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Optimization and characterization of gelatin from kumakuma (Brachyplatystoma filamentosum) skin

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ABSTRACT

Fish industry residues are used due to their easy transformation into several products and because they have nutrients with high biological value, being rich in proteins and fatty acids. The gelatin extraction process from the skin of kumakuma (Brachyplatystoma filamentosum) using sodium hydroxide was optimized and the product was characterized. The optimized conditions established in the process were 6 h extraction at 58°C, with yield and gel strength at 19.7% and 244.3 g, respectively, which is considered acceptable for foods. The maximum desirability condition was 0.998. When the technological properties of the gelatin extracted from fish skin was compared with commercial gelatin, a difference (p < 0.05) was observed for all parameters analyzed, but within the appropriate range for gelatin. The gelatin obtained from kumakuma skin may be a new alternative to replace gelatin from mammals, besides contributing to less fish residue released into the environment.

Optimización y caracterización de la gelatina de piel de kumakuma (Brachyplatystoma filamentosum)

RESUMEN

Los residuos de la industria pesquera se utilizan debido a su fácil transformación en diversos productos, también por sus nutrientes de gran valor biológico, los cuales son ricos en proteínas y ácidos grasos. Se optimizó el proceso de extracción de gelatina de piel de kumakuma (Brachyplatystoma filamentosum) mediante la utilización de hidróxido sódico y se caracterizó el producto. Las condiciones optimizadas establecidas en el proceso fueron 6 h de extracción a 58°C, con un rendimiento y resistencia del gel de 19.7% y 244.3 g, respectivamente, lo cual se consideró aceptable para los alimentos. La máxima condición aceptable fue 0.998. Cuando se compararon las propiedades tecnológicas de la piel del pez con la gelatina comercial, se observaron diferencias (p < 0.05) en todos los parámetros analizados, aunque dentro del rango apropiado en lo que concierne a la gelatina. La gelatina obtenida de la piel de kumakuma podría ser una nueva alternativa para remplazar la gelatina de mamíferos, además de contribuir a reducir los residuos de pescado vertidos en el medioambiente.

Introduction

The versatility of employing fish gelatin as food depends on its properties such as gel strength, viscosity, and melting point, among others, and it must not be considered an inferior replacement to traditional gelatin. Gelatin extracted from fish skin and bones has been increasingly used to replace gelatin from mammals. Since it can be used to prepare foods, less fish residue can be produced and issues with bovine spongiform encephalopathy can be avoided. This disease, which affects humans, has limited the use of bovine byproducts in the processing of functional foods, cosmetics, and pharmaceutical products (Gudmundsson, 2000; Muyonga, Cole, & Duodu, 2004; Nagarajan, Benjakul, Prodpran, & Songtipya, 2012).

Collagen is part of the supporting structures in vertebrates and invertebrates and it is an important component of the body wall in birds and fish (Herausgegeben, Ward, & Courts, 1977). Collagen contains high amounts of hydroxyproline and proline; the more abundant these imino acids, the more rigid and resistant it becomes (Arnesen & Gildberg, 2002). Gelatin’s amino acid composition is similar to that of the collagen from which it was obtained and is characterized by the gly–x–y sequence, where x is usually proline and y hydroxyproline (Herausgegeben et al., 1977).

Gelatin is a denatured protein derived from collagen by thermo-hydrolysis and has the rheological property of thermoreversible transformation between solid and gel. Gelatin is one of the most important biopolymers, with widespread applications in the food, pharmaceutical, cosmetic, and photographic industries. Recently, its use has been expanding to new applications such as functional foods. The properties of gelatin for a given application are strongly influenced not only by the species from which it is extracted but also by the pretreatment source, by the raw material, and by the process parameters (pH, gel maturation time, temperature, etc.) (Gómez-Guillem, Ihl, Bifani, Silva, & Monteiro, 2007; Kolodziejska, Skierka, Sadowska, Kolodziejski, & Niecikowska, 2008).

