UV Spectrophotometry Applied to the Quantification of Omega-3, -6 and -9 in Fresh Tissues of Wild and Farmed Tambaqui

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Abstract

The demand for healthy foods has also increased the demand for wild and farmed tambaqui since it is a fish rich in omega-3, -6 and -9. To determine which of the two types of fish has the best nutritional quality and thus test the hypothesis that there are no nutritional differences between the groups of fish evaluated, the method of ultraviolet absorption spectrophotometry was used. For this, tambaqui from different environments (wild and farmed) were obtained in the states of Amazonas and Rondonia, Brazil. The fish groups showed differences in the concentrations of omega-3, -6 and -9 (ANOVA, $F_{(8.30)} = 16.213$, and $p < 0.01$), both between states and between environments. The wild fish of the Amazonas state presented the best quality meat, and exhibited the highest concentrations of omega-3 (0.223 g $\pm$ 0.05 g) and omega-9 (0.208 g $\pm$ 0.04 g), which also implies the presence of omega-6, while the other group of fish exhibited the lowest values of omega in their composition.

Keywords: Fatty acids; alpha-linolenic acid; linoleic acid; oleic acid; absorption spectrophotometry; unsaturated fatty acid.

1. Introduction

The human body needs the intake of certain nutrients for proper functioning (Vidal et al., 2012), among these stand out lipids, which are organic chemical compounds that act as an energy source for the cellular maintenance of living beings, mainly in the form of fatty acids (Suárez-Mahecha et al., 2002).

Fatty acids at room temperature are classified into saturated (solid state) and unsaturated (liquid state), and these can be used for energy by the cells of animals and plants, where they are found, respectively (Bolzan, 2013). They belong to the group of carboxylic acids (COOH) of the aliphatic chain, to which belong omega-3 (alpha-linolenic acid, ω-3) (Figure 1A), -6 (linoleic acid, ω-6) (Figure 1B) and -9 (oleic acid, ω-9) (Figure 1C) (Pubchem, 2020).
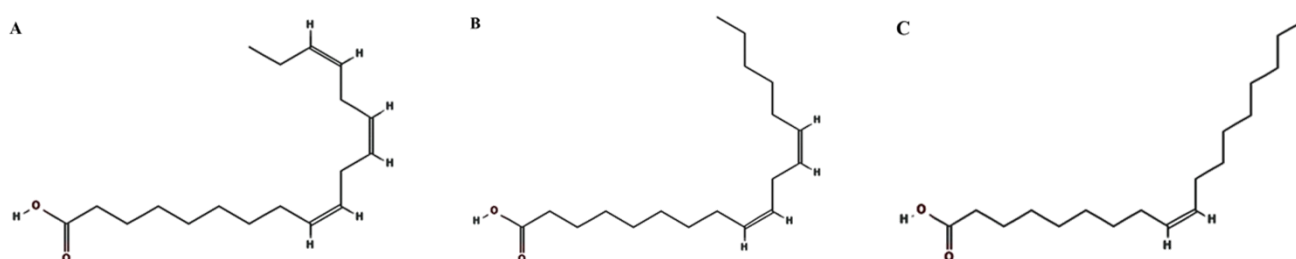

Figure 1. Two-dimensional images of the respective omegas, A = ω-3; B = ω-6; C = ω-9 (PUBCHEM, 2020).

The fatty acids ω-3 and ω-6 are considered essential because they are not synthesized by the human body and for this reason they must be ingested through food (Souza et al., 2007; Kus and Mancini-Filho, 2010). In addition, they have distinct physiological functions,though act together for the regulation of biological processes (Suárez-Mahecha et al., 2002; Kromhout and Goede, 2014). On the other hand, ω - 9 is produced through endogenous processes, but, for this to occur, it is necessary that the omega-3   and 6 have been previously ingested (Suárez-Mahecha et al., 2002; Asif, 2011).

