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Artigo

Constituents and antioxidant activity of two varieties of coconut water (*Cocos nucifera* L.)

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RESUMO: "Constituintes químicos e atividade antioxidante de duas variedades de água de coco (*Cocos nucifera* L.)". Uma análise dos componentes da água-de-coco (*Cocos nucifera* L.) de duas variedades da fruta (verde e amarelo) por hidrodestilação e extração com solvente, mostrou a presença de álcoois, cetonas, tióis, ácidos carboxílicos, fenóis, e ésteres. Significativa atividade antioxidante foi observada, usando o método DPPH, para as amostras obtidas por hidrodestilação e extração de éter de petróleo para ambas as variedades do coco.

Unitermos: *Cocos nucifera*, Arecaceae, água-de-coco, composição química, atividade antioxidante.

ABSTRACT: An analysis of the constituents of coconut (*Cocos nucifera* L.) water from two fruit varieties (green and yellow) by hydrodistillation and solvent extraction showed the presence of alcohols, ketones, thiols, carboxylic acids, phenols, and esters. Substantial antioxidant activity was observed, using the DPPH assay, for the samples obtained by hydrodistillation and petroleum ether extraction of both coconut varieties.

Keywords: *Cocos nucifera*, Arecaceae, coconut water, chemical composition, antioxidant activity.

INTRODUCTION

Cocos nucifera L. (Arecaceae) is a 20-30 m high tropical palm tree cultivated, preferentially, on beach coastal areas. Its fruits are popularly known as "coco", forming a bunch or a cluster of fruits in different stages of development (Reddy et al., 2005).

In the northeast of Brazil, State of Ceará, the coconut tree is available as two main varieties, giant and dwarf, being subdivided in green, yellow, and red varieties. The water from the green variety of coconut is widely consumed as a soft drink, and is known to possess beneficial health properties (Farr, 1994; Campbell-Falck et al., 2000; Pummer et al., 2001; Mepba and Achinewhu, 2003; Agra et al., 2007 and 2008), whereas the liquid produced from the yellow and red coconut varieties type is little used as a drink, because it is not so sweet as the green variety, and thus it is less cultivated.

Coconut water has a *sui generis* flavor, being sweet and slightly acid (Borse et al., 2007). It contains proteins, fats and minerals such as sodium, potassium, magnesium and calcium electrolytes (Chumbimuni-Torres and Kubota, 2006; Jirovetz et al., 2003). The nutritional composition of coconut water has been well documented (Santoso et al., 1996), but to the best of our knowledge there is no report comparing the chemical compositions of the water produced by the green and yellow varieties of coconuts.

In recent years, there has been considerable interest in finding sources of natural antioxidants, especially from fruits and vegetables which have provided a measure of protection against the process of oxidative damage in the human species (Jacob and Burri, 1996). Antioxidant compounds from plants, particularly flavonoids and other polyphenols, have been reported to inhibit the propagation of free radical reactions, and to protect the human body from disease (Kinsella et al., 1993). Moreover, antioxidant activities have been associated with acids (Loki and Rajamohan, 2003), aromatic compounds (Agnaniet et al., 2005; Letizia et al., 2000), aldehydes (Yi and Kim, 1982), and esters (Narain et al., 2007). Coconut water also possesses antioxidant properties (Leong and Shui, 2002; Skrede et al., 2004). This property was verified mainly in the coconut water *in natura*, and it decreases drastically with the use of thermal treatments, or the actions of

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acids or alkaline on coconut water, and with the degree of maturation of the fruits (Mantena et al., 2003).

The present work reports a study of the low molecular weight, hydrodistillable or petroleum ether extractable constituents of the coconut water from the green and yellow varieties of coconut (Figure 1) collected in Fortaleza, Ceará, Brazil, and their antioxidant activities using the DPPH assay.

Figure 1. Photography of two types of coconut fruits.

