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Research Article

Application of Hemicellulose in Restructured Tilapia Meat Product

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Abstract

Xyloglucan is a polysaccharide found in species such as H. courbaril var. courbaril, known as Jatobá, and has been used for different functions in food, proving to be an excellent food additive of natural origin. On the other hand, products such as nuggets can be processed from mechanically separated meat, and these require additives that act as emulsifiers. Therefore, in this study, xyloglucan from H. courbaril was applied to nuggets made from mechanically separated tilapia meat. Physico-chemical analyses such as pH, moisture, protein, as well as color parameters such as luminosity (L*), chromaticity (a* and b*), intensity (C*), hue angle (H*) and cooking parameters such as yield, retention and shrinkage capacity and texture were carried out with cut and hardness analysis. The results confirm that the prepared product has the right pH, low humidity and high protein content. The color characteristics emphasize that the addition of xyloglucan favored a brightness, saturation and intensity of yellowing, with favorable yield, retention capacity and shrinkage, where the force exerted for cutting is lower, presenting a soft texture during storage. The conclusion is that the application of xyloglucan as a natural ingredient to replace commercial starch represents a favorable alternative, with added value and quality to the product, giving the possibility of using and industrializing this polysaccharide.

Keywords: Emulsifier; Fish; Xyloglucan; Polysaccharide; Nuggets

Introduction

In recent times, studies on various polymeric materials of plant origin, such as polysaccharides, have attracted the attention of researchers because they are natural and renewable sources.

Xyloglucan is one of these polysaccharides that has a structural and energy storage function in seeds, such as that of the species H. courbaril, known as Jatobá, which belongs to the Caesalpiniaceae family and is abundant throughout the Brazilian forest, and in the food industry, it has been applied as a thickener, stabilizer, antimicrobial agent, crystallization inhibitor in foods such as juices, ice creams and desserts [1], (Kumar; Bhattacharya, 2008), but there are no records of applications in products based on mechanically separated fish meat.

Mechanically separated fish meat can be used as a raw material for nuggets in order to add value, but there are few studies in the literature on fish-based products and, despite knowledge of the nutritional properties of this fish, there is not enough information on the potential for using its meat, such as that of tilapia fish (Oreochromis niloticus) to produce these food formulations.

In this study, the xyloglucan from H. courbaril was applied to nuggets made from mechanically separated tilapia meat in order to assess its influence on the physicochemical, texture and cooking characteristics of the products produced.

Methodology

Extraction of xyloglucan from the seeds of Hymenaea courbaril var.

The plant material was collected in the municipality of Carnaubal, located in the Serra da Ibiapaba, in the state of Ceará. The polysaccharide was extracted according to the methodology [2] described by Farias., *et al*. (2018). The seeds were removed from the pods by hand and boiled in distilled water at 100°C for 30 minutes, and when the shells were slightly loose they were removed with a knife. The seeds were then dried at 60°C until constant weight and ground with 0.1 M NaCl [15% (w/v)] at 25°C. The impure extract obtained was centrifuged for 20 min at 1,500 rpm. The supernatant was filtered through a Voil cloth, followed by a new filtration using the screen printing cloth (90 threads). Ethanol was then added at 46% [1:3 (v/v)] and left in contact with the supernatant for 16 hours. The precipitate was filtered through a screen-printing cloth (110 threads), washed with 100% ethanol [1:3 (m/v)] for 30 minutes and twice with acetone PA [1:3 (m/v)] for 30 min, then filtered through a screen-printing cloth (110 threads) between each wash and dried at 60°C to constant weight. The precipitate (purified polysaccharide) was pulverized and stored in amber glass vials.