Studies have pointed out that omega fatty acids are able to help in the control and prevention of diseases (Saravanan et al., 2010; Weiser et al., 2016), such as postnatal depression (Kaviani et al., 2014) and cardiovascular diseases (Saravanan et al., 2010). They can also act as anti-inflammatories in neurodegenerative diseases, among other health benefits (Hu et al., 1999; Skulas-Ray, 2015). For these and other reasons, the demand for "healthy" foods, containing omegas -3, -6 and -9, has increased.

The foods most sought out for having high concentrations of omega-3 and -6 are fish oils (Vilarta, 2007; Bentes et al., 2009) and vegetables (Gebauer et al., 2006). Among the freshwater fish, the tambaqui (*Colossoma macropomum*) is rich in ω-3, especially because it is a plankton filter, which is one of the primary sources that are rich in fatty acids (Kus and Mancini-Filho, 2010). It is also one of the most consumed fish in the northern region of Brazil, where it leads production, whether farmed or wild (Instituto Brasileiro de Geografia e Estatistica, 2019).

With the increasing supply and demand for fish for human consumption, a question has arisen between commercial fishermen and fish farmers, in which it is alleged that, due to it coming from a natural environment, the meat of wild fish has better quality than that of captive fish in relation to nutritional characteristics (Oliveira et al., 2020). However, determining which is the best meat in a nutritional sense

requires complex and in-depth analysis, since there are few methods used for this.

There are a number of techniques that can be used to determine the occurrence and concentrations of omegas present in food, including gas chromatography (Bentes et al., 2009; Reis, 2015), infrared spectroscopy (Pantoja, 2013) and ultraviolet absorption spectrometry (Pavia et al., 2010). The latter is widely used since it is low cost and since it can detect the presence of certain functional groups in small samples (Pavia et al., 2010; Pantoja, 2013).

Thus, in this study, we applied the spectrophotometry technique allied to the method of extraction of fatty acids in order to evaluate whether rearing in the wild or the farming of tambaqui influences the levels of omega-3, -6 and -9, in other words, if the meat of wild fish or farmed fish differ in their fatty acid content. For this, the following hypotheses were tested: i) there are no differences between the absorption values for omegas-3, -6 and -9 between the meats of the groups of wild and farmed tambaqui; ii) the previous parameters do not differ for the fish groups between the states of the northern region (Rondonia and Amazonas states).

2. Materials and Methods

2.1 Fish samples

The specimens of tambaqui used in this research were acquired in wild and farmed environments, in the states of Amazonas and Rondonia, since these two are the largest producers of this species in Brazil (Brazilian Institute of geography and Statistics, 2019).

A total of 28 slaughtered tambaqui were obtained, seven were wild and obtained in the Lago do Cacau (03º09'22''S 60º06'42''W) and seven were from fish farms (03º17'06''S 60º11'09''W), both in the municipality of Iranduba (Amazonas). In the state of Rondônia, the same quantities were acquired in the Madeira River, near the city of Porto Velho (08º42'56''S 63º55'23''W) (wild fish) and from a fish farm in the municipality of Presidente Medici (11º09'37''S 61º54'22''W).

All fish, when obtained, were stored in isothermal boxes, in alternating layers of ice and fish, in a ratio of 1:1 (kg of ice:kg of fish), labelled and transported to a laboratory facility, where they were eviscerated, descaled, boned, packaged, labeled and frozen. From each fish, three samples of meat with skin were randomly collected for further procedures and analysis in the Nanomaterials Laboratory-Nanobiomagnetism, Federal University of Rondonia. The methodological procedures for the collection and processing of the data of this research were approved by the Ethics Committee of the Federal University of Rondonia under the registration number: 82882817.5.0000.5300.

2.2 Chemical Reagents

Chloroform compounds (P.A. 99.8% Alphatec), methanol (P.A. 99.8% Alphatec), oleic acid (P.A. 99.5% Synth), linoleic acid (P.A. 99% Sigma) and linolenic acid (P.A. 99% Sigma) were used for the solubility of the samples.

