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Gelatin extraction from Kumakuma (*Brachyplathystoma filamentosum*) skin using the liming method

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Gelatin extraction process from Kumakuma (*Brachyplathystoma filamentosum*) skin was optimized using a calcium hydroxide solution. The gelatin obtained was characterized through scanning electron microscopy and analyses of yield, gel strength, color, viscosity, amino acid profile, melting point, foaming capacity, and emulsifying capacity. The optimized conditions were defined over ten days of pre-treatment at 50°C. This condition resulted in desirability of 0.965 and yield and gel strength values of 20.24% and 221 g, respectively. Glycine was the main amino acid both in the fish skin (11.68%) and in the gelatin obtained (23.39%). Gelatin had extendable and consistent gel characteristics and its microstructure showed even threads with small gaps throughout, which is favorable for the food industry.

Key words: Residue, fish, pre-treatment, gelatin, gel strength.

INTRODUCTION

Gelatin is a valuable protein derived from animal by-products obtained through partial hydrolysis of collagen originated from cartilages, bones, tendons and skins of cold-water (cod, king weakfish, salmon, among others), and warm-water fish (tuna, catfish, tilapia, among others) (Sakr, 1997). However, no study has been carried out with the Amazon species Kumakuma (*Brachyplathystoma filamentosum*), a large-size fish that can reach over 1.5 m in the overall length and weigh 100 kg, which is widely used in the filleting industry (Gonçalves et al., 2003).

The procedures used to extract collagen from fish normally involve chemical pre-treatment of the raw material and mild temperature during the process (Karim and Bhat, 2009). Depending on the method employed in the pre-treatment, two types of gelatin with different characteristics can be produced. Type-A gelatin (isoelectric point at pH 6 to 9) is produced by acid treatment of the collagen, while type-B gelatin (isoelectric

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point at pH 5) is produced by alkaline treatment (Stainsby, 1987). Gelatin is a protein derived from the partial hydrolysis of collagen and is made up of approximately 19 amino acids (Osborne et al., 1990). Collagen's thermal stability is related to its imino acid content (proline and hydroxyproline). The higher this content, the greater the stability of the bonds among proteins. Collagen is denatured at temperatures above 40°C, which produces a mix of species with one, two, or three randomly weaved polypeptide chains. On the other hand, the controlled cooling of collagen (below its melting point) causes the recovery of the helicoidal structure (Wong, 1994).

The liming process, which uses calcium hydroxide ($Ca(OH)_2$) in the collagen pre-treatment, was especially designed to extract gelatin from the skin and bones of mammals. This type of process normally takes days or months depending on the calcium hydroxide concentration and temperature employed (Schreiber and Gareis, 2007). An alkaline extraction process of type-B gelatin from fish was patented by Stanley (2002). This process consists of an initial extraction through liming for 42 days followed by an acid extraction process. The use of $Ca(OH)_2$ is usually preferred for its ability to regulate alkalinity, which does not allow the collagen to elongate and lose firmness (Ockerman and Hansen, 1999). In addition, the gelatin yield and bloom strength are much higher (Jamilah et al., 2011). Response surface methodology (RSM) has been an effective tool to control food processes. It is an important experimental design and a critical technology in process optimization (Cho et al., 2004).

The worldwide production of gelatin was approximately 326,000 metric tons, 46% which is from pig skins, 29.4% from bovine hide, 23.1% from bones, and 1.5% from other parts (GME, 2008). However, the common occurrence of bovine spongiform encephalopathy (BSE) has caused problems to human health and, therefore, the use of by-products from mammals has been limited in the processing of foods, cosmetics, and pharmaceuticals (Cho et al., 2005). Thus, research on processes to obtain gelatin from fish, particularly from skin and bones, has increased the aim to obtain a product with properties equivalent to those of gelatin from mammals (Gudmundsson, 2002). The present study aimed to obtain gelatin from fish skin using the liming method, as well as to determine the ideal processing conditions using the response surface methodology, besides assessing desirability function and characterizing the properties of the gelatin obtained.

MATERIALS AND METHODS

The study used fresh skins from Kumakuma (*B. filamentosum*) fish purchased at the Ver-o-Peso fish market in the city of Belém, PA, Brazil. The raw material was transported under refrigeration (2 to 4°C) in isothermic boxes to the laboratory of products of animal origin (Laboratório de Produtos the Origem Animal – LAPOA) of the Federal University of Pará (UFPA), where the trials were carried out.

