Effect of the gelatin extraction method from tilapia skin and its application as a coating

Efeito do método de extração de gelatina da pele de tilápia e sua aplicação como revestimento

DOI:10.34117/bjdv9n2-121

Recebimento dos originais: 23/01/2023
Aceitação para publicação: 20/02/2023

Monique de Oliveira Maia
Doctor in Food Science and Technology
Institution: Instituto Federal de Educação, Ciência e Tecnologia do Ceará - Campus Limoeiro do Norte
Address: Rua Estevão Remígio de Freitas, 1145, Monsenhor Otávio, Limoeiro do Norte – Ceará, CEP: 62930-000
E-mail: nickmaia3@gmail.com

Juliana Nascimento da Costa
Doctor in Food Science and Technology from Universidade Federal do Ceará
Institution: Bolsista do Instituto Federal de Educação Ciência e Tecnologia do Ceará - Campus Sobral (PDCRT)
Address: Avenida Dr. Guarany, 317, Jocely Dantas de Andrade Torres, Sobral - CE, CEP: 62042-030
E-mail: julianacosta31@gmail.com

Renata Chastinet Braga
PhD in Biochemistry from the Universidade Federal do Ceará
Institution: Instituto Federal de Educação, Ciência e Tecnologia do Ceará - Campus Limoeiro do Norte
Address: Rua Estevão Remígio de Freitas, 1145, Monsenhor Otávio, Limoeiro do Norte - Ceará, CEP: 62930-000
E-mail: rchastinet@ifce.edu.br

Rinaldo dos Santos Araújo
PhD in Chemistry from the Federal Universidade Federal do Ceará
Institution: Instituto Federal de Educação, Ciência e Tecnologia do Ceará - Campus Fortaleza
Address: Av. Treze de Maio, 2081, Benfica, Fortaleza – CE,CEP: 60040-531
E-mail: rinaldoifce@gmail.com

Antônio Silva do Egito
PhD in Biochemistry from the Université Henri Poincaré
Institution: Empresa Brasileira de Pesquisa Agropecuária
Address: Rua Oswaldo Cruz, 1143 Centenário, Campina Grande - PB, CEP: 58428-095
E-mail: antoniosilvio.egito@embrapa.br

Marlene Nunes Damaceno
PhD in Food from the Universidade de Santiago de Compostela, Espanha
Institution: Instituto Federal de Educação, Ciência e Tecnologia do Ceará - Campus Limoeiro do Norte
Address: Rua Estevão Remígio de Freitas, 1145, Monsenhor Otávio, Limoeiro do Norte – Ceará, CEP: 62930-000
E-mail: marlene@ifce.edu.br

Bartolomeu Warlene Silva de Souza
PhD in Chemical and Biological Engineering from the Universidade do Minho, Braga, Portugal
Institution: Universidade Federal do Ceará
Address: Av. Mister Hull, s/n, Pici, Fortaleza – CE, Departamento de Engenharia de Pesca - Campus do PIC, CEP: 60455-760
E-mail: souzabw@gmail.com

Daniele Maria Alves Teixeira Sá
PhD in Biochemistry from Universidade Federal do Ceará
Institution: Instituto Federal de Educação, Ciência e Tecnologia do Ceará - Campus Sobral
Address: Avenida Dr. Guarany, 317, Jocely Dantas de Andrade Torres, Sobral – CE, CEP: 62042-030
E-mail: danielemaria@ifce.edu.br

ABSTRACT
The objective of this study was to analyze gelatins extracted from tilapia (*Oreochromis niloticus*) skin and evaluate its effect as a coating on fish fillets of the same species. Four gelatin extractions from tilapia skins were prepared in acidic and alkaline conditions, followed by filtration and freeze drying. The samples were analyzed for moisture, lipids, ash, proteins, color parameters, viscosity, spectrophotometry and the coatings made with fish gelatin (FG) and commercial gelatin (CG) were sensorially tested. The protein content and the viscosity of the FG were influenced by the extraction method. The fillets coated with FG showed less moisture than those coated with CG. The sensorial acceptance of the fillets was not influenced by the use of the coatings. Therefore, the use of fish gelatin appears as a sustainable alternative to use tilapia skin after disposal in industrial processing.

Keywords: fish gelatin, tilapia, fish protein, gel properties, acceptance test.

RESUMO
O objetivo deste trabalho foi analisar gelatinas extraídas da pele de tilápia (*Oreochromis niloticus*) e avaliar seu efeito como revestimento em filés de peixe da mesma espécie. Quatro extrações de gelatina de pele de tilápia foram preparadas em condições ácidas e alcalinas, seguidas de filtração e liofilização. As amostras foram analisadas quanto à umidade, lipídios, cinzas, proteínas, parâmetros de cor, viscosidade, espectrofotometria e os revestimentos feitos com gelatina de peixe (FG) e gelatina comercial (CG) foram testados sensorialmente. O teor de proteína e a viscosidade do FG foram influenciados pelo método de extração. Os filés revestidos com FG apresentaram menor umidade do que os revestidos com CG. A aceitação sensorial dos filés não foi influenciada pelo uso dos revestimentos. Portanto, o uso de gelatina de peixe surge como uma alternativa

sustentável de aproveitamento da pele de tilápia após descarte no processamento industrial.

Palavras-chave: gelatina de peixe, tilápia, proteína de peixe, propriedades do gel, teste de aceitação.

