CHEMICAL ENGINEERING TRANSACTIONS
VOL. 64, 2018
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Enrico Bardone, Antonio Marzocchella, Tajalli Keshavarz

ISBN 978-88-95608- 56-3; **ISSN** 2283-9216
DOI: 10.3303/CET1864040

Chemical Characterization of Oils and Fats from Amazonian Fruits by ^{1}H NMR

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Native plants from the Brazilian Amazon Biome are rich in nutrients, oils and fats and, therefore, represent a very important regional food resource. There is a global interest in the nutritional potential of tropical fruits, the potential of Amazonian fruits as source of essential fatty acids is still under exploited, since their composition is still understudied. ^{1}H Magnetic Resonance Nuclear (^{1}H NMR) is a robust spectroscopic tool to profile fatty acids components in oils. The objectives of this work were to use ^{1}H NMR to determine the fatty acids profile and the physicochemical properties of crude oils and fats obtained from fruits of Brazilian Amazon: *açaí*, *ata-brava*, *bacaba*, *buriti*, *buritirana*, yellow *murici*, red *murici*, *piaçaba braba*, *tucumanzinho*, *pupunha* red and yellow varieties. Oils were obtained by Soxhlet extraction using hexane as a solvent. After solvent removal by rotoevaporation, the resulting oils and fats were analyzed by ^{1}H NMR spectroscopy. Unsaturated fatty acids oleic (ω9), linoleic (ω6) and linolenic (ω3) acids were quantified in the oils and their physicochemical properties were determined. It was observed that the oils have predominately ω9 fatty acids, followed by ω6 and ω3 representatives. The physicochemical properties confirmed edibility and good quality of oils and fats present in the aforementioned Amazonian fruits.

1. Introduction

Mediterranean diet is an icon of health food. This diet focuses on the abundant consumption of some fruits and vegetables along with use of olive oil as the main source of lipids. Exclusive olive oil consumption has been reported to be directly linked to lower risk of coronary artery disease (Dimitriou et al., 2016). Plants from Amazonian region have a diversity of fruits like *açaí* (Yamaguchi et al., 2015), bacaba (Dos Santos et al., 2017; Neves et al., 2015; Finco et al., 2012), buriti (Koolen et al., 2013; Silva et al., 2011; Manhães et al., 2011), pupunha (Dos Santos et al., 2017; Rojas-Garbanzo et al., 2016; Yuyama et al., 1999) that can be used as source of functional oils due to their high contents of phenolic compounds, as well as fat soluble vitamins and fatty acids. Many of these fruits present pulp and seeds rich in oils (Dimitriou et al., 2016) containing saturated and unsaturated fatty acids with nutritional value that may comparable to ingredients of Mediterranean diet. The potential of Amazonian fruits as source of essential fatty acids is still under exploited.

Please cite this article as: Carvalho Dos Santos R., Alves Chagas E., Melo Filho A.A., Takahashi J.A., Montero Fernandez I., Dos Santos F. G., Cardoso Chagas P., Goncalves Reis De Melo A.C., 2018, Chemical characterization of oils and fats from amazonian fruits by 1h nmr, Chemical Engineering Transactions, 64, 235-240 DOI: 10.3303/CET1864040

Analysis of oil composition is traditionally carried out using chromatographic techniques such as gas chromatography (GC). Nevertheless very useful, this methodology requires hydrolysis of the triacylglycerides to release free fatty acids followed by fatty acids derivatization (Fernandez et al., 2016). Complex sample handling and need of expensive derivatization agents are among the disadvantages of using GC for fatty acids analysis. Besides, sample cannot be recovered after the experiment. Fatty acids identification can also be carried out using Nuclear Magnetic Resonance (NMR), a technique that preserves oils integrity and can be used to verify authenticity and quality of oils (Salinero et al., 2012). This technique speeds up control quality process since is able to analyze the oils without need of derivatization. Sample can be recovered after the experiment and data obtained enable determining physicochemical properties of the oils (Knothe and Kenar, 2004; Vigli et al., 2003).

