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Original Article

Evaluation of physicochemical properties of Nile tilapia skin collagen extracted in acid médium

Avaliação das propriedades físico-químicas do colágeno da pele de tilápia do Nilo extraído em meio ácido

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Abstract

Tilapia has high-temperature tolerance, can breed in captivity, grow fast, and have excellent cost-benefit. Because of these characteristics, this species is of great interest in aquaculture and, currently, the most produced fish in Brazil. However, by increasing tilapia production, there was also a rise in the amount of organic waste, mainly from filleting, which discards 70% of waste. There are many studies on collagen extraction from tilapia skin as an alternative to reduce these residues and add commercial value. In this work, the extraction of protein concentrate was tested using an acid protocol, in which the tilapia skins underwent a pre-treatment in an acid medium and saline precipitation, with variations in time and concentration. After its extraction, the skin was evaluated for ash, moisture, protein, solubility, and pH. The protein concentrate obtained showed low ash contents, and the humidity is within those presented by the literature. The protein concentrate showed levels from 68.73 to 80.58% of protein and a low solubility between 4.03 to 6.93%. In conclusion, acid extraction is a possible means of collagen extraction, and tilapia skin is a good alternative to reuse waste generated in the fish industry.

Keywords: tilapia, protein concentrate, extraction, acid medium.

Resumo

A tilápia tem tolerância a altas temperaturas, pode se reproduzir em cativeiro, crescer rápido e tem excelente custo-benefício. Por essas características, esta espécie é de grande interesse na aquicultura e, atualmente, o pescado mais produzido no Brasil. No entanto, com o aumento da produção de tilápias, houve também um aumento na quantidade de resíduos orgânicos, principalmente da filetagem, que descarta 70% dos resíduos. Existem muitos estudos sobre a extração de colágeno da pele de tilápia como alternativa para reduzir esses resíduos e agregar valor comercial. Neste trabalho, a extração do concentrado protéico foi testada utilizando um protocolo ácido, no qual as peles de tilápia foram submetidas a um pré-tratamento em meio ácido e precipitação salina, com variações de tempo e concentração. Após sua extração, a pele foi avaliada quanto a cinzas, umidade, proteína, solubilidade e pH. O concentrado protéico obtido apresentou baixos teores de cinzas, e a umidade está dentro dos apresentados pela literatura. O concentrado protéico apresentou teores de 68,73 a 80,58% de proteína e uma baixa solubilidade entre 4,03 a 6,93%. Em conclusão, a extração ácida é um possível meio de extração de colágeno, e a pele de tilápia é uma boa alternativa para reaproveitar os resíduos gerados na indústria de pescados.

Palavras-chave: tilápia, concentrado proteico, extração, meio ácido.

1. Introduction

In the last six years, the production of farmed fish increased by 31% in Brazil, with 578,800 tons in 2014, rising to 758,006 tons in 2019. In the last year, there was a 4.9% growth over 2018, of which 432,149 tons refer if tilapia production, equivalent to 57% of the national fish production, reinforcing its position as the 4th largest tilapia producer in the world with a difference of 90,000 tons for the 5th place, Thailand (Peixe BR, 2020). With this demand, problems that involve waste generation have emerged since tilapia has a yield of only 30% for fillets, resulting in the formation of 70% of residue, of waste that include: head (14%), carcass (35%), viscera (10%), skin (10%) and scales (1%) (Marques Bueno et al., 2011). These residues, because they have no added economic value, are deposited in the environment in any way, causing the bad smell and attraction of insects, which confers risks to the public health

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of riverside dwellers and in several other environments (Marques Bueno et al., 2011). One of the ways to take advantage of these discarded residues is the production of proteins, for example obtaining collagen from the skin of Nile tilapia. The reuse reduces environmental impact and adds value to the production process, factors highly accepted by most consumers who value a more conscious and sustainable production, and is an alternative to help company economy (Marques Bueno et al., 2011).

In Brazil, most of the collagen comes from the by-products of the beef industry, due to its high production for export. However, the outbreak of Bovine Spongiform Encephalopathy (BSE) and religious issues are recurrently obstacles to the continued use of collagen originating from these sources (Silva and Penna, 2012). Thus, in recent years the collagen extracted from fish skin and by-products generated by chicken meat has been gaining space and attention, mainly due to the growing concern of companies in relation to waste reduction (Lu & Chen, 2004). According to Lee et al. (2001) collagen has natural properties that include low immune response, low toxicity and the ability to promote cell growth and the reconstruction (in vitro and in vivo) of the microfibrillary structure found in natural tissues, which can be applied in food, pharmaceutical and cosmetics industries.

There are different methods of collagen extractions however the most common uses acid / alkaline solutions and enzymatic hydrolysis. These methods provide the elimination of cross-linking of the polypeptide chains, increasing the amount of soluble collagen, it is worth mentioning that if the hydrolysis or extraction is very vigorous, the final product may be a degraded protein, being closer to a gelatin than to the real characteristics of a collagen (Rebouças et al., 2017). Acid and alkaline extraction (NaOH) is more active, as it requires better temperature control, due to the use of high temperatures reaching 75 °C, while acid and enzymatic extraction requires chemical reagents such as acetic acid, pepsin and NaCl (Huang et al., 2016). The chemical pretreatment removes calcium, fat and maximum impurities without the protein becoming denatured (Oliveira et al. 2017).