1 INTRODUCTION

Tilapia (*Oreochromis niloticus*) is one of the fish species grown in more than 140 countries and with a significant position in the world market. With considerable nutritional value, is rich in proteins, enzymes and unsaturated fatty acids, low fat content, easy to process into fish fillets and has good acceptance by consumers (ZHANG *et al*., 2020). In view of this perspective, there has been an increase in tilapia processing and consequently an increase in the generation of its by-products such as skin, scales and bones (SUN *et al*., 2018; SHI *et al*., 2018; MENEZES *et al*., 2020). Thus, new applications of this waste have appeared as an opportunity to use them, generate economic profit and protect the environment, providing an increase in its use (LV *et al*., 2019).

One of these applications is the use of collagen from by-products (skin, scales, bones, fins, etc.), as gelatin sources, being applied as a flavoring and functional ingredient in the food industry and thus reducing waste generation and adding value to fish waste (HONG *et al*., 2019; MENEZES *et al*., 2020; MIRZAPOUR-KOUHDASHT *et al*., 2020).

Gelatin is a biopolymer solid, colorless, hydrocolloid generated from partial collagen hydrolysis and containing different types of amino acids, with high nutraceutical value for the food production area (LI *et al*., 2020). It is classified according to its source which can be bovine, swine or fish gelatin, and the latter has become an alternative to the other gelatins due to its functional properties, good film formation (LV *et al*., 2019).

Gelatin made from skins of aquatic organisms has recently been the subject of several studies which have been investigating results on the advantages of perfecting extraction processes, as well as comparing them with bovine gelatins. One of these studies was the production of fish gelatins from skin collagen and scales of the tilapia species and its peptides used as ingredients for application in food and cosmetics (YAN *et al*., 2020). Another study showed the effectiveness of FG in forming films which can be applied in the area of medicines and food packaging (HOSSEINI GÓMEZ-GUILLÉN, 2018). Therefore, it is important that more studies and analysis on the extraction methods of FG

be conducted as an alternative to mammalian gelatin in order to meet most consumer needs and the global demand for gelatin (LIN *et al*., 2017). The application of fish gelatin to replace mammalian sources has been increasing in recent years, so evaluating the nutritional and physicochemical properties is essential to direct its application (SOW; YANG, 2015).

Tilapia (*Oreochromis niloticus*) is considered a perishable product due to its susceptibility to chemical and microbiological reactions which accelerate its deterioration, reducing its quality (ZHAO *et al*., 2019). Therefore, it is important to use conservation technologies which favor increasing the useful life of these products. The application of edible gelatin coatings on products such as fish has recently been studied to reduce changes that affect the quality of these products during storage (ZHAO *et al*., 2019). Thus, the use of tilapia skin-based gelatin as a coating on fillets of the same species can be an alternative for the preservation of these products during storage.

This study aimed to extract gelatine from the skin of tilapia (*Oreochromis niloticus*) by different methods and characterize its physicochemical, colorimetric, spectrophotometric properties and its effect as a coating on tilapia fillets.

2 MATERIAL AND METHODS

2.1 MATERIALS

The material used for the gelatin extraction was Nile tilapia skin of the *Oreochromis niloticus* species provided by the Aplages company, located in the city of Jaguaribara, Ceará, Brazil. Gelatin (analytical grade) was used for the spectrophotometric analysis, while flavored, food grade gelatin, without flavor (Dr. Oetker®) was used in the sensory study. The other products used in the extraction and physical-chemical analysis had an analytical degree.

2.2 SAMPLE PREPARATION, EXTRACTION AND CHARACTERIZATION OF *OREOCHROMIS NILOTICUS* SKIN GELATIN

2.2.1 Obtaining Gelatin

Tilapia skin residues from the filleting process were stored in thermal boxes (5 ºC ± 2 ºC) and transported to the fish processing laboratory, where they were washed in running water to remove surface material. Next the skins were cut (4 × 4 cm) and hydrated in 0.2% NaCl solutions for 5 minutes with stirring in a magnetic stirrer. The skins were left on boards to remove the water excess for one hour and then samples of 100 g were

weighed for each extraction. The samples were extracted using four chemical methods (T1, T2, T3 and T4) according to Alfaro (2008) e Niu *et al*. (2013), com modificações. Each treatment was initially submitted to immersion of the skin in alkaline solution for 80 min. at room temperature (25 °C ± 2 °C), then, stabilization to pH 7 was carried out by washing the skins in running water. After this step, the samples were submitted to the acid solution and again remained at rest for 80 min. (5 °C ± 2 °C). Finally, each treatment was washed with tap water to adjust pH 4.8-5.2. The solutions and concentrations used in each treatment are shown in Table 1.

Table 1 – Chemical treatments applied in the extraction of gelatin from *Oreochromis niloticus* skin

Treatments	Solutions	Concentrations (Mol.L^{-1})
T_1	NaOH (Sodium Hydroxide)	0.70
	H_2SO_4 (Sulfuric Acid)	0.03
	$C_6H_8O_7$ (Citric Acid)	0.05
T_2	NaOH (Sodium Hydroxide)	0.30
	HCl (Hydrochloric Acid)	0.04
T_3	NaOH (Sodium Hydroxide)	0.30
	$C_6H_8O_7$ (Citric Acid)	0.10
T_4	NaOH (Sodium Hydroxide)	0.30
	CH_3COOH (Acetic Acid)	0.03

After each chemical treatment, gelatin extraction was performed in a water bath at 50 °C ± 2 °C (2 mL of deionized water/1 g of skin (2: 1 v / p)) with continuous agitation for a period of 3 hours. Then, the gelatin was filtered through gauze to remove suspended residues, and the supernatant subjected to the lyophilization process for 30 hours through the lyophilizer (MARK) and finally crushed to obtain gelatin powder, then packaged and stored at vacuum for subsequent analysis.

