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Fatty chemical composition and antioxidant activities of coconut oils (*Cocos nucifera* L)

Aluísio M. da Fonseca[1*] Délcio D. Marques[2] Telma Leda G. Lemos[2] Gisele R. Aguiar[2] and Ayla Márcia C. Bizerra[2]

[1]Instituto de Ciências e da Natureza, Campus da Liberdade, Avenida da Abolição 3, Centro, CEP: 62.790-000, Redenção – CE, Brazil.
[2]Departamento de Química Orgânica e Inorgânica, Campus do Pici, s/n, Avenida Humberto Monte, Pici, CEP: 60.455-760, Fortaleza – CE, Brazil.

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Fixed oils from two cultivars (green and yellow) of solid albumen in the coconut palm (*Cocos nucifera*), were obtained by a solvent extraction using hexane in three different maturation stages, such as: unripe; ripe and dried. The aforesaid oils were saponified and methylated. Fatty acids and methyl esters were analyzed by the gas chromatography, mass spectrometry (CG-MS). The oils from unripe (green and yellow) coconuts, as major constituents, were identified with hydrocarbons, thioesters and carboxylic acids, as well as the major compounds being 69 and 65%, respectively. The oils for the ripe coconut, having major compounds, corresponded to 74 and 70% for the green and yellow variation, respectively. In these said experiments, a common set of fatty acids were detected. Therefore, for the dried coconut, the main constituents corresponded to 99.98 and 98.11% (green and yellow) variations. The results of the free radical scavenging effects in the fixed oil from the coconuts of the both cultivars, showed a concentration-dependent activity with IC_{50} varying from $5.2x10^{-6}$ to $1.1x10^{-4}$.

Key words: Coconut oil, fatty acid, chemical composition, antioxidant activity.

INTRODUCTION

The coconut tree is one of the most important palm trees cultivated in the tropics (Laureles et al., 2002); it belongs to the *Arecaceae* and it is characterized by three drupaceous ovaries confined in the fruit with a rigid endocarp of three germinal pores, as well as the fruit being usually a seed (Ejedegba et al., 2007). These species, in turn, are composed of some varieties, whereby the most important are the “typica” (Giant variety) and “nana” (dwarf) varieties). The latter, is composed by yellow and green varieties, Malaysia´s red and Camerron´s red cultivars (Aragão et al., 1999). The *natura* for daily delicious and nutritional beverage. This palm tree plays an important role as a subsistence crop thought its commercial oil (Aragão et al., 2002). The dried coconut is only a stage where the shell undergoes dehydration and simultaneously, the liquid albumen decreases and the water

*Corresponding author. E-mail: aluisiomf@unilab.edu.br. Tel: +55 (85) 8811-2586

the water juice dries (Cascudo, 1983). The coconut oil called “oleo-de-coco” is divided into two categories: virgin and refined. The vigin oil type is obtained starting from fresh fruits (ripe coconut). On the green cultivar is very common in Brasil and it is used *in* flip side, the refined oil is obtained typically from the dried coconut, named “Copra” (Kabara, 1990). According to the ethnobotanical knowledge, the coconut for oil extraction, is chosen based on their weight and the amount of water it has. In order to see if it is in good condition, it is good enough to hit it with a coin in its shell. If it is cool, the sound is loud; if the sound is hollow, it indicates that the fruit is rotten and has oxidized. Daily published reports showed that a considerable percentage of the coconut oil, is constitute carbon chain C6-C12 (Lópes-Villalobos et al., 2001).

The main constituents of the coconut fixed oil are the triacylglycerols and the carbon chain do not exhibit an unsaturation degree. In this particular case, the major portion is due to the lauric acid and the myristic acids (dodecanoic acid), followed by a small amount of the linoleic acid (Pham et al., 1998; Nevin and Rajamohan, 2006; Laureles et al., 2002). Concerning the biological benefits, the comparison between virgin and Copra oils showed that the former presents higher benefits than the Copra oil, such as a reduction of the levels in the total cholesterol, phospholipids and LDL-c, as well as an increase in the tissular levels and the series HDL-c (Guo et al., 2006), antithrombotic effect, fibrinogen, factor V, 6-ketoPGF1E, Prothrombin time (Nishi et al., 2005), besides showing an antioxidant activity. Despite the antioxidant activity in the virgin oil promoting a reduction in the lipid peroxidation *in vitro,* as well as *in vivo* (Nevin and Rajamohan, 2006), and a reduction of the abdominal fat in the men, besides the loss of corporal weight and the reduction of the total fat mass (Guo et al., 2006; Nishi et al., 2005; Nevin and Rajamohan, 2008; Stonje et al., 2003; Stonge and Bosarge, 2008; Stonge and Jones, 2002; Hargrave et al., 2005).