2.3 Preparation procedures

Initially, a sample of tambaqui meat was randomly selected and subjected to milling with the aid of an

ultrasonic sonicator (homogenizer disperser), using 200 mg of fish meat sample for each of the respective chemical compounds: chloroform (3 ml), formaldehyde (2 ml), methanol (1 ml), hydrochloric acid (1 ml) and ethylene glycol (0.5 ml), in order to verify in which solution the sample presented the best solubility. At the end of the tests, the solutions of chloroform and methanol showed better solubility in the dilution of the samples.

After homogenization, the resulting mixtures of the samples were used for two-hour incubation in the standard extraction solution of chloroform: methanol (2: 1). The preparation of the samples were in the following proportions: 300 µl (30%), 150 µl (15%), 50 µl (5%), 10 µl (1%) and 5 µl (0.5%) of sample dissolved in a total volume of 3 ml for each sample generated. The standard solution was also used to prepare the fatty acids Ѡ -3, Ѡ-6 and Ѡ-9, in their respective percentages.

The sample solutions were submitted to spectrophotometery (NOVA, 2102 UVPC), with the aid of Win-sp5 UV work station software, in the region of 190 to 1100 nanometers (nm). For spectrophotometry measurements, chloroform solvent solution (CHCL3) was used in the proportion of 1% of the sample, which was observed as being the best resolution of the spectra (Figure 2). For the analyses, 3 ml of each sample was used. This was added to a quartz cuvette with an optical path of 5 mm and an optical dispersion width of 0.2 mm. Between each measurement, the cuvette was cleaned with distilled water to avoid contamination.
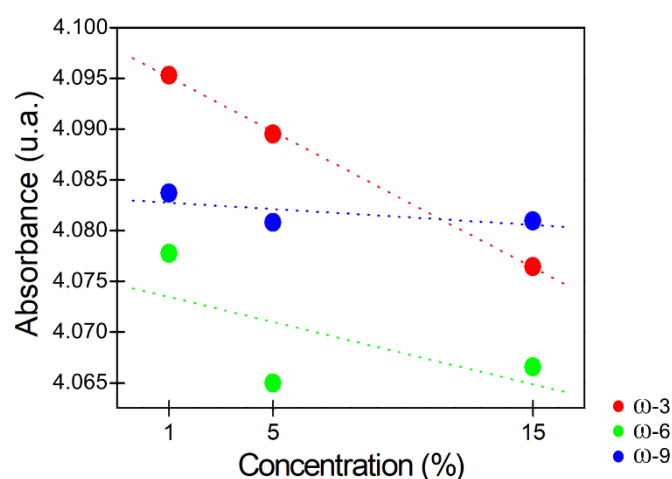

Figure 2: Calibration of UV/VIS absorbance spectroscopy in arbitrary units (a.u.) as a result of the concentration of omegas, which was dispersed in standard solution medium of omegas in chloroform/methanol. Dotted lines are the linear regressions for the experimental data of the maximum absorbance value of each UV/VIS spectrum. (Ѡ = Omega)

2.4 Statistical analyses

The data treatment for the analyses was based on the range of the electromagnetic spectrum of the ultraviolet light that extended from 190 to 400 nm, displayed with the aid of Origin Microcal (TM) software (Version: 6.0, serial number: G73S5-9478-7063326), where the absorption peaks were identified in regards to the wavelength of each sample, resulting in a dimensional graph that showed these variables (Pavia et al., 2010; Pantoja, 2013). The standard value of each omega, Ѡ-3 = 278.43 g/mol, Ѡ-6 = 280.45 g/mol and

ω-9 = 282.47 g/mol, was used in the quantification of omegas present in each fish group, determined from the number of pi transitions present in each sample. For this, the Lorentz distribution (Equation 1) was applied to both fish samples and omegas, in order to find out the value of the area under the peak absorption (A). Then, the A values of the samples were used to quantify the Omegas -3, -6 and -9 present in each individual (Equation 2).

Equation 1

$$y = y_0 \; \frac{2Aw}{\pi(x - xc)^2 + w^2}$$

Where:

y = Spectrum intensity;
y_0 = Initial spectrum intensity constant;
x = Independent variable. Fixed wavelength;
π = 3.14;
A = Area under peak absorption;
w = Line width at half height; and
xc = Floating wave length.

