees of hydroxylation of proline between BSG (32.72±0.15 %) and PSG (34.24±0.17%) were significant. Generally, the imino acid (proline and hydroxyproline) content of gelatin extracted from different fish species was lower than that of mammalian gelatin, such as channel catfish gelatin [20], tuna gelatin[23], grass carp gelatin [12], Nile tilapia gelatin [24], Nile perch gelatin [25], and lizardfish gelatin [10], especially these cold water fish gelatin [26]. The hydroxyproline may contribute to the cross-linking structures, and the content of proline and hydroxyproline can be associated with the stability of the triple helical structure [8]. Both of BSG and PSG contained a low content of tyrosine and histidine.

Dynamic viscoelastic properties: The elastic modulus (G2; Pa) and the viscosity modulus (G3; Pa) indicated the gelling ability of the gelatin, and the phase angle (rad) showed the phase change of the

Table 2. Amino anid composition of bighead carp scale gelatin and porcine skin gelatin.

Amino acid	BSG%	PSG%
Hydroxy proline	6.79±0.24	8.22±0.20
Aspartic acid	6.25±0.17	6.06±0.08
Threonine	2.93±0.06	1.90±0.19
Serine	3.56±0.06	3.58±0.10
Glutamic acid	11.16±0.59	11.42±0.40
Proline	13.96±0.58	15.79±0.50
Glycine	21.37±0.05	22.32±0.30
Alanine	10.06±0.36	8.20±0.30
Valine	2.00±0.06	2.25±0.21
Methionine	1.84±0.22	0.66±0.23
Isoleucine	1.30±0.17	1.58±0.16
Leucine	2.73±0.23	3.04±0.10
Tyrosine	0.54±0.15	0.26±0.05
Phenylalanine	2.24±0.11	2.04±0.10
Lysine	3.72±0.10	3.92±0.10
Histidine	1.13±0.04	1.00±0.08
Arginine	8.40±0.04	7.76±0.34
totle	100.00	100.00

gelatin [2]. Figs 2-4 show the evolution of the elastic modulus (G2; Pa) , the viscosity modulus (G3; Pa), and the phase angle (rad) during cooling (from 40°C to 5°C) and heating (from 5°C to 40°C) of the gelatin solutions, respectively. G2 and G3 of BSG began to increase at a lower temperature than these of PSG when the temperature decreases from 40°C to 5°C, while G2 and G3 of BSG exceed those of PSG at 12°C and 18°C, respectively. This situation was similar to gelatin from grass carp fish [12]. G3 of BSG showed greater rising range during cooling (Fig 3) which was similar to channel catfish [20]. This indicated that BSG had better gelling ability than PSG. At the beginning of heating G2 and G3 in BSG were greater than those in PSG, whereas they decreased more obviously



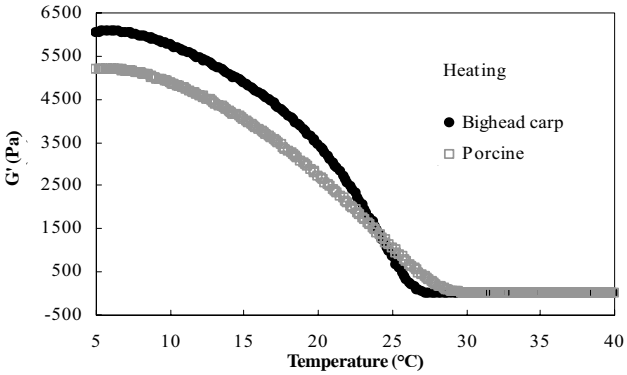
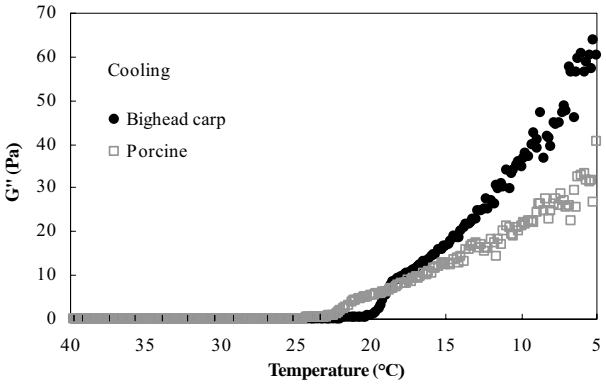