In the extraction of collagen by the alkaline method, the raw material is subjected to a pretreatment with basic pH for several weeks having a slow transformation of the collagen structure. In the acid treatment, the raw material is immersed in an acid solution at a controlled temperature, with the solution penetrating the raw material, promoting an expansion of two to three times its initial volume, giving the cleavage process of the inter and intra non-covalent bonds molecular (Silva and Penna, 2012). Collagen solubility in an acidic environment plays a fundamental role in the efficiency of the extraction since there is an increase in H+ ions, facilitating the access of water in the fibers present in the collagen (Peña-Saldarriaga et al. 2020). In the extraction by the enzymatic method, different enzymes are used, such as pepsin, trypsin and collagenase. Pepsin is the most used in the extraction of collagen and can act alone after a previous extraction of collagen with acetic acid or in conjunction with the acetic acid solution, generating a soluble collagen with PSC pepsin (Caldato et al., 2019).

Based on these considerations, this study sought to optimize the process of obtaining collagen from fishing residues using the skin of the Nile tilapia (Oreochromis niloticus) and employing low complexity and costly methodology for industrial application, even more than the results found in the literature are scarce in these conditions.

2. Material and Methods

2.1. Material

The equipaments used were the analytical scale (Bioscale FA2204B-BI), scissors (Tramontina Extracort), shaker incubator (C-Mag HS 7), lyophilizer (Gamma 1-16 / 2-16 LSC), laminar flow hood (Veco FUV-12), DSC (Shimadzu DCS 50), distiller (Quimis Q341-22), digester (DK20/26 VELP Scientifica), centrifuge (Hoffmannlab HCL-4), muffle oven (Quimis Q318A24), oven (Memmert BKD-20N) and desiccator (Schott - LABDEL). The materials used were the skins, 0.1M sodium hydroxide solution, 0.5M EDTA solution, 10% butanol solution, acetic acid, NaCl solution, hydrochloric acid, boric acid, dialysis membrane, Erlenmeyer flask, beaker, and porcelain crucibles were purchased from Sigma-Aldrich Co.

2.2. Methods

The extraction methodology used was modified from the method described by (Yan et al., 2015). The meat-free skins were washed in running water and frozen at -18 °C for seven days. To begin the extraction procedures, the skins were weighed on an analytical scale and cut into small pieces using scissors. They were subsequently washed in a 0.1M sodium hydroxide (NaOH) solution in the proportion 1:20 w/v (20 mL of NaOH for each 1g of tilapia) for 24 hours in the shaker incubator. This step was necessary to remove pigments and proteins that did not contain collagen. The skins were removed from the incubator and immersed in a 0.5M EDTA solution, in a ratio of 1:10 w/v (1g of sample to 10 mL of EDTA). The pH was corrected with NaOH to 7.5. The solution was renewed every 24 hours for five days to descale the sample. After this period, the samples were washed with distilled water at 5 ºC. This temperature was chosen throughout the process as a way to avoid/protect the protein from possible denaturation steps (Damodaran et al., 2010; Fennema, 2000).

The skin was degreased in the following stage by emerging them in a 10% butanol solution at a 1:20 w/v ratio (1g of sample to 20 mL of butanol) for 24 hours. The solution was changed every 8 hours, after 24 hours, and the skins washed with distilled water at 5 ºC.

Subsequently, the samples were submerged in an acetic acid solution in the shaker incubator with varying concentrations and times, as shown in Table 1.

The skins were added to a 0.9M NaCl solution, the pH was corrected to 3.5 with HCl, and the sample was left under refrigeration for 24 hours to remove the precipitate. The sample was then dissolved in 0.5M acetic acid and dialyzed against 0.1M acetic acid, changing the solution every eight hours, for one day. Afterward, the 0.1M acetic

acid solution was exchanged for distilled water for three days, renewing the water every eight hours.

The samples taken from dialysis were lyophilized for four days with a temperature of -52 °C according to the required adaptation of the methodology. The analyzes were then performed to evaluate the collagen.

2.2.1. Experimental planning and statistical treatment of the samples

The assays established in Table 1 are from the evaluation of possible factors that can interfere in the collagen yield. To do this, complete factorial design with three levels and two factors (3^2) and two replicates at the central point was elaborated, following a quadratic regression model with the variables of acetic acid concentration and time, according to Equation 1.

$$y = b_0 + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_{22}x_2^2 + b_{12}x_1x_2 \quad (1)$$

where:
y = response variable;
b_0 = intercept;
b_1 = coefficient of regression for the linear effects of the concentration;
b_2 = coefficient of regression for the linear effects of time;
b_{11} = coefficient of regression for the quadratic effects of the concentration;
b_{12} = coefficient of regression for the interaction effects of concentration and time;
b_{22} = coefficient of regression for the quadratic effects of time;
x_1 = concentration;
x_2 = time.

Table 2 presents the independent variables, with real and coded values, for extraction in an acid medium.

The experimental data were analyzed using the Statistica 12 software, employing the response surface methodology with quadratic model adjustment. The analysis of variance with Tukey's test at a 5% significance level (p-value <0.05) were also used to verify whether there were differences between the means and the desirability function methodology.

The desirability function was used to verify which conditions of time and concentration of acetic acid would be ideal to obtain the extraction of the collagen considering the solubility and the protein derived from the skin of the Nile tilapia. This method consists of applying a dimensionless value for each response variable that represents their individual desirability (*di*). According to Novaes et al. (2017), the desirability can vary from 0 (for an undesired response) to 1 (for the desired response). The global desirability (D) can be obtained using the global average of individual desires, expressed by Equation 2:

$$D = \sqrt[m]{d_1 d_2 d_3 \ldots d_m}, \quad (2)$$

where:
D is the global desirability;
d_1, d_2, d_3, and d_m are the individual desirabilities;
m is the number of response variables.