2.2.2 Yield Determination

The yield of each extraction was calculated from the weight of gelatin obtained from the wet weight value of the skins used, according to the equation:

$$\text{Yield}\ (\%) = \frac{\text{Gelatin (Dry base)}}{\text{Wet skin weight}} \text{x}\ 100 \qquad (01)$$

2.2.3 Proximate Composition And Color Measurement

The skinfish gelatin samples from each treatment were evaluated for moisture, fat, ash and protein content according to AOAC (2002). The color of FG was performed by reflectance in a colorimeter Minolta Chroma Meter CR 400/410, obtaining data for the

parameters of L* (luminosity), a* (varies from green (-60) to red (+60)) and b* (varies from blue (-60) to yellow (+60).), for each sample with six different measurements.

2.2.4 Viscosity

Viscosity was determined following the method described by Schrieber and Gareis (2007). The gelatin was dissolved in distilled water (6.67%) and the viscosity was measured by a Capillary Viscometer 520-01 under controlled temperature 45°C. The viscosity measurement was determined according to the equation 1:

$$V = K.t \qquad (02)$$

In which:
V: viscosity
K: 0.004893 mm^2/s
t: Time in seconds used by the solution to traverse the system.

2.3 CARACTERIZAÇÃO AND COATING APPLICATION

The FG which showed the better results among the tested treatments in relation to the protein value, color (parameter L - luminosity) and viscosity parameters were applied as a coating on fish fillets of the *Oreochromis niloticus* species and then the fillets were sensorially evaluated.

The tilapia fillets were obtained from filleting the fish which were purchased whole at a local market and transported in thermal boxes (5 °C ± 2°C) to the fish processing laboratory. The following ingredients were added to the fillets obeying the following proportions for 1Kg of fillet: refined salt (20g) and garlic (4.8g). The coating applications and concentration (CG and FG) on fillets followed a method adapted from Niu *et al*. (2013). Three fillet formulations were made: C (uncoated), CG (6.67% commercial gelatin and 1.8% glycerol), FG (made with 6.67% fish gelatin and 1.8% glycerol). The gelatin solutions (CG and FG) were dissolved (6.67% w/v) in distilled water under continuous agitation for 15 minutes at temperature ± 25 °C. Glycerol was subsequently added as a plasticizer at a concentration of 1.8% (w/v). The fillets were immersed in the coating solutions for 5 minutes, and for each treatment, three fillet formulations were prepared (Ou *et al*., 2002). Then they were removed and placed in plastic containers with lids and stored under refrigeration (5 °C ± 2 °C) for 24h until the time of analysis in of proximate composition. The samples referring to each formulation (C, CG and FG) were

evaluated for approximate composition of lipids, moisture, ash and protein (AOAC, 2002). The specific method for samples of Bligh-Dyer fish described by Bligh and Dyer (1959) was followed for lipid analysis.

2.3.1 Sensory Acceptance

For sensory acceptance, the coated files were fried in a pan with hot oil at a temperature of ± 160 °C, and then they were cut into uniform sizes of 4 × 4 cm. The tests were carried out with 120 untrained tasters (of both genders) but who had a habit of consuming the product, aged between 18 and 65 years old, and they evaluated three samples of tilapia fillets, namely: Control (without coating), CG (coated with commercial gelatin) and FG (coated with fish gelatin). The acceptance test was performed in individual booths and the samples were served in polyethylene pratos encoded with random three-digit numbers, servidos de forma monádica. Sensory response was measured using a structured 9-point hedonic scale, anchored at the ends corresponding to 9 "I liked it a lot" and 1 "I really did not like it". The tasters' purchase intention in relation to the product was determined through a 5-point structured scale ranging from "certainly would buy it" to "certainly would not buy it", as described by Stone and Sidel (2004). The study was approved by the Research Ethics Committee (CEP) under the opinion of No. 1.527.808.

2.4 STATISTICAL ANALYSIS

The results were analyzed by ASSISTAT 7.7 (SILVA; AZEVEDO, 2009) through analysis of variance (ANOVA) at the significance level of 5%. Spectrophotometric data were evaluated using the Origin© 8.0 software program. All analyzes were performed in triplicate.

3 RESULTS AND DISCUSSION

3.1 PERFORMANCE AND PHYSICO-CHEMICAL CHARACTERIZATION OF THE *OREOCHROMIS NILOTICUS* SKIN GELATIN

The results of extraction yield showed a statistical difference ($p \leq 0.05$) between treatments. A lower extraction yield was observed for the T1 formulation (15%), followed by T2 and T3 (17%) and with higher yield than the other samples, the T4 formulation with 21% gelatin extraction. T1 (Sodium ydroxide, citric and sulfuric acid) had a lower gelatine extraction yield, possibly due to incomplete hydrolysis during the process or loss

of collagen by leaching during the series of washing steps, or due to the high concentration of acids providing a low gelatine yield (MUYONGA *et al*., 2004; NIU *et al*., 2013)

T4 (Sodium hydroxide and acetic acid) showed a higher yield than the other treatments, with values close to those found in the literature (21.55% - 24.35%) (NIU *et al*., 2013). It is suggested that the acetic acid extraction method has obtained greater yield, due to its small molecular size and reduced ionization constant, boosting a more efficient collagen swelling before conversion to gelatin (MONTERO, P.; GÓMEZ-GUILLÉN, 2001).

