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Research Article

Obtaining Gelatin from the Skin of Gilthead Bream (*Brachyplathystomarousseauxii*) using Two Pre-treatment

[1]Elen Vanessa Costa da Silva, [2]Lúcia de Fátima Henriques Lourenço and [3]Rosinelson da Silva Pena
[1]Food Technology Department, Estadual University of Pará, UEPA, Postal Code: 66095-015,
[2]Faculty of Food Engineering,
[3]Faculty of Food Engineering, Technology Institute, Federal University of Pará (UFPA), Rua Augusto Côrrea, 01, P.O. Box 479, Postal code: 66075-110, Belém, PA, Brazil

Abstract: Different pre-treatment and extraction processes have been researched to provide information on the properties of gelatin from fish skin and conversion rate of collagen into gelatin.Two methodologies were employed to extract collagen and obtain gelatin from the skin of gilthead bream (*Brachyplathystomarousseauxii*). Pre-treatment with sodium hydroxide (NaOH) and calcium hydroxide ($Ca(OH)_2$) was used and the gelatin obtained was characterized through analyses of yield, gel strength, color, viscosity, amino acid profile, melting point, emulsifying capacity and scanning electron microscopy. Both gelatins were classified as type A, which is attributed to the chemical treatment with acid employed in the collagen pre-treatment step. When the characteristics of the gelatins obtained were compared, the one that used NaOH had greater ($p<0.05$) technological potential due to higher yield, greater amount of imino acids and better properties (gel strength, viscosity, melting point, emulsifying power). However, the gelatin extracted with $Ca(OH)_2$ had weak gels and lower melting point, which is appropriate for refrigerated products that require low gelling temperatures. When gelatins obtained with NaOH and $Ca(OH)_2$were compared, the structure of the former has more empty protein filaments possibly correlated with the low aggregation of peptide chains during gelling It is concluded that the two gelatins obtained can be used in several applications in products according with the characteristic desired.

Keywords: Collagen, fish, gel strength, hydroxyproline, residue

INTRODUCTION

Gelatin is a soluble polypeptide derived from the rupture of cross-bonds among collagen chains. When collagen is treated with an acid or base followed by thermal treatment, its fibrous structure is irreversibly hydrolyzed and forms gelatin (Hou and Regenstein, 2004; Zhou and Regenstein, 2005).

Gelatin properties are influenced by two main factors, i.e., initial collagen characteristics and pre-treatment process. The latter is key in the preparation of collagen for effective gelatin extraction (Johnston-Banks, 1990). Different pre-treatment and extraction processes have been researched to provide information on the properties of gelatin from fish skin and conversion rate of collagen into gelatin. The gelatin properties are directly related to pH, temperature and pre-treatment and extraction times (Montero and Gómez-Guillén, 2000; Hou and Regenstein, 2004).

The pre-treatment step to obtain gelatin from mammals can employ hydrochloric, sulfuric and phosphoric acids and sodium and calcium hydroxides (Cho *et al*., 2005). The collagen obtained from skin or bone treated with an acidic or alkaline solution easily hydrolyzes in hot water due to its high soluble collagen content, however, the pre-treatment of fish skin must be gentle by using sodium or calcium hydroxide as shown by Cho *et al*. (2006).

Fish skins are alternative materials for gelatin extraction since they use processing by-products and meet sociocultural needs (Montero and Gómez-Guillén, 2000) given that Judaism and Islam prohibit the consumption of any product containing pork, while Hindus do not consume cattle (Karim and Bhat, 2009). Moreover, the gelatin from aquatic sources does not transmit Bovine Spongiform Encephalopathy (BSE), also known as mad-cow disease (Sadowska *et al*., 2003).

Nonetheless, in order to be applied in the food and/or pharmaceutical industries, fish gelatin depends mainly on its rheological properties, particularly gel strength and viscosity, as well as transparency,

Corresponding Author: Elen Vanessa Costa da Silva, Food Technology Department, Estadual University of Pará, UEPA, Belém, PA, Brazil, Postal Code: 66095-015, Belém, PA, Brazil, Tel.: +5591981140062

solubility and melting point (Gómez-Guillén *et al*., 2007).