Obtaining gelatin

The gelatin was obtained by the liming method based on the methodology proposed by Jamilah et al. (2011). The fish skins were washed in running water to remove undesirable materials, and after excess water was drained, the skins were immersed in a saturated $Ca(OH)_2$ solution at a concentration of 27 g/L at 20°C. For each kilogram of wet skin, 2 L saturated solution were used as impregnation medium. After pre-treatment (6 to 14 days), the skins were removed and washed in ten parts of water (m/m) to remove excess alkali, maintaining the skins at pH 10. For each 20 g of skin, 100 ml distilled water were added and the system was maintained in a water bath (36 to 64°C) for collagen extraction. Next, hydrochloric acid was used to lower the solution's pH to approximately 5. The solution was then filtered in Whatman no. 4 filter paper and the denatured collagen (gelatin) collected was placed in trays, frozen at -50°C, and lyophilized for 30 h. The lyophilized product (gelatin) was vacuum packaged in polyethylene bags, stored at -22°C, and later subjected to the assays in the experimental design.

Analytical determinations

Analyses were performed for moisture (method no. 950.46), total proteins with correction factor 5.55 (method no. 928.08), lipids (method no. 960.39), and ashes (method no. 920.153) according to the AOAC (2002). Skin pH was determined through AOAC method no. 981.12 and gelatin pH, using the methodology proposed by Schrieber and Gareis (2007). The amino acid profile was determined using a Waters-PICO Tag™ high-performance liquid chromatograph (Waters model 712 WISP, Watford, Herts, UK) according to White et al. (1986). Water activity was determined with an Aqualab 3TE electronic hygrometer (Decagon Devices Inc., USA). All analyses were performed in triplicate. Instrumental color was determined with a CR 310 colorimeter (Minolta, Japan) using the Commission Internationale de L'Éclairage (CIE) L*, a*, and b* space, where L* is luminosity, a* is red color intensity, and b* is yellow color intensity. The chroma index (c*) and hue angle (h°) were calculated (Hunterlab, 2008).

Determining the technological properties

The total yield and gelatin yield (%) were calculated from the ratio between gelatin weight and skin wet weight (Binsi et al., 2009). Gel strength (Bloom) was determined in a texture analyzer using a cylindrical Teflon probe 12.5 mm in diameter pressing 4 mm into the gelatin at 1 mm/s (Choi and Regenstein, 2000). The morphological analyses were carried out in a LEO-1430 (LEO, USA) scanning electron microscope. The samples were metallized with gold using a coating time of 1.5 min. The analysis conditions for the secondary electron images were: electron beam current = 90 µA, constant acceleration voltage = 10 kV, and work distance = 15 mm. The melting point was investigated based on the methodology by Choi and Regenstein (2000). The foaming capacity (FC) was determined in gelatin solutions at different concentrations (1, 2, and 3%) and homogenized at 1,750 rpm for 1 min at room temperature (24°C). FC was calculated from the ratio between the volumes before and after homogenization (Shahiri et al., 2010).

The emulsifying capacity (EC) was determined according to Shahiri et al. (2010), with modifications. Twenty milliliters of 3.3% gelatin solution were mixed with 20 ml soybean oil. The mix was homogenized at 1,750 rpm for 30 s and then centrifuged at 2,000 *g*

Table 1. 2^2 experimental design matrix with the results obtained for yield (%) and gel strength (g).

Test	Coded		Real		Response	
	A	B	Time (h)	Temperature (°C)	Yield (%)	Gel strength (g)
1	-1.00	-1.00	7.0	40.0	14.0	70.0
2	-1.00	1.00	7.0	60.0	30.0	39.0
3	1.00	-1.00	13.0	40.0	10.0	78.0
4	1.00	1.00	13.0	60.0	23.5	57.0
5	-1.41	0.00	6.0	50.0	21.0	89.0
6	1.41	0.00	14.0	50.0	16.0	163.0
7	0.00	-1.41	10.0	36.0	12.0	80.0
8	0.00	1.41	10.0	64.0	26.0	75.0
9	0.00	0.00	10.0	50.0	25.1	243.0
10	0.00	0.00	10.0	50.0	21.0	240.0
11	0.00	0.00	10.0	50.0	24.9	200.0

for 5 min. EC was calculated as the ratio between the volume of the emulsified portion and the initial volume. Viscosity was determined according to Yang et al. (2008). The sample was placed in a water bath at 45°C and transferred to the Ostwald-Fensk Viscosimeter (no. 100), which was placed in a water bath at 60°C for 10 min for temperature stabilization. The reading was expressed in centipoise (cP).

Experimental design

A central rotatable composite design (CRCD) and the surface response methodology (SRM) were used to define the best conditions for the responses of total process yield and gel strength allied to the appropriate viscosity conditions for the product's commercial purposes. Eleven (11) assays (Table 1) were performed, four were factorial (combination among the levels ±1), three in the central point (two variables at level 0), and four axial (one variable at level ±α and the other variable at level 0). For each response, variable significance or interactions were verified using the polynomial equation described in Equation 1.

$$Y = f(X) = \beta_0 + \beta_1(A) + \beta_{11}([A])^2 + \beta_2(B) + \beta_{22}(B)^2 + \beta_{12}(AB) \quad (1)$$

where Y is the dependent variable (gel yield and strength); β_0 is the constant; β_i, β_{ii}, and β_{ij} are regression coefficients; X_i and X_j are the level of the independent variables.