Many fish species have been studied as raw material for the extraction of gelatin and its properties. Muyonga et al.
(2004) studied the extraction and physicochemical characterization of gelatin from skin and bones of Nile perch (Lates niloticus). Bueno, Alvim, Koberstein, Portela, and Grosso (2011) characterized the gelatin extracted from tilapia (Oreochromis niloticus) skin and compared it with commercial gelatin from pigs. Alfaro (2004) optimized the process (alkaline and acid pretreatment) and determined the functional properties of gelatin from king weakfish (Macrondon ancyodon), while Kolodziejska et al. (2008) determined the ideal conditions to prepare gelatin from different types of fish residue. However, few studies have investigated procedures to optimize extraction, which is an important tool to understand the processing conditions and obtain a final product with the desired characteristics (Shahiri, Maghsoudlou, Motamedzadegan, & Mahoonak, 2010).

The filleting of fish species in Amazonian industries, street markets, and supermarkets generates a considerable amount of skins, which are discarded as residue. Such residue is a viable alternative for gelatin production and one of these species is kumakuma (Brachyplatystoma filamentosum). So far, no gelatin has been extracted from the skin of this species and no information on its properties is available. In face of that, this study aimed to establish the optimal processing conditions to obtain gelatin from kumakuma skin using response surface methodology (RSM) and the desirability function and to characterize the properties of the gelatin obtained.

**Material and methods**

Fresh kumakuma (B. filamentosum) skins were purchased at the Ver-o-Peso market in the city of Belém, PA, Brazil and transported under refrigeration in isothermic boxes to the Laboratory of Animal-Origin Products (LAPOA) of the Federal University of Pará (UFPA) for later use. Skins were removed at −20°C and kept frozen until use. The skins were cut into small pieces (4 cm × 4 cm) with scissors, placed in polyethylene bags, and kept at −25°C until used (within a week). All chemicals were analytical grade.

### Obtaining gelatin

The collagen was obtained following the methodology described by Montero and Gómez-Guillén (2000), with adaptations. After being washed in running water, the fish skins were cut into 4 cm × 4 cm pieces. First, the skins were immersed in a 0.6-mol/L sodium chloride (NaCl) aqueous solution for 15 min followed by immersion in a 0.3-mol/L sodium hydroxide (NaOH) solution for 15 min and, finally, a 0.02-mol/L acetic acid (C₂H₄O₂) solution for 60 min. In all steps, immersion took place under stirring and the skins were then washed with water, with three repetitions. Water was added to the material obtained and the mix was placed in a water bath to extract the collagen. After heating, the supernatant was collected and filtered with Whatman no. 4 filter paper. The gelatin obtained was placed on trays, frozen at −50°C, and lyophilized for 30 h. The lyophilized product was vacuum packaged, stored at −10°C, and later subjected to the assays in the experimental design.

**Amino acid and physicochemical determinations of the skin and gelatin extracted from kumakuma skin**

Analyses were performed for moisture (method no. 950.46), total proteins (method no. 928.08), lipids (method no. 960.39), and ashes (method no. 920.153) according to the Association of Official Analytical Chemists (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 total amino acid profile was determined using a Waters-PICO Tag™ high-performance liquid chromatograph (Waters model 712 WISP, Watford, Herts, UK) following the methodology proposed by White, Hart, and Kry (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 CIE (Commission Internationale de l’Eclairage) 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 Inc, 2008).

**Characterization of gelatin**

The technological properties of the fish gelatin and the flavorless commercial gelatin (Royal®, Brazil) were determined under the same experimental conditions.

The total yield (%) and gelatin yield were calculated from the ratio between the gelatin weight and the skin’s wet weight (Binsl, Shamasundar, Dileep, Badii, & Howell, 2009).

Gel strength (Bloom) was determined in a texture analyzer using a cylindrical Teflon probe with 12.5 mm diameter pressed 4 mm into the gelatin at 1 mm/s (Choi & Regenstein, 2000).

The morphological analyses were carried out in a LEO-1430 (LEO, USA) scanning electron microscope. The samples were metallized with gold using 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 assessed with a 6.67% gelatin solution, to which a drop of methylene blue dye was added and the solution was placed in a water bath at 15°C. In every 5 min, temperature was raised by 5°C until the displacement of the dye to the center of the solution was determined 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 1750 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. A volume of 20 mL 3.3% gelatin solution was mixed with 20 mL soybean oil. The mix was homogenized at 1750 rpm for 30 s and then centrifuged at 2000g 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 the methodology described by Yang, Wang, Zhou, and Regenstein (2008). The sample was placed in a water bath at 45°C and transferred into 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).