MATERIAL AND METHODS

General procedures

GC-FID: The quantitative analysis was carried out on a Shimadzu GC-17A gas chromatograph using a dimethylpolysiloxane DB-5 fused silica capillary column (30 m x 0.25 mm, film thickness 0.25 μm). H2 was used as the carrier gas at a flow rate of 1 mL/ min and 30 psi inlet pressure; split, 1:30; temperature program: 35-180 °C at 4 °C/ min, then heated at a rate of 17 °C/ min to 280 °C and held isothermal for 10 min; injector temperature, 250 °C; detector used FID, detector temperature, 250 °C.

GC/MS: analysis of the oils was performed on a Hewlett-Packard 5971 GC/MS instrument employing the following conditions: dimethylpolysiloxane DB-1 fused silica capillary column (30 m x 0.25 mm, 0.1 μm film thickness); carrier gas: He (1 mL/ min); injector temperature: 250 °C; detector temperature: 200 °C; column temperature: 35-180 °C at 4 °C/ min, then 180-250 °C at 10 °C/ min; mass spectra: electronic impact 70 eV. Individual components were identified by two computer library MS searches using retention indices as a pre-selection routine (Stenhagen et al., 1974) and visual inspection of the mass spectra from the literature for confirmation (Adams, 2001).

Plant material

The samples of *Cocos nucifera* fruits were harvested, from Icarai (Caucaia) County, State of Ceará, Brazil, in February, 2007. The two varieties were authenticated by Professor Edson P. Nunes, and the voucher specimens (N°35458 and Nº 35459) were deposited at the Herbarium Prisco Bezerra (EAC) of Departamento de Biologia, Universidade Federal do Ceará.

Extraction of the constituents from samples of coconut water by hydrodistillation

The coconut water (2 L) from the two varieties was separated from the coconut fruit in a glass container (several fruits from the same tree). The essential oil of the coconut water was extracted by steam distillation in a Clevenger-type apparatus for two hours. Hydrodistillation was carried out in triplicate. The hydrolyte (20 mL) was extracted with petroleum ether (3 x 10 mL), and the solvents were evaporated under vacuum, yielding 32 mg and 45 mg for the green (sample 1) and yellow (sample 2) coconut water varieties, respectively. The extracts obtained from the two varieties were analyzed by GC/MS and GC-FID and the results are presented in Table 1. In addition, the extracts were submitted to the DPPH assay, and the results are presented in Table 2.

Extraction of the constituents from samples of coconut water using petroleum ether

Coconut waters from the two varieties (green and yellow) (2 L) were extracted with petroleum ether (3 x 300 mL). The solvents were dried with anhydrous Na_2SO_4 and concentrated under vacuum yielding residues of 0.26 g (sample 1') from the green variety and 0.30 g (sample 2') from the yellow variety. The extracts obtained from the two varieties were submitted to silica gel column chromatography using petroleum ether as eluent. Fractions were pooled according to their TLC profile, resulting in 30 mg of sample 1' and 56 mg of sample 2'. Samples were analyzed by GC/MS and GC-FID and the results are presented in Table 1. The extracts were also submitted to the DPPH assay, and the results are presented in Table 2.

Antioxidant assay

The hydrogen or electron donation abilities of the compounds were measured from the bleaching of the purple-colored ethanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH). This spectrophotometer assay uses the stable radical DPPH as a reagent (Bandeira et al., 2006; Burits and Bucar, 2000; Hegazi and El Hady, 2002). The sample solution of material (500 μL) at four concentrations (1.0, 0.5, 0.25, and 0.125 μg/μL) was mixed with the same volume of 60 μM DPPH solution

Table 1. Constituents identified from the hydrodistillation or petroleum ether extraction of samples of the water from the green and yellow varieties of coconut.