The deviations and relative deviations between the experimental values and those predicted by the models for each response variable at the optimal condition were calculated by Equations 2 and 3, respectively:

$$\text{Deviation} = Y - \hat{Y} \quad (2)$$

$$\text{Relative deviation} = Y\text{-}\hat{Y}/Y \times 100 \quad (3)$$

where Y is the experimental response and $\hat{Y}$ is the response predicted by the model.

Statistical analysis

The experimental data of the design and analysis of variance (ANOVA) and the determination of the optimal point of the design through the desirability function were analyzed using the software Statistica 7.0 for Windows.

RESULTS AND DISCUSSION

Optimizing the gelatin obtention process

The last two columns of Table 1 presented the experimental results obtained for yield (%) and gel strength (g). The estimated coefficients of the factors for the model of each response assessed are shown in Table 2. The effects in bold indicated that the variable had a significant effect ($p \leq 0.05$). Obtaining higher yields in gelatin extraction processes was the key to enable its use as a potential source of production. The effect on total yield was significant and positive for linear extraction temperature (B), that is, the higher this factor, the greater the yield. Figure 1 shows higher yields in the ranges in which higher extraction temperatures were employed, which may have allowed for more pronounced collagen hydrolysis and led to higher yields.

For gel strength (Table 2), the effects were significant and negative for quadratic pre-treatment time (AA) and quadratic extraction temperature (BB), that is, the higher these factors, the lower the gel strength will be. It can also be seen that the linear pre-treatment time (A) had the greatest effect on gel strength. The increase in temperature lead to greater gel strength, however, as temperature increased, gel strength tended to decrease with the consequent formation of low-molecular-weight peptides. It had been known that gel strength and the other functional properties of gelatins (viscosity, melting point, and gelling point) were dependent on the gelatins' molecular weight distribution and amino acid composition (Johnston-Banks, 1990). The highest gel strength values were obtained when temperatures around 50°C were employed (Figure 2).

ANOVA resulted in R^2 values of 0.93 and 0.92 for gel

Table 2. Estimate of the variables of second-order polynomials (eq. 1) associated with the significance for each response studied (pure error).

Factors	Yield (%)		Gel strength (g)	
	Effects	p-Value	Effects	p-Value
Constant	**23.622**	**0.003**	**229.032**	**0.003**
A	-4.544	0.114	33.000	0.199
AA	-5.216	0.132	**-136.306**	**0.025**
B	**12.301**	**0.017**	-15.639	0.454
BB	-4.143	0.164	**-167.531**	**0.013**
AB	-1.250	0.642	5.000	0.854

A = Extraction time (h); B = Extraction temperature (°C). The effects in bold indicate the variable had a significant effect ($p < 0.05$).