Figure 2. Evolution of the elastic modulus (G'; Pa) of the gelatin solutions (6.67%, w/v) during cooling (from 40°C to 5°C) and heating (from 5°C to 40°C) at the rate of 1°C/min.



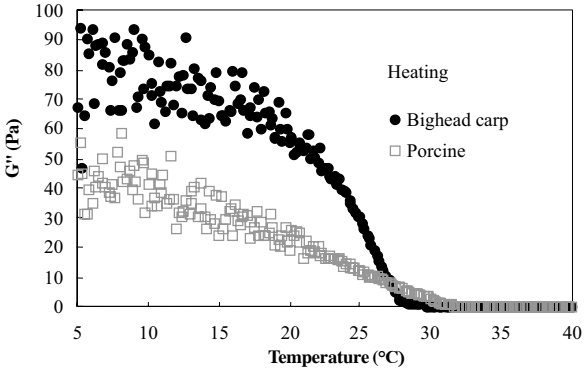
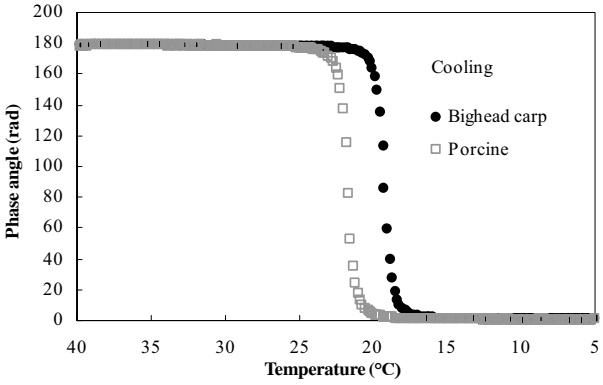


Figure 3. Evolution of the viscosity modulus (G''; Pa) of the gelatin solutions (6.67%, w/v) during cooling (from 40°C to 5°C) and heating (from 5°C to 40°C) at the rate of 1°C/min.



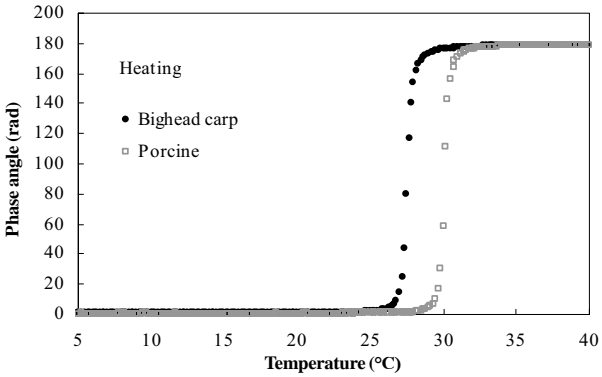


Figure 4. Evolution of the phase angle (rad) of the gelatin solutions (6.67%, w/v) during cooling (from 40°C to 5°C) and heating (from 5°C to 40°C) at the rate of 1°C/min.

than those of PSG during heating (Figs 2-3). This indicated that BSG had lower thermo-stability than PSG. According to the sharp decrease of the phase angle (Fig. 4), the solution of BSG and PSG became gel at 21°C and 24°C, then melted at 26°C and 29°C, respectively [27]. One reason that both of the gelling and melting temperatures of BSG were lower than those of PSG was the amino acid composition. High content of proline and hydroxyproline could contribute to the viscoelastic properties [26].