Compounds	RT	Sample 1	Sample 2	Sample 1'	Sample 2'	RI	Identification
				%			
n-Propyl ethanoate	3.05	15.3		53.5	16.7	750	RI, MS, Lit
1-Methylpropyl methanoate	3.18			4.1		752	RI, MS, Lit
3-Hydroxybutan-2-one	3.24			4.5	6.2	753	RI, MS, Lit
Methyl 2-hydroxypropanoate	3.30		0.9			754	RI, MS, Lit
4-Methylpentan-2-one	3.65	12.0		29.0		759	RI, MS, Lit
Ethyl 2-hydroxypropanoate	4.00			0.7		764	RI, MS, Lit
Butane-1,3-diol	4.02			1.7	67.7	765	RI, MS, Lit
Butane-1,2-diol	4.04			3.1	3.0	766	RI, MS, Lit
Butane-2,3-diol	4.13			3.2	3.6	767	RI, MS, Lit
Ethyl carbonate	4.17	2.1				768	RI, MS, Lit
Hexan-2-ol	4.67		1.0			774	RI, MS, Lit
n-Butyl ethanoate	4.83	2.0				776	RI, MS, Lit
Furfural	5.18	14.5	0.8			781	RI, MS, Lit
4-Hydroxy-4-methylpentan-2-one	5.41	30.5				784	RI, MS, Lit
2-Hydroxy-2-methylpentane	6.73	3.2				803	RI, MS, Lit
p-Menth-8-en-1-ol acetate	11.60	1.4				873	RI, MS, Lit
Phenyl acetaldehyde	11.87		0.9			877	RI, MS, Lit
4-Methoxybenzyl acetate	25.21		5.3			1069	RI, MS, Lit
7,9-Di-*ter*-butyl-oxaspiro[4.5] deca-6,9-diene-2,8-dione	36.79		4.5			1235	RI, MS, Lit
Methyl 6,9,12-octadecatrienoate	37.21		4.2			1241	RI, MS, Lit
n-Hexadecanoic acid (palmitic acid)	37.46		18.4			1244	RI, MS, Lit
3,6-Dioxane-1,8-diol	38.64		5.0			1262	RI, MS, Lit
3-Mercaptodecane	39.47		46.2			1274	RI, MS, Lit
9-Octadecenoic acid (oleic acid)	39.66		5.3			1276	RI, MS, Lit
9-Octadecen-1-ol	41.58		6.1			1300	RI, MS, Lit
Dioctyl adipate	41.62	11.2				1304	RI, MS, Lit

RT: retention time in minutes; Samples 1 and 2: constituents isolated by the hydrodistillation process; Samples 1' and 2': constituents isolated by the solvent extraction process; RI: retention indices on DB-5 column; MS: mass spectra; Lit: literature. Samples 1 and 1': From the water from the green variety of coconut; Samples 2 and 2': From the water from the yellow variety of coconut.

Table 2. The DPPH free radical scavenging activity of the constituents obtained by hydrodistillation (samples 1 and 2), and by extraction using petroleum ether (samples 1'and 2'), from the green and yellow varieties of coconuts.

Sample	Concentration (μg/μL)								
	1.0		0.5		0.25		0.125		
	Abs	%	Abs	%	Abs	%	Abs	%	IC_{50}(μg/μL)
Control	0.2380	00.0	0.2380	00.0	0.2380	00.0	0.2380	00.0	0.0
1	0.0490	79.4	0.0557	76.6	0.0838	64.8	0.1399	41.2	0.19
2	0.0393	83.5	0.0490	79.4	0.0781	67.2	0.1607	32.5	0.24
1'	0.1630	31.5	0.1780	25.2	0.1990	16.4	0.2300	3.4	>1.0
2'	0.1550	34.9	0.1690	29.0	0.1780	27.7	0.1930	18.9	>1.0
Trolox	0.0336	85.9	0.0583	75.5	0.0616	74.1	0.0928	61.0	0.063
BHT	0.0450	81.1	0.1085	54.4	0.1126	52.7	0.1345	43.5	0.17

Samples 1 and 2: Constituents obtained by hydrodistillation; Samples 1' and 2': Constituents obtained by extraction using petroleum ether.

and allowed to stand for 30 min at room temperature. The absorbance was then measured at 520 nm using a spectrophotometer and the inhibition of free radical DPPH in percent (I%) was calculated using the formula below; where Ablank is the absorbance of the control reaction (containing all of the reagents except for the test compound), and Asample is the absorbance of the test compound. The compound concentration demonstrating 50% inhibition (IC_{50}) was calculated from the