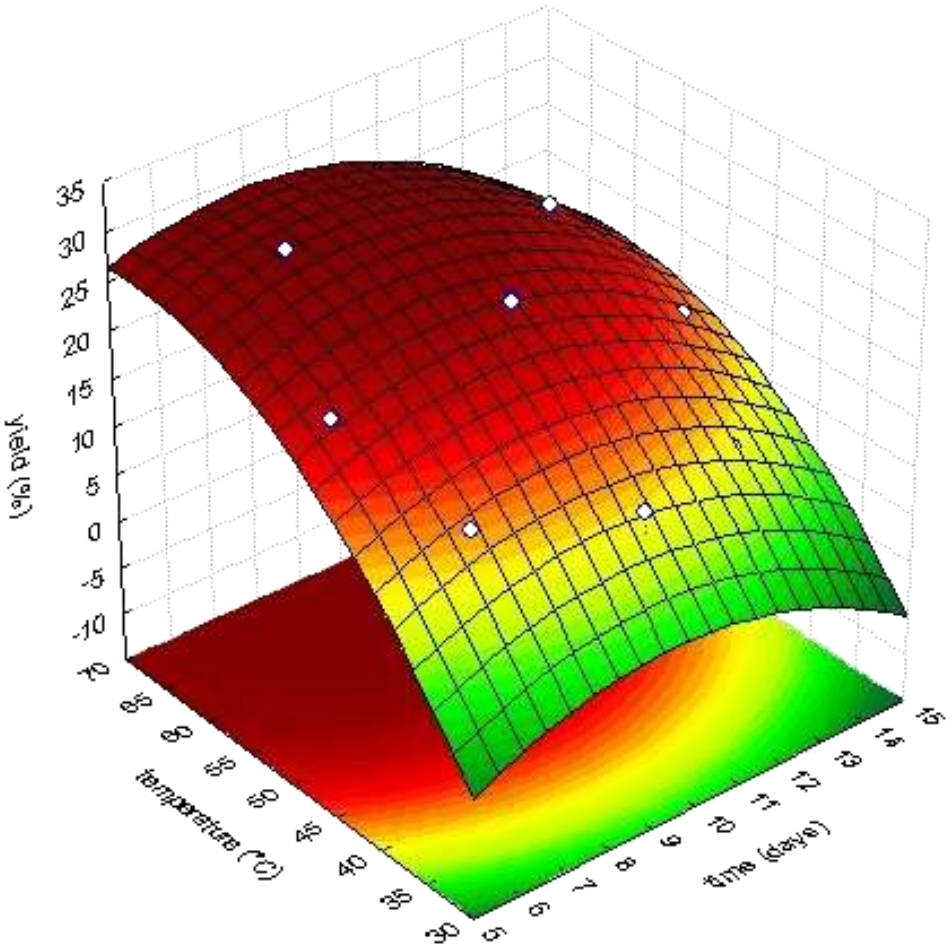

Figure 1. Yield response surface as a function of time (days) and temperature (°C).

yield and gel strength, respectively (Table 3), which showed the model adequately predicted the process behavior by explaining over 92% of the experimental data. The lack-of-fit for the equation of gel yield and gel strength response was not significant (Table 3) since Fcal>Ftab, which suggested that the predictive equation can be used for any combination of the values of the variables studied. The increase in yield was proportional to the increase in extraction temperature, which caused greater collagen hydrolysis and reduced gel strength. The severity in the extraction treatment is crucial for the gelatin's functional properties (Montero et al., 2002). Gel

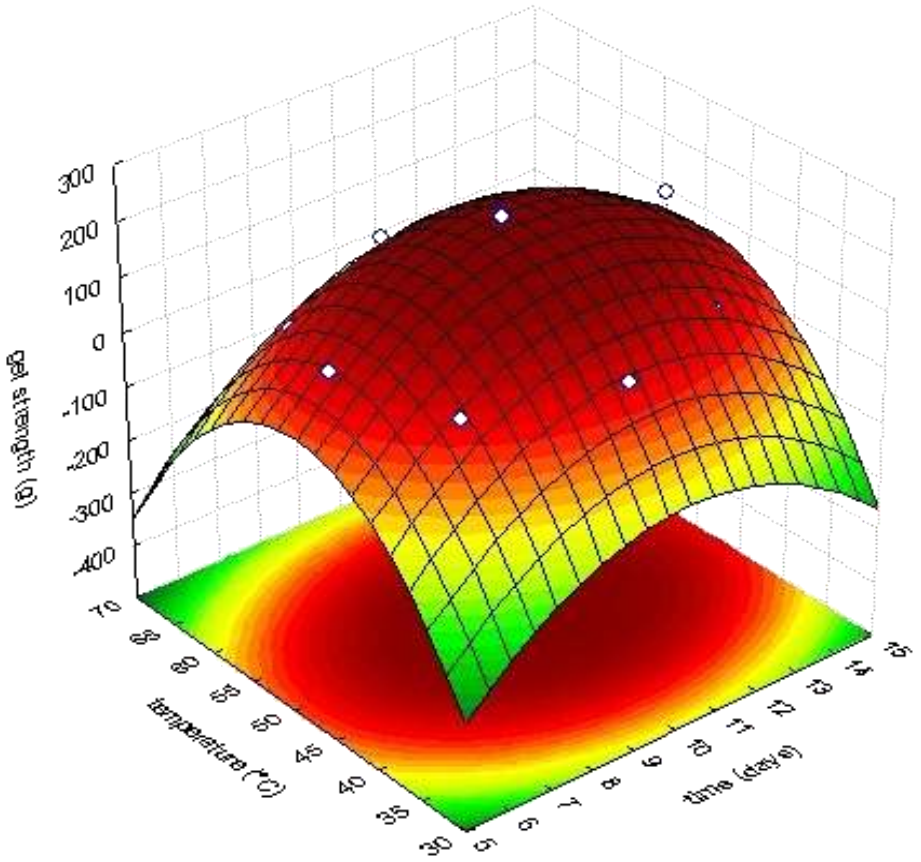

Figure 2. Gel strength response surface as a function of time (days) and temperature (°C).

Table 3. Analysis of variance (ANOVA) for gel yield and strength as functions of the independent variance (pre-treatment time and temperature), F test, and R^2.