The results for moisture, lipids, ash and proteins are shown in Table 2. There was no significant difference ($p>0.05$) between the treatments for moisture and ash data, being statistically different in the other tested parameters.

Table 2 – Centesimal composition of *Oreochromis niloticus* gelatin powder obtained by lyophilization

Treatments	**Component Averages (%)**			
	Moisture	Lipids	Ashes	Protein
T1	7.98 ± 0.06 ^{a}	1.88 ± 0.01 ^{b}	0.81 ± 0.03 ^{a}	88.55 ± 0.6 ^{bc}
T2	7.99 ± 0.14 ^{a}	2.32 ± 0.43 ^{a}	0.89 ± 0.02 ^{a}	86.81 ± 1.05 ^{c}
T3	7.50 ± 0.04 ^{a}	2.30 ± 0.08 ^{a}	0.76 ± 0.24 ^{a}	90.56 ± 1.63 ^{ab}
T4	6.78 ± 0.90 ^{a}	1.98 ± 0.56 ^{ab}	1.06 ± 0.51 ^{a}	91.58 ± 0.51 ^{a}

T1 (Sodium hydroxide 0.70 Mol.L^{-1}; sulfuric acid 0.03 Mol.L^{-1}, citric acid 0.05 Mol.L^{-1}) T2 (Sodium hydroxide 0.30 Mol.L^{-1}, hydrochloric acid 0.04 Mol.L^{-1}) T3 (Sodium hydroxide 0.30 Mol.L^{-1}, citric acid 0.10 Mol.L^{-1}) T4 (Sodium Hydroxide 0.30 Mol.L^{-1}, Acetic Acid 0.03 Mol.L^{-1}). Values expressed as mean ± standard deviation (n = 3). Different letter in the same column indicate difference ($p < 0.05$) by Anova and the Tukey Test.

The average varied moistureined from 6.78 to 7.99%, values different from those reported by Liao *et al* (2021), in which the values found ranged from 7.37 to 12.93%, in skin fish gelatins. Gelatine presented humidity above 7%, showing itself to be hygroscopic, thus, it favors the absorption of humidity from the environment (Cole, 2000). Ash contents for the four treatments varied from 0.81 a 1.06% which can be suggested that it was presented to be of good quality (LIAO *et al*., 2021).

For the average lipid content, treatments T2 (NaOH and HCl extraction) and T3 (NaOH and $C_6H_8O_7$ extraction) were superior to T1 extracted with sodium hydroxide, sulfuric acid and citric, which differed statistically ($p\leq0.05$). This fact indicates that during the extraction, in treatments T2 and T3, the lipid material was not completely removed during the washings or performed in an unsatisfactory way, which contributed to high percentages of lipids in the gelatins (Bueno *et al*., 2011). In fact, the percentage of fat in the tilapia skin is high, so it is essential that the treatments referring to the

extraction of gelatin are efficient in the complete removal of the lipid material (ALFARO; SILVA, 2010).

Regarding protein content, T4 showed percentage of 91, 58% differed significantly from T1 and T2, those with lower protein percentages, but were similar to T3 with 90.56%. Possibly, higher levels for T4 extraction are due to the lower concentration of acetic acid (Table 1), compared to other formulations. Thitipramote and Benjakul (2013) reported that more acidic conduction (low pH) was obtained when using low concentration citric acid combined with high concentrations (0.4%) of sodium hydroxide and/or sulfuric acid. More acidic gelatin extraction conditions can over-hydrolyze collagen molecules, resulting in protein loss, affecting its yield (NIU *et al*., 2013; LIAO *et al*., 2021). The T4 treatment showed protein contents relatively higher than those reported by Liao *et al*. (2021) with protein percentage for tilapia skin gelatin from 79.04 to 90.70%. Therefore, it is suggested that the percentage of proteins was influenced by the extraction and acid method used. However, it may have influenced the final protein content sincce they have found a lower value of protein (84.28%) than the maximum value (91.58%) found by other authors. Gelatins extracted from Nile tilapia skins generally have a high protein percentage and low moisture, fat and ash contents (JONGJAREONRAK *et al*., 2010).

3.2 DETERMINATION OF COLOR PARAMETERS

The fish skin gelatin color obtained from Nile tilapia skins are shown in Table 3. For the L*, the T3, showed the lowest result, differing statistically from the other treatments ($p \leq 0.05$), favoring the production gelatin darker and less luminous.

Table 3 – Color parameters of skin fish gelatin of Oreochromis *niloticus*.