Application of xyloglucan in mechanically separated tilapia meat nuggets

Mechanically separated meat (MSM) from fresh tilapia (Oreochromis niloticus) was obtained from a fish processing industry. Two formulations of Pepitas: Nc (control - no xyloglucan added); Nx (xyloglucan added), with three batches of each formulation. The CMS (83%) was homogenized in a slicer with the ingredients of [3] Veit et. al. (2011). The ingredients include ice water (4.0%), corn starch (2.5%), textured soy protein (2.0%), soy protein isolate (2.0%), salt (1.5%), dehydrated onion (1.5%), soybean oil (1.2%), dehydrated garlic (0.8%), dehydrated spring onions (0.6%), dehydrated parsley (0.6%), monosodium glutamate (0.2%), white pepper (0.1%) weighed on a semi-analytical balance and mixed until a homogeneous mass was obtained. Xyloglucan was weighed out in a proportion of 2.5% to replace cornstarch in the Nx formulation. Before molding, the dough was kept refrigerated for three hours (at 4°C) so that the breading acquired the desired consistency. The breading operation comprised three stages. In the first phase, the portion was subjected to a soaking called Pre-dusting, which consisted of wrapping the portions with a thin layer of crushed and sieved cassava flour, before applying the batter (second phase), which is a mixture of wheat flour, starch, powdered milk, water and salt, forming a viscous liquid in order to evenly cover the portions and promote the adhesion of the covering flour. Once the product was covered with the batter, the third stage was carried out, which is the baking stage, by dusting the portions with flour.

The formulations were evaluated by studying their storage under freezing conditions (-18°C) at 0, 30 and 60 days. They were thawed according to their time and baked in an oven for 15 minutes to carry out the analyses.

Physico-chemical characteristics

As described in the methodologies in the following topics. Instituto Adolfo Lutz (2008).

pH

5g of each crude sample was weighed, 50ml of distilled water was added and the contents were shaken for 30 minutes in an automatic shaker and left to stand for 10 minutes. The pH was determined using a digital pH meter.

Moisture

Moisture content was determined gravimetrically by drying in an oven at 105°C until a constant weight was obtained, using an analytical balance and crucibles.

Protein

Protein was quantified using the Kjeldahl method [4] (Association of Official Analytical Chemists - AOAC). For the determination of ammoniacal nitrogen, digestion, distillation and titration were used. A specific conversion factor of 6.25 was used in the calculation.

Color characteristics

The surface color of the raw Pepita samples was measured using the Cie L*, a*, b*, C* and H* systems obtained by a previously calibrated portable colorimeter (Colo-rium, Delta color, Brazil). In the color system used in this analysis, L* represents luminosity (L* = 0 black and L* = 100 white) and a* and b* are the color coordinates responsible for chromaticity: (+a* is red and - a* is green, +b* is yellow and -b* is blue). C* refers to the intensity of the color which ranges from 0 to 100, and the hue angle (H*) is defined as hue and starts on the +a* axis and is expressed in degrees: 0° would be +a* (red), 90° would be +b* (yellow), 180° would be -a* (green) and 270° would be -b* (blue).

Cooking characteristics

Cooking performance

After thawing the samples, the Pepitas were cooked in an electric oven for about 15 minutes, turning every 5 minutes, until they reached an internal temperature of 74ºC. Pepitas were calculated by the difference between the weight of the raw and cooked [5,6] samples Berry (1992) and Seabra., *et al*. (2002), as shown in Equation 1.

% yield = (weight of cooked sample) (weight of raw sample) x 100 Eq. 1

Water retention capacity (WRC)

To obtain the result of the water retention capacity of the samples, it was necessary to obtain the moisture content of the samples. The percentage water retention capacity (WRC) (6) will be calculated according to Seabra., *et al*. (2002), as shown in Equation 2.

%WRC= 1 - A - D x 100 U (Eq. 2)

Where, A = sample weight (g) after heating; D = sample weight (g) after heating and centrifugation; U = sample humidity (%).