Sources	SS	DF	MS	F cal.	F tab.	R^2
			Yield			
Regression	292.10	1	292.1	21.36	18.51	0.93
Residue	123.05	9	13.67	-	-	-
L. F	112.37	7	16.05	3.0	19.35	-
Error	10.68	2	5.343	-	-	-
Total	415.15	10	41.51564	-		-
Model			23.6228+6.1505(B)			
			Gel strength			
Regression	50126.82	2	25063.41	28.26	19.0	0.92
Residue	7093.36	8	886.67		-	-
L. F	5940.69	6	990.11	1.71	19.3	-
Error	1152.67	2	576.33	-	-	-
Total	57220.18	10	5722.01	-	-	-
Model			229.03 -68.15(AA)-83.76(BB)			

consistency decreased for extraction temperatures above 50°C, which matches Ledward (1986), Norman et al. (2000), and Cho et al. (2004). According to these authors, higher extraction temperatures cause the

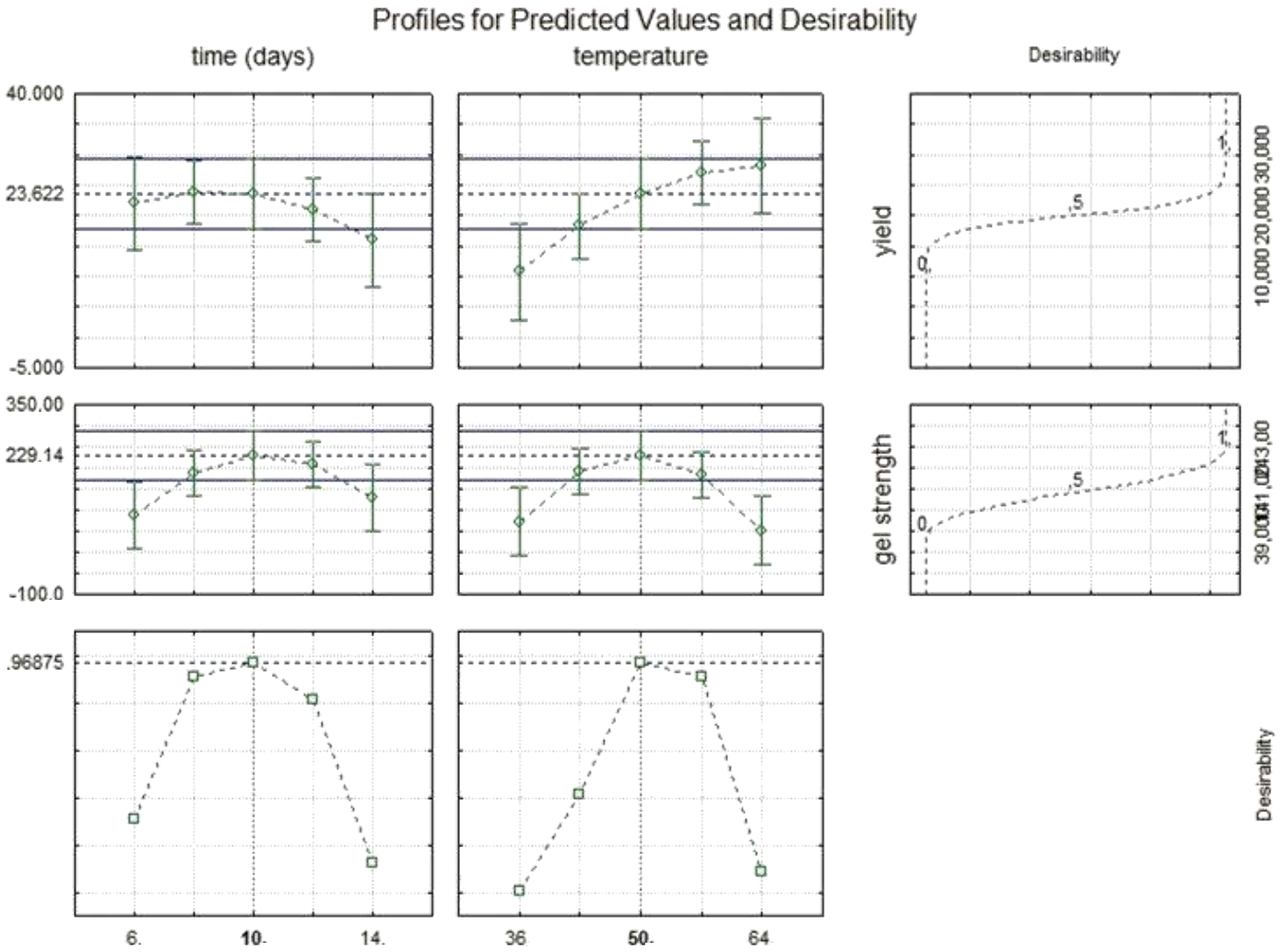

Figure 3. Desirability function for yield (%) and gel strength (g).

