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Characterization of Bioactive Molecules in Palm Oil Pulp (*Elaeis Guineensis* Jacq.)

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Oil palm (*Elaeis guineensis* Jacq.) is a crop that has great economic potential, since its productive potential is extremely high. It is grown in several countries in Asia, Africa and South America, with the main purpose of producing biodiesel raw material for the cosmetics and food industry. In Brazil, its cultivation is concentrated in only a few small regions, distributed mainly in the Northeast and North of the country. However, in spite of the few areas with commercial cultivation, the oil palm has potential of planting in other diverse areas such as the plantations of São João da Baliza in the of Roraima state. Sample collection was in São João de Baliza city, Roraima (Brazil). The pulp of fruit was taken out and dried in an oven with air circulation, material was milled and sieved to 20-40 Mesh. The lipid was obtained by Soxhlet and hexane as solvent. The lipid yield was 21.34%. The lipid analysis was on HP5890 gas chromatograph equipped with flame ionization detector, GC-FID. Eleven fatty acids were identified, where the majority were UFA were ω-9 (32.68%), ω-6 (8.34%) and ω-3 (0.29%) and the majority SFA were palmitic (44.16%) and palmitoleic acid (0.17%). The concentration of total carotenoids in the pulp from where were 587±0.21 mg kg^{-1} and the antioxidant activity by the DPPH method was 44.17 ± 0,12 % and by the iron reduction method of 117.31 ± 0.21 μmol $FeSO_4 100g^{-1}$ and the total phenol compounds in the palm oil pulp was 4.19 ± 0.32 mg GAE g^{-1}.

Keywords: *Arecaceae*, ω-3, Carotenids, Antioxidant Activity.

1. Introduction

African palm (*Elaeis guineensis* Jacq.) is native to West Africa and the Northwest Region of South America, whose plant has become one of the largest oil production palms worldwide (Carr, 2011; Myint et al., 2019). In Brazil, the production of this oil is 416,000 t $year^{-1}$ (Brazil, 2015). This oil has a high nutritional value derived from both its mesocarp and the seed and on the other hand, according to Corley (2009), it would be an alternative route to oil in the production of biofuels, since there is a growing trend in oil consumption, producing 240 million tons by 2050.

There are different types of methodologies for the extraction of fatty acids in foods, such as conventional extraction techniques such as cold pressing, percolation, maceration or solvent extraction, being techniques that sometimes have limitations such as degradation by part of the sample when using high temperatures and solvents, other less destructive techniques being modernly used for the samples such as ultrasonic extraction,

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microwave and supercritical fluid extraction (di Sanzo et al., 2018). When we do not have more sophisticated techniques in the laboratory, the most common technique for extracting oils is the use of solvents because of their speed compared to mechanical extraction but with the danger that these solvents and extraction system used can cause explosion (Berbel, 2010).

Among the bioactive compounds in palm oil pulp, fatty acids stand out, since they play diverse roles in plants, since they are the constituents of plant cell walls, providing a large energy reserve, as well as in translation of signals (Falcore et al.,1994). Among these fatty acids, the palmitic acid (16: 0) and oleic acid (18: 0) stand out in the mesocarp (Dussert et al., 2013). In addition to fatty acids, authors such as Sambanthamurthi et al., (2000), other beneficial constituents in African palm standout such as phenolic compounds, tocopherols, tocotrienols and carotenoids. These phenolic compounds are both bound to both esterified and insoluble fatty acids (Neo et al., 2010). Such antioxidant compounds are also found in another part of the palm such as the leaves (Soundarian and Sreenivasan (2013).

The objective of this work was to characterize the bioactive compounds in palm oil pulp from a production in the northern region of Brazil, including carotenoids, phenolic compounds as well as determine antioxidant activity and on the other hand the quantification of fatty acids in the oil extracted from said pulp in order to characterize the nutritional importance of said fruit.