The aim of this work was to determine the contents of oleic (ω9), linoleic (ω6) and linolenic (ω3) acids as well as some physicochemical properties, iodine index (II), acidity (AI) and saponification index (IS) of Amazonian fruit oils and fats through ^{1}H NMR spectroscopy. Fruits studied were *açaí (Euterpe oleracea)*, *ata brava (Annona hypoglauca)*, *bacaba (Oenocarpus bacaba)*, *buriti (Mauritia flexuosa)*, *buritirana (Mauritia aculeata)*, yellow *murici (Byrsonima crassifolia)*, red *murici (Byrsonima coccolobifolia)*, yellow *pupunha* (*Bactris gasipaes* var. yellow) and red *pupunha (Bactris gasipaes* var. red*)*, *piaçava brava* (*Barcella odora*) and *tucumanzinho (Astrocaryum acaule)*.

2. Material and Methods

2.1 Samples preparation and oil obtaining

The fruits screened in this work *açaí* (pulp), *ata brava* (seeds), *bacaba* (pulp), *buriti* (pulp), *buritirana* (pulp), yellow *murici* (seeds), red *murici* (seeds), *piaçaba braba* (pulp), *tucumanzinho* (pulp), red (pulp) and yellow *pupunha* (pulp) were obtained in different regions and in the market from Roraima State, Brazil.

The seeds and pulps were dried in an oven with circulating air at 50 °C for 48 h. Then, the plant materials were milled and sieved (20-40 Mesh), for obtaining a homogenized powder that was extracted from hexane in a Soxhlet extractor, to obtain the raw oils and fats. Solvent was removed on a rotary evaporator, the extracts were placed in amber bottles under nitrogen atmosphere until further analysis (Santos et al., 2015).

2.2 ^{1}H NMR Spectroscopy and Determination of percent amount of omega 3 (ω3), 6 (ω6) and 9 (ω9)

^{1}H NMR spectra were recorded on a Bruker Avance DPX 200 spectrometer, operating 4.7 Tesla, corresponding to the resonance frequency of 200.13 MHz for the ^{1}H core, equipped with a direct detection probe head operating at 300 K. The samples were analyzed in 5 mm NMR tubes Wilmad ®. NMR samples were prepared by dissolution of 0.5 mL of each oil in 0.6 mL of $CDCl_3$. Chemical shifts are presented in ppm, using TMS as internal standard. ^{1}H NMR spectra of samples were processed in the software SpinWorks 4.2 (SpinWorks, 2015). Chemical shifts were analyzed according to Guillén and Ruiz (2003) for non-Amazon fruits. The chemical shifts showed signs characteristic of the fatty acids before mentioned, and were used to calculate their physical properties. The following mathematical equations used to determine the amount in % of omegas 3 (ω3), 6 (ω6) and 9 (ω9), as well as the physicochemical properties. To quantify the concentration of unsaturated fatty acids oleic (ω9), linoleic (ω6) and linolenic (ω3) acids according to the equations proposed by Garcia (2006). Calculi of percent amounts of ω3 (18:3), ω6 (18:2) and ω9 (18:1) of fatty acids, in Amazon fruits oils, were obtained based on characteristic NMR signals found in the ^{1}H NMR spectra and named A, B, C and D (Figure 1) according to methodology proposed by Garcia (2006).

Percentage of ω3 (18:3) (%):

[(B/A)/2.25].100

where A: Glycerol hydrogen; B: Linolenic acid; 2.25: Maximum theoretical ratio of trilinolenin (Garcia, 2006)

Percentage of ω6 (18:2) (%):

$D = D_{18:3}+D_{18:2}RD_{18:3}/A = [(\%18:3)*3]/100$

$[(D_{18:2}/A)/1.5]*100$

where D: Bis-allyl; 1.5: Maximum theoretical ratio of trilinolein (Garcia, 2006)

Percentage of ω9 (18:1) (%):

$C = C_{18:3} + C_{18:2} + C_{18:1}$

$RD_{18:3}/A = [(\%18:3)*3]/100$

$D_{18:3} = (RD_{18:3}/A)*ARC_{18:2}/A = [(\%18:2)*3]/100$

$C_{18:2} = (RC_{18:2}/A)*A$

$C_{18:3} + C_{18:2} + C_{18:1}$

$[(C_{18:1}/A)/3]*100$

Where C: Allylic hydrogen; 3: Maximum theoretical ratio of triolein (Garcia, 2006)

2.3 Physicochemical Properties by ^{1}H NMR spectra

The physicochemical properties according to the equations proposed by Carneiro et al. (2005) and Garcia (2006), as Iodine Index (II), Saponification Index (SI), Acidity Index (AI) and Proportion of olefinic/aliphatic hydrogen atoms ($R_{o,a}$), corresponding signal in the NMR spectra (Figure 1).