breakdown of properties and the protein fragments produced decrease the gelling capacity and, consequently, gel strength. Thus, employing high temperatures and long pre-treatment times is desirable to obtain higher yields, but cause the fragmentation of alpha (α) chains in the collagen, which reduces gel strength (Johnston-Banks, 1990; Cho et al., 2004). The increase in pre-treatment time close to the optimal point (10 days) caused an increase both in yield and gel strength. A similar result was found by Johnston-Banks (1990) and Poppe (1992), who reported that the number of cross bonds must be weakened enough to convert the collagen into a form appropriate for extraction. According to the desirability function for the gel yield and gel strength responses (Figure 3), over 10 days of pre-treatment and extraction temperature of 50°C can be obtained for maximum desirability condition (0.96), 23.62% yield and 229.14 g gel strength. According to Jamilah and Harvinder (2002), the yield of gelatin from fish varies from 6 to 19% and gel strength, from 250 to 260 g. Assays were carried out at the optimal temperature (50°C) and pre-treatment time (10 days) so that the experimental values of gel yield and strength were compared to those predicted by the regression models. The difference between the experimental and predicted values resulted in a relative deviation of -3.6% for yield and -16.6% for gel strength, which shows that the method can be used to predict the yield and gel strength of gelatin within the experimental domain.

Characterization of the skin and gelatin

Table 4 shows the values of composition and physicochemical parameters determined for the skin and the gelatin obtained. Skin moisture was at the same magnitude observed for corvina (*Johnius dussumieri*, 62.3%) and shortfin scad (*Decapterus macrosoma*,

Table 4. Physicochemical properties of the skin and gelatin extracted from kumakuma skin.*

Components	Skin*	Gelatin*
Moisture (%)	58.83 ± 0.57	11.37 ± 0.98
Lipid (%)	14.25 ± 0.22	5.39 ± 0.21
Protein (%)	31.08 ± 0.99	81.67 ± 0.83
Ash (%)	0.37 ± 0.04	0.34 ± 0.06
pH	6.72 ± 0.01	4.50 ± 0.08
Water activity	0.98 ±0.06	0.25 ± 0.01

*3 replicate.

60.4%) (Cheow et al., 2007). For the gelatin, moisture was within the range observed for commercial gelatins (9 to 14%) (Eastoe and Leach, 1977). The fat content in the skins was high (14.25 ± 0.22%) since the fish used had a high fat content. Ribeiro et al. (2013) found 14.5 ± 0.3% fat in Kumakuma muscle. Shahiri et al. (2012) found 13.12 ± 0.20% fat in the skin and 0.31 ± 0.07% in the collagen of rainbow trout (*Onchorhynchus mykiss*). The fat content of 5.39 ± 0.21% found in the gelatin shows that the treatments prior to the extraction with Ca $(OH)_2$ was efficient in reducing this component. The protein contents in the skin (31.08 ± 0.99%) and gelatin (81.67 ± 0.83%) were similar to those observed in the skin (30.6 ± 0.9%) and gelatin (84.28 ± 5.39%) of Nile tilapia (Rawdkuen et al., 2013). Jamilah et al. (2011) found proteins contents of 31.01 ± 0.48 and 80.02 ± 0.33%, respectively, in the skin and gelatin extracted from the skin of freshwater fish using the liming method. The ash content in the skin was lower than that reported by Bueno et al. (2011) (1.9 ± 0.3%) in tilapia skin. In turn, the ash content of the gelatin was close to that found by Ratnasari et al. (2013) (0.20%) in the gelatin from red tail catfish (*Phractocephalus hemioliopterus*) extracted with $Ca(OH)_2$ and by Sarbon et al. (2013) for gelatin from chicken skin (0.37%). According to Jones (1977) and Muyonga et al. (2004), the maximum ash content recommended for gelatins is 2.6%. However, Benjakul et al. (2009) indicated that high-quality gelatin must not have over 0.5% ashes. Jongiareonrak et al. (2006) suggested that the high protein content and the lower content of moisture, ashes, and fat in gelatin are determined by the raw material or by the residual chemical products after processing. The low pH of the gelatin obtained (4.5 ± 0.08) is attributed to the extraction process, in which the pH of the gelatin solution was reduced with a strong acid. Cheow et al. (2007) also reported low pH values for the gelatin extracted from corvina (3.35) and shortfin scad (4.87). The low water activity value suggests good gelatin stability regarding microbiological degradation.

Table 5 shows the amino acids profile in Kumakuma skin and gelatin. The imino acids proline (Pro) and hydroxyproline (Hyp), which represented 13.59 and 14.40% of the total amino acids in the skin and gelatin, were directly correlated with gel strength (Holzer, 1996) since they play a role in the stabilization of the triple helix (Ramachandran, 1988). Actually, the amino acid composition is key for the physical properties of the gelatin. Gelatins with limited imino acid content tend to have lower melting points (Ratnasari, 2013). The values of hydroxyproline in the skin (8.40%) and in the gelatin (9.35%) matched those reported by Nalinanon et al. (2008) of hydroxyproline representing 7 to 10% of the total amino acids. Glycine was the main amino acid in the skin (23.77%) and in the gelatin (24.97%), which matches the results observed by Cho et al. (2004) and Silva et al. (2014), who found, respectively, 27.54% glycine in gelatin from shark cartilage and 30.7% in gelatin from cobia. According to Nagarajan et al. (2012), one third of collagen is made up of glycine. In order to close the triple helix structure, the small glycine molecules are needed to occupy every third position (Te Nijenhuis, 1977). According to Piez and Sherman (1970), the formation and effective stabilization of the triple helix structure in the collagen requires the repetition of the sequence Gly X-Y, in which X and Y can be any amino acid with at least one proline or hydroxyproline in any other triplet. Thus, GLY-PRO-Y, X-GLY-HYP, and GLY-PRO-HYP are important for the stabilization of the collagen structure (Privalov, 1982).