Gelatin extraction using pre-treatment with sodium hydroxide has been performed with squids (Kim and Cho, 1996), blue shark (Yoshimura *et al*., 2000), tilapia (Jamilah and Harvinder, 2002), cod, king weakfish, megrin and sole (Gómez-Guillen *et al*., 2002), albacore tuna (Cho *et al*., 2005), catfish (Yoshimura *et al*., 2000) and corvina (Cheow *et al*., 2007). Pre-treatment with calcium hydroxide has been employed to extract gelatin from the skins of catfish (*Ietaluruspunetaus*) (Liu *et al*., 2008), Nile tilapia (*Oreochromisniloticus*), walking catfish (*Clariasbatrachus*) and striped catfish (*Pangasiussutchi fowler*) (Jamilah *et al*., 2011), pangas catfish (*Pangasiuspangasius*), Asian redtail catfish (*Hemibagrusnemurus*), striped snakehead (*ChannaStriata*) and Nile tilapia (*Oreochromisnilotica*) (Ratnasari *et al*., 2013) and pangascatfish (*Pangasiuspangasius*) (Ratnasari *et al*., 2014).

This research aimed to show the effect of extraction processes using pre-treatment with calcium hydroxide ($Ca(OH)_2$) and sodium hydroxide (NaOH) on the properties of gelatin from the skin of gilthead bream (*Brachyplathystomarousseauxii*).

MATERIALS AND METHODS

Fresh gilthead bream (*B. rousseauxii*) skins were purchased in the market and transported under refrigeration in isothermic boxes to the Laboratory for later use. Skins were taken off at-20°C and kept frozen till use.The skins were cut into small pieces (4×4 cm^2) with scissors, placed in polyethylene bags and kept at -25°C until used (within a week). All chemicals were of analytical grade.

Obtaining gelatin by the NaOH method: 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 M NaCl aqueous solution for 15 min followed by immersion in a 0.3 M NaOH solution for 15 min and, finally, a 0.02 M acetic acid ($C_2H_4O_2$) solution for 60 min. In all steps, immersion took place under stirring and the skins were then washed with water, with three repetitions. The material resulting from the steps above was added with water and placed in a water bath at 64°C for 6 h to extract the collagen. After heating, the supernatant was collected and filtered with Whatman no. 4 filter paper. The denatured collagen obtained was placed on trays, frozen at -50°C and lyophilized for 30 h. The lyophilized product (gelatin) was vacuum packaged and stored at 22°C.

Obtaining gelatin by the $Ca(OH)_2$ method: 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 removed, they were immersed in a saturated $Ca(OH)_2$ solution at 27 g/L and 20°C. For each kg of wet skin, 2 L saturated solution were used as impregnation medium. After the pre-treatment time (12 days), the skins were removed and washed in ten parts water (m/m) to remove excess alkali while maintaining the skins at pH 10. For every 20 g of skin, 100 mL distilled water were added and the mix was kept in a water bath at 64°C for 6 h to extract the collagen. Next, hydrochloric acid was used to lower the pH of the solution. The solution was then filtered in Whatman no. 4 filter paper and the denatured collagen (gelatin) recovered was placed on trays, frozen at -50°C and lyophilized for 30 h. The lyophilized product (gelatin) was vacuum packaged in polyethylene bags and stored at 22°C.

Physicochemical determinations: Analyses were performed for moisture (method no. 950.46), total proteins with correction factor 5.5 (method no. 928.08), lipids (method no. 960.39) and ashes (method no. 920.153) according to the Horwitz (2002). Skin pH was determined through Horwitz (2002) 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 *et al*. (1986). Water activity was determined with an Aqualab 3TE electronic hygrometer (Decagon Devices Inc., Pullman, 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'Éclairage) 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 technological properties: The technological properties of the gelatins were determined under the same experimental conditions. Total yield (%) and gelatin yield were calculated from the ratio between the gelatin weight and the skin's wet weight (Binsi *et al*., 2009). Gel strength (Bloom) was determined in a texture analyzer using a cylindrical Teflon probe with 12.5 mm diameter pressed for 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 determined based on the methodology by Choi and Regenstein (2000).