The oxidative process is associated in several diseases, such as the cardiac and the Alzheimer in our continuous research for natural antioxidants. The activities of oils from the coconut were evaluated. The coconut juice was previously reported to be a source of antioxidants. The present work has the purpose to determine and compare oil compositions in the coconut from two varieties, each one in three different maturation stage: unripe, ripe and dried, besides the analysis of antioxidant activities in these oils.

MATERIALS AND METHODS

Plant material

The fruits samples in the *Cocos nucifera,* were harvested in the city of Icarai (Geographical Coordinates: 3° 41' 0" South, 38° 40' 0" West), State of Ceará, Brazil in February, 2011. The plant identification was done by Edson P. Nunes and the voucher specimens # 30848 and 30849 have been deposited in the Herbario Prisco Bezerra’s, Biology Department of the Federal University of Ceara in Brazil.

Assays

Extraction of the Fixed Oil from the Solid Coconut Albumen

The unripe, ripe and dried of the green and yellow coconuts (albumen, Figure 1), were cut in small pieces of about 1cm. The cut pieces (200g) were placed in an Erlenmeyer 500mL and extracted with the hexane (200mL) for 48 hours in room temperature. The solid material was then filtrated in vacuum and solvent, as well as evaporated under a reduced pressure. The duly obtained weights (g) and yields (%) respectively were: unripe 1G (2.0/1.0), 1Y (2.2/0.4), ripe 2G (3.9/2.0), 2Y (2.7/1.4) and dried 3G (7.0/2.2), 3Y (5.0/1.6). The residue were named 1G, 2G, 3G, 1Y, 2Y and 3Y oils for the green/yellow coconut in the stages as unripe, ripe and dried, respectively.

Saponification/ methylation of the coconut oils

Each of the oil samples, 2.0g (1G, 2G, 3G, 1Y, 2Y and 3Y), were dissolved in 15.0mL of MeOH (11.85g/0.37mol) with a stoichiometric amount of the NaOH (0.37mol/14.8g) and left to react in room temperature for two hours. The solvent residue evaporated until dryness. The yielded saponified compounds duly obtained are summarized as follows: unripe 1G (2.4 g), ripe 2G (4.3g), dried 3G (5.2g), unripe 1Y (2.1g), ripe 2Y (3.8g) and dried 3Y (5.4g). The methylation from each oil was carried out using the MeOH (10mL) in acidic medium (2mL of HCl) heating during 1.5h. The reacted products were submitted to a chromatography column in silica gel, using hexane and chloroform (9:1) as eluents. The products were analyzed in the GC/MS. (Table 1).

Antioxidant activity

This spectrophotometer assay used the stable radical DPPH, as a reagent (Burits and Bucar, 2000; Hegazi and El Hady, 2002). These aforementioned experiments were carried out with oil samples, before the derivatization. The experiments consisted in preparing seven concentrations, each fixed with oil and mixed with the same volume of 60μM DPPH solution (e.g., 500μL). This particular analysis was measured in the spectrophotometer at 520nm and the inhibition of the free radical DPPH (in percent, I%) was calculated using the formula below:

$$I\,\% = [1 - \frac{A_{sample}}{A_{blank}}] \cdot 100$$

Where the A_{blank} is the absorbance in the control reaction (containing all the reagents, except the oil sample), while the A_{sample} is the absorbance of the test sample. The IC_{50} value was calculated from the plot of the inhibition percentage against the sample concentration. These said tests were carried out in triplicate. Six samples (oil unripe green, oil unripe yellow, oil ripe green, oil ripe yellow, oil dried green and oil dried yellow), as well as the DPPH were dissolved in ethanol. The Trolox and the BHT were used as a positive control and the results are presented on Table 2.

Statistical analysis

The results are expressed as mean ± S.E.M. However, the one-way analysis of the variance (ANOVA) was used. Whereas, in the

Figure 1. Photography of albumen of two cultivars of coconut: yellow and green skin fruit.

antioxidant activity assay, the one-way ANOVA test was used, followed by the Tukey test ($P < 0.001$).