Treatments	L*	a*	b*
T1	52.87 ± 4.91 [a]	-0.92 ± 0.40 [a]	7.78 ± 1.07 [a]
T2	53.21 ± 0.80 [a]	- 0.21 ± 0.13 [a]	5.93 ± 0.75 [a]
T3	43.80 ± 2.66 [b]	0.82 ± 0.17 [a]	2.70 ± 0.50 [b]
T4	54.64 ± 2.63 [a]	- 0.18 + 0.12 [a]	3.41 ± 0.78 [b]

T1 (Sodium hydroxide 0.70 Mol.L^{-1}; sulfuric acid 0.03 Mol.L^{-1}, citric acid 0.05 Mol.L^{-1}) T2 (Sodium hydroxide 0.30 Mol.L^{-1}, hydrochloric acid 0.04 Mol.L^{-1}) T3 (Sodium hydroxide 0.30 Mol.L^{-1}, citric acid 0.10 Mol.L^{-1}) T4 (Sodium Hydroxide 0.30 Mol.L^{-1}, Acetic Acid 0.03 Mol.L^{-1}). Values expressed as mean ± standard deviation (n = 3). Different letter in the same column indicate difference ($p < 0.05$)

The other formulations were clearer, which may be a result of the loss of related substances during the lyophilization process (ZHANG *et al*., 2020). Color does not generally have an influence over functional properties. However, clearer colors are

preferred because it is easier to implement gelatins into any dietary system without transmitting strong colors to the product (SHYNI *et al*., 2014). Higher luminosity values are preferred since they relate to gelatin purity and make them clearer. Negative values for the a* coordinate indicate that the sample tends to green and red when they are positive (SAHIN; SUMNU, 2006). No differences were found in the a* value ($p > 0.05$) between treatments. Treatments 1 and 2 showed higher values of b*, equal to each other ($p>0.05$), but significantly differing with treatments 3 and 4. This indicates that T1 and T2 obtained a gelatin with yellow intensity. This coloration is possibly associated with protein-aldehyde interactions favoring the Maillard reaction, influenced by the material composition, temperature and pH (NUANMANO; PRODPRAN; BENJAKUL, 2015).

Gelatin colors become darker with acidic treatments, especially if carried out in lower temperature and shorter times. Dark colors may also be caused by inorganic and mucous substances, as well as protein components which are not removed during extraction. The general aspect of our gelatin was whitish, slightly yellow and shiny, similar to the color of commercial gelatins (ZHANG *et al*., 2007).

3.3 VISIBLE ABSORPTION SPECTRA

The visible absorption spectra in standard gelatin (for analysis) and the ones obtained from skin fish in the extractions of the treatments 1, 2, 3 and 4 are represented in Figure 1. All samples registered higher absorption rates with the wavelength range of 220-240 nm represented in the figure, showing the presence of peptide bonds in the gelatin polypeptide chain. Other short peaks were detected between 270 and 280 nm. Chandra and Shamasundar (2015) observed the same registers in gelatin made of the *Catla catla* fish species. Similar to Shandra, Shamasundar and Kumar (2013), registered absorptions in the wavelength range of 220-230 nm, while studying bones of *Cirrhinus mrigala*.

3.4 VISCOSITY

Viscosity is one of the main rheological characteristics observed in products such as gelatin. The averages for the viscosity values (mPa.s) for the treatments tested are shown in Table 4.

Table 4 - Average values and standard deviation of viscosity (mPa.s) of *Oreochromis niloticus* gelatin.

Treatments	Viscosity (mPa.s)
T1	10.82 ± 0.43 [b]
T2	18.34 ± 0.63 [a]
T3	9.28 ± 1.90 [b]
T4	20.16 ± 0.77 [a]

T1 (Sodium hydroxide 0.70 Mol.L^{-1}; sulfuric acid 0.03 Mol.L^{-1}, citric acid 0.05 Mol.L^{-1}) T2 (Sodium hydroxide 0.30 Mol.L^{-1}, hydrochloric acid 0.04 Mol.L^{-1}) T3 (Sodium hydroxide 0.30 Mol.L^{-1}, citric acid 0.10 Mol.L^{-1}) T4 (Sodium Hydroxide 0.30 Mol.L^{-1}, Acetic Acid 0.03 Mol.L^{-1}). Values expressed as mean ± standard deviation (n = 3). Different letter in the same column indicate difference ($p < 0.05$).

The viscosity of gelatins could possibly have been affected by the initial protein yield. It was observed that treatments 1 and 3 did not differ statistically, however they showed a significant difference ($p<0.05$) in relation to treatments 2 and 4, which did not differ from each other. It is suggested that these reduced values for T1 and T3 were caused by the citric acid used in the extraction. According to Niu *et al*. (2013), a high concentration of citric acid (≥0.05 M) or HCl (≥0.07 M), or with weak acetic acid (0.01, 0.03 M) or HCl (0.01 M) favor low gelatin viscosity. Niu *et al*. (2013) evaluated the extraction of gelatine from tilapia skin with citric, acetic and hydrochloric acids at different concentrations. The authors showed that high concentrations and type of acids influence the viscosity of gelatin samples, in which they reported that citric acid concentrations of 0.05 and 0.1 Mol.L-1 had lower viscosities compared to formulations extracted with 0.04 Mol hydrochloric acid.L-1

The T2 and T4 samples showed higher values (Table 4), possibly due to changes in the structure of the samples from the elimination of water around the protein molecule (ALFARO, FONSECA, PRENTICE–HERNÁNDEZ, 2013). Therefore, the extraction method does not interfere with the viscosity of these treatments and can be characterized as gelatins with good consistency (BORAN, LAWLESS, REGENSTEIN, 2010).