2. Material and methods

2.1 Preparation of samples

The samples of *Elaeis guineensis* Jacq. they were collected in the municipality of São Jõao de Baliza, in the state of Roraima (Brazil) and subsequently taken to the Agricultural Sciences Center of the Federal University of Roraima, Cauamé campus, where they were washed with 1% sodium hypochlorite solution, separated those that presented an optimal appearance and subsequently the pulps were extracted, lyophilized and ground being preserved in an amber bottle until the moment of making the analyzes.

2.2 Fatty acid profile

The extraction of fatty acids was performed according to the Soxhlet extraction procedure, where cartridges with 100 grams of lyophilized material were placed and extracted with hexane for 4 hours, being performed in triplicate, subsequently, were dissolved in 2.0 mL cryogenic tube approximately 12 mg of sample lipid in 100 L of a solution of ethanol (95%) / Potassium hydroxide 1 mol L^{-1} (5%). After vortexing for 10 s, oil was hydrolyzed in domestic microwave oven (Panasonic Piccolo), a power of 80 W for 5 min. After cooling, 400 mL of hydrochloric acid 20% was added a spatula tip of NaCl (approximately 20 mg) and 600 mL of ethyl acetate. After vortexing for 10 s rest for 5 min. An aliquot of 300 mL of the organic layer was removed, placed into microcentrifuge tubes, dried by evaporation, thus obtaining free fatty acids (Christie, 1989). Subsequently, the free fatty acids were methylated with 100 µL of BF_3 / methanol (14%), by heating for 10 min in water bath at 60 ^{o}C. These samples were diluted in 400 mL of methanol and analyzed by gas chromatography.

The analyzes were performed on a HP- 7820A chromatograph (Agilent equipped with a gas flame ionization detector. As data acquisition program was used EZChrom Elite Compact (Agilent). HP- INNOWax column 15m x 0.25 mm x 0.20 microns with temperature gradient was used: 120 °C, 0 min 7 ºC.min^{-1} to 240 °C; injector (Split 1/50) detector at 250 °C and 260 °C. Hydrogen was used as carrier gas (3.0 mL.min^{-1}) and injection volume of 1 µL. Identification of the peaks was performed by comparison with standards of methylated fatty acids C14-C22 FAME (Supelco cat no 18917) (Christie, 1989).

2.3 Total phenolic compounds and antioxidant activity

The determination of total phenolic compounds (CFT) was made according to the methodology proposed by Wolfre et al. (2013) modified where methanolic extracts prepared from the extraction of 4.0 grams of lyophilized material with 35 mL of 80% (v/v) methanol acidified with 0.5% (v/v) hydrochloric acid were used, in falcon tubes and then placed in a 90 °C water bath for 30 minutes, the supernatant was separated and 35 ml were added again and treated under the same conditions as above. The fractions were then pooled and centrifuged at 6000 rpm for 30 minutes. The samples were placed in amber glass and were stored in a refrigerator at 2ºC until the moment of analysis. According to Singleton et al. (1999), to make the readings, gallic acid (G.A.) was applied as a reference standard, using the Shimadzu UV-1800 spectrophotometer. The reagents used were analytical grade.

The method involves the reduction of Folin's reagent by the phenolic compounds present in the blue complex-forming sample. A 0.1 mL aliquot of the extracts was transferred to a 10 mL test tube and added 3 mL of distilled water followed by 0.25 mL of Folin Ciocalteau reagent. The mixture was allowed to stand for 3 minutes and finally 2 mL of 7.5% (w/v) sodium carbonate solution (Na_2CO_3) was added. A blank test was also

used under the same conditions, so 0.1 mL of distilled water was used to replace the samples. They were incubated in a bath at 37 ºC for half an hour and readings were taken in a spectrophotometer at 765 nm, and the total phenols quantification was expressed as mg GA 100 g^{-1} sample.
The antioxidant activity in the different extracts was determined by different methods: the method of extinction of the absorption of 1,1-diphenyl-2-picrylhydrazyl radical (DDPH); and the iron reduction method. The DDPH method was developed by visible ultraviolet molecular absorption spectrophotometry, measured at 515 nm (MIRANDA; FRAGA, 2006) in Shimadzu UV-1800 model. For the antioxidant activity by the DPPH method, 300 μL of the methanolic extract with 2.7 mL of the 0.06 mM DPPH solution were added, waiting 60 minutes in a dark environment for further analysis at 515 nm. The calibration curve was made by preparing diluted standards from the 60 mM stock concentration in the range 10 to 50 μM and at the same time the white with methanol was made.