SDS-PAGE: The electrophoretic patterns of BSG and PSG are shown in Fig. 5. The subunit components of gelatin are γ-components, β-components, and α-chains. The γ-components were very week both in PSG and BSG. The β-components (around 200 kDa) were clearly observed in BSG. But PSG exhibited weak bands around 200 kDa. The molecular weights of α-chains from BSG were lower than these from PSG. There were also some small

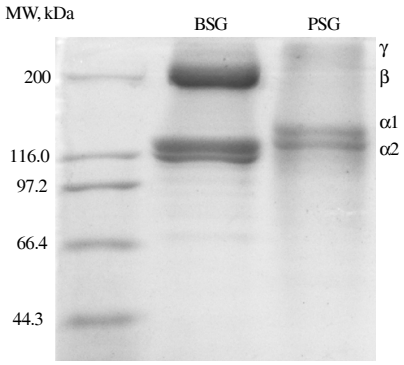


Figure 5. SDS-PAGE patterns of BSG and PSG.

chains whose molecular weights were less than 116 kDa both in BSG and PSG. The higher content of low molecular weight peptides were associated with lower melting point [25], and the content of α-chain and β-component may be associated with the gel strength of gelatins [12]. That may explain that BSG had lower melting point and higher gel strength than PSG had.

Conclusions

Response surface method was used to determine the optimum operating conditions of extracting gelatin from bighead carp fish with an objective to improve gelling strength of the extracts. The gel strength of BSG under the optimum extracting conditions was 318±7 g, significantly higher than the strength of similar gelatin extracted with conventional process and that of PSG. The amino acid composition of bighead carp scale gelatin was basically similar to that of PSG, with a slightly lower imino acid content. The degree of hydroxylation of proline of BSG was also slightly lower than that of PSG. Compared with PSG, BSG had better gelling ability and lower thermo-stability. In conclusion, our experimental results suggested that bighead carp scale is a good source of high quality fish gelatin for potential application in food industry.

Acknowledgements

The authors would like to thank the Foundation for the Program of Key Innovative Research Team of Zhejiang Province (2009R50036).

References

[1]Choi, S. S. and Regenstein, J. M. 2000. Physicochemical and sensory characteristics of fish gelatin. J. Food Sci. **65**(2):194-199.

[2]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**(2):221-229.

[3]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. J. Food Sci. **71**(4):202-207.

[4]Zhou, P., Mulvaney, S. J. and Regenstein, J. M. 2006. Properties of Alaska pollock skin gelatin: A comparison with tilapia and pork skin gelatins. J. Food Sci. **71**(6):C313-C321.

[5]Kasankala, L. M., Xue, Y., Wei, L.Y., Hong, S.D.and He, Q.2007. Optimization of gelatine extraction from grass carp (*Catenopharyngodon idella*) fish skin by response surface methodology. Bioresource Technol. **98**(17):3338-3343.

[6]Yang, H., Wang, Y., Jiang, M., Oh, J.H., Herring, J. and Zhou, P. 2007. 2-Step optimization of the extraction and subsequent physical properties of channel catfish (*Ictalurus punctatus*) skin gelatin. J. Food Sci. **72**(4):C188-C195.

[7]Bae, I., Osatomi, K., Yoshida, A., Osako, K.,Yamaguchi, A.and Hara, K. 2008. Biochemical properties of acid-soluble collagens extracted from the skins of underutilised fishes. Food Chem. **108**(1):49-54.

[8]Benjakul, S., Oungbho, K., Visessanguan, W., Thiansilakul, Y. and Roytrakul, S. 2009. Characteristics of gelatin from the skins of bigeye snapper, *Priacanthus tayenus* and *Priacanthus macracanthus*. Food Chem. **116**(2):445-451.

[9]Wang, Y. and Regenstein, J. M. 2009. Effect of EDTA, HCl, and citric acid on Ca salt removal from Asian (silver) carp scales prior to gelatin extraction. J. Food Sci. **74**(6):C426-C431.

[10]Wangtueai, S. and Noomhorm, A. 2009. Processing optimization and characterization of gelatin from lizardfish (*Saurida* spp.) scales. LWT - Food Sci. and Technol. **42**(4):825-834.