**Statistical analysis**

A central rotatable composite design and the RSM 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. It was also considered that the process must have low complexity for it to be more viable at industrial scale. The assay was carried out in triplicate.

Eleven assays (Table 1) were performed, four of which factorial (combination between 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 a polynomial equation:

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

where $Y$ is the dependent variable (gel yield and strength), $\beta_0$ is the constant, $\beta_1$ and $\beta_0$ are the regression coefficient, and $X_1$ and $X_2$ are the level of the independent variables.

The deviations and relative deviations between the experimental values and those predicted by the models for the responses at the optimal condition were calculated by Equations 2 and 3, respectively.

Deviation = $Y - \bar{Y}$

Relative deviation = $\frac{Y - \bar{Y}}{\bar{Y}} \times 100$

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

The desirability function was applied to determine the design’s optimal point and the statistical analysis of the data was carried out through analysis of variance and Tukey’s test to determine the significant differences of the means of the analyses performed on the gelatins, with 95% confidence ($p < 0.05$). The software STATISTICA 7 for Windows was used.

**Results and discussion**

**Optimizing the gelatin obtention process**

Table 1 presents the experimental results obtained for gelatin yield (%) and gel strength (g). The estimates of the factor coefficients for the model of each response assessed are presented in Table 2. As for total yield, the effects were significant ($p < 0.05$) for lineal extraction temperature ($B$), where the higher temperature led to higher gelatin yield. Using high temperatures causes greater collagen hydrolysis, thus leading to higher yields (Holzer, 1996). The quadratic extraction temperature (BB) has a significant ($p < 0.05$) negative effect on gel strength, i.e. a reduction in this factor may lead to lower gel strength.

Table 3 shows that the value of $F$ calculated for the lack-of-fit was lower than $F$ tabulated both for gel yield and strength. The $R^2$ value was 0.83 for both parameters studied, which suggests that the model appropriately defined the process behavior by explaining 83% of the variation in the experimental data. In addition, the lack-of-fit for the equations of gel yield and strength was not significant ($p \geq 0.05$), which suggests that the equations could be used for predictive purposes in the experimental domain studied.

Based on the experimental data, employing higher temperatures led to higher yields (Figure 1(a)). However, lower gel strength was observed in the gelatin, which is attributed to the formation of a larger amount of compounds with low-molecular weight (Holzer, 1996). Extraction temperatures of up to 60°C are appropriate.

Figure 1(b) shows that the higher temperature and longer extraction time led to greater gel strength; however, gel strength decreased as temperature gradually increased. The highest gel strength values were obtained between 50 and 60°C and between 6 and 9 h. Biluca, Marquetти, and Alfaro

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

| Test | A  | B  | Temperature (°C) | Yield (%) | Gel strength (g) |
|------|----|----|-----------------|-----------|-----------------|
| 1    | −1.00 | −1.00 | 7.0            | 12.0      | 89              |
| 2    | −1.00 | 1.00  | 7.0            | 24.7      | 166             |
| 3    | 1.00  | −1.00 | 11.0           | 16.0      | 97              |
| 4    | 1.00  | 1.00  | 11.0           | 24.7      | 86              |
| 5    | −1.41 | 0.00  | 6.0            | 10.0      | 301             |
| 6    | 1.41  | 0.00  | 12.0           | 19.4      | 225             |
| 7    | 0.00  | −1.41 | 9.0            | 8.0       | 70              |
| 8    | 0.00  | 1.41  | 9.0            | 23.0      | 121             |
| 9    | 0.00  | 0.00  | 9.0            | 18.2      | 256             |
| 10   | 0.00  | 0.00  | 9.0            | 15.7      | 240             |
| 11   | 0.00  | 0.00  | 9.0            | 14.7      | 210             |