3.5 PROXIMATE COMPOSITION OF COATED THREADS

The proximate composition of the coated fillet and control samples is shown in Table 5. The results demonstrate significant difference ($p \leq 0.05$) in the analysis of moisture, lipids and ash.

Table 5 – Centesimal composition of fillets of *Oreochromis niloticus* coated with and without gelatin.

Treatments	Component averages (%)			
	Moisture	Lipids	Ashes	Proteins
C	77.04 ± 0.06 [ab]	0.23 ± 0.01 [b]	2.49 ± 0.01 [b]	17.84 ± 1.32 [a]
CG	78.37 ± 0.38 [a]	0.41 ± 0.04 [a]	2.81 ± 0.03 [a]	17.29 ± 0.14 [a]
FG	74.19 ± 3.13 [b]	0.37 ± 0.21 [a]	2.79 ± 0.04 [a]	18.25 ± 0.09 [a]

C (control), CG (6.67% commercial gelatin and 1.8% glycerol) and FG (6.67% gelatin and 1.8% glycerol). Values expressed as mean ± standard deviation (n = 3). Different letter in the same column indicate difference ($p < 0.05$).

As for the percentage of proteins, there was no difference between the formulations. It is observed that the ash and lipids content increased in the coated samples, and this increase is attributed to the added coating itself. Possibly the presence of glycerol in the film formulation influenced the increase in the lipid content of the coated samples. For the moisture analysis, it was observed that the CG significantly differed ($p \leq 0.05$) from the FG presenting higher average values, which suggests that the coating with tilapia gelatin favored less water absorption of the product. This corroborating the study by Lin *et al*. (2017), in which fish gelatins showed excellent film and barrier formation properties and presenting greater permeability to water vapor than films with mammalian gelatin. According to Eça, Sartori and Menegalli (2014), edible coatings in food must act as a protection against molecule losses such as water vapor which can alter the composition of food and cause undesirable effects. The results of this study were similar to those reported by Alcântara *et al*. (2019). Thus, it appears that the use of tilapia skin-based gelatin coating presented similar results to those made with CG, and therefore is an option to be used as an edible coating for use on tilapia fillet surfaces.

3.6 SENSORY EVALUATION

For the sensory tests, T4 (NaOH 0.3 Mol.L-1 and CH_3COOH 0.03 Mol.L-1) was selected for application as a coating on tilapia fillets, due to its better presentation in terms of yield, color and viscosity. The averages obtained in the acceptance and purchase intention tests are shown in Table 6.

Table 6. Sensory evaluation for coated tilapia fillets

Attributes	Samples		
	Control	CG	FG
Appearance	7.78 ± 1.12[a]	7.80 ± 1.01[a]	7.79 ± 1.15[a]
Aroma	7.78 ± 1.18[a]	7.77 ± 1.18[a]	7.68 ± 1.29[a]
Texture	7.79 ± 1.07[a]	7.76 ± 1.20[a]	7.79 ±1.15[a]
Flavor	8.06 ± 1.04[a]	8.11 ± 1.08[a]	8.05 ± 1.15[a]
Overall acceptance	7.97 ± 0.99[a]	7.94 ± 0.87[a]	7.84 ± 1.05[a]
Purchase Intention	4.33 ± 0.84a	4.36 ± 0.83a	4.30 ± 0.77a

Values expressed as mean ± standard deviation (n = 3). Where: C (control, fillet without coating), CG (file coated with 6.67% commercial gelatin and 1.8% glycerol) and FG (6.67% fish gelatin and 1.8% glycerol). Different letter in the same column indicate difference ($p < 0.05$). (n = 120 panel participants; hedonic scale of 9 points: 1 - disliked extremely, 9-liked extremely and Overall acceptance of 5 points: 1-certainly not buy, 5-certainly buy.

There was no statistical difference ($p>0.05$) between the formulations of coated *Oreochromis niloticus* fillets and the control for all evaluated sensory attributes (Figure 2). The results showed excellent acceptance of the coated fillets, since the average values of the appearance, aroma and texture attributes ranged from 7.68 to 7.80, corresponding to "I liked it moderately" and "I liked it a lot" on the scale, respectively. The flavor parameter obtained for all average formulations ranged from 8.05 to 8.11, referring to "I liked it a lot". Therefore, it is suggested that the coated samples did not modify the sensory aspects of the tilapia fillets, being similar to the uncoated sample (control). Therefore, these coatings did not transfer any residual flavor or odor to the fillets to the point of being perceived by the tasters. Thus, the gelatin-based coating appears as a barrier against environmental conditions (absorption of water and O_2), as well as preserving it against micro-organism attacks, without altering the nutritional composition of the tilapia file. It was noted that the FG coating did not differ significantly ($p> 0.05$) from the CG, thus concluding that it is possible to substitute the use of commercial gelatin without altering the sensory quality of tilapia fillet samples, and its application can help increase the fish gelatin industry. According to Lin *et al*. (2017), fish-based gelatins have similar characteristics to mammalian gelatins, and therefore they have the potential to replace pork and beef gelatins in many applications. Regarding the purchase of the product, the treatments did not differ statistically ($p>0.05$), presenting average values according to the term "would probably buy", therefore they obtained approval of the tasters.