Iodine Index (II)

From the data provided in the ^{1}H NMR spectra it was possible to calculate the molecular weight (MW) (g mol^{-1}) and thus the iodine index (Eq1). The II parameter indicates the presence of double bonds in oils and fats, and may be expressed in grams of iodine which will react with the double bonds in 100 grams of sample (g I_2 100 g^{-1}):

$$II = \frac{126.91 \times 100 \times V}{MW} \quad (1)$$

Where the value of 126.91 is related to the atomic mass of iodine; V corresponds to the vinyl protons and can be calculated using Eq(2):

$$V = \frac{(J + K) - Ap}{Ap} \quad (2)$$

Where Ap corresponds to the integral of one hydrogen and is calculated using Eq(3):

$$Ap = \frac{(I + H)}{4} \quad (3)$$

Saponification Index (SI)

The SI represents the number of milligrams of potassium hydroxide per gram of oil (mg KOH g^{-1}) (Eq4, 5 e 6):

$$SI = [MW \times (-0.2358)] + 398.42 \quad (4)$$

$$MW = 119.7 + (7.036 \times T) + (5.983 \times V) \quad (5)$$

$$T\ (\text{Total hydrogen area}) = \frac{(I + J) + A + B + D + E + C + F + G + (B + H)}{Ap} \quad (6)$$

Where the value of 398.42 is relative to the average mass of the fatty acids.

Acidity Index (AI)

This parameter is calculated in order to get the acidity index of vegetable oils. This parameter also indicates the degree of unsaturation and oxidative condition of the oil (Carneiro et al., 2005).

$$AI = 3.0597 \times (R_{o,a})^2 - 6.3181 \times (R_{o,a}) + 3.3381 \quad (7)$$

Proportion between olefinic and aliphatic hydrogen atoms ($R_{o,a}$)

The $R_{o,a}$ property verifies the ratio of olefinic to aliphatic hydrogen atoms, so it can be used to measure the degree of unsaturation present in oils and fats, in addition to indicating the oxidation of samples:

$$R_{o,a} = \frac{V}{A + B} \quad (8)$$

3. Results and Discussion

All ^{1}H NMR spectra obtained for the oils are similar to the ^{1}H NMR spectrum from *piaçava brava* oil (Figure 1).

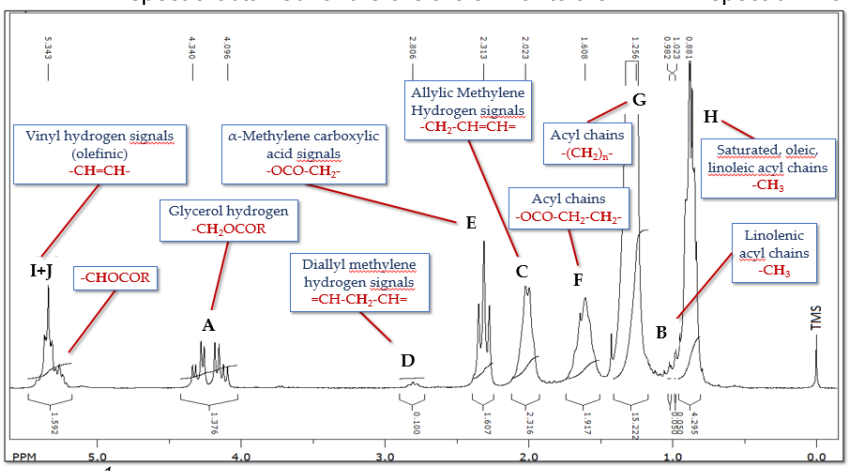

Figure 1: ^{1}H NMR spectrum from piaçava brava oil in the region of 0-5.5 ppm (200 MHz, $CDCl_3$).

The major components of the oils present in the respective ^{1}H NMR spectra were used to quantify the amounts of linolenic, linoleic and oleic acids (ω3, ω6 and ω9, respectively) and to determine some physicochemical properties of such oils. The results are shown in Table 1.