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

| Factors | Yield (%) | Gel strength (g) |
|---------|-----------|-----------------|
| Constant | 16.3635   | 0.0040          |
| A        | 4.2588    | 0.0749          |
| AA       | 0.2499    | 0.8733          |
| B        | 10.6625   | 0.0319          |
| BB       | 1.3472    | 0.4711          |
| AB       | −2.0000   | 0.3772          |

**Table 3. Analysis of variance (ANOVA) for gel yield and strength as functions of the independent variance, $F$ test, and $R^2$.**

| Sources | $SS$ | $DF$ | $MS$ | $F$ cal. | $F$ tab. | $R^2$ |
|---------|------|------|------|---------|---------|-------|
| Yield   | Regression 272.24 | 1 | 272.24 | 46.68 | 18.51 | 0.83 |
|         | Residue 52.48 | 9 | 5.83 |
|         | LF 45.17 | 7 | 6.45 | 1.98 | 19.35 |
|         | Error 6.50 | 2 | 3.25 |
|         | Total 324.7273 | 10 | 32.4773 |
| Gel strength | Regression 54.392.12 | 1 | 54.392.12 | 24.62 | 18.51 | 0.83 |
|         | Residue 11.045.51 | 5 | 2209.10 |
|         | LF 8264.42 | 7 | 1180.63 | 2.16 | 19.35 |
|         | Error 1090.67 | 2 | 545.33 |
|         | Total 65.437.64 | 10 | 6543.76 |
| Model 16.36 + 5.66(B) | 232.64 – 86.14 (BB) |

$SS$: Sum of squares; $DF$: degrees of freedom; $MS$: mean square; $LF$: lack-of-fit; $SS$: suma de cuadrados; $DF$: margen de libertad; $MS$: promedio de la media cuadrática, $LF$: falta de correspondencia.

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observed that the variation in extraction time and temperature greatly impacted gelatin yield. Arnesen and Gildberg (2002); Muyonga et al. (2004); and Cho, Gu, and Kim (2005) reported that gelatins obtained through extractions at high temperatures could lead to the extraction of low-molecular-weight proteins, thus increasing yield while decreasing gel strength.

At 6 h of extraction at 58°C, the maximum desirability conditions were found (0.998), with gel yield and strength of 19.7% and 244.3 g, respectively, both of which desirable. Jamilah and Harvinder (2002) found yields of 6% and 19% and gel strength between 250 and 260 g for gelatin from two species of tilapia.

Assays were carried out to obtain gelatin at the optimal temperature (58°C) and extraction time (6 h) conditions 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 low relative deviation (2% for yield and 7% for gel strength), which shows that the method can be used to predict the yield and gel strength of gelatin obtained under these conditions.

### Chemical characterization and amino acid content of the skin and gelatin

Table 4 presents the physicochemical properties of kumakuma skin and of the gelatin extracted from it. Skin moisture is in accordance with the value found in corvina (62.3%) and shortfin scad (Decapterus macrosoma) (60.4%) (Cheow, Norizah, Kyaw, & Howell, 2007). Likewise, gelatin moisture was similar to that observed by Cho and Kim (2004) in shark cartilage (7.98%) and lower than what Eastoe and Leach (1997) reported for commercial gelatins (9–14%).

The skin’s fat content was high (14.25 ± 0.22%), a characteristic of the fish species studied, which was classified as a fatty fish by Souza, Baccarin, Viegas, Macedo, and Kronka (2004). Shahiri, Maghsoudlou, Motamedzadean, Sadeghi, and Rostamzad (2012) observed similar lipid content (13.12%) in rainbow trout (Onchorhynchus mykiss) skin. The gelatin’s high lipid content (29.7 ± 2.48%) suggests the need for the extraction of the lipid fraction from the fish skin prior to collagen extraction. The protein content in kumakuma skin (31.08 ± 0.99%) was similar to the value found by Bueno et al. (2011) (28.5 ± 1.8%). It is important to point out that the protein content in the skin represents the amount of collagen in the gelatin and, consequently, the extraction yield (Biluca et al., 2001). The gelatin obtained had lower protein content (72.76 ± 2.02%) than that observed by Alfaro, Fonseca, Balbinot, Machado, and Prentice (2013) (81.16 ± 2.15%) for gelatin from tilapia skin. The ash content in the skin and gelatin was lower than that reported by Bueno et al. (2011) for tilapia skin (1.9%) and by Haug, Draget, and Smidsrod (2004) for gelatin from cold-water fish (0.82%).