With this, the simultaneous optimization process is reduced to the simple task of calculating the levels of variables that maximize global desirability. There are different types of transformations possible to obtain individual desirabilities. If the target value, *Ui* for the response y_i, for example, is a maximum, the individual desirability (*di*) is described by the following function:

$$d_i\left(y_i(x)\right) = \begin{bmatrix} 1 \text{ se } y_i(x) < L_i \\ \left[\dfrac{U_i - y_i(x)}{U_i - L_i}\right]^t \\ 0 \text{ se } y_i(x) > U_i \end{bmatrix} \quad (3)$$

where:
y_i is the response obtained for the studied variable;
$d_i(y_i)$ is the individual desirability of response y_i;
U_i is the highest acceptable value adopted for the response;
L_i is the lowest acceptable value adopted for the response;
t is the factor that determines how desired it is that y_i be close to the minimum, ranging from 0 to 1.

Table 1. Concentration of acetic acid and time for each essay.

Essay	Concentration of acetic acid (M)	Time (hours)
1	0.25	12
2	0.25	24
3	0.25	36
4	0.50	12
5	0.50	24
6	0.50	36
7	0.75	12
8	0.75	24
9	0.75	36
10	0.50	24
11	0.50	24

Table 2. Real values of the variables for the experimental planning and their respective coded levels.

Variables	-1	0	1
	Extraction in acid medium		
Concentration of acetic acid (M)	0.25	0.50	0.75
Time (hours)	12	24	36

Functions for calculating individual desirabilities can also be established for other situations, such as the target value being a minimum or located between a minimum value and a maximum value. The application of the desirability function to optimize analytical methods brings numerous advantages such as efficiency, economy, and objectivity to the optimization of procedures with multiple responses (Vera Candioti et al., 2014).

2.3. Analyses

2.3.1. Differential scanning calorimetry (DSC)

To determine the thermal stability of collagen in the skin of Nile tilapia Differential scanning calorimetry analysis was carried out. The DSC analyzes were performed in a Q20 equipment (from TA Instruments), in a hermetically sealed aluminum pan, with about 50 mg of each sample. The experiment was carried out with a heating rate of 20 ºC/min, at an initial temperature of 0 ºC and a final temperature of 300 ºC. The data obtained were plotted using the OriginPro software (Zeng et al., 2009).

2.3.2. Ash

The ash analysis was done according to the methodology of the Instituto Adolfo Lutz (IAL, 2008). This methodology is based on the carbonization of the sample and subsequent heating in a muffle at 550 ºC for four 4 to 6 hours (until obtaining light-colored ash). To analyze the ashes, 3 to 5 grams of sample were weighed, and their percentage was obtained.

2.3.3. Humidity

The humidity of the lyophilized collagen was determined using the Physical-Chemical Method for Food Analysis of the Adolfo Lutz Institute (IAL, 2008). It consists of drying the samples in an oven at 105 ºC for six hours and weighing the dried samples in crucibles to obtain the weight.

2.3.4. pH

The Adolfo Lutz Institute methodology (IAL, 2008) was used to determine the pH. The samples were dissolved in distilled water at a ratio of 1:10 (1 gram of sample to 10 mL of water), and the pH was measured using a pH meter.

2.3.5. Proteins

Protein extraction was performed based on the methodology of Kjeldahl (Saputri et al., 2019), acidic digestion was carried out, in which the nitrogen contained in the sample was transformed into ammonia, then separated by distillation. Then, the sample was titrated to determine the amount of ammonia present in the solution.

First, a catalytic mixture was made at a ratio of 100:10:1 of sodium sulfate, copper, and selenium sulfate, with 2 g in each sample. For digestion, 0.2 g of the sample and 2 g of the catalytic mixture were weighed and placed on parchment paper and later inserted into the digestion tube. After this, 5 mL of sulfuric acid was added, and then the mixture was placed in the digester block.

Solutions of 4% boron, 40% NaOH, and 10% HCl were prepared for the distillation. With the samples at room temperature, 10 mL of distilled water was added to the digestor tube. The 125 mL Erlenmeyer flask was filled with 15 mL of the boric acid solution and six drops of the indicator. Subsequently, 25 mL of NaOH was added to the dosing funnel, and, after boiling the water, the digester tube was fitted to the distillation block, and the Erlenmeyer flask fitted to the other end of the distiller before opening the valve. The valve was closed after the sample was neutralized and filled into the Erlenmeyer flask, up to 75 mL. The sample was titrated with HCl solution.

2.3.6. Solubility

Protein solubility was determined as performed by Chalamaiah et al. (2010) with some adaptations. For this, 300 mg of sample were diluted in 30 ml of distilled water and the pH of the solution adjusted to 3, 5, 7, 9 and 12 with 0.5 N hydrochloric acid (HCl) or 0.5 N sodium hydroxide (NaOH). The solution was stirred at room temperature (25 ± 2 °C) for 30 min and centrifuged at 6000 rpm for 30 min at 4 °C. The protein content in the supernatant was determined using the Biuret method and the total protein was determined by the MicroKjeldahl method Instituto Adolfo Lutz (IAL, 2008). The protein solubility was calculated as follows the Equation 4.

$$\text{Solubility}(\%) = \frac{\text{Protein in the supernatant}}{(\text{Total protein in the sample})\ *100} \quad (4)$$

3. Results and Discussion

In the DSC analysis, the denaturation temperatures (Td) for each of the 11 tests and for the standard collagen samples are shown in Table 3. And even presenting values below

Table 3. Denaturation temperatures of collagen samples.