The emulsifying capacity (EC) was determined according to Tabarestani *et al*. (2010), with modifications. 20 mL 3.3% gelatin solution were mixed with mL soybean oil. The mix was homogenized at 1,750 rpm for 30 s and then centrifuged at 2,000 g 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 *et al*. (2008). The sample was placed in a water bath at 45°C and transferred to a Ostwald-Fenskviscosimeter (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: Data statistical analysis was carried out through Analysis of Variance (ANOVA) 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

Chemical characterization of the skin and gelatin: The results in Table 1 for moisture and proteins in gilthead bream skin match those in researches carried out with skin of different fish species: albacore tuna (*Thunnusalbacares*) (60.1% and 28.8%) (Rahman *et al*., 2008), moontailbullseye (*Priacanthushamrur*) (52.79% and 25.19) (Binsi *et al*., 2009) and skipjack tuna (*Katsuwonuspelamis*) (56.5% and 27.7%) (Shyni *et al*., 2014), respectively. The latter authors found 18.3% lipids, 0.68% ashes and pH 6.3 in tuna (*Katsuwonuspelamis*) skin, similar to the values in the present study.

In turn, moisture and protein values of the gelatin obtained using NaOH as pre-treatment were similar to those obtained for albacore tuna (*Thunnusalbacares*) (8.3% and 78.1%) (Rahman *et al*., 2008). Koli *et al*. (2012) found values of moisture, proteins and ashes of 8.73%, 72.63% and 0.30%, respectively, for Japanese threadfin bream (*Nemipterusjaponicus*) skin. In gelatin pre-treated with $Ca(OH)_2$, values of moisture, protein and ashes of 7.29%, 77.88% and 0.18%, respectively, were found (Jamilah *et al*., 2011).

Both gelatins were classified as type A, which is attributed to the chemical treatment with acid employed in the collagen pre-treatment step. The low concentration of acid (0.02 M) in the pre-treatment with NaOH was not enough to promote hydrolysis and to cause deamination of glutamine and asparagine into glutamic acid and aspartic acid (Johnston-Banks, 1990) and pH did not drop to 5. For the gelatin pre-treated with $Ca(OH)_2$, a small amount (0.5 mL) of acid was added to lower pH since, when it was added at higher amounts (1,0 mL), pH dropped to 3.0, which reduced gel strength. According to FIB (2001), gel strength is independent from pH in a broad range of values above approximately 5.0. That is particularly important in acid food systems such as those found in certain confectionery products, water-based gelatinous desserts and products that use dairy cultures, for example. Norziah *et al*. (2014) found pH 8 for type-A gelatin obtained from surimi residue.

The amino acid composition (Table 2) of the gilthead bream skin and gelatins obtained with NaOH and $Ca(OH)_2$ had a high proportion of glycine at 13.27%, 16.46% and 17.71, respectively (Table 2). Glycine is the most prevalent amino acid in gelatin (Arnesen and Gildberg, 2002). However, amino acid composition in the present study had low contents of methionine, taurine, histidine and tyrosine, which are characteristic of gelatins.

The imino acid contents (Pro + HPro) in the skin and gelatins with NaOH and $Ca(OH)_2$ pre-treatment were 16.04, 18.12 and 17.86%, respectively. It is believed that gelatin's structural stability greatly depends on the amount of hydroxyproline (Cho *et al*., 2005), which was higher in the gelatin pre-treated with NaOH (9.23%). Several authors have reported imino acid content in gelatins obtained from fish skin with NaOH pre-treatment: Grossman and Bergman (1992) found 17.0% in gelatin from cod skin, Muyonga *et al*. (2004) found 21.5% in Nile perch and Kasankala *et al*. (2007) found 19.47% in carp. Liu *et al*. (2008) reported imino acid content of 20% in gelatin from catfish skin pre-treated with $Ca(OH)_2$.