Equation 2

A=A(mean) $\pm$ ΔA(error)

The total sum of A multiplied by the standard of each omega (g/mol) results in the total amount of omega present in each fish (Equation 3).

Equation 3

ai = m(ΣA)pi

ai = Total amount of omega per individual
m = Standard of each omega
pi = Weight of each individual

The omega values obtained for each group of fish were submitted to the Levene and Shapiro-Wilk test, in order to verify the assumptions of homocedasticity required by the analysis of variance (ANOVA), then the Tukey test was used to verify the significant difference (honestly significant difference - HSD) between the different groups. A correspondence analysis (CA) along with the Tukey test showed the differences between fish groups in relation to the amount of omegas. These tests were performed using the Statistica 9.0 program (Statsoft 2009), where $p < 0.01$ was considered statistically significant.

3. Results

The values of absorption spectra in the ultraviolet region, obtained from triplicates and omegas, exhibited

maximum absorbances in wavelengths ranging from 190 to 320 nm. The spectra of the omegas showed up to three characteristic bands of absorption. Omega-3 showed maximum absorption at wavelengths 201, 224 and 268 nm, omega-6 at 211, 234 and 278 nm, and omega-9 at wavelengths 203, 227 and 271 nm (Figure 3). However, band 2 showed higher absorbance intensity when compared to bands 1 and 3, this behavior is due to the transitions of N $\rightarrow$ π^* and π $\rightarrow$ π^*, because of the carbonyl group and the polyenes present in the omegas (Valeur, 2005), and was therefore considered for the quantitative calculations of the omegas.

The samples of the tambaqui presented different behavior in the absorption spectra for each individual and respective environments. A wild specimen from the Amazonas state showed predominance of omega-3 and -9 in its spectrum. When the highest intensity absorption bands were observed in the samples, omega-3 band 1 was present in four individuals, while band 2 was identified in 3 individuals. Omega-6 band 2 was present in the 7 fish of the wild group of the Amazonas state, but with greater absorbance in only 3 specimens. Omega-9 band 1 was present in all individuals, while band 2 was present in only 3. The band 3 of the omegas was not found in any of the samples tested (Figure 3A).

On the behavior of the absorption spectra of the wild fish from Rondonia state, five specimens presented the bands 1 and 2 of omega-3, -6 and -9, and only one individual exhibited greater intensity in wavelength. Two individuals presented band 3 of omega-6 and five fish were present in band 3 of omega-3 and -6 (Figure 3B).

The fish from the Amazonas state farms presented the bands 1 and 2 of the omegas in practically all individuals, and only 2 exhibited different intensities in the absorption bands, one fish presented band 2 greater than band 1, while the other showed the reverse (Figure 3C). Omega-6 showed a low incidence in all animals. Omega-3 bands 1 and 2 were present in 6 individuals, except for one fish that did not present band 2. As for omega-6, band 1 was evident in four individuals and band 2 in six. For omega-9, bands 1 and 2 were present in 6 of the 7 individuals tested. All animals showed jumps to the smaller wavelengths and did not show band 3 in their spectra (Figure 3C).