Shrinkage percentage (shrinkage)

The percentage of shrinkage will be calculated (5.6) according to Berry (1992) and Seabra., *et al*. (2002), using Equation 3:

% shrinkage = (diameter of raw sample-diameter of cooked sample) x100

Diameter of raw sample(Eq. 3)

Instrumental texture

The analysis was carried out according to the American Meat Science Association - AMSA and National Live Stock and Meat Board [7-9]. (1995), Scheeder., *et al*. (2001) and Jeong., *et al*. (2007) on TA-xT2i texture equipment (Stable Micro Systems, Surrey, UK). The samples were cut on standard Warner-Bratzler shear blades, 1.27 cm in diameter parallel to the muscle fibers, and were sampled using a manual cutter. The shear force will be measured on a TA-XT2i texture analyzer (Texture Technologies Cor-poration/Stable Micro Systems, Hamilton, UK), equipped with a 1 mm thick Warner-Bratzler blade. The equipment will be previously calibrated with a weight of 5 kg. The ascent/descent speed will be 200 mm/min and the distance to the platform will be 25 mm. Each cylinder will be cut only once and the result expressed in N.

Texture analysis

For this cutting analysis, a Warner Bratzler probe was used, with enough distance to cut the entire sample (35 mm) and a test speed of 1 mm/s, where the samples were divided, totaling ten repetitions.

For this hardness or firmness analysis, a cylindrical aluminum probe with a diameter of 36 mm, 50% compression of the sample, a test speed of 1 mm/s and 5 seconds between the two compressions [10] (Szczézniak, 2002) were used.

Statistical analysis

The data obtained was subjected to analysis of variance (ANOVA) to check for significant differences between the samples and the means were compared using the Tukey test at 5% significance, using the statistical program Estatística [11] (2004).

It is worth noting that the project initially envisaged sensory and microbiological analyses, but due to Ministry of Health guidelines in relation to the Covid-19 pandemic, these were not carried out.

Results and Discussions

Physico-chemical characteristics

Table 1 shows the average values (mean ± standard deviation) of the control (Nc) and xyloglucan (Nx) samples on days 0, 30 and 60 in relation to the values of the physicochemical parameters.

During storage, under freezing, the pH depends on the storage temperature, composition, physiological state, buffering power of the proteins and enzymatic action [12] (Gryschek., 2003). In the present study, the addition of this type of polysaccharide probably altered the pH of the samples during storage and caused significant changes in pH from day 30 and remained until the end of the study (day 60). According to RIISPOA [13] (2017), the pH of fish meat should be less than 7.00, considering fresh, chilled or frozen fish, however, as it is a by-product of fish, with the addition of other ingredients, there may be changes in pH, as in the case of nuggets.

Moisture is related to stability, quality and composition and can affect the storage, packaging and processing of the product [14] (Izidoro et. al, 2008). According to Table 1, the samples showed significant differences on all the study days, but there was an increase over the storage time. It is known that among the centesimal com-

Study days	0		30		60	
Analysis	**NC**	**NX**	**NC**	**NX**	**NC**	**NX**
ph	6.01a ± 0.05	6.06a ± 0.05	5.82a ± 0.05	5.69b ± 0.05	5.75a ± 0.05	5.64b ± 0.05
Moisture (%)	75.53a ± 0.56	74.46b ± 0.16	76.31a ± 0.03	75.12b ± 0.95	77.55 ^{a} ± 0.03	76.22b ± 0.05
Protein (%)	14.51b ± 0.09	17.23a ± 0.02	11.33b ± 0.11	13.96a ± 0.03	14.82b ± 0.05	11.94a ± 0.02

Table 1: Results (médias standard deviation) of the physico-chemical analysis of the nuggets.

*NC (Control Nugget with 2.5% preparation) and NX (Nuggets with xyloglucan with 2.5% preparation). The same letters on the same line indicate that there were no significant differences between the samples.

position of fish, moisture is the component that varies the most, with values ranging from 53 to 80% [15] (Kirschnik, 2007).