Apparatus/ Chemical analysis

The quantitative analysis of all the oil samples was performed in a Shimadzu GC-17A gas chromatography, using a dimethylpolysiloxane DB-5 fused silica capillary column (30m × 0.25mm, film thickness 0.25µm) and a flame ionization detector (FID). The H_2 was used as the carrier gas at a flow rate of 1mL/ min and with a 30psi inlet pressure; split 1:30; temperature program: 35 to 180 °C at 4 °C/ min, then heated at a rate of 17°C/ min to 280°C and held in isothermal for 10 min; injector temperature 250°C and the detector temperature was at 250°C. The fatty oils compositions were obtained from the GC/MS analysis, while the analysis of the samples were performed with a Hewlett-Packard 5971 GC/MS instrument, using the following conditions: dimethylpolysiloxane DB-1 fused silica capillary column (30m x 0.25mm, 0.1µm film thickness); carrier gas: He (1mL/ min); injector temperature: 250 °C; detector temperature: 200°C; column temperature: 35° to 180°C at 4°C/ min, then 180 to 250°C at 10°C/ min; mass spectra: electronic impact 70 eV. Each component was identified by two computers combined with library microsoft searches, using their retention indices followed by a visual inspection of the mass spectra from the literature (Adams, 2007) and the standards, NIST 98 and Wiley MS Libraries (Wiley 275). The retention index was calculated using reference, the retention times of a series in the standards for hydrocarbons (C11-C-28), under the same conditions, according to the calculations of the literature (Porte, 2000).

RESULTS AND DISCUSSION

The unripe oil of the green variety (1G), showed in the CG-MS analysis nine compounds as: 38.05% of carboxylic acids and 48.46% as hydrocarbons. The major compounds for this stage were methyl palmitate (19.23%); methyl elaidate (11.55%); isotetradecane (15.20%) and isohexadecane (14.37%). The unripe oil of the yellow variety (1Y) showed 67.32% of hydrocarbon and only 3.68% of the carboxylic acid. The major compounds for this stage were the isotetradecane (20.64%) and the isohexadecane (18.15%). In this particular stage, the coconut juice (1G) is used as a delicious soft drink, however, the juice from the yellow (1Y) coconut is not consumed, since the taste is not pleasant. The CG-MS analysis in the ripe oil from the green coconut (2G), showed 8.43% of hydrocarbons and 93.44% of the carboxylic acid, and the major compounds were the methyl laurate (38.27%); methyl mirystate (19.37%) and the methyl palmitate (12.38%). In the chemical composition of the ripe yellow oil (2Y), it was identified as having 4.62% of hydrocarbons and 68.26% of the carboxylic acid, when major compounds were identified as the same as the green variety in the ratio of 30.21, 12.34 and 9.00% respectively. The yield of oils and the presence of the carboxylic acid in this stage increased the composition with the unripe stage. At this point, the green coconut (2G) is used in the production of foods such as: cake, ice-cream and sweets. The dried stage of the green coconut (3G) is composed by 9.52% of hydrocarbons and 90.46% of the carboxylic acids, where the methyl laurate (57.80%) and the methyl mirystate (17.55%) were the major compounds. The yellow variety (3Y) yield with 9.39% of hydrocarbons and 88.72% of the carboxylic acids, were the major compounds and being the same of the green coconut and the ratios were 40.49 and 21.06% respectively. The yield of oils as well as the major constituents such as the lauric acid increased as the coconut became older. By now, the albumen of the coconut from the green variety (3G) is used with the extraction of oils and commercialized. The results, from the free radical scavenging effect with the fixed oil from the coconut of both cultivars, showed concentration-dependent activities. Concerning both cultivars, the free radical scavenging effect of the sample had the fixed oil of the ripe coconut and it was better compared to others stages (unripe and dried). The sample of the ripe stage showed significant antioxidant activity compared to the IC50 of Trolox and the BHT, which concludes that this composition may have increased the activity (Table 2).

Conclusion

Hydrocarbons, thioesters and the carboxylic acids were detected in the fixed oil of the two specimens with the solid albumen from the coconut in the three phases of maturity

Table 1. Chemical composition of coconut oils as methyl esters from the green (1G, 2G, 3G) and yellow (1Y, 2Y, 3Y) cultivars.