As for the intensity of the absorption bands of farmed fish from Rondonia state, one of the specimens presented the bands 1, 2 and 3 of the omega-3, -6 and -9 in its entire spectrum, the other samples exhibited the lower absorbance intensity (Figure 3D). In four fish, band 1 and 2 of omega-3, -6 and -9 were present with the exception of 2 fish that did not present band 3 of omega-6 (Figure 3D).
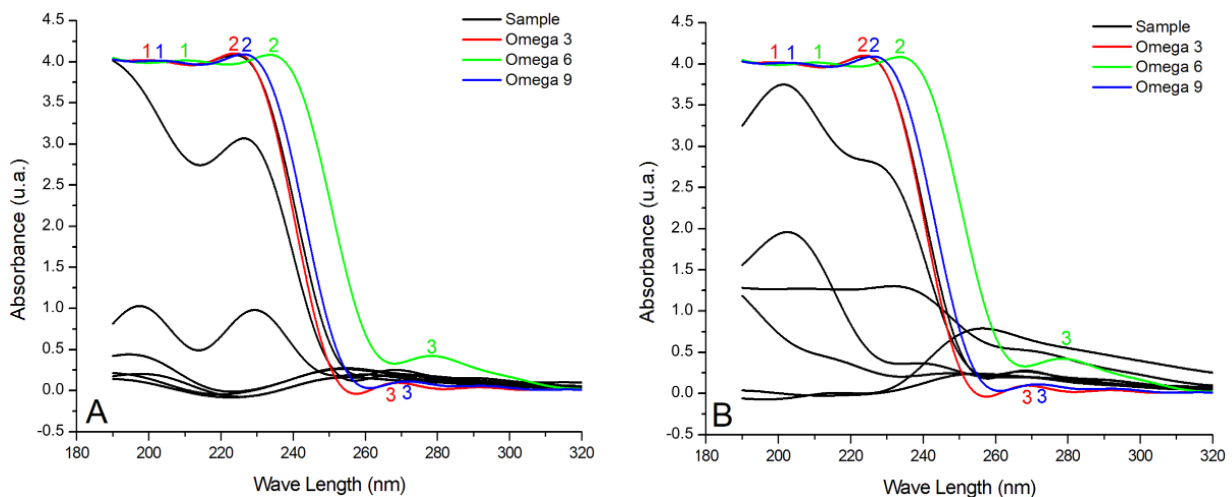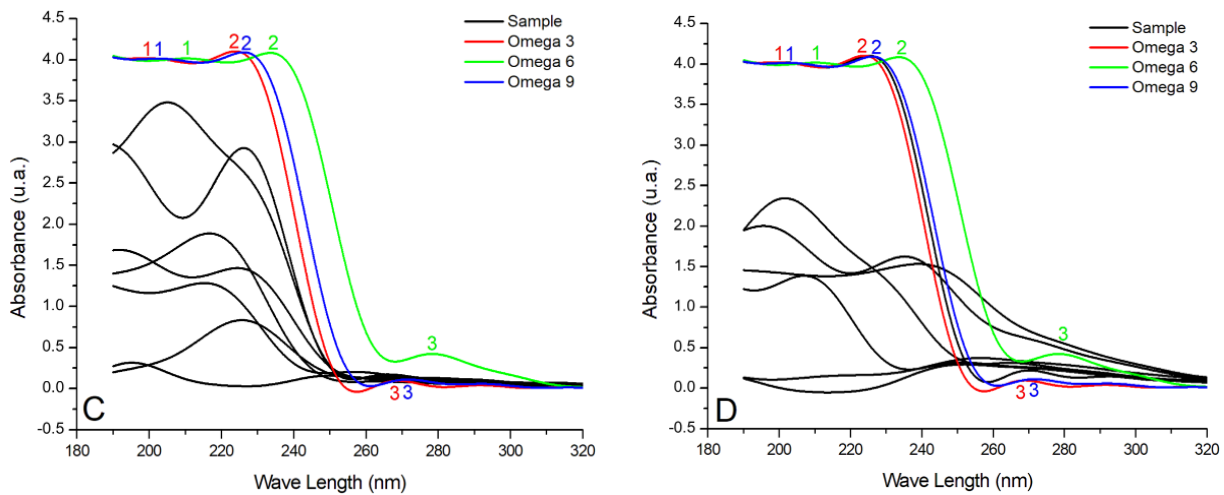
Figure 3. Representation of absorption spectra in the ultraviolet region of tambaqui samples together with the spectra of omega-3, -6 and -9. Where: A = wild Amazonas fish, B = Wild Rondonia fish, C = farmed Amazonas state fish, and D = farmed Rondonia state fish.