To determine the percentage of antiradical activity (%), equation 1 is used.

$$\% \text{ Antioxidant activity} = \left(\frac{A_{DPPH} - A_S}{A_{DPPH}}\right).100 \quad (1)$$

where:
A_{DPPH} = Control absorbance
A_S= Sample Absorbance

The second method to evaluate total antioxidant activity was by Fe3+ → Fe2+.reduction method. According to Sánchez-Moreno et al. (2008), the parameter IC50 corresponds to the concentration of the methanolic extract capable of inhibiting any oxidative process, including the corresponding free radical chain lipid peroxidation process.

$$IC_{50} = ((x_2-x_1)/(y_2-y_1))x(50-y_1)+x_1 \quad (2)$$

Where: X_1 = concentration (mg mL^{-1}) of the extract with inhibition percentage is less than 50%. X_2 = concentration (mg mL^{-1}) of the extract with percentage higher than 50%. Y1 = inhibitory percentage less than 50%. Y_2 = inhibitory percentage greater than 50%. The iron reduction methodology employed was described by Barros et al. (2010), using different concentrations of methanolic extracts. 0.5 mL aliquots of each concentration were mixed with 0.5 mL sodium phosphate buffer (200 mmol. L^{-1}, pH 6.6) and 0.5 mL potassium ferrocyanide (1% w /v in water). The mixture was incubated for 20 minutes at 50 ° C using 0.5 mL trichloroacetic acid (10% w/v) to stop the reaction.

$$2n(Fe(CN)_6)^{3-} + Ar(OH)_n \rightarrow 2n\ (Fe(CN)_6)^{4-} + Ar\ (=O)_n + 2_nH^+ \quad (3)$$

$$(Fe(CN)_6)^{4-} + Fe^{3+} \rightarrow (Fe(CN)_6)^{-} \quad (4)$$

Subsequently, a 1.5 mL aliquot of the mixture was transferred to a test tube with 1.5 mL deionized water and 0.16 mL iron chloride (0.1% w/v), taking absorbance readings. at 690 nm on the ultraviolet-visible molecular absorption spectrophotometer.

2.4 Total carotenoids

The determination of total carotenoids was carried out using the technique described by Lichtenthaler and Buschmann (2001) with modifications using the UV-visible molecular spectrophotometry where 18 mL of acetone was added to 1 gram of lyophilized palm oil pulp, being carotenoids removed by shaking for 20 minutes in the absence of light. Samples are filtered and absorbance readings are carried out at concentrations of 661 nm, 644 nm and 470 nm respectively, to calculate the concentration of carotenoids using equations 5-7.

$$C\ carotenoids\ (\mu g\ mL^{-1}) = (1000\ A470 - 1.90\ Ca - 63.14\ Cb) / 214 \quad (5)$$

$$Ca\ (\mu g\ mL^{-1}) = 11.24\ A661 - 2.04\ A644 \quad (6)$$

$$Cb\ (\mu g\ mL^{-1}) = 20.13\ A664 - 4.19\ A661 \quad (7)$$

3. Results and Discussion

3.1. Fatty acid profile in palm oil pulp oil

In Table 1, the results of the fatty acid profile for the oil extracted from the Palm oil pulp are presented.

Table 1: Fatty acids in Eleasis guineansis Jacq.