4 CONCLUSION

Gelatin made from tilapia skin (FG) and its application as an edible film in fillets was analyzed. The physical and physical-chemical data show similarities between gelatins made with different extraction methods. The use of sodium hydroxide solution acetic acids (T4) provided greater preservation of protein contents. The extraction type also interfered with the gelatin viscosity, however it was possible to obtain gelatin of good extensibility and consistency. When analyzing the coated fillets, the moisture content for FG treatment was significantly lower than the others and the ashes and lipids were higher compared to the control. These findings indicate that the use of tilapia skin gelatin as an edible coating was effective in preserving the proximate composition of fillets of the same species, so its use is recommended. The sensory data suggest that the quality attributes of the coated fillets were not affected by the treatments. Therefore, the extraction and application of tilapia skin gelatins can be a trend as an active packaging in the preservation of the chemical composition and useful life of fish and favor the reuse of industrial waste.

ACKNOWLEDGMENTS

The authors would like to thank CAPES, FUNCAP and CNPq for financial support [DC8-0145-00018.01.00/18]. BWSS is senior investigator of Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

REFERENCES

ALCÂNTARA, L. O.; MARTINS, M. E. O.; SOUSA, J. R.; CERQUEIRA, M. Â.; SILVA, A. L. C.; SOUZA FILHO, M. S. M.; SOUZA, B. W. S. Wettability of edible coatings on Nile tilapia fillets (*Oreochromis niloticus).* **Journal of Food Engineering**, v. 247, p.152-159, 2019.

ALFARO, A. T.; FONSECA, G. G.; PRENTICE–HERNÁNDEZ, C. Enhancement of functional properties of Wami tilapia (*Oreochromis urolepis hornorum*) skin gelatin at different pH values. **Food and Bioprocess Technology**, v. 6, p. 2118-2127, 2013.

ALFARO, A. T.; SILVA, E. F. Propriedades reológicas da gelatina obtida de pele de tilápia (*Oreochromis niloticus*). **Revista Instituto Adolfo Lutz**, v. 69, n.4, p. 555-561, 2010.

Association of Official Analytical Chemists. 2002. **Official methods of analysis of AOAC international**. 17. ed., Washington, 2002.

BORAN, G.; LAWLESS, H. T.; REGENSTEIN, J. M. Effect of extraction conditions on the sensory and instrumental characteristics of fish gelatin gels. **Journal of Food Science**, v. 75, p. 469-476, 2010.

BUENO, C. M.; ALVIM, IZABELA D.; KOBERSTEIN, T. C. R. D.; PORTELLA, M. C.; GROSSO, C. Produção de gelatina de pele de tilápia e sua utilização para obtenção de micropartículas contendo óleo de salmão. **Brazilian Journal of Food Technology**, v.14, p. 65-73, 2011.

CHANDRA, M. V.; SHAMASUNDAR, B. A. Rheological properties of gelatin prepared from the swim bladders of freshwater fish *Catla catla.* **Food Hydrocolloids**, v. 48, p. 47-54. 2015.

EÇA, K. S.; SARTORI, T.; MENEGALLI, F. C. Films and edible coatings containing antioxidants – A review. **Brazilian Journal of Food Technology**, v. 17, p. 98-112, 2014.

HONG, H.; FAN, H.; CHALAMAIAH, M.; WU, J. Preparation of low-molecular-weight, collagen hydrolysates (peptides): Current progress, challenges, and future perspectives. **Food Chemistry**, v. 301, p. 125222, 2019.

HOSSEINI, S. F.; GÓMEZ-GUILLÉN, M. C. A state-of-the-art review on the elaboration of fish gelatin as bioactive packaging: Special emphasis on nanotechnology-based approaches. **Trends in Food Science & Technology**, v. 79, n. 125-135, 2018.

JONGJAREONRAK, A.; RAWDKUEN, S.; CHAIJAN, M.; BENJAKUL, S.; OSAKO, K.; TANAKA, M. Chemical compositions and characterisation of skin gelatin from farmed giant catfish (*Pangasianodon gigas*). **Food Science and Technology**, v.43, n. 1, p. 161-165, 2010.

KTARI, N.; JRIDI, M.; NASRI, R.; LASSOUED, I.; AYED, H. B.; BARKIA, A.; NASRI, M., Characteristics and functional properties of gelatin from zebra blenny (*Salaria basilisca*) skin. **Food Science and Technology**, v. 58, p. 602-608, 2014.

LI, X.; LIU, X.; LAI, K.; FAN, Y.; LIU, Y.; HUANG, Y. Effects of sucrose, glucose and fructose on the large deformation behaviors of fish skin gelatin gels. **Food Hydrocolloids**, v. 101, p. 105537, 2020.

LIAO, W.; ZHU, Y.; LU, Y.; WANG, Y.; DONG, X.; XIA, G.; SHEN, X. Effect of extraction variables on the physical and functional properties of tilapia gelatin. **Food Science and Technology**, v.146, p. 111514, 2021.

LIN, L.; REGENSTEIN, J. M.; LV. S.; LU, J.; JIANG, S. An overview of gelatin derived from aquatic animals: Properties and modificatio. **Trends in Food Science & Technology**, v. 68, p. 102-112. 2017.

LV, L. C.; HUANG, Q. Y.; DING, W.; XIAO, X. H.; ZHANG, H. Y.; XIONG, L. X. Fish gelatin: The novel potential applications. **Journal of Functional Foods**, v. 63, p.103581, 2019.