The quantification of omegas -3, -6 and -9 in relation to meat mass of fish was obtained using the Lorentz distribution function for the spectra of Figure 3, and we performed the sum of the derivatives of the acquired absorption areas. In this case, the distribution function was applied to the identity spectrum of each individual and determined the areas under the spectra. The areas are equivalent to the amount of omega that responded to a given wavelength. This amount was correlated with the standard molar weight of each omega, along with the percentage of the material dissolved in the medium in which the spectrophotometry measurement was performed (Folch et al., 1956; Bligh and Dyer, 1959; Rodríguez et al., 2010).

The analysis of variance showed significant differences between the quantitative omega by groups of tambaqui (F (8.30) = 16.213, and p < 0.01) and also varied with the Tukey post hoc test (Table 1). The tambaqui that presented the highest amounts of omega-3 and -9 in their meat were the wild fish of the Amazonas state, with an average of 0.223 g and 0.208 g per fish, respectively. The individuals that exhibited the lowest amount of omega-3 were from the farmed group of the Amazonas state (0.045 g), and the lowest values of omega-9 was equal to tambaqui farmed in Rondonia state (0.051 g). The wild tambaqui of Rondonia (0.075 g) had a higher concentration of omega-6 when compared to the wild tambaqui of the Amazonas, and the specimens from farms in Rondonia (0.028 g) presented lower amounts of omega-6 compared to those of cultivation from the Amazonas state (Table 1).

Table 1. Mean and standard deviation values of the amount of omega extracted from each tambaqui individual per group, in different environments and states, followed by the Tukey test (p > 0.01). Identical letters in the same column do not show significant differences between the means of the variables presented. PM = mean weight of individuals per group.

Sample location	PM (g)	ω-3 (g)	ω-6 (g)	ω-9 (g)
Amazonas (wild)	1381.4 ± 294.2	0.223 ± 0.047 a	0.059 ± 0.012 a	0.208 ± 0.044 a
Rondonia (wild)	1803.5 ± 721.3	0.075 ± 0.030 b	0.075 ± 0.030 a	0.071 ± 0.028 b
Amazonas (farmed)	1490.6 ± 581.8	0.045 ± 0.008 c	0.058 ± 0.010 b	0.060 ± 0.008 c
Rondonia (farmed)	2351.1 ± 623.6	0.098 ± 0.025 d	0.028 ± 0.007 c	0.051 ± 0.013 d

The correspondence analysis explained the distribution of the values of the omegas within the coordinate matrix with 86.91% inertia. There is an evident separation between the groups of tambaqui and their concentrations of fatty acids, in which in dimension 1 the omegas 3 and 9 were grouped on the left side of the x axis for the wild fish of the Amazonas and Rondonia farms, while the wild fish of the Amazonas state and farmed fish from Rondonia were grouped on the right side with the highest values of omega-6. In dimension 2, the separation of fish groups by state was noted, in which the groups of the Amazonas were in the upper part and the groups of Rondonia in the lower part of the Y axis (Figure 4). This demonstrates that the wild tambaqui of the Amazonas presented the highest amounts of ω-3 and ω-9, followed by the farmed individuals holding equal place for ω-6. The group of Rondonia tambaqui remained in the lower part of the y-axis, where they exhibited the lowest concentrations of ω-3 and ω-6, for farmed and wild fish, respectively (Figure 4).
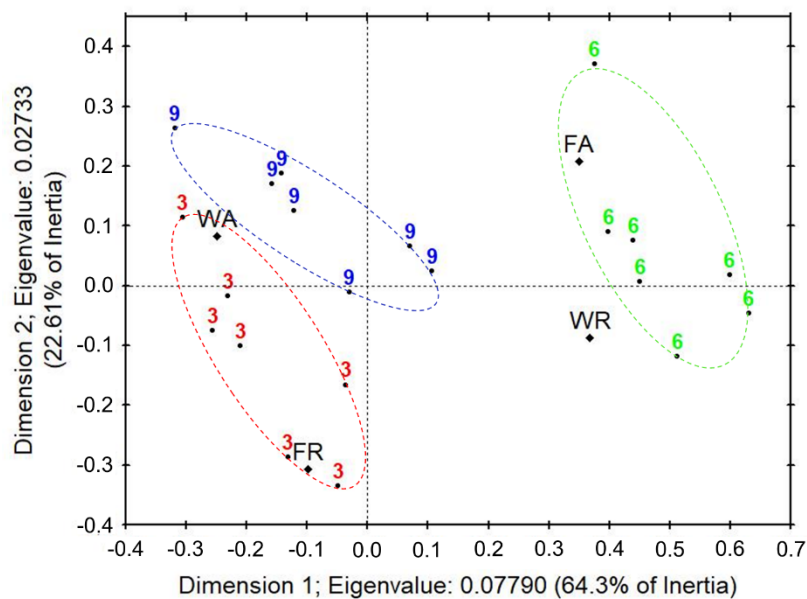