The stability of collagen and gelatin is proportional to its imino acid and glycine contents (Lehninger *et al*., 1993). In order to complete the triple helix bond, glycine molecules are required to occupy the third position (Te Nijenhuis, 1977). Amino acids such as tryptophan and cysteine are normally absent in

Table 1: Physicochemical properties of the skin and gelatin extracted from Gilthead Bream skin*

Propriedade*	Pele	Gelatina NaOH	Gelatina $(CaOH)_2$
Moisture (%)	54.26±0.38	7.01±1.84[a]	6.32±0.19[a]
Lipid (%)	17.59±1.90	25.09±0.24[a]	20.87±0.70[b]
Protein (%)	28.68±2.20	70.93±1.57[a]	74.43±0.90[a]
Ash (%)	0.36±0.03	0.07±0.02[a]	0.19±0.17[a]
pH	6.52±0.04	10.06±0.04[a]	9.30±0.21[b]
Wateractivity	0.96±0.02	0.25±0.05[a]	0.23±0.03[a]

* 3 replicates; values in the same row followed by same letter are not significantly different

Table 2: Total amino acid profile in Gilthead Bream skin and gelatin (mg/g protein)

Aminoácidos (mg/g)	Skin	Gelatin NaOH	Gelatin $(CaOH)_2$
Asparticacid	3.70	2.56	3.28
Glutamicacid	5.95	5.16	6.40
Hydroxyproline	9.17	9.23	8.08
Serine	2.51	2.85	2.81
Glycine	13.27	16.46	17.71
Histidine	0.73	0.80	1.30
Taurine	0.08	Not detected	Not detected
Arginine	4.16	5.27	5.71
Threonine	1.95	2.02	1.65
Alanine	4.56	6.03	7.04
Proline	6.87	8.89	9.78
Tyrosine	0.71	0.63	0.43
Valine	1.88	1.43	1.43
Methionine	0.99	1.22	1.27
Cysteine	0.66	1.05	0.68
Isoleucine	1.33	1.20	1.14
Leucine	2.33	1.80	1.83
Phenylalanine	1.44	1.03	1.04
Lysine	2.61	2.30	2.30
Pro + HPro	16,04	18,12	17,86

Table 3: Technological property

Property	Gelatin NaOH	Gelatin $Ca(OH)_2$
Yield (%)	30.6 ± 1.24^a	21.33 ± 0.24^b
Gel strength (g)	312 ± 16.5^a	157 ± 13.58^b
Viscosity (cP)	3.4 ± 0.16^a	2.5 ± 0.16^b
Melting temperature (°C)	29.6 ± 0.47^a	23 ± 0.14^b
Emulsifyingcapacity (%)	48.7 ± 0.55^a	43.6 ± 2.36^b
L* (lightness)	65.02 ± 1.11^b	85.01 ± 2.21^a
a* (green to red)	0.40 ± 0.17^a	-0.79 ± 0.42^b
b* (blue to yellow)	5.94 ± 0.75^b	10.53 ± 1.07^a
c* (chroma)	5.95 ± 0.74^b	10.57 ± 1.10^a
h* (hueangle)	85.92 ± 2.12^b	94.09 ± 2.06^a

Values in the same row followed by same letter are not significantly; different Tukey's test with 95% confidence interval ($p<0.05$).; Means of three determinations

conventional gelatin (FIB, 2001). However, the presence of cysteine at low concentration both in the skin and in the gelatins was similar to that reported by Badii and Howell (2006) and Cheow *et al*. (2007) in gelatins from Atlantic horse mackerel and corvina.

Technological characterization of gelatin: The gelatin yields differed ($p<0.05$) and were considered high for gelatin from fish skin (Table 3) since, according to Karim and Bhat (2009), the yield of gelatin extraction from fish ranges from 6% to 19%.