MIRZAPOUR-KOUHDASHT, A.; MOOSAVI-NASABA, M.; KRISHNASWAMY, K.; KHALESI, M. Optimization of gelatin production from Barred mackerel by-products: Characterization and hydrolysis using native and commercial proteases. **Food Hydrocolloids**, v. 108, p. 105970, 2020.

MONTERO, P.; GÓMEZ-GUILLÉN, M. C. Extracting conditions for Megrim (*Lepidorhombus boscii*) skin collagen affect functional properties of the resulting gelatin. **Journal of Food Science** v. 65, p. 434-438, 2001.

MENEZES, M. L. R.; RIBEIRO, H. L.; ABREU, F. O. M. S.; FEITOSA, J. P. A.; SOUSA FILHO, M. D. S. Optimization of the collagen extraction from Nile tilapia skin (Oreochromis *niloticus*) and its hydrogel with hyaluronic acid. **Colloids and Surfaces B: Biointerfaces,** v. 189, p.110852, 2020.

MUYONGA, J. H.; COLE, C. G. B.; DUODU, K. G. Extraction and physicochemical characterisation of Nile perch (*Lates niloticus*) skin and bone gelatin. **Food Hydrocolloids**, v.18, p. 581–592, 2004.

NIU, L.; ZHOU, X.; YUAN, C.; BAI, Y.; LAI, K.; YANG, F.; HUANG, Y. Characterization of tilapia (*Oreochromis niloticus*) skin gelatin extracted with alkaline and different acid pretreatments. **Food Hydrocolloids**, v. 33, p. 336-341, 2013.

NUANMANO, S.; PRODPRAN, T.; BENJAKUL, S. Potential use of gelatin hydrolysate as plasticizer in fish myofibrillar protein film. **Food Hydrocolloids**, v. 47, p. 61-68, 2015.

SAHIN, S.; SUMNU, S. G. **Physical properties of foods. Springer:** New York. 2006.

SCHRIEBER, R.; GAREIS, H. **Gelatine handbook, theory and industrial practice.** Ed. digital, Wiley-Vch, 2007, 347 p.

SHI, C.; HAN, S.; YANG, X.; QIAN, J.; FAN, B.; WU, X. Developing a machine vision system for simultaneous prediction of freshness indicators based on tilapia (*Oreochromis niloticus*) pupil and gill color during storage at 4 °C. **Food Chemistry**, v. 243, p. 134-140, 2018.

SHINY, K.; HEMA, G. S.; NINAN, G.; MATHEW, S.; JOSHY, C. G.; LAKSHMANAN, P. T. Isolation and characterization of gelatin from the skins of skipjack tuna (*Katsuwonus pelamis*), dog shark (*Scoliodon sorrakowah*), and rohu (*Labeo rohita*). **Food Hydrocolloids**, v. 39, p. 68-76, 2014.

SILA, A.; MARTINEZ-ALVAREZ, O.; HADDAR, A.; GÓMEZ-GUILLÉN, M. C.; NASRI, M.; MONTERO, M. P.; BOUGATEF, A. Recovery, viscoelastic and functional properties of barbel skin gelatine: Investigation of anti-DPP-IV and anti-prolyl endopeptidase activities of generated gelatine polypeptides. **Food Chemistry**, v. 168, p. 478-486, 2015.

SILVA, F.; AZEVEDO, C. Assistat 7.7 Versão Beta: Principal Components Analysis in the Software Assistat-Statistical Attendance. In: World Congress on Computers in Agriculture, 7, Reno-NV-USA: American Society of Agricultural and Biological Engineers, 2009.

SOW, L. C.; YANG, H. Effects of salt and sugar addition on the physicochemical properties. **Food Hydrocolloids**, v. 45, p. 72-82, 2015.
STONE, H.; SIDEL, J. L. 2004. **Sensory evaluation practices.** 3rd ed. New York: Academic Press.

SUN, L.; QIUMING, L.; JIAN, F.; XIAO, L.; YONGLIANG, Z. Purification and characterization of peptides inhibiting MMP-1 activity with C-terminate of Gly-Leu from simulated gastrointestinal digestion hydrolysates of tilapia (*Oreochromis niloticus*) skin gelatina. **Journal of Agricultural and Food Chemistry**, v. 66, p.593-601, 2018.

TAN, C. C.; KARIM, A. A.; UTHUMPORN, U.; GHAZALI, F. C. Effect extraction temperature on the emulsifying properties of gelatin from black tilapia (*Oreochromis mossambicus*) skin. **Food Hydrocolloids**, v. 108, 2020.

YAN, M.; JIANG, X.; WANG, G.; WANG, A.; WANG, X.; WANG, X.; ZHAO, X.; XU, H.; AN, X.; LI, Y. Preparation of self-assembled collagen fibrillar gel from tilapia skin and its formation in presence of acidic polysaccharides. **Carbohydrate Polymers**, v. 233, p.115831, 2020.

ZHANG, T.; SUN, R.; DING, M.; TAO, L.; LIU, L.; TAO, N.; WANG, X.; ZHONG, J. Effect of extraction methods on the structural characteristics, functional properties, and emulsion stabilization ability of Tilapia skin gelatins. **Food Chemistry**, v. 328, p. 127114, 2020.

ZHAO, X.; WU, J.; CHENA, L.; YANGA, H. Effect of vacuum impregnated fish gelatin and grape seed extract on metabolite profiles of tilapia (*Oreochromis niloticus*) fillets during storage. **Food Chemistry**, v. 293, p. 418-428, 2019.