Fatty acids	Composition	Retencion time (min)	%
Lauric acid	C12:0	1.563	0.61
Myristic acid	C14:0	2.862	0.84
Pentadecylic acid	C15:0	3.735	0.04
Palmitic acid	C16:0	4.742	44.16
Palmitoleic acid	C16:1	4.903	0.17
Estearic acid	C18:0	6.867	5.21
Oleic acid	C18:1	7.071	32.68
Linoleic acid	C18:2	7.562	8.34
Linolenic acid	C18:3	8.197	0.29
Araquic acid	C20:0	8.977	0.31
Total acids	-	-	92.65
UFA[1]	-	-	48.83
SFA[2]	-	-	51.17
Others	-	-	7.35

[1]UFA – unsaturated fatty acids and [2]SFA - saturated fatty acids.

In total, 10 fatty acids were determined, of which 51.17% of SFA and 48.83% of UFA correspond. The determined fatty acids are all medium-chain and long-chain, with the highest concentration of saturated than unsaturated, the majority being palmitic acid with 44.16%. These saturated fatty acids are transferred to the bloodstream and in that transport they are bound to the albumin protein where they are metabolized by the l increasing serum cholesterol levels (Patterson et al., 2011). Studies developed by Sambanthamurthi and Sreenivasan (2013), highlight the antioxidant properties of this palm, especially in leaf extracts.
On the other hand, the determined unsaturated fatty acids were palmitoleic acid (0.17%), oleic acid (32.68%), linoleic acid (8.34%) and linolenic acid (0.29%). Among the majority UFA, oleic acid stands out, being the one responsible for the prevention of cardiovascular diseases, being able to reduce serum levels of triacylglycerol's that contribute to the reduction of inflammation of the blood vessels (Cunnane et al., 2009).

3.2. Antioxidants, phenolic compounds and carotenoids in palm oil pulp

In Table 2, the values of total phenolic compounds, antioxidant activity by means of the DDPH method and by the iron reduction method as well as the total carotenoids in the palm oil pulp are presented.

Table 2: Phenolic compounds, antioxidant activity and total carotenoids

Total Carotenoids (mg kg^{-1})	587 ± 0.21
Total phenolic compounds mg GAE g^{-1}	4.19 ± 0.32
Antioxidant Activity	
DPPH (%)	44.17 ± 0.12
Iron reduction methods μmol $FeSO_4 100g^{-1}$.	117.31 ± 0.21

The concentration of total carotenoids in this work was 587 ± 0.21 mg kg^{-1}, these values are within the determined concentrations of carotenoids for said palm since the range of concentrations for carotenoids varies between 500-700 ppm (Zeb and Mohmeed, 2004). These molecules have biotechnological interest since they have pro-vitamin A activity (Deming et al., 2002). In comparison with other studies conducted in Amazonian palm trees such as the work carried out by Santos et al., (2015), these values are close to those found for the *Mauritia flexuosa* pulp (540.81 ± 34.50 mg kg^{-1}) but lower at the values obtained for the *Astrocaryum aculeatum* pulp (1222.33 ± 36.09 mg kg^{-1}) the latter palm being one of the Amazonian palms with the highest concentration in carotenoids.
On the other hand, the determination of the total phenolic compounds was expressed in mg of gallic acid equivalents GAE g^{-1} of sample, where the value obtained for the pulp of this fruit, are within the values

determined by Tsouku et al., 2019 for different parts of the African palm. Phenolic compounds are present in higher plants, also known as phenylpropanoids, synthesized from the shikimic acid route whose main property is the behavior as an antioxidant (Jovanovic, 2006). The antioxidant activity was evaluated by two different methods: in the DPPH reduction method an antioxidant activity of 44.17 ± 0.32 mg GAE g^{-1} was obtained and by the iron reduction method of 117.31 ± 0.21 μmol $FeSO_4 100g^{-1}$. According to the classification performed by Vasco et al. (2008), the African palm pulp has a moderate antioxidant activity and inferior to other Amazonian fruits studied by Montero et al., 2018.

4. Conclusions

This paper highlights the importance of Palm oil as a functional food due to the amount of bioactive compounds that it possesses in its chemical composition such as carotenes, antioxidants, phenolic compounds and unsaturated fatty acids that give these plant nutraceutical properties with biotechnological interest.

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