The difference in yield between the samples may be related to the pre-treatment time. The short (2 h) pre-treatment with NaOH at extraction temperature of 64°C favored greater yields. According to Schrieber and Gareis (2007), extraction yield increases with higher temperatures (between 50°C and 70°C). Meanwhile, the long (12 days) pre-treatment with $Ca(OH)_2$ used may have contributed to higher yield, which matches the results by Cho *et al*. (2005). According to Yoshimura *et al*. (2000), collagen dissolves in the pre-treatment solution and results in a loss of gelatin yield, which may also be caused by collagen leaching during washing (Jamilah and Harvinder, 2002) in the pre-treatment with $Ca(OH)_2$.

Rahman *et al*. (2008) found gelatin yield of 18.0% from skin on albacore (*Thunnusalbacares*). Sinthusamran *et al*. (2014) reported higher yields when collagen was extracted at 55°C compared to at 45°C and observed that extraction longer than 6 h did not increase yield significantly.

Several researches using pre-treatment with $Ca(OH)_2$ showed yield results very close to those found for gilthead bream: 21.28 and 21.93% for Asian redtail catfish and Nile tilapia, respectively (Ratnasari *et al*., 2013) and 23.12% for pangas catfish (*Pangasiuspangasius*) (Ratnasari *et al*., 2014). Higher yield results were published for Nile tilapia (39.97%), walking catfish (32.06%) and striped catfish (26.23%) (Jamilah *et al*., 2011).

The gel strength of the gelatin with $Ca(OH)_2$ pre-treatment (157±13.58 g) was lower than and significantly different ($p<0.05$) from the gelatin extracted with NaOH (312±16.5 g). The hydroxyl groups of hydroxyproline act to stabilize the α helix through the hydrogen bridge bond with the water molecule, as well as direct hydrogen bonds with the carbonyl group (Wong, 1989), while gelatin's structural stability greatly depends on the amount of hydroxyproline (Cho *et al*., 2005), which was higher in the gelatin pre-treated with NaOH (9.23%) compared with the gelatin pre-treated with $Ca(OH)_2$ (8.08%).

The lower gel strength in the gelatin extracted with $Ca(OH)_2$ may be related to the addition of acid in the pre-treatment to lower pH since this acid hydrolysis may affect the cross-bond of collagen as reported by Zhou and Regenstein (2005), thus reducing gel strength. It may also be attributed to the presence of low-molecular-weight proteins that lower the gel-forming ability (Ledward, 1986; Normand *et al*., 2000).

The gel strength of fish gelatin ranges between 124 g and 426 g, while gelatin from cattle or pig ranges from 200 g to 300 g (Karim and Bhat, 2009). This value may be considered low (<150 g), medium (150 g to 220 g), or high (220 g to 300 g) (Johnston-Banks, 1990). Gudmundsson and Hafsteinsson (1997) suggested that gel strength may depend on the isoelectric point and can be controlled, to a certain extent, by adjusting pH.

The gelatin with NaOH pre-treatment may be considered as having high gel strength and is advantageous for a host of applications, e.g., it enables shorter drying time of the final product and is used in smaller amounts. They are more effective and used in several products to provide the ideal texture in chewing gum, pâtés, drops, etc. Gelatins with low gel strength are used in yoghurt as a colloidal protector to prevent syneresis and adjust consistency**,** from creamy to almost solid, in dairy desserts such as flan and milk jelly, in which it acts as a gelling and stabilizing agent to provide smooth and soft texture, besides being also used as a foaming agent in mousses (FIB, 2001).