Test	Td (ºC)
1	82.29
2	90.20
3	91.96
4	81.34
5	85.31
6	85.02
7	83.31
8	84.32
9	85.78
10	86.43
11	87.45
Nova_prom collagen	107.92
Pharmacy collagen	98.32

those indicated in the literature, it presents a good thermal stability. For the other analyzes carried out, a significance level of 5% for Anova was considered. The ash, humidity, pH, protein, and solubility assays were performed for the eleven formulations shown in Table 1 and determined according to the complete factorial design 3^2 with three levels and two factors, using two replicates at the central point. The ash, humidity, pH, and protein assays were performed in triplicate and solubility in duplicate. The results of the means and standard deviations are presented in Table 4.

The results show that there were no significant differences for the eleven samples regarding the ash and humidity analyses. The residue obtained from the incineration or the ash itself is the residue obtained from heating a sample at approximately 550 °C (IAL, 2008). The ash is a vital identification measure since it is widely used as a food identification criterion. Furthermore, its composition in food shows its nutritional value in advance, indicating the quantity of minerals in the sample (Dotto et al., 2019).

The ash results obtained ranged from 1.16 to 1.82%, as shown in Table 4, and showed no significant differences between the 11 samples analyzed. The values are below 2.0%, which is the result recommended by Ockerman and Hansen (1999). The result presented by samples A3 and A7 is similar to the values found by Caldato et al. (2019), which was 1.44%.

The humidity shows the amount of weight loss the sample underwent when heated when water is removed from the food. The humidity content is essential for food preservation (IAL, 2008).

The percentages of humidity in the samples are shown in Table 4. The values ranged between 10.93 and 18.49%, and, according to the Analysis of Variance, the samples from A1 to A11 did not differ.

The values obtained for samples A1, A2, and A4 were close to those obtained by Tavares Alves and Prudencio-Ferreira (2002), which was 15.06% humidity for non-lyophilized collagens. The other samples, except for sample A7, presented values for lyophilized collagen humidity superior to the value established by the authors, which was 11.29%.

Regarding the pH values, the samples differed significantly, and samples A9 and A4 presented the highest and lowest pH values, respectively, ranging from 5.52 to 6.85%. Therefore, the pH values of samples A4, A5, A6, A10, and A11 are below the range of pH values obtained by Prestes et al. (2013) for bovine collagens. Samples A1, A2, A3, A7, A8, and A9 were within the range highlighted by the authors, which was from 6.11 to 8.41%.

The protein determination of samples A5, A10, and A11 did not differ from each other. The same occurred with samples A1, A3, A4, A6, A7, A8, and A9, which have no significant difference from any of the samples shown in Table 3. Sample A2 only differs from samples A5, A10, and A11, with A10 presenting the highest amount of protein extracted and A2 the lowest.

The protein levels found are inferior to the values in the literature. Basso et al. (2013) report 91.24%. The result is also inferior to that found by Tavares Alves and Prudencio-Ferreira (2002), which was 85.59%. However, samples A5, A9, A10, and A11 fall within the range of values obtained by Olivo & Shimokomaki (Olivo et al., 2001), which is from 76.27 to 81.41%.

Samples A2 and A3, as well as samples A4, A5, A6, A7, A8, A10, and A11, showed no significant difference. Samples A1 and A9 do not differ from any of the other samples and is considered similar to both groups. The lowest solubility value was found for samples A4 and A11, with A11 presenting the highest protein value.

The values presented by samples A2 and A3 are slightly superior to the value obtained by Tavares Alves and Prudencio-Ferreira (2002), which was 6.45% for lyophilized collagen with a concentration of 1:40. However, samples A4, A5, A6, A7, A8, A10, and A11 were very close to the value obtained by the authors when the concentration in the acid medium was 1:150, with 4.0% solubility for the collagens extracted from chicken legs.

The high protein content and a low solubility content obtained indicate the possibility of collagenous proteins

Table 4. Values of the physical-chemical variables for all formulations.

Samples	Ash (%)	Humidity (%)	pH	Proteins (%)	Solubility (%)
A1	1.77^{a}±0.06	15.98^{a}±0.50	6.40^{abc}±0.02	71.52^{ab}±2.60	5.72^{ab}±0.40
A2	1.40^{a}±0.24	15.44^{a}±2.29	6.48^{ab}±0.09	68.73^{b}±3.95	6.74^{a}±0.32
A3	1.46^{a}±0.23	13.63^{a}±2.76	6.19^{abc}±0.32	72.18^{ab}±1.13	6.93^{a}±0.02
A4	1.65^{a}±0.23	12.05^{a}±0.79	5.52^{c}±0.29	71.26^{ab}±1.05	4.03^{b}±0.19
A5	1.69^{a}±0.12	13.62^{a}±3.25	5.73^{bc}±0.12	79.96^{a}±0.72	4.07^{b}±0.41
A6	1.17^{a}±0.08	18.49^{a}±0.35	5.71^{bc}±0.05	74.73^{ab}±2.22	4.33^{b}±0.66
A7	1.45^{a}±0.27	10.93^{a}±0.48	6.34^{abc}±0.15	71.26^{ab}±3.21	4.70^{b}±0.30
A8	1.51^{a}±0.18	15.50^{a}±1.56	6.18^{abc}±0.10	73.50^{ab}±2.17	4.14^{b}±0.20
A9	1.16^{a}±0.16	13.34^{a}±2.31	6.85^{a}±0.28	77.80^{ab}±0.23	5.42^{ab}±0.47
A10	1.79^{a}±0.01	12.81^{a}±0.30	5.81^{bc}±0.16	80.58^{a}±0.35	4.15^{b}±0.27
A11	1.82^{a}±0.19	12.91^{a}±1.06	5.82^{bc}±0.06	80.24^{a}±1.11	4.04^{b}±0.21

Means in the same column, followed by distinct letters differ from each other by the Tukey test at a significance of 5%.

since, according to the literature, collagen is poorly soluble as a protein removed from connective tissue, as was shown in this experiment (Dotto et al., 2019).