Small amounts of cysteine (0.01%) and methionine (1.77%) were identified in the gelatin, similarly to what was reported by Hou et al. (2009). These amino acids play a crucial role in the formation of disulfide bonds (Foegeding et al., 1996). The presence of cysteine might indicate that the gelatin contained a small amount of protein from the stroma (Duan et al., 2011; Bougatef et al., 2012). Lysine stabilizes the gelatin structure by forming structures between the cross-link chains. The percentage of lysine in the gelatin was 3.02%, a result close to that found by Cho et al. (2004) in gelatin from shark cartilage (2.27%) and from pigs (2.32%).

The yield (Table 6) obtained for gelatin from Kumakuma skin pre-treated with Ca $(OH)_2$ was 20.24 ± 0.02%. The results were close to those observed by Ratnasari et al. (2014) (23.12%), who used $Ca(OH)_2$ in pre-treatment. Jamilah et al. (2011), using the extraction process with liming for 14 days, obtained yield of 39.97%.

Table 5. Total amino acid profile in kumakuma skin and gelatin (g/100 g protein).

Amino acids (g/100 g)		Skin*	Gelatin*
Aspartic acid	Asp	5.91	5.07
Glutamic acid	Glu	9.30	9.07
Hydroxyproline	Hyp	8.40	9.35
Serine	Ser	1.85	1.84
Glycine	Gly	23.77	24.97
Histidine	Hys	0.36	0.28
Taurine	Tau	2.74	2.93
Arginine	Arg	5.13	5.06
Threonine	The	10.60	10.57
Alanine	Ala	10.36	11.70
Proline	Pro	5.19	5.05
Tyrosine	Tyr	0.63	0.33
Valine	Val	2.70	2.38
Methionine	Met	1.61	1.77
Cysteine	Cys	0.04	0.01
Isoleucine	Ileu	1.34	1.13
Leucine	Leu	3.86	3.06
Phenylalanine	Phe	2.42	1.34
Lysine	Lys	3.48	3.02
Triptofane	Trp	0.08	0.18
Total		99.77	99.11

Table 6. Technological characterization.

Components	Gelatin $Ca(OH)_2$
Yield (%)	20.24 ± 0.02
Gel strength (g)	221.00 ± 9.93
Viscosity (cP)	3.10 ± 0.26
Melting temperature (°C)	23.16 ± 0.23
Emulsifying capacity (%)	51.35±0.02
Foaming capacity	
Solution 1%	106±0.04
Solution 2%	110±0.02
Solution 3%	116±0.01
L* (lightness)	83.41 ± 2.42
a* (green to red)	-4.93 ± 0.19
b* (blue to yellow)	9.63 ± 0.10
c* (chroma)	10.82 ± 0.15
h* (hue angle)	117.09 ± 0.80

Tukey's test with 95% confidence interval ($p < 0.05$). Means of three determinations.

The use of $Ca(OH)_2$ to condition the skin prior to extraction resulted in great gelatin recovery in water. The type of skin, the concentration of the acid in the pre-treatment, the pH conditions, the collagen to be solubilized, the washing treatment, and the swelling process are among the factors that may impact gelatin extraction yield (Ratnasari et al., 2013). Gomez-Guillen et al. (2001) observed that gelatins from different fish species had different structures and physical properties. That is due to the differences in collagen molecules in the skin (Jamilah and Harvinder, 2002). Karim and Bhat (2009) observed that gelatin productivity and quality are influenced by fish species and age, extraction process, and pre-treatment temperature. Gel consistency is one of the most important functional properties of gelatin. The data found suggest that the gel strength of the gelatin pre-treated with $Ca(OH)_2$ (221 ± 9.93 g) favors a firm and resistant gel within the desirable range for foods, which is between 50 and 300 g (GMIA, 2003). A similar result was observed for Nile tilapia (*Oreochromis niloticus*, 263 g) (Grossman and Bergman, 1992), carp (*Cyprinus carpio*, 267 g) (Kansakala et al., 2007), and catfish *(Ictalurus punctatus*, 252 g) (Yang et al., 2007). The viscosity of the gelatin treated with $Ca(OH)_2$ (3.1 ± 0.262 cP) favored a consistent gelatin and expandable gel. The increase in viscosity is followed by the increase in gel strength, melting temperature, and pH (Sperling, 1985; Stainsby, 1987). Grossman and Bergman (1992) observed viscosity of 6.28 cP for gel from red tail catfish (*Phractocephalus hemioliopterus*), whereas Yang et al. (2007) found viscosity below 3.0 cP in gelatin from channel catfish. The results indicate that variations in viscosity may also be related to the different freshwater fish species and to the extraction method.