The gel strength values obtained with NaOH pre-treatment for gelatin from skin of king weakfish, cod and megrin ranged from 100 g to 200 g (Gudmundsson and Hafsteinsson, 1997; Montero and Gómez-Guillén, 2000; Fernandez-Diaz *et al*., 2001). Gel strength results of 254.10 g were reported by Benjakul *et al*. (2009) for gelatin from the skin of tuna (*P. macracanthus*) and of 282 g to 369 g for gelatin from the skin of sea bass by Sinthusamran *et al*. (2014). When $Ca(OH)_2$ pre-treatment was used, gel strength of 276.5 g was reported by Liu *et al*. (2008) for gelatin extracted from the skin of catfish (*Ietaluruspunetaus*). For Nile tilapia and pangas catfish, gel strength of 191.20 g and 343.18 g, respectively, were reported (Ratnasari *et al*., 2013, 2014).

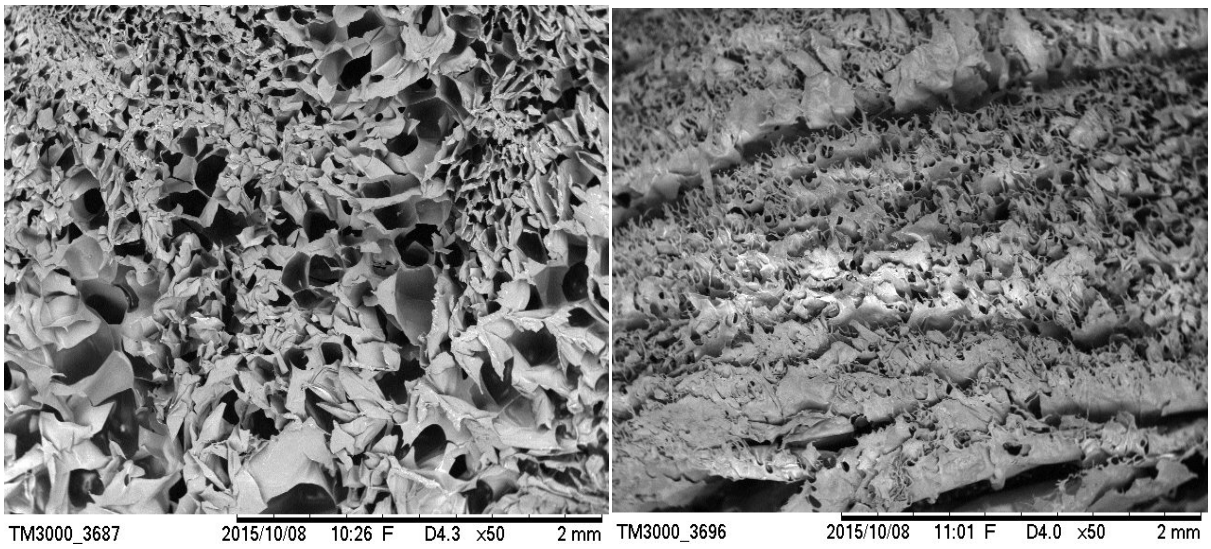
The viscosity of the gelatin obtained with NaOH was higher than that in the gelatin with $Ca(OH)_2$ (Table 3). The values differed ($p<0.05$), but were within the range of 2 to 7 cP observed in commercial gelatins (Johnston-Banks 1990; Jamilah and Harvinder, 2002). Changes in pH are related to the increase in gelatin viscosity and the minimum viscosity is found at alkaline pH (above 10) (Stainsby, 1987). The reduction in viscosity may be advantageous during the development and preparation of new products such as dairy beverages (Karim and Bhat, 2009) and in the molded starch candy industry, in which the high working speed of modern production equipment requires the use of low-viscosity gelatin to keep the ends from hardening and allow the rapid distribution in the molds. Viscosity of 3.2 cP for gelatin from Nile tilapia and catfish was reported by Jamilah and Harvinder (2002) and Yang *et al*. (2007). Ratnasari *et al*. (2014) found 3.3 cP for gelatin from pangas catfish (*Pangasiuspangasius*) pre-treated with lime.