In the literature, some studies have shown the moisture content of different formulations, which were lower than the results of the present study, such as [16] Uchida et. al (2007) when making nuggets from tilapia filleting waste, and found a moisture value of 50.48% ± 1.1, unlike the present study, which showed values between 61.55% ± 0.05 and 77.53% ± 0.56 in the samples. Still on the moisture content results obtained during the analysis over the 60-day period, a decrease in values was observed in both the control sample (Nc) and the sample with added xyloglucan (Nx), but when comparing them on the different days, it can be seen that the Nx sample differs significantly from the control sample (Nc), This shows that the addition of xyloglucan may have interfered in the reduction of free water in the product in question, i.e. it suggests that this xyloglucan performed better for the moisture parameter than the starch added to the control sample (Nc).

Tilapia muscle contains from 10.75% to 17.74% high nutrition standard proteins [17-19] Bernadino Filho and Xavier, 2019; Vendas and Maia, 2012; Rebouças., *et al*. 2012), while tilapia CMS contains 15.9% high nutrition standard proteins [20] Fogaça., *et al*. 2015). Normative Instruction No. 6 (NI No. 06) of February 15, 2001 [21] (Brazil, 2001) establishes 10% as the minimum protein content for breaded food items. The protein content in fish is subject to certain fluctuations that depend on the biological state of the fish, just as the fat content can be influenced by factors such as age, biological period, type of feeding and nutritional status [3] Veit et. al, 2011). In similar studies, [15] Kirschnik (2007), when evaluating the stability of nuggets, obtained a result of 8.93% for protein, which is below that proposed by current legislation, noting that the author's samples in question came from mechanically separated meat and in which the washing process caused losses in their protein content [22] (2007), who produced nuggets from filleting waste, obtained a protein percentage of 16.04%. The high content represented in the present study with the use of xyloglucan can be explained by the presence of texturized soy protein added at 4% for both, and also the washing process does not take place, only the removal of thawing.

Color Analysis

The color of the meat is the first parameter to be evaluated by the consumer at the time of purchase, and it is from this that the quality of the meat in general is attributed [23] Campêlo., *et al*. 2017.

Table 2 shows the average values (mean ± standard deviation) in the color analysis of raw nuggets.

The color parameter *L refers to the luminosity and brightness of a sample, so it can be seen that on all days of the study the samples (Nc and Nx) showed significant differences, where at time 0, the control sample showed a statistically higher brightness, but this result changed with storage time, as on the 30th and 60th days of the study, the sample with xyloglucan (Nx) showed greater brightness compared to the control (Nc). This can be attributed to water retention (WRC), which was higher in Nx and sufficient to increase the gloss of this sample. In the work [24] by Biassi., *et al*. (2016) who developed a product called tilapia strips, higher L* values were obtained (53.8 ± 1.0), compared to the commercial sample (45.4 ± 0.8), which is different to what was obtained in this research, and this is due to the fact that they are different products with different raw materials and ingredients.

In the a* parameter, the samples showed a reddish tone at all times, but there were significant differences between the samples, where Nx showed slight redness at the start of the study (0 days), but on the remaining days of the study this tone increased, compared to the control sample (Nc).

Dias de estudo	0		30		60	
Parâmetros de cor						
	Nc	*Nx*	*Nc*	*Nx*	*Nc*	*Nx*
L*	48.63a ± 0.64	47.61b ± 0.35	40.99b ± 0.02	43.31a ± 0.04	36.33b ± 0.5	39.71a ± 0.41
um*	1.44a ± 0.46	0.88b ± 0.28	1.13b ± 0.0	1.62a ± 0.02	1.30b ± 0.13	1.97a ± 0.03
b*	6.04b ± 6.04	6.52a ± 0.12	5.95 b ± 0.01	7. 10a ± 0.03	5. 30b ± 0.07	7.43a ± 0.03
C*	5. 22b ± 0.94	6.54a ± 0.09	5.95 b ± 0.01	7. 10a ± 0.03	6.91b ± 0.46	8.01a ± 0.02
h*	81.23a ± 1.15	79.96b ± 2.63	79.2a ± 0.05	77.11b ± 0.12	75.41a ± 4.38	76.21a ± 0.77

Table 2: Results (mean standard deviation) of the color parameters of raw nuggets.