Higher pH was observed in the gelatin than in the skin, which is attributed to the chemical treatment with NaOH employed in the collagen extraction step (Gudmundsson & Hafsteinsson, 1997). According to Shahiri et al. (2012), collagen solubility is minimum at pH 7 and 9, which makes dissolving the gelatin in water more difficult.

The total imino acid contents (proline + hydroxyproline) in the skin and in the lyophilized gelatin were 13.67% and 13.53%, respectively (Table 5). A higher imino acid content was found by Alfaro et al. (2013) (19.5%) in gelatin from the skin of Nile tilapia (Omnhus dussumier). The imino acid content is the main difference between the gelatin from fish and from mammals since these imino acids stabilize the structures during gel formation and confer better viscoelastic properties to gelatin from fish skin (Foegeding,

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

| Components   | Skin          | Gelatin        |
|--------------|---------------|----------------|
| Moisture (%) | 58.83 ± 0.57  | 7.51 ± 0.40    |
| Lipid (%)    | 14.25 ± 0.22  | 14.25 ± 0.22   |
| Protein (%)  | 31.08 ± 0.99  | 72.76 ± 2.02   |
| Ash (%)      | 0.37 ± 0.04   | 0.13 ± 0.04    |
| pH           | 6.72 ± 0.01   | 9.06 ± 0.07    |
| Water activity | 0.98 ± 0.06 | 0.25 ± 0.01    |

*Three replicates.

* 3 réplicas.
Caracterización de gelatina

La Tabla 5 muestra los resultados del análisis de aminoácidos en gelatina de kumakuma y gelatina comercial. La comparativa entre las dos muestra que los valores de la gelatina de kumakuma son significativamente diferentes (p < 0.05) de los de la gelatina comercial. En particular, los valores de los aminoácidos hidroxiprolina, tirosina, valina y metionina son significativamente diferentes en las dos muestras. Los valores de alanina, proline y hidroxiprolina también son diferentes, pero no de manera significativa.

La Tabla 6 muestra los resultados del ensayo de emulsificación. Se observa que la capacidad de emulsificación de la gelatina de kumakuma es superior a la de la gelatina comercial. Esto se puede deber a la presencia de aminoácidos hidroxilados en la gelatina de kumakuma, que facilitan la emulsificación.

La tabla 7 muestra los resultados de la caracterización de la gelatina de kumakuma y la gelatina comercial sobre la base de la solución al 2%.

La caracterización de la gelatina de kumakuma y la gelatina comercial muestra que las dos muestras presentan diferencias significativas en varios parámetros. Por ejemplo, la gelatina de kumakuma presenta una menor densidad y una mayor viscosidad que la gelatina comercial. Estos resultados sugieren que la gelatina de kumakuma podría tener aplicaciones diferentes en productos alimenticios.

La caracterización de la gelatina también muestra que la gelatina de kumakuma es más estable térmicamente que la gelatina comercial. Esto se puede deber a la presencia de aminoácidos hidroxilados en la gelatina de kumakuma, que facilitan la emulsificación.

En conclusión, la caracterización de la gelatina de kumakuma y la gelatina comercial muestra que existe una diferencia notable entre las dos muestras. Las aplicaciones potenciales de la gelatina de kumakuma en productos alimenticios podrían ser diferentes de las de la gelatina comercial debido a estas diferencias.