The melting point of the gelatin treated with $Ca(OH)_2$

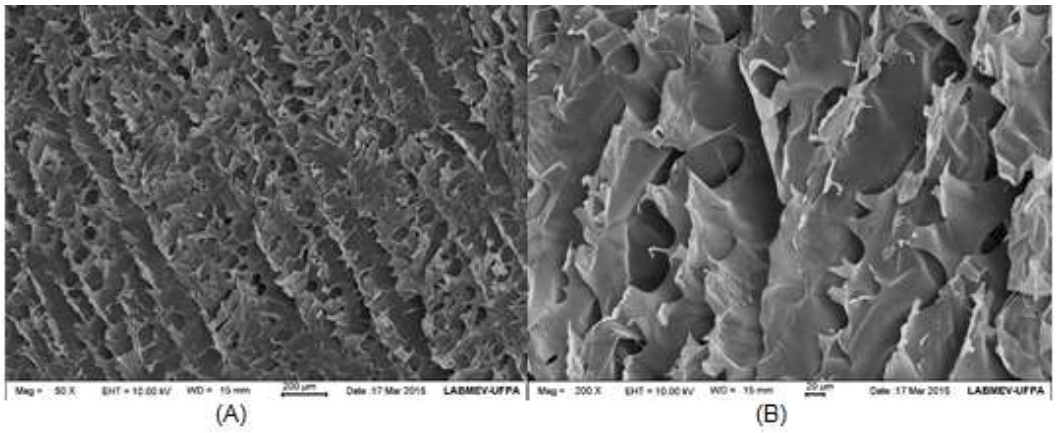

Figure 4. Electron micrographs of kumakuma gelatin at 50 (A) and 200x (B) magnification.

was 23.16 ± 0.23, which was directly related to the Amino acid content in the original collagen molecule. Melting point in this range results in quick-dissolving gelatin, which defines the possible applications in the food industry. Cho et al. (2005) observed melting points of 24.3°C for gelatin from tuna (*Thunnus albacares*), 33.8°C for gelatin from bovines, and 36.5°C for gelatin from pigs. The gelatin had emulsifying power, a parameter that is dependent on the level of exposition of hydrophobic residues in the gelatin's interior (Shyni et al., 2014). The content of the hydrophobic amino acid tyrosine (Table 5) in gelatin from Kumakuma skin was 0.33%. Shyni et al. (2014) mentioned values of 0.25 and 0.26% in gelatin from rohu and tuna skin, respectively. The amount of tyrosine is likely responsible for the high emulsifying power of the gelatins. The solutions of the gelatins obtained for the same protein concentration had foaming capacity, which increased with higher protein concentrations in all gelatin solutions. The hydrophobic surfaces of the peptide chain are responsible for the gelatin's emulsifying and foaming properties (Galazka et al., 1999; Cole, 2008). The gelatin extracted through the liming method had a shiny whitish color. Jamilah et al. (2011), when performing extraction using the liming method, found values of L* were 79.45 ± 1.10, a* were -0.71± 0.09, and b* were 5.75 ± 0.14 in gelatin from striped catfish skin. The c* value far from zero means clear color. The h° defines the hue itself, and when positive, indicates a tendency for clear color (Cheow et al., 2007; Hunterlab Inc, 2008). Such values correspond to the gelatin color, however, this does not impact other functional properties. Figure 4 shows the electromicrographies of gelatin from Kumakuma skin obtained through scanning electron microscopy (MEV). The gelatin's microstructure had even thin threads and small gaps throughout. Overall, the arrangement and combination of protein molecules in the gel matrix directly contributes to the gelatin's gel strength since the microstructure of the gel tissue is related with the gelatin's properties (Yang et al., 2008; Benjakul, 2009). Gels with fewer inter-chain bonds or thinner chains may be more easily degraded by applying force, which results in lower gel strength (Kaewruang et al., 2014).

Conclusion

According to the model proposed, the optimal extraction conditions were established at 10 days of pre-treatment and extraction temperature of 50°C. The use of high temperatures was desirable for obtaining higher yields, but caused a decrease in the gelatin's gel strength. Glycine was the amino acid found in the highest amount both in the skin and gelatin. Gelatin had expandable and consistent gel characteristic and its microstructure showed even threads with small gaps throughout, which is favorable for the food industry.

Conflict of Interests

The authors have not declared any conflict of interests.

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