*NC (Control Nugget with 2.5% preparation) and NX (Nuggets with xyloglucan with 2.5% preparation). The same letters on the same line indicate that there were no significant differences between the samples.

As for the b* variable, the results in both samples (Nc and Nx) refer to yellowing, and in this study there were statistical differences ($p < 0.05$) between them, where the values were higher in the sample with the presence of xyloglucan (Nx) on all the study days. When compared with studies carried out on other types of products that use fish meat as a raw material, such as sausages which had an overall average b* value of 15.01 [25] (Oliveira, 2009) and fish burgers made from tilapia meat with an average B* value of 17.5 [26] (Bainy., *et al*. 2015), it can be seen that the control Pepitas or those with the addition of xyloglucan had lower values, and this can be explained by the fact that they are products with different ingredients.

Chroma (C*) indicates the saturation of the colors that were determined in the previous parameters (a* and b*), verifying that statistically the two samples are different and showed a low saturation, already expected for fish-based products [27] (Farias., *et al*. 2018), and that Nx showed greater saturation and intensity on all study days, compared to the control (Nc), that is, the addition of xyloglucan altered this parameter.

On the other hand, the hue angle (h*) proves the tendency of the samples to yellow, as this is predicted in fish products due to their coloring, and showed significant differences up to the 30th day, when the averages for both formulations decreased at all times. Changes in color can occur as a result of lipid oxidation, and are difficult to control over time due to the complexity and variability of the reactions involved [28,29] Shimokomaki; Olivo, 2006).

Cooking characteristics

The yields obtained for Pepitas were 95.94 ± 0.45 for the sample with xyloglucan (Nx) and 87.91 ± 0.71 for the control sample with commercial starch (Nc). The values found were favorable, adding value and quality to the product, where a high yield of the prepared product may be related to the coating system that acts as a barrier in the product, contributing to a lower loss of components such as water and lipids, since the greater weight loss in cooking fish burgers is due to the lack of ingredients capable of retaining water [30] Sá Vieira., *et al*. 2015).

A Table 3 mostra os valores médios (média ± desvio padrão) das amostras controle e xiloglucano nos dias 0, 30 e 60 dias em relação aos valores dos parâmetros de cozimento.

Water retention capacity (WRC) is an important property in terms of quality, both in meat intended for direct consumption and meat intended for industrialization [31] Oliveira, 2011. The samples in this study (Table 3) showed high water retention compared to other experiments. However, they were significantly different during the study days (0, 30 and 60 days), which can be explained by the structural differences of the two polysaccharides used in both samples and by the addition of the other ingredients used. Galvão et. al (2010) observed that the addition of wheat fiber and corn oil to fishburgers made with mechanically separated tilapia meat increased the product's water retention capacity from 79% to 87%, demonstrating beneficial effects, since products with low

Dias de estudo	0		30		60	
Análise	***Nc***	***Nx***	***Nc***	***Nx***	***Nc***	***Nx***
CRA (%)	94.54b ± 0.19	98.10a ± 0.32	94,71 ± 0,17b	98.71a ± 0.16	93.49b ± 0.63	96.30a ± 0.18
Encolhimento (%)	7.04a ± 0.57	3,59b ± 0.40	5.57a ± 0.37	3.73b ± 0.71	3.44a ± 0.91	2.18pt ± 0.41

Table 3: Resultados (média desvio-padrão) da análise físico-química das pepitas (Nc e Nx).

*NC (Control Nugget with 2.5% preparation) and NX (Nuggets with xyloglucan with 2.5% preparation). The same letters on the same line indicate that there were no significant differences between the samples.

water retention capacity generally cause large quantitative and qualitative losses that are undesirable for marketing [32,33] Almeida, 2011).