The melting point of the gelatin samples extracted with NaOH was significantly higher ($p<0.05$) than that of the gelatin extracted with $Ca(OH)_2$ (Table 3). That may be related to the higher hydroxyproline content, gel strength and viscosity of the latter. According to Choi and Regenstein (2000), melting point increases with maturation time and hydroxyproline content. With high melting point, the gel can be maintained for longer, which confers better feeling during tasting and consumption (Gómez-Guillén *et al*., 2002). Authors have reported melting points at 28.9°C for black tilapia (Jamilah and Harvinder, 2002), 27.1°C for silver carp (Boran *et al*., 2010) and 26.3°C to 27.0°C for sea bass (Sinthusamran *et al*., 2014). Melting point of 25.1°C was reported for gelatin from catfish (Liu *et al*., 2008), 24.9°C for walking catfish (Jamilah *et al*., 2011) and 25°C for Nile tilapia (Ratnasari *et al*., 2013), all obtained with $Ca(OH)_2$ pre-treatment.

The emulsifying power of the gelatin from gilthead bream skin with NaOH pre-treatment was higher and significantly different ($p<0.05$) from that observed for gelatin obtained with $Ca(OH)_2$. The level of exposure to hydrophobic residues and the higher tyrosine content (Table 2) may have been responsible for the higher emulsifying capacity of the fat (Ninan *et al*., 2011). The amphoteric nature and hydrophobic zone in the peptide chain makes the gelatin behave as an emulsifier that may be used to make caramels and water-oil emulsions such as margarine with low fat content, salad dressings and whipped cream (Baziwane and He, 2003).

Koli *et al*. (2012) found emulsifying capacity in gelatin from the skin and bone of corvina (*Otolithesruber*) of 55.70% of 40.50%, respectively. Those same authors found values of 47.50% and 35.50%, respectively, for Japanese threadfin bream (*Nemipterusjaponicus*). Lassoued *et al*. (2014) found emulsifying power of 36.42% for gelatin from the skin of thornback ray (*Raja clavata*).

Gelatin color depends on the raw materials used and on the extraction method (Ockerman and Hansen, 1999), but, overall, color does not impact functional properties. L*, b*, c* and h* values in the gelatin extracted with NaOH was statistically different ($p<0.05$) and lower compared to the gelatin extracted with $Ca(OH)_2$, the latter having a clearer and shinier color. Due to its high transparency and shine, gelatin confers an attractive appearance, besides providing characteristics texture and elasticity while maintaining its structure and preventing sugar crystallization (FIB, 2001).



(a) (b)

Fig. 1: Electron micrographs of Gilthead Bream gelatin at 50x (a) Pretreatment with NaOH and (b) Pretreatment with $Ca(OH)_2$

Shyni *et al*. (2014) found L* value of 82.8±0.40 in gelatin from shark and classified the color as pearly white. Muyonga *et al*. (2004) stated that the efficiency of the filtration process during gelatin extraction impacts clarity of the gelatin solution, however, color does not impact functional properties (Ockerman and Hansen, 1999). Jamilah *et al*. (2011) found values of L*, a* and b* of 79.45, -0.71 and 5.75 for gelatin from Nile tilapia with pre-treatment with $Ca(OH)_2$.

Variations in gel pre-treatment and microstructure are directly related to gelatin properties (gel strength) (Yang *et al*., 2008). When gelatins obtained with NaOH and $Ca(OH)_2$ were compared, the structure of the former has more empty protein filaments possibly correlated with the low aggregation of peptide chains during gelling. Usually, the arrangement and association of protein molecules in the gel matrix directly contribute to gel strength in gelatin. Ratnasari *et al*. (2014) found an uneven tissue with slightly thick thread and small gaps in the microstructure of gelatin obtained with $Ca(OH)_2$ pre-treatment (Fig. 1).

CONCLUSION

The gelatin extracted using NaOH had higher technological potential due to its higher yield, greater imino acid content and better properties (gel strength, viscosity, melting point and emulsifying power) and can be used in a broad range of products. The gelatin extracted with $Ca(OH)_2$ had weak gels and lower melting point, which are appropriate characteristics for refrigerated products that require low gelling temperatures.

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