Figure 4. Analysis of correspondence with the distribution of the groups of tambaqui, according to the concentrations of the groups of omega-3, -6 and -9 and their respective capture sites. Where: FA = Amazonas farmed fish; FR = Rondonia farmed fish; WA = wild Amazonas fish; and WR = wild Rondonia fish.

4. Discussion

Spectrophotometry is a technique used to measure the presence of functional chromophore groups of a sample (Gonçalves, 2001; Pantoja, 2013). When the statistical equations are added, these results can efficiently report the exact amount of the investigated substance (Waite, 1976), as well as provide information on compounds that have double bonds in their structure (Gonçalves, 2001; Pantoja, 2013; Pavia et al., 2010).

Unsaturated fatty acids have these double bonds in their molecules, and physico-chemical characteristics, such as boiling points and melting points that are different from saturated fatty acids (Assif, 2011; Bolzan, 2013), as is the case of omega-3, -6 and -9, which showed absorption in wavelengths of between 200 to 300 nm, and which occurs due to the diene system present in their structures. (Instituto Adolf Lutz, 2008).

These acids can be found in oily seeds, such as flaxseed and Brazil nuts, in corn and sunflower vegetable oils, olive oils and almonds, and fish oils, such as salmon (*Salmo salar*) and trout (*Oncorhynchus mykiss*). In addition, fish are also considered as the main source of omega (Sugano and Hirahara, 2000; Kus and Mancini-FILHO, 2010).

Omega-3 and -6 are usually found in greater quantities (up to seven times more) in fish that inhabit cold regions, when compared to fish from warmer environments (Visentainer et al., 2000; Souza et al., 2007; Torres et al., 2012), and the main source of these compounds are   plankton and algae (Souza et al., 2007; Asif, 2011). However, in the Amazon basin (Fishbase, 2020), well known for being a tropical region, with temperatures ranging from 20 °C to 35 °C (Ruffino and Roubach, 2009), there is the tambaqui, a native fish species, considered rich in ⍵-3 (Kus and Mancini-Filho, 2010). These individuals also have as their food base the phyto and zooplanktons (Oliveira and Sousa, 2017), which have large concentrations of fatty acids in their structure.

Thus, it is noted that fish in general have distinct behaviors and characteristics, which may be linked to the environmental conditions in which they live and consequently the feeding in these different places (Sugano and Hirahara, 2000; Bentes et al., 2009), and therefore, the amounts of omegas present in the meat of these individuals may vary (Sugano and Hirahara, 2000; Moreira et al., 2003; Bentes et al., 2009). This pattern was observed in the results presented here, where the groups of fish from different environments (wild and farmed) showed differences between themselves.

However, studies point out that both the human organism and the fish do not have the ability to produce the double bonds existing in the fatty acids ⍵-3 and ⍵-6. However, they can be lengthened and desaturated by the enzyme system (Martin et al., 2006; Assif, 2011), although this conversion is performed in low percentages, since there is a physiological competition between these omegas, which in turn, are only able to be altered one at a time (Suárez-Mahecha et al., 2002; Bentes et al., 2009). This factor may explain the fact that the wild tambaqui of the Amazonas presented high values of ⍵-3 and ⍵-9 and low amounts of ⍵-6.