The analysis of variance of the interest responses concerning the independent variables of the collagen extraction process also aimed to analyze the significance of their effects on the response variables and adjust the experimental data to a regression model that mathematically expressed the extraction. The responses found in this work are commented on below.

3.1. DCS analysis

In the analysis of differential exploratory calorimetry (DSC), the samples of bovine collagen acquired from a handling pharmacy, collagen nova_prom and collagen extracted from the tilapia coproduct were heated concomitantly and submitted to a controlled temperature program. DSC analysis is generally used to determine the thermal stability of collagen (Zeng et al., 2009). The DSC curves obtained for each assay of collagen samples and standard collagen samples are shown in Figure 1.

Denaturation temperatures (Td) for each test from 1 to 11 and for standard collagen samples are shown in Table 3.

It is observed that the curves related to the pharmacy manipuladed control and nova_prom industrializaded control and the curves of the tests from 1 to 11 present a transition around 100 ºC, which can be attributed to the collagen denaturation (Monterrey-Quintero and Sobral, 2000). According to De Batista et al. (2009), the longer the sample treatment time, the lower the desnaturation temperature (Td). The data collected by the DSC analysis indicate that collagen has good thermal stability, which means that it can be used as a polymeric matrix for the formulation of composites (Samouillan et al., 2011).

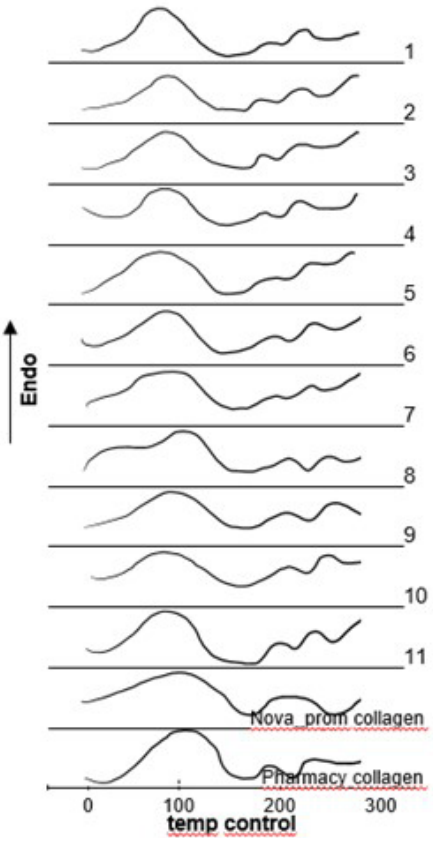

Figure 1. DSC curves.

3.2. Ash analysis

Table A1 (Supplementary material) shows the analysis of variance for the experimental data obtained for ash. Table A2 (Supplementary material) shows the adjustment coefficients to the quadratic model for this response (the effects that were significant in Anova are highlighted in red). The terms of acetic acid concentration, as well as the interactions between the concentration and time, were eliminated since they did not meet the level of significance and aiming at a better value for R^2adj. As a result, an excellent fit for the model was 98.275%. Figure B1 (supplementary material) shows the relationship between the experimental values and the values predicted by the adjusted model.

Time showed a significant negative effect on ash. That is, the decrease in time contributed to the increase in ash, as shown in Figure 2. That the lower the concentration and time, the higher the ash values. This trend begins between the levels 0.0 and 1.0 for both variables.

3.3. Humidity analysis

Table A3 (supplementary material) presents the analysis of variance for the experimental data obtained for humidity. Table A4 (supplementary material) shows the adjustment coefficients to the quadratic model for this response (the effects that were significant in Anova are highlighted in red). Both the time and concentration were significant for the adjustment in the model to be satisfactory: 99.125%. Figure B2 (supplementary material) shows the relationship between the experimental values and the values predicted by the adjusted model.

Higher values for humidity are predominant in intermediate levels for concentration (0.0) and higher levels for time (+1.0), as presented in Figure 3.

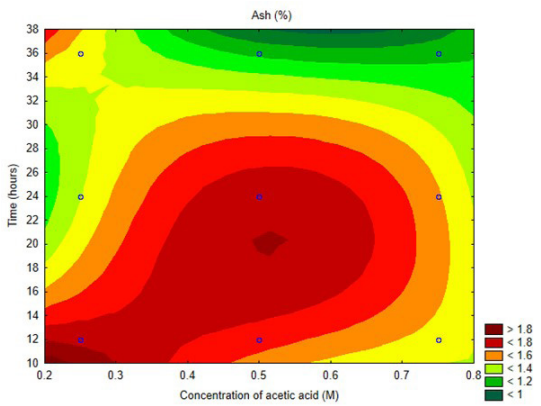

Figure 2. Outlining graph of the ash variable.

3.4. pH analysis

Table A5 (supplementary material) shows the analysis of variance for the experimental data for pH. Table A6 (supplementary material) shows the adjustment coefficients to the quadratic model for this response, according to a 5% significance level. The concentration and interaction between concentration and time were significant, so that the adjustment in the model was entirely satisfactory: 99.723%. Figure B3 (supplementary material) shows the relationship between the experimental values and the values predicted by the adjusted model.

Figure 4 illustrates that the higher the concentration and time, the higher the pH values. This trend begins between the levels 0.0 and +1.0 for both variables.

3.5. Protein analysis

Table A7 (supplementary material) provides a summary of the analysis of variance for the experimental data for protein, and Table A8 (supplementary material) shows the adjustment coefficients to the quadratic model for this response with a 5% significance level. The concentration, time, and interaction of concentration and time were very significant for the adjustment of the model to be excellent: 99.893%. Figure B4 (supplementary material) shows the relationship between the experimental values and the values predicted by the adjusted model.

Figure 5 shows the influence of the concentration, time, and the interaction, for the increase of proteins obtained in the extraction. The increase in the response of this variable is clear from the center point (0.0; 0.0) to the highest level (+1.0; +1.0).

3.6. Solubility analysis

Tables A9 and A10 present a summary of the analysis of variance for the experimental data and the adjustment coefficients to the quadratic model for solubility at a significance level of 5%, respectively. The concentration, time, and interaction of concentration and time were significant for the adjustment of the model to be excellent: 99.943%. Figure B5 (supplementary material) shows the relationship between the experimental values and the values predicted by the adjusted model.

Figure 6 shows that intermediate concentrations (0.0) of acetic acid and intermediate time (0.0) contribute to the decrease in the response of the solubility variable.

Based on the responses obtained, the aim was to determine the ideal concentration of acetic acid and the ideal time, especially considering that, for the collagen

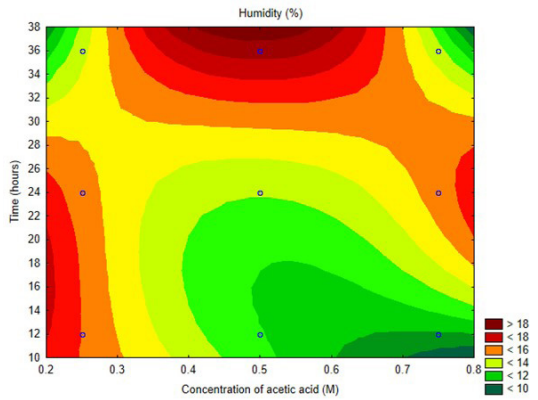

Figure 3. Outlining graph for the humidity variable.

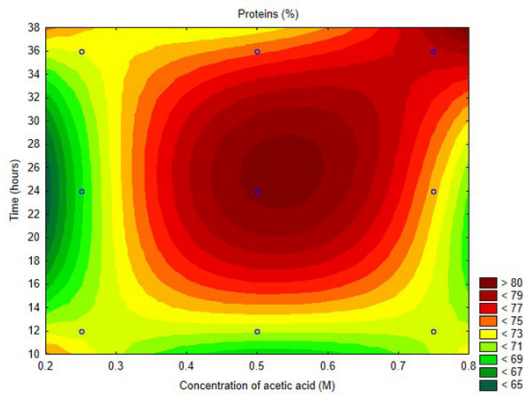

Figure 5. Outlining graph for the protein variable.

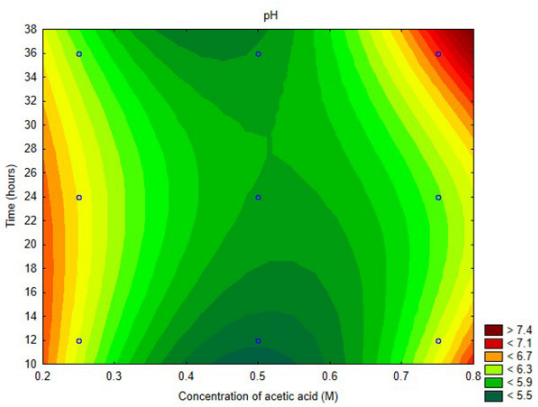

Figure 4. Outlining graph for the pH variable.

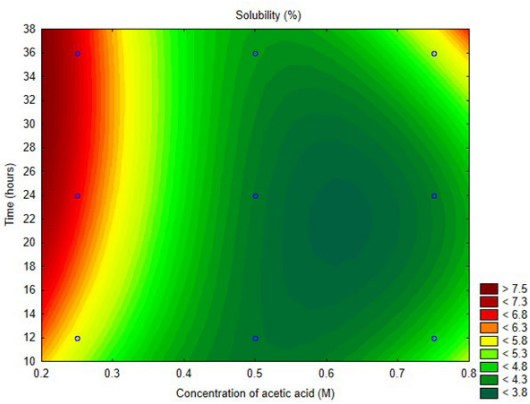

Figure 6. Outlining graph for the solubility variable.

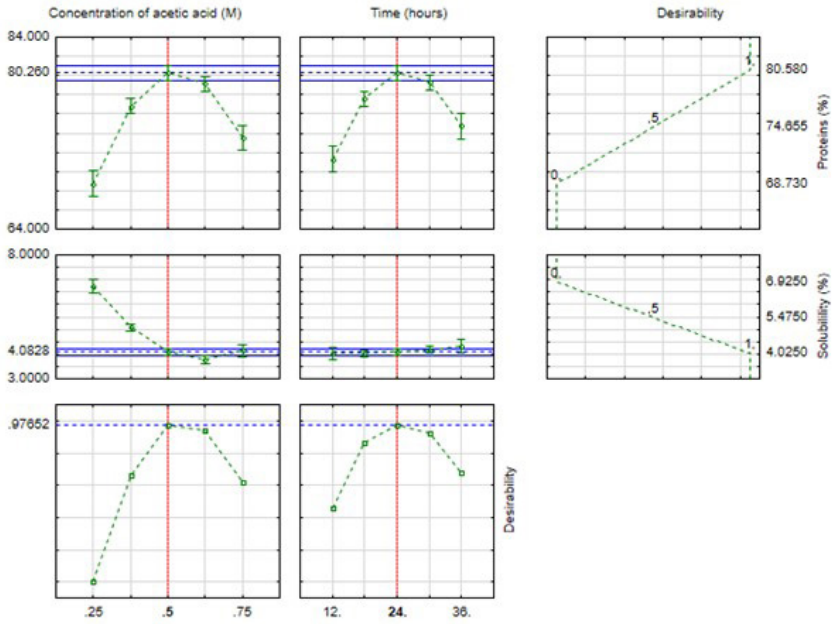

Figure 7. Graph of the influence of the concentration of acetic acid (M) and time (hours) to obtain protein and solubility.

extraction process, high values of proteins associated with low solubility values are ideal.

Figure 7 shows that optimal values for solubility and proteins are obtained when the central values of the previously proposed design are chosen, that is, a concentration of 0.5 M acetic acid and a time of 24 hours. Thus, sample A5, as well as samples A10 and A11, replicates at the central point, showed the best values (97.652%) for the determination of the collagen extracted from tilapia skin.

4. Conclusion

The results of protein determination were consistent with the literature. They indicated a direct influence of the concentration and time variables and an interaction between them, promoting an increase in proteins obtained in the extraction. The low solubility values found in the experiment and the high levels of protein demonstrate a high chance that the extracted protein is collagen since it is an insoluble protein.

The results of ash adjust to those discussed in the literature and vary in quantity according to the increase in concentration and decrease in time. The humidity results were superior to the theoretical values, obtaining higher values at intermediate concentration levels, and a longer time. The pH showed a direct influence of the concentration and time variables since higher pH values were found for a higher concentration and longer time.

In conclusion, the results obtained in the extraction of collagen from tilapia skin employing an acid protocol were satisfactory, considering that the values found are not discrepant concerning those presented in the literature. However, the values were discrepant compared with the collagen obtained in bovine and chicken. Furthermore, the condition that most favored the extraction of collagen was 0.5M concentration and 24 hours with desirability of 97.652%.

References

ASSOCIAÇÃO BRASILEIRA DA PISCICULTURA – PEIXE BR. (2020). *Anuário 2020: Peixe BR da Piscicultura*. São Paulo: PEIXE BR, pp. 136.

BASSO, T.R., URNAU, R.M., BRANDALIZE, C. and SIMÕES, M.R., 2013. Extração e caracterização de colágeno obtido de peles do processamento de Tilápia. In *Anais do III Encontro Paranaense de Engenharia e Ciência*, 28-30 de Outubro de 2013, Toledo, Paraná. Cascavel: UNIOESTE.

BATISTA, T.M., MARTINS, V.C.A. and DE GUZZI PLEPIS, A.M., 2009. Thermal behavior of in vitro mineralized anionic collagen matrices. *Journal of Thermal Analysis and Calorimetry*, vol. 95, no. 3, pp. 945-949. http://dx.doi.org/10.1007/s10973-007-8897-7.

CALDATO, K., NAVES, F.K. S. and ZATTA, L., 2019. Gelatina extraída de escamas da tilápia do Nilo (Oreochromis niloticus) produzidas no município de Pato Branco: caracterizações e comparações com amostras comerciais. *Revista Brasileira de Tecnologia Agroindustrial*, vol. 13, no. 1, pp. 2730-2751. https://doi.org/10.3895/rbta.v13n1.8386.

CHALAMAIAH, M., RAO, G.N., RAO, D.G. and JYOTHIRMAYI, T., 2010. Protein hydrolysates from meriga (Cirrhinus mrigala) egg and evaluation of their functional properties. *Food Chemistry*, vol. 120, no. 3, pp. 652-657. http://dx.doi.org/10.1016/j.foodchem.2009.10.057.

DAMODARAN, S., PARKIN, K.L. and FENNEMA, O. R., 2010. *Química de alimentos de Fennema*. Porto Alegre: Artmed.

DOTTO, J., MATEMU, A.O. and NDAKIDEMI, P.A., 2019. Nutrient composition and selected physicochemical properties of

fifteen Mchare cooking bananas: a study conducted in northern Tanzania. *Scientific African*, vol. 6, pp. e00150. https://doi.org/10.1016/j.sciaf.2019.e00150.

FENNEMA, O. R., 2000. *Química de los alimentos*. España: Editorial Acribia, S.A.

HUANG, C.Y., KUO, J.M., WU, S.J. and TSAI, H.T., 2016. Isolation and characterization of fish scale collagen from tilapia (Oreochromis sp.) by a novel extrusion-hydro-extraction process. *Food Chemistry*, vol. 190, pp. 997-1006. http://dx.doi.org/10.1016/j.foodchem.2015.06.066. PMid:26213067.

INSTITUTO ADOLFO LUTZ – IAL, 2008. *Métodos físico-químicos para análise de alimentos*. São Paulo: IAL.

LEE, C.H., SINGLA, A. and LEE, Y., 2001. Biomedical applications of collagen. *International Journal of Pharmaceutics*, vol. 221, no. 1-2, pp. 1-22. http://dx.doi.org/10.1016/S0378-5173(01)00691-3. PMid:11397563.

LU, Y.X. and CHEN, M.Z., 2004. Immunotherapeutic effects of soluble chicken collagen type II on collagen type II induced arthritis in rats. *Zhongguo Yaolixue Yu Dulixue Zazhi*

MARQUES BUENO, C., DUTRA ALVIM, I., RIBEIRO DIAS KOBERSTEIN, T.C., PORTELLA, M.C. and GROSSO, C., 2011. Produção de gelatina de pele de tilápia e sua utilização para obtenção de micropartículas contendo óleo de salmão. *Brazilian Journal of Food Technology*, vol. 14, no. 1, pp. 65-73. http://dx.doi.org/10.4260/BJFT2011140100009.

MONTERREY-QUINTERO, E.S. and SOBRAL, P.J.A., 2000. Preparo e caracterização de proteínas miofibrilares de tilápia-do-nilo para elaboração de biofilmes. *Pesquisa Agropecuária Brasileira*. https://doi.org/10.1590/s0100-204x2000000100020.

NOVAES, C.G., YAMAKI, R.T., DE PAULA, V.F., DO NASCIMENTO JÚNIOR, B.B., BARRETO, J.A., VALASQUES, G.S. and BEZERRA, M.A., 2017. Otimização de métodos analíticos usando metodologia de superfícies de resposta - Parte I: variáveis de processo. *Revista Virtual de Quimica*, vol. 9, no. 3, pp. 1184-1215. https://doi.org/10.21577/1984-6835.20170070.

OCKERMAN, H.W. and HANSEN, C.L., 1999. *Animal by-product processing & utilization*. Boca Raton: CRC Press. http://dx.doi.org/10.1201/9781482293920.

OLIVEIRA, V.M., ASSIS, C.R.D., HERCULANO, P.N., CAVALCANTI, M.T.H., BEZERRA, R.S. and PORTO, A.L.F., 2017. Collagenase from smooth weakfish: extraction, partial purification, characterization and collagen specificity test for industrial application. *Boletim do Instituto de Pesca*, vol. 43, no. 1, pp. 52-64. http://dx.doi.org/10.20950/1678-2305.2017v43n1p52.

OLIVO, R., SCARES, A.L., IDA, E.I. and SHIMOKOMAKI, M., 2001. Dietary vitamin E inhibits poultry PSE and improves meat functional properties. *Journal of Food Biochemistry*, vol. 25, no. 4, pp. 271-283. http://dx.doi.org/10.1111/j.1745-4514.2001.tb00740.x.

PEÑA-SALDARRIAGA, L., FERNÁNDEZ-LÓPEZ, J. and PÉREZ-ÁLVAREZ, J. (2020). Quality of Chicken Fat by-Products: Lipid Profile and Colour Properties. *Foods*, vol. 9. pp. 1046-1047. doi: 10.3390/foods9081046.

PRESTES, R.C., GOLUNSKI, S.M., TONIAZZO, G., KEMPKA, A.P. and LUCCIO, M., 2013. Caracterização da fibra de colágeno, gelatina e colágeno hidrolisado. *Revista Brasileira de Produtos Agroindustriais*, vol. 15, no. 4, pp. 375-382. http://dx.doi.org/10.15871/1517-8595/rbpa.v15n4p375-382.

REBOUÇAS, L.O.S., FIGUEIREDO, A.C.N., SANTOS, J.J., ASSIS, A.P.P., CAMÊLO, M.C.S., SILVA, J.B.A. and LIMA, P.O., 2017. Qualidade física e sensorial da tilápia. *Boletim de Indústria Animal*, vol. 74, pp. 116-121.

SAMOUILLAN, V., DELAUNAY, F., DANDURAND, J., MERBAHI, N., GARDOU, J.-P., YOUSFI, M., GANDAGLIA, A., SPINA, M. and LACABANNE, C., 2011. The use of thermal techniques for the characterization and selection of natural biomaterials. *Journal of Functional Biomaterials*, vol. 2, no. 3, pp. 230-248. http://dx.doi.org/10.3390/jfb2030230. PMid:24956305.

SAPUTRI, G. R., TUTIK, & PERMATASARI, A. I. (2019). Determination of protein levels in young and old leaves (moringaoleifera l.) Leaves using the kjeldahl method. *Jurnal Analis Farmasi*, vol. 4, no. 2, pp. 108-116.

SILVA, T.F. and PENNA, A.L.B., 2012. Colágeno: características químicas e propriedades funcionais. *Revista do Instituto Adolfo Lutz*

TAVARES ALVES, S.G. and PRUDENCIO-FERREIRA, S.H., 2002. Propriedades funcionais de material colagenoso de pés de frango. *Archivos Latinoamericanos de Nutricion*

VERA CANDIOTI, L., DE ZAN, M.M., CÁMARA, M.S. and GOICOECHEA, H.C., 2014. Experimental design and multiple response optimization. Using the desirability function in analytical methods development. *Talanta*, vol. 124, pp. 123-138. https://doi.org/10.1016/j.talanta.2014.01.034.

YAN, M., QIN, S. and LI, J., 2015. Study on the self-assembly property of type I collagen prepared from tilapia (Oreochromis niloticus) skin by different extraction methods. *International Journal of Food Science & Technology*, vol. 50, no. 9, pp. 2088-2096. http://dx.doi.org/10.1111/ijfs.12870.

ZENG, J.L., CAO, Z., YANG, D.W., XU, F., SUN, L.X., ZHANG, L. and ZHANG, X.F., 2009. Phase diagram of palmitic acid-tetradecanol mixtures obtained by DSC experiments. *Journal of Thermal Analysis and Calorimetry*, vol. 95, no. 2, pp. 501-505. http://dx.doi.org/10.1007/s10973-008-9274-x.

Supplementary Material

Supplementary material accompanies this paper.

Table A1. Anova for the ash response model
Table A2. Coefficients of the regression model for ash in the extraction
Table A3. Anova for the humidity response model
Table A4. Coefficients of the regression model for humidity in the extraction
Table A5. Anova for the pH response model
Table A6. Coefficients of the regression model for pH in the extraction
Table A7. Anova for the protein response model
Table A8. Coefficients of the regression model for proteins in the extraction
Table A9. Anova for the solubility response model
Table A10. Coefficients of the regression model for solubility in the extraction
Figure B1. Experimental values vs. predicted values for ash
Figure B2. Experimental values vs. predicted values for humidity
Figure B3. Experimental values vs. predicted values for pH
Figure B4. Experimental values vs. predicted values for proteins
Figure B5. Experimental values vs. predicted values for solubility

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