Table 1: Linolenic, linoleic and oleic acids contents and physicochemical properties of oils present in Amazonian fruits as determined by ^{1}H NMR.

Amazonian fruits	Oil yiel (%)	Concentration (%)			Physicochemical properties				
		ω3	ω6	ω9	II (g I_2 100 g^{-1})	AI (mg KOH g^{-1})	SI (mg KOH g^{-1})	MM (g mol^{-1})	$R_{o,a}$
Ata brava	15.0	0.6	29.4	43.2	89.6	0.14	177.34	937.58	1.2
Açaí	35.4	0.8	7.8	65.2	78.5	0.16	175.34	901.21	1.1
Bacaba	43.9	0.6	4.3	44.8	63.6	0.19	212.67	787.73	0.8
Buriti	23.2	1.0	2.9	67.1	71.8	0.10	206.62	813.42	0.9
Buritirana	26.0	1.4	1.8	69.4	81.1	0.15	178.79	858.09	0.9
Yellow *Murici*	46.5	0.3	29.9	38.1	90.3	0.15	207.68	808.89	1.2
Red *Murici*	38.0	0.5	29.5	33.6	85.2	0.10	212.39	788.94	1.1
Piaçaba brava	12.0	1.6	1.6	52.9	63.6	0.19	227.76	723.73	0.8
Yellow *Pupunha*	17.1	0.6	4.9	38.4	33.7	0. 22	244.99	650.67	0.7
Red *Pupunha*	27.3	2.4	10.7	26.2	70.5	0.12	168.63	974.50	0.9
Tucumanzinho	33.1	0.7	2.6	75.4	84.2	0.09	178.24	956.65	1.1

Oleic acid is an unsaturated fatty acid (ω9) and characteristic signals of its structure observed in the oils ^{1}H NMR spectra are in accordance with data described in the literature (Guillén and Ruiz, 2003). The contents of this acid, as estimated by the evaluation of corresponding signals in the NMR spectra of the oils analyzed, varied between 26.2% (red *Pupunha*) to 75.4% (*Tucumanzinho*) in samples studied (Table 1). This variation in oleic acid content, observed for oils of Amazonian fruits, was also observed for non-Amazonian fruit oils as reported by Guillén and Ruiz (2003). Concentration of ω9 in *tucumanzinho* oil is comparable to the average concentration of olives residue oil (76%) and slightly lower than the average concentrations of monounsaturated extra virgin olive (80.8%), olive (79.4%), nut virgin (82.4%) and refined (78.1%) oils.

The benefits of oleic acid are reported to be associated to synergy with other components obtained from the diet when there is frequent consumption of olive oil (rich in oleic acid), fish (rich in linolenic acid) and red wine (or olives) (rich in polyphenols) (Lou-Bonafonte et al., 2012; Pauwels EK, 2011). In addition, a great highlight is given to the presence of oleic acid in the diet, as it has been associated to beneficial effects on cancer (Sales-Campos et al. 2013), autoimmune (Sales-Campos et al. 2013) and rheumatic diseases (Sales, Oliviero e Spinella, 2009), anti-inflammatory (Carrillo, Cavia, Alonso-Torre, 2012), beneficial effects on diabetes mellitus (Vassiliou et al., 2009), reduction of coronary heart disease (Lou-Bonafonte et al., 2012), among others. Lipidic macromolecules can be fragmented by the action of bile and pancreatic secretions, entering into the bloodstream (Barrett et al., 2012).

Signals for linoleic acid (Guillén and Ruiz, 2003), a polyunsaturated fatty acid (ω6) were detected in the ^{1}H NMR spectra obtained for the Amazonian oils. Regarding to the contents of this component, the Amazonian fruits studied were classified in three groups: the first one, comprising samples with low yields of this fatty acid (*bacaba*, *buriti*, *buritirana*, *piaçaba brava*, yellow *pupunha* and *tucumazinho* (values lower than 4.9%). The second class, with two representants, *açaí* (7.8%) and red *pupunha* (10.7%), with intermediate contents, and the third class, with the fruits with the most expressive amounts of linoleic acid: *ata brava* (29.4%), yellow *murici* (29.9%), and red *murici* (29.8).