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)   | 5.92 | 5.11    |
| Glutamic acid (Glu)   | 9.10 | 9.44    |
| Hydroxyproline (Hpr)  | 8.48 | 8.76    |
| Serine (Ser)          | 1.85 | 1.86    |
| Glycine (Gly)         | 23.77| 25.33   |
| Histidine (Hys)       | 0.36 | 0.26    |
| Taurine (Tau)         | 2.74 | 2.34    |
| Arginine (Arg)        | 5.13 | 5.98    |
| Threonine (Ter)       | 10.60| 10.63   |
| Alanine (Ala)         | 10.36| 11.17   |
| Proline (Pro)         | 5.19 | 4.77    |
| Tyrosine (Tyr)        | 0.63 | 0.49    |
| Valine (Val)          | 2.70 | 2.41    |
| Methionine (Met)      | 1.60 | 1.64    |
| Cysteine (Cys)        | 0.94 | 0.01    |
| Isoleucine (Ileu)     | 2.00 | 1.00    |
| Leucine (Leu)         | 3.36 | 3.21    |
| Phenylalanine (Phe)   | 2.42 | 1.60    |
| Lysine (Lys)          | 3.08 | 3.43    |
| Tryptophan (Trp)      | 0.06 | 0.17    |
| Total                 | 98.60| 99.91   |

Tyro's test with 95% confidence interval (p < 0.05). Means of three determinations.
higher protein concentrations. This is in line with the study of Kaewruang, Benjakul, and Prodpran (2013), who reported that increasing concentrations of gelatin extracted at different temperatures from the skin of leatherjacket lead to the increase in foam expansion levels. The hydrophobic surfaces of the peptide chain are responsible for the gelatin’s emulsifying and foaming properties (Cole, Cad, & Benameur, 2008; Galazka, Dickinson, & Ledward, 1999). Rawdkuen et al. (2013) observed that gelatin from fish skin had greater FC compared to commercial gelatin.

A significant difference \( p < 0.05 \) was observed in all color parameters analyzed between the gelatin from kumakuma skin and the commercial gelatin. Fish gelatin’s color was clear and shiny, whereas the commercial gelatin was bright yellow. The color of commercial gelatins usually ranges from pale yellow to dark amber (Cole & Roberts, 1997). The color difference between the gelatins may be due to the presence of the pigment melanin, which is dark brown, produced by the melanocytes present in fish skin (Junqueira & Carneiro, 2008) or due to the manufacturing process. The thermal extraction of gelatin causes Maillard reactions (Wang, Qian, & Yao, 2011); thus, the color intensity depends on the extraction temperature.

The electronmicrographies of gelatin from kumakuma skin (Figure 2(a,b)) clearly show the formation of interconnected pores, capillary canals, and cross-links in the pores’ periphery. In gelatin processing, during lyophilization, ice crystals are formed due to freezing, followed by sublimation during vacuum drying, which causes pore formation (Frydrych, Wan, Stengler, O’Kelly, & Chen, 2011). The differences in gelatin microstructure were probably associated with the length of extracted gelatins, as well as the disruption of the interchain hydrogen bonds stabilizing the triple helix structure (Jidi et al., 2013). Large empty and uneven spaces were observed in the gelatin’s structure. Usually, the arrangement and association of protein molecules in the gel matrix directly contribute to the gelatin’s gel strength (Benjakul, Oungbho, Visessanguan, Thiansilakul, & Roytrakul, 2009). The gel’s microstructure is related to the gelatin’s physical properties (Yang et al., 2008). Gels with thicker chains are harder to be unstructured by an applied force, which results in greater gel strength. Similar results were observed by Soottawat, Kwunchit, Wonnop, Yaowapa, and Sittiruk (2009); Bhat, Tripathi, and Kumar (2011); Liu, Li, Zhou, Fan, and Fan (2012); and Henderson et al. (2012) in gelatin from fish skin.

**Conclusion**

According to the model proposed, the optimal extraction conditions were established at 6 h and 58°C, the maximum desirability conditions were found (0.998), with gel yield and strength of 19.7% and 244.3 g, respectively, both of which were desirable. The gelatin from kumakuma skin had chemical and functional properties similar to those of commercial gelatin, clear and shiny color, and lower amino acid content than what is reported in the literature for warm-water fish. The electronmicrographies of gelatin from kumakuma skin show large empty and uneven spaces in the gelatin’s structure. Based on the functional properties, gelatin from kumakuma skin can be used as one more alternative in the food industries and areas alike.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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Figure 2. Electron micrography of kumakuma gelatin at 50 (A) and 200× (B) magnification.

Figura 2. Micrografía electrónica de la gelatina de kumakuma a una magnificación de 50 (A) y 200× (B).
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