[11]Ngo, D.-H., Qian, Z. J., Ryu, B.M., Park, J.W. and Kim, S.K. 2010. *In vitro* antioxidant activity of a peptide isolated from Nile tilapia (*Oreochromis niloticus*) scale gelatin in free radical-mediated oxidative systems. J Funct. Foods **2**(2):107-117.

[12]Zhang, F., Xu, S. and Wang, Z. 2011. Pre-treatment optimization and properties of gelatin from freshwater fish scales. Food Bioprod. Process. **89**(3):185-193.

[13]Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., Nagai, T. and Tanaka, M. 2005. Characterisation of acid-soluble collagen from skin and bone of bigeye snapper (*Priacanthus tayenus*). Food Chem. **89**(3):363-372.

[14]Jamilah, B. and Harvinder, K. G. 2002. Properties of gelatins from skins of fish-black tilapia (*Oreochromis mossambicus*) and red tilapia (*Oreochromis nilotica*). Food Chem.**77**(1):81-84.

[15]Zhang, Y., Liu, W., Li, G., Shi, B., Miao, Y. Q. and Wu, X. H. 2007. Isolation and partial characterization of pepsin-soluble collagen from the skin of grass carp (*Ctenopharyngodon idella*). Food Chem. **103**(3):906-912.

[16]Chen, S., Hirota, N., Okuda, M., Takeguchi, M., Kobayashi, H., Hanagata, N. and Ikoma, T. 2011. Microstructures and rheological properties of tilapia fish-scale collagen hydrogels with aligned fibrils fabricated under magnetic fields. Acta Biomater. **7**(2):644-652.

[17]Singh, P., Benjakul, S., Maqsood, S. and Kishimura, H. 2011. Isolation and characterisation of collagen extracted from the skin of striped catfish (*Pangasianodon hypophthalmus*). Food Chem. **124**(1):97-105.

[18]Kołodziejska, I., Skierka, E., Sadowska, M., Kołodziejski, W. and Niecikowska, C. 2008. Effect of extracting time and temperature on yield of gelatin from different fish offal. Food Chem. **107**(2):700-706.

[19]Bradbury, E. and Martin, C. 1952. The effect of the temperature of preparation on the mechanical properties and structure of gelatin films. P. Royal Soc. **214**(4):183-192.

[20]Liu, H. Y., Li, D. and Guo, S. D. 2008. Extraction and properties of gelatin from channel catfish (*Ietalurus punetaus*) skin. LWT - Food Sci. and Technol. **41**(3):414-419.

[21]ISO 3496. 1978. Meat and Meat Products. Determination of L(-)-hydroxyproline. International Organization for Standardization, Switzerland.

[22]Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227**:680-685.

[23]Gómez-Estaca, J., Montero, P., Fernández-Martín, F. and Gómez-Guillén, M.C. 2009. Physico-chemical and film-forming properties of bovine-hide and tuna-skin gelatin: A comparative study. J. Food Eng. **90**(4):480-486.

[24]Zeng, S., Yan, X., Cao, W., Hong, P., Zhang, C. and Li, L. 2010. Optimisation of extraction conditions and characteristics of skin gelatin from Nile tilapia (*Oreochromis niloticus*). Int. J. Food Sci. Technol. **45**(9):1807-1813.

[25]Muyonga, J. H., Cole, C. G. B. and Duodu, K.G. 2004. Extraction and physico-chemical characterisation of Nile perch (*Lates niloticus*) skin and bone gelatin. Food Hydrocolloids **18**(4):581-592.

[26]Haug, I. J., Draget, K. I. and Smidsrød, O. 2004. Physical and rheological properties of fish gelatin compared to mammalian gelatin. Food Hydrocolloids **18**(2):203-213.

[27]Fernández, M. D., Montero, P. and Gómez-Guillén, M.C. 2003. Effect of freezing fish skins on molecular and rheological properties of extracted gelatin. Food Hydrocolloids **17**(3):281-286.