The main importance of linoleic acid consumption relies in the reduction of cardiovascular risks. According to Poli and Visioli (2015) the bigger consumption of ω6 fatty acids, the lower are the risks of cardiovascular diseases. Besides, linoleic acid has bioactivity on dermatitis and eczema, rheumatoid arthritis, premenstrual syndrome and prevention of stroke (Knowles and Watkinson, 2014).

Regarding to the presence of linolenic acid (ω3) in the fruits studied, contents varied from 0.3 (yellow *murici*) to 2.4% (red *pupunha*) (Table 1). Low contents of oleic acid is common in several oils of vegetal origin (Guillén and Ruiz, 2003), except for canola, soya, nuts and linseed. Simopoulos (2004) cites a series of benefits attributed to ω3 fatty acids in the treatment of hypertension, diabetes, arthritis, coronary, autoimmune, inflammatory diseases among others.

Linoleic and linolenic acids are essential to human metabolism and must be obtained from the diet, since humans cannot endogenously synthesize them (Zivkovic et al., 2011). However, intake must be done in a balanced manner and so that the ingestion of ω6 is not very high, since excess is associated with cardiovascular and other diseases (Simopoulos, 2006). For this reason, FAO (2010) recommends ingestion of ω-6 between 2.5-9% and ω3 between 0.5-2%. Appropriate consumption of those fatty acids helps regulating the inflammatory process, oxidative stress and endothelial function (Yang et al., 2015).

Contents of unsaturated in the oils were confirmed by determining their iodine index (II), a parameter used to express the degree of unsaturation and the proportion of different acyl groups in the samples (Table 1). Levels

of oleic, linoleic and linolenic acids found in the oils studied are in accordance with contents reported for non-Amazonian oils (Guillén and Ruiz, 2003), as for linseed (183.1 g I_2 100 g^{-1}), which is rich in linolenic acid (three unsaturations). For soy oil, the value reported for II is lower (127.6-129.9 g I_2 100 g^{-1}), which is compatible with its major constituent (linoleic acid, two unsaturations). For olive oil, iodine index is lower (77.4-80.7 g I_2 100 g^{-1}) since it is richer in the monounsaturated oleic acid.

$R_{o,a}$ values (Table 1) of the Amazonian oils studied varied from 0.71 to 1.2, and are in accordance with data reported for vegetable oils, in which this value should not be inferior to 0.7 (Carneiro et al., 2005). In this way, the values found in this work indicate that the fruits, already consumed in Roraima state (Brazil) are indeed adequate for human consumption. Carneiro et al. (2005) report that this property can be used to measure the degree of unsaturation present in oils and fats, in addition to indicating the oxidation state of samples, the latter with more precision than the peroxide index.

Acidity index indicates the good quality in obtaining and handling the oil, as well as oil degradation and adulterated, so the value assigned to the index should be inferior than or equal to 0.8% for olive oils extra virgin. All values found (Table 1) are below this value (Mailer and Beckingham, 2006).

In the saponification index, it is observed that the crude oils from the Amazon (Table 1) are in consonance, since the saponification index is inversely proportional to the average molecular weight of the fat (MW). This index is used to observe the size of the fatty acid chain (Carneiro et al., 2005).

Therefore, all the physicochemical parameters determined by ^{1}H NMR indicated the quality of the edible oils and fats present in the fruits studied, and can be used to detect adulteration or oxidation. In addition, this is a fast, practical and non-destructive technique for oils identification.

4. Conclusion

The Amazonian crude oils and fats here in studied presented high concentrations of ω9 fatty acids, followed by small concentrations of ω3 and ω6. *Tucumanzinho* presented ω9 contents close to that of olive oil, indicating that this oil can offer many benefits to the human health. As for the physical-chemical characterization, all samples are within the allowed values for human consumption.

This study shows that Amazonian biodiversity is able to provide fruits with essential fatty acids. This knowledge may contribute to the popularization of the consumption these fruits, as part of a heath diet or as components of novel gastronomic dishes inspired in healthy fruits.

Acknowledgments

We are grateful to CAPES for a fellowship and FAPEMIG for a grant, the CNPq for the financial support and to the Research Group Oleoquímicos UFRR.

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