Therefore, it is necessary that the intake of omega-3 and -6 through food occurs in a balanced way, since the excess of one induces the deficiency of the other (Suárez-Mahecha et al., 2002) and this may promote disease pathogenesis or suppressive effects (Renaud, 2002; Suárez-Mahecha et al., 2002; REIS, 2015). On the other hand, ⍵-9 is beneficial to humans and also to fish, as it is naturally produced by the organisms of these individuals in the optimal amount (Martin et al., 2006). There are recommendations regarding the amount of omega-3 and -6 that should be ingested, however there is no exact amount proven and pre-established for consumption (Almeida and Franco, 2006). The balance between the omegas is essential to promote a good functioning of the body, however omega-3 is the most important among the studied omegas (Assif, 2011), since this fatty acid, among other benefits, is also responsible for the reduction in the formation of clots that occur in the bloodstream (Connor, 2000; Suárez-Mahecha et al., 2002; Almeida and Franco, 2006).

The results of this study showed that the wild tambaqui of the state of Amazonas stood out in relation to the wild tambaqui of Rondonia, with individuals rich in ⍵-3 and ⍵-9. As for farmed fish, Rondonia individuals had more ⍵-3 than Amazonas fish, which in turn exhibited the highest rates of ⍵-6 and ⍵-9. Some studies have indicated that farmed fish have higher omega-3 and -6 levels than wild fish (Suarez-

Mahecha et al., 2002), while other studies report the reverse (Moreira et al., 2001). In the case of farmed carp (*Cyprinus carpio*), the omega-6 values (16.1%) were higher than those of wild individuals (13.5%) and for omega-3 the reverse occurred, farmed fish presented 9.6% and wild fish 15.8% of these fatty acids in their meat (Suarez-Mahecha et al., 2002). In the Amazonas basin, wild specimens of piramutaba (*Brachyplastystoma vaillantii*) did not present ꞷ-6 values in their flesh, while farmed piraputanga (*Brycon microlepis*) exhibited a higher rate of omega-6 (11.86%) when compared to wild specimens (5.27%) (Moreira et al., 2003; Bentes et al., 2009). For the wild tambaqui of the state of Amazonas, the highest concentrations of ꞷ-3 and ꞷ-6 were found compared to farmed fish. While wild tambaqui from Rondonia exhibited the highest values of ꞷ-6 and lowest rates of ꞷ-3 in relation to farmed fish.

The distribution of different levels of omegas in the tissues of the fish studied may be related to the different environments and types of food to which they were subjected (Almeida and Franco, 2006). The states of Amazonas and Rondônia have distinct geographical and environmental characteristics, where the former still contains large areas of floodplains and well preserved forests (Bittencourt and Amadio , 2007; Pacheco, 2009), thus providing favorable conditions for the healthy development of the existing tambaqui.

The state of Rondonia, however, presents a history of economic growth based on the production of cattle with large areas of pasture and bean production (coffee and soy) (Melo Filho et al., 2005; Silva, 2014), which, added to the large areas of deforestation, lead to the anthropization of river beds, causing siltation and environmental modification (Fearnside, 2006). These factors may have influenced the low values of ꞷ-3 and ꞷ-9 found in wild tambaqui of this region, and this may be a consequence of food shortages.

Therefore, the maintenance and preservation of the natural flooded areas in which the tambaqui live is necessary, since they are still the most suitable spaces for the acquisition of healthy food and rich in nutrients for these individuals, which act as a source of protein indispensable for human diets. On the other hand, the proper management of fish in confined environments can also be achieved, but for this to occur a diet with higher amounts of omega-3 and -6 must be offered to the animals being reared.

5. Conclusion

The groups of tambaqui evaluated showed differences in the concentrations of omegas-3, -6 and -9, both between the states (Amazonas and Rondôinia), and between the environments in which they were obtained (wild and farmed). However, the wild fish of the Amazonas were the ones that presented the best nutritional quality in their meat, since they exhibited the highest concentrations of omega-3 and -9, which also implies the presence of omega-6.

6. Acknowledgments

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