The sample with the presence of xyloglucan had a higher water retention capacity, i.e. due to the presence of xyloglucan, which can also act as a dietary fiber [34] Picout., *et al*. 2003), this would lead to the absorption of more water [35,36] Assis et. al., 2009; Perez, Germany, 2007) which would possibly bind to the product.

The shrinkage percentage is mainly caused by protein denaturation during cooking and partially by water evaporation and melting of meat fats, where these factors can be causes of variability [37,38] Choi et. al., 2012; Yildiz-Turp et. al, 2010). The low shrinkage in the present study corroborates the results of productivity and WHC, where the addition of xyloglucan (Nx) increased water absorption, which consequently increased productivity and reduced water loss during shrinkage.

Textural features table 4 shows the values (mean ± standard deviation) of the control (Nc) and xyloglucan (Nx) samples on the 30th and 60th days in relation to the values of the cutting and hardness analyses.

Study days	30		60	
Analysis	***Nc***	***Nx***	***Nc***	***Nx***
Cut (N)	5.88a ± 1.38	5.03a ± 1.15	9.773a ± 2.356	7.339b ± 1.903
Hardness or firmness (N)	45.12a ± 4.215	40.97a ± 10.46	120A ± 14.09	59.67b ± 34.68

Table 4: Results (means ± standard deviation) of the cutting analyses.

*NC (Control Nugget with 2.5% preparation) and NX (Nuggets with xyloglucan with 2.5% preparation). The same letters on the same line indicate that there were no significant differences between the samples.

According to [39] Guitarra and Guitarra (2005), texture can be defined by physical characteristics that are perceptible to the touch and are related to deformation, disintegration and flow when a force is applied, such as the combination of sensations derived from the lips, tongue, oral mucosa, teeth and ear. In this way, breaded products provide greater texture and crispiness, as this breading technique offers the meat protection against dehydration and freezer burn during the freezing process [40] Dill., *et al*. 2009).

According to Table 4, the samples did not present significant differences in the cutting analysis on the 30th day, but on the 60th day the control sample presented a higher result, suggesting that the force exerted to cut or slice the sample was lower for Nx (Sample with xyloglucan) as the days of storage passed. This result corroborates those found [41] in Signor's (2018) research when developing the DMC for the production of breading, using pre-gelatinized starch, thus suggesting that xyloglucan had a similar behavior to this type of starch. In the hardness analysis, the behavior of the samples was similar to that of the cut, verifying that as the days went by, the Nx sample presented significantly lower results, that is, the hardness or firmness was lower when compared to the control sample (from the 60^{th} day). [42] Bainy., *et al*. (2015) verified the texture profile of tilapia burger and found that during storage (for 6 months) the hardness increased, since the water provided lower resistance to compression. Another study points out that higher hardness and cut values are related to the increase in texture and reduction in moisture content that occur both with the frying process of breaded products and with storage time [43] (Teruel., *et al*. 2014).

Conclusions

The application of xyloglucan as a substitute for starch in the formulation of Nuggets made with tilapia CMS demonstrated physical-chemical parameters such as pH within the current legislation for fresh fish, in addition to emphasizing a reduction in moisture and a high protein content, suggesting that the free water of the product may have bound to the polysaccharide in question. The color characteristics emphasize that the addition of xyloglucan favored brightness, saturation and intensity of yellowing. Another piece of evidence is that with the addition of xyloglucan (Nx) increased water absorption (WRA) which consequently increased productivity and decreased water loss during shrinkage. In the analysis of the texture, it was concluded that the force exerted to cut the sample with the presence of xyloglucan was lower and that the product became softer over the days of storage.

These conclusions allow us to emphasize that the use of xyloglucan as a natural ingredient to replace commercial starch represents a favorable alternative, with added value and quality to the product, providing the possibility of using and industrializing this polysaccharide.

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