Compound	RT	Unripe (1G,1Y)		Ripe (2G, 2Y)		Dried (3G, 3Y)		Identification
		Green	Yellow	Green	Yellow	Green	Yellow	
Methyl hexanoate (methyl caproate)	3.10	-	-	-	0.90	-	0.24	RT, MS
Methyl octanoate (methyl caprilate)	6.25	-	-	2.10	1.20	1.98	4.51	RT, MS
Tridecane	10.40	5.96	8.41	0.18	0.10	-	-	RT, MS
Methyl decanoate (methyl caprinate)	10.96	-	-	3.87	2.42	7.54	4.64	RT, MS
2,6,11-trimetildodecane	12.34	4.20	-	-	-	-	-	RT, MS
Isotetradecane	12.91	15.20	20.64	-	-	-	-	RT, MS
2,8-dimetilundecane	14.47	8.73	11.51	-	-	-	-	RT, MS
Isohexadecane	15.40	14.37	18.15	-	-	-	-	RT, MS
Methyl dodecanoate (methyl laurate)	16.13	-	-	38.27	30.21	57.80	40.49	RT, MS
Dimyristyl tiodipropionate	20.12	3.95	-	-	-	-	-	RT, MS
n-heneicosane	20.13	-	6.28	-	-	-	-	RT, MS
Methyl tetradecanoate (methyl mirystate)	20.73	-	-	19.37	12.34	17.55	21.06	RT, MS
n-eicosane	23.64	-	2.33	-	-	-	-	RT, MS
Methyl hexadecanoate (methyl palmitate)	24.05	19.23	3.68	12.38	9.00	7.85	11.57	RT, MS
Methyl 9,12-octadecadienoate (methyl linoleate)	26.13	7.27	-	4.08	1.07	-	1.86	RT, MS
Methyl 9-octadecenoate (methyl elaidate)	26.23	11.55	-	16.37	7.90	4.90	8.44	RT, MS
Methyl ocadecanoate (methyl estereate)	26.50	-	-	2.97	3.12	2.36	5.30	RT, MS

RT – retention time; 1, 2, 3 - maturation stages unripe, ripe and dried; MS – mass spectrum.

Table 2. The DPPH free radical scavenging activity of oils samples[a,b]

Oil of coconut albumen	Concentration (µg/ml)								IC_{50} (µg/ml)
	0.1		0.001		0.00001		0.000001		
	Abs	Percent	Abs	Percent	Abs	Percent	Abs	Percentage (%)	
Unripe									
Green (1G)	0.0849±0.00005	68.9	0.1825±0.00003	33.2	0.2133±0.00002	21.9	0.2483±0.00003	9.1	5.2×10^{-3}
Yellow (1Y)	0.0937±0.00002	65.7	0.1992±0.00004	27.1	0.2240±0.00002	18.0	0.2532±0.00003	7.3	9.5×10^{-3}
Ripe									
Green (2G)	0.0713±0.00004	73.9	0.1571±0.00005	42.5	0.1882±0.00005	31.1	0.2314±0.00003	15.3	1.3×10^{-3}
Yellow (2Y)	0.0805±0.00003	70.5	0.1606±0.00004	41.2	0.1915±0.00002	29.9	0.2275±0.00005	16.7	1.7×10^{-3}
Dried									
Green (3G)	0.1377±0.00003	49.6	0.1912±0.00002	30.0	0.2221±0.00003	18.7	0.2478±0.00005	9.3	1.1×10^{-1}
Yellow (3Y)	0.1300±0.00003	52.3	0.1928±0.00006	29.4	0.2183±0.00003	20.1	0.2393±0.00005	12.4	5.9×10^{-2}
Trolox	0.0110±0.00003	96.0	0.0585±0.00002	78.6	0.1439±0.00002	47.3	0.2043±0.00005	25.2	1.9×10^{-5}
BHT	0.0052±0.00003	98.1	0.0538±0.00007	80.3	0.1210±0.00003	55.7	0.1909±0.00002	30.1	7.4×10^{-6}
Control	0.2732±0.00003	0.0	0.2732±0.00005	0.0	0.2732±0.00005	0.0	0.2732±0.00005	0.0	0.0

[a]The free radical scavenging effect was measured by the absorbance radical at 520 nm in a reaction containing the test sample and 60 µM DPPH; [b]Results are expressed as mean ± S.D.

duly analyzed therefore, are probably responsible in part, for the viscosity and odor of this particular oil. Furthermore, the palmitic acid has been present and increasing the concentration of each maturity phase for the skin yellow coconut, on the flip side, the palmitic acid has been present and decreasing the concentration of each maturity phase for the skin green coconut, probably it is a compound common to all. It was also observed in relation to the maturity, that a lot of the green species especially the yellow type had the amount of fixed oil increased, in other words, the composition was richer in the fat acids. In addition, it was observed as well that when it is unripe their majority constituents are formed by ramified hydrocarbons, which means that they can probably be oxidized in the following carboxylic acids when becoming ripe or dried. A high scavenging activity was found in samples obtained from the ripe coconut and a moderate activity was observed in the unripe and the dry samples. The authors of this study suggest that the data from the antioxidant activities in the coconut oil, can be used as a natural antioxidant additive.

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Conflict of Interest

We declare that we have no conflict of competing interest.

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