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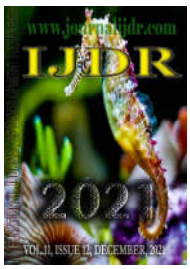

RESEARCH ARTICLE OPEN ACCESS

COLLAGEN EXTRACTION OF TILAPIA SCALES (OREOCHROMIS NILOTICUS) FOR DEVELOPMENT OF NUTRICAMISTIC AND DERMATOLOGICAL PRODUCT

Estéfane Rodrigues[1], Vanessa Caroline Cardoso Silva[1], Brunna Évellyn Dias Ribeiro[1], Maria Tereza Carneiro Paschoal Bernardes[1], Gérsika Bitencourt Santos Barros[1] and Diogo Gontijo Borges[2]

[1]Department of Pharmacy, José do Rosário Vellano University (Unifenas) - Alfenas, Minas Gerais
[2]Químico Industrial e Doutor em Engenharia Química pela Universidade Federal de São Carlos

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Corresponding author:
Gérsika Bitencourt Santos Barros

ABSTRACT

The development of products obtained from tilapia scales, besides being innovative for the pharmaceutical industry, can result in numerous benefits for the environment, since they are low cost and are generally discarded in dumps and landfills, and that can generate environmental impacts. The present work aimed to develop a dermatological and nutricosmetic product from tilapia scales, as well as bibliographic survey of its benefits for human health. This research has an experimental and investigative character, which includes the extraction of gelatin from tilapia scales, which have been undergone to several acid and basic baths, heating and drying. Subsequently, two products were developed, one for dermatological and the other nutricosmetic use, called with the fancy name of revitalizaderm and gummy BEV, respectively. After obtaining the products, pharmacotechnical and microbiological tests were performed. The microorganisms investigated were *Escherichia coli,Salmonella*and *Staphylococcus aureus,* presenting negative results for the culture. The physical-chemical tests performed in the samples were: organoleptic characteristics, pH, spalmability and relative density. It should be noted that the developed products were not used *in vivo,* so their possibility of effectiveness is based on literary data. Then, further studies will be carried out to evaluate their activity.

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INTRODUCTION

Brazil is one of the countries that has one of its main activities the fishing industry, generating a high rate of waste, and in some cases can reach more than 50% of the initial weight of fish (MOREIRA, 2003). The fish is highlighted in the present research, because in addition to being used in food, it presents numerous important components such as proteins, iodo, phosphorus, calcium, vitamins A, E, B and D complex, besides omega 3, among others, resulting in products of high commercial value in the cosmetics industry (BOSCOLO *et al.,* 2009). Among these residues, the following stand out: viscera, tail, spine, fin, meat remains and mainly scales. (FELTES *et al.,* 2010). In order to help reduce these environmental impacts, it is important to apply innovative and emerging technologies to the use of these wastes for the development of products which seek an improvement in human health (GONÇALVES, *et al*., 2011). Gelatin is one of several products that can be developed through these fish residues. Its procurement process takes place in different stages, which guarantees its quality.

Among the organic residues obtained through fish, the skin and the flaking are important sources for the production of gelatine. Besides having a high nutritional quality, these residues are also rich in collagen (VIDOTTI and GONÇALVES, 2006). Collagen is a protein constituted of connective tissue and it is the most abundant in the heterotrophs organisms, having as its main function to maintain the resistance of the tissue, its structure and also its flexibility. It is widely used in the pharmaceutical, cosmetic and biomedical industries (ACOSTA, VELARDE, VEGA; 2015). Burns can cause great damage to the individual, such as physiological changes on the skin, healing difficulty, due to the fact that it is an organ of the human body, the skin loses its abilities, facilitating the entry of microorganisms causing an infection, in addition to the total destruction of the sweat and sebaceous glands (COSTA *et al.,*2017).
The type I collagen of tilapia skin stimulates factors of fibroblasts. These fibroblasts express and release the factor of keratinocytes, both are important cytokines and indispensable for wound closure (TANG; SAITO, 2015). The production of gelatin through marine substances gain prominence due to the lack of disease that can be found in

gelatins obtained from bovine and swine residues. This makes fish scales a more accessible and favorable substance, thus being produced on a large scale (KARIM & BHAT, 2009). After oral administration, hydrolyzed collagen is digested and absorbed into the digestive tract, recognized by its constituent peptides in the blood and reaches the skin in four days. Because it is similar to collagen, especially type I collagen in the dermis, its function is not only to replace, but also to promote the synthesis of type I collagen, which is effective in aging and even in other skin diseases, such as tissue repair (ZAGUE, *et al.,*2011).

MATERIAL AND METHODS

The present work is experimental and investigative. Initially, gelatin was developed from the tilapia scales. The process took place in the pharmaceutical handling laboratories of the pharmacy course of the University José do RosárioVellano, following all safety and ethics standards. Subsequently, two products were elaborated: one for dermatological and the other nutricosmectic use, and only *in vitro* tests were performed.

Scale collection: Tilapia scales were provided by a producer in the municipality of Carmo do Rio Claro, located in the south of the state of Minas Gerais, Brazil, in 2020 and 2021. Once extracted, the scales were frozen and then processed in the laboratory, where they were washed in running water to remove excess residues, sifted and, after, the gelatin was extracted.

Gelatin extraction process: It was adapted to what was reported in the literature (CALDATO *et al.,* 2019). After thawed, the scales were washed and dried in an oven at 40 °C for 48 hours with a variation of +/- 2. Then, for the demineralization process, a mass of 100g was separated and mixed to a concentrated solution of 10% (m/v) of sodium chloride (NaCl, 99%), in the ratio of 1:10 (m/v) for 24 hours at room temperature. Then, the scales were washed with distilled water, sieved and mixed, in the proportion 1:10 (m/v), to a solution of hydrochloric acid (HCl, Reatec, 37%) of concentration 0.4 mol L-1 for 24 hours. The resulting material was washed with distilled water, dried in an oven at 40 °C, with a variation of +/- 2, for 72 hours and crushed in a Wiley TE-650 mill. For acid and alkaline hydrolysis processes, the demineralized and dried scales were mixed with a solution of acetic acid (CH_3COOH, 99.7%) with a concentration of 0.1 mol L-1 in the proportion 1:10 (m/v) for 1 hour at room temperature. Then, the resulting material was mixed with sodium hydroxide solution (NaOH, 99%) of concentration 0.1 mol L-1 in the proportion of 1:3 (m/v) for 1 hour, followed by treatment with 0.1 mol L-1 solution of sulfuric acid (H_2SO_4, 98%) in the proportion of 1:3 (m/v) for 1 hour. For each solution change step, the solid material was filtered and washed with distilled water. The extraction of hydrolyzed collagen from demineralized and hydrolyzed scales was caused by immersion of the material in distilled water, in the proportion of 1:4 (m/v) for 2 hours under agitation and heating at 60 °C. The solid residue (scales) was separated and stored for further research and the obtained solution (hydrolyzed collagen) was stored in a refrigerator for 24 hours and then frozen.

Yield: It was calculated according to Martins *et al.*(2015), which calculated the percentage of gelatin obtained after the extraction process in relation to the initial mass of dry scales.

$$\frac{R\ (\%) = mgelatin}{m\text{scales}} \times 100$$

R (%) is the extraction yield; m gelatin is the mass of gelatin obtained at the end of the extraction process and m scales is the mass of dry scales at the beginning of the process.

Gel manipulation process: Several tests were performed from the gelatin obtained previously, until reaching the ideal pharmacotechnical aspects. For this process, the following were used:

- Gelatin obtained from the tilapia scales, which was used at the concentration of 7%;
- Glycerin, at a concentration of 15%; glycerin has emollient properties, hydration that aids in the absorption of the skin promoting hydration;
- Propylene glycol, at a concentration of 3%; presents as a wetting purpose used for moisturizing, protecting the skin;
- Aristoflex in the concentration of 2%, which allowed the removal of the gel from the refrigerator, since it has the purpose of gelling the formulation and generating a uniform gel, allowing the formation of gels, and synthetic polymers;
- Methylparaben, at a concentration of 0.15%; it is a preservative used in food and topical formulations;
- Distilled water, sufficient quantity for 100%, which is the vehicle of the formulation;

Which underwent to a controlled heating until the gel was forming.

Edible gum handling process: Several tests were performed from the gelatin obtained previously, until reaching the ideal pharmacotechnical aspects. For this process, the following were used:

- Gelatin obtained from tilapia scales, at a concentration of 15%;
- Animal gelatin at the concentration of 25%, which allowed the removal of the gum from the refrigerator and left the formation firmer and gelled, responsible for maintaining the structure of the gum leaving it firm and elastic;
- Glycerin, at a concentration of 10%, which left the gum with a softer and sweeter appearance;
- Aspartame, at a concentration of 0.2%; it has characteristics of an artificial sweetener and sweetener, has the purpose of sweetening the foods replacing sugar;
- Sodium benzoate at a concentration of 0.15%; its purpose is to be a preservative and antifungal;
- Flavoring of gooseberry, at the concentration of 4%; offers organoleptic features pleasing to gum where it makes it palatable and with a fruit odor;
- -Citric acid at the concentration of 3%; it is a natural preservative, which offers a slightly acidic, refreshing and fruity taste;
- Distilled water, sufficient quantity for 100%;

Physical-chemical tests: PH tests were performed in triplicate, and then the average of the tests was performed, relative density which was performed on the metal pycnometer and then the following calculation was performed:

$$\text{Relative density} = \frac{\text{PA - PS g/ml}}{\text{PW – PS}}$$

Being, PS the weight of the pycnometer clean and dry; PW, the weight of the pycnometer containing purified water at 25 °C; weight of the pycnometer with the sample at 25°C.

The spread ability was also observed in triplicate and then the average was performed among them. And the appearance, color and odor were evaluated. All these tests were used for gel evaluation. To perform the organoleptic tests on the gum, appearance, color, flavor, texture and odor and pH in reactive tape were evaluated.

Microbiological test: Gel, gum and gelatin samples were analyzed in relation to the identification of pathogens such as *Escherichia coli, Staphylococcus aureus* and *Salmonella.* The preparation mode for *Escherichia coli* consisted on information provided by the Brazilian Pharmacopeia, being weighed 1 gram of the sample and diluted in 1:10, using 10 ml of dilution for 90 ml of enriched broth, incubated and plated for microorganism count.

The test for *Staphylococcus aureus* followed the parameters mentioned in the Brazilian Pharmacopeia, and the dilution prepared 1:10 and 1 gram of the product was examined and all samples were incubated and plated. The preparation of the sample for *salmonella*

identification consisted on the dilution 1:10 in which 10 grams were diluted in 90 ml. With the presence of turbidity, it was necessary to subculture and transfer 0.1 ml of the content to 10 ml of Salmonella Rappaport Vassiliadi enriched broth. Then, the dilutions were prepared and the samples were plated.

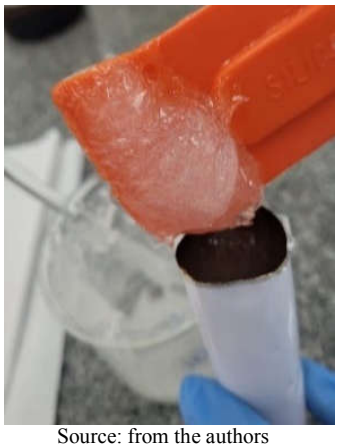
RESULTS

The solution (hydrolyzed collagen) obtained a yield of 364.1 grams for 100 grams of dry scales. Being a transparent gelatin, without lumps, odor, consistently, with pH 6.5.

Source: from the authors

Figure 1. Gelatin esand developed from tilapia scales

The transparent color gel, free of lumps and/or particles, without odor, presented pH of 6.37, relative density of 1.019 and spalmability of 29.66 mm x 31.33 mm.



Source: from the authors

Figure 2. Gel developed for dermatological use

The gum is reddish in color, with sweet and citrus flavor, consistent and gooseberry odor with pH5.

Source: from the authors

Figure 3. Nutricosmetic gums

For the microbiological tests, a negative result was obtained for the three pathogens surveyed. *Escherichia coli* clouded into MacConkey broth with a negative result in MacConkey agar when compared to the control.

Source: from the authors

Figure 4. Sample platingin Negative **MacConkey**

The sample directed to *Salmonella* clouded the broth Rappaport and had growth in THE XLD. After this, the confirmive test for Gram or blade agglutination test was performed according to Figure 6, which obtained a negative *result for Salmonella* when compared to the control.

Source: from the authors

Figure 5. Platingthe sample to identify the possible presence of Salmonella

Source: from the authors

Figure 6. Blade agglutination test

Staphylococcus aureus had turbidity in casein-soybean broth (TSA). Thus, a subculture was carried out, where a raised was transferred to plate containing agar mannitol salt, obtaining growth according to figure 7. Then, confirmed tests were performed for GRAM and obtained positive catalase that can be identified in Figure 8 and negative coagulase in Figure 9. Under the microscope, the presence of GRAM-positive rods was observed, which proves that there is no presence of Salmonella *in* the samples.

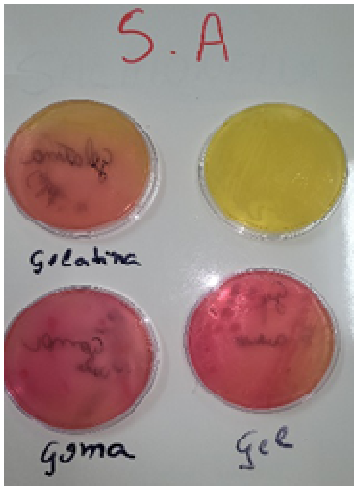


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Figure 7. Platingthe sample to identify possible presence of dand *Staphylococus aureus*

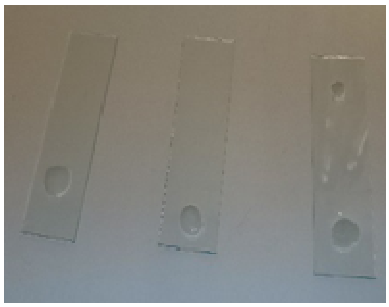

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Figure 8. Positive Catalase

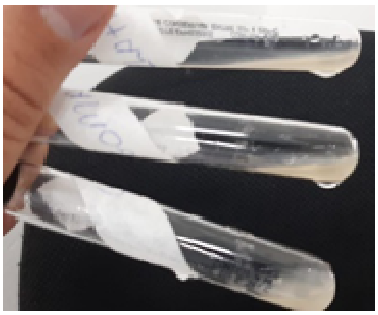

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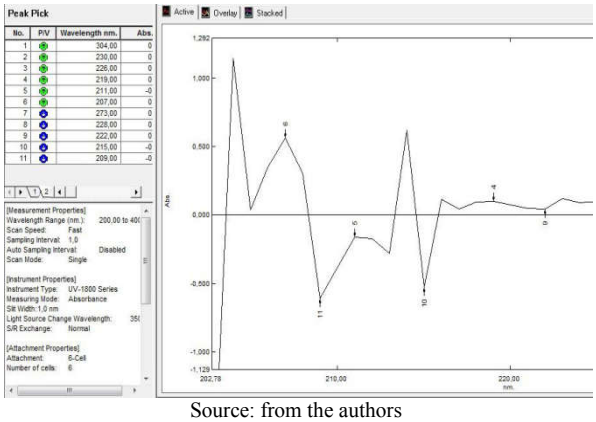
Figure 9. Coagulase negative

DISCUSSION

Gums have high technological potential and contain hydrolyzed collagen, in which the smaller size of collagen molecules improves their absorption, which begins in the stomach. In it there are some pepsin enzymes that initiate the breakdown of collagen protein into polypeptides. In the small intestine, pancreatic enzymes break polypeptides into tri (three amino acids) and dipeptides (two amino acids). And finally, in the intestinal lumen, there is the last stage of digestion, more specifically in enterocyte microvilli, where peptidases break the triand dipeptides into amino acids, completing collagen absorption (GUYTON, 2006). Rona and Berardesca (2008) say that the treatment of the skin where the problem is installed is based on the synergistic effect of functional substances applied locally, associated with other agents with internal action that restore and or correct functions. Therefore, the importance of the association of both products developed. The results obtained in spectroscopy with 1% DMSO, shown in Figure 10, corroborate the results of Li, Liu, Gao and Chen (2004). It is known that positive peak around 223 nm and a negative peak close to 204 nm is characteristic of triple helix collagen (PIEZ, 1984). The positive peak obtained closer to 223 nm was 226 nm and the negative closest to 204 nm, the 209 nm. The other peaks are unknown, since collagen was not isolated for the respective analysis.



Source: from the authors

Figure 10. Spectroscopy

According to Pelczar Jr (1997), microbiological tests in food are very common, because they offer excellent growth conditions for microorganisms. Among the microbiological tests, the identification of *Escherichia coli* stands out, as it is one of the best identifiers for fecal coliforms, according to silva's literature (1996), assuring the consumer at all stages, both of preparation and handling of the products mentioned above. The method of *identification of Staphylococcus aureus* is common and when it presents a positive result shows failures in the associated hygiene in the development of the product, processing of samples, which can cause a food surge in the intake of positive samples. Based on the studies, we can affirm that the intoxication of this microorganism is caused by enterotoxin intake (HENNEKINNE *et al.,* 2012). It is known that *Salmonella* is an intestinal bacterium that can cause severe food poisoning and is considered one of the main pathogens of outbreaks recorded throughout the country. This pathogen is related to fish from its handling, hygiene and especially in contaminated waters. Therefore, the importance of not having its presence in the products (FERNANDES *et al.,* 2018).

FINAL CONSIDERATIONS

For the development of this work, gelatin, gum and gel from tilapia scales were elaborated, with gel for dermatological use and gum for nutricosmetic use. These are called Revitaliza Derm and Gummy BEV gel, respectively. The performance of physical-chemical and microbiological tests showed that the products are suitable to follow the *tests in vivo.* It should be noted that Revitaliza Derm and Gummy BEV were not used in humans, so their possibility of efficiency is based on literary data. Further studies will be applied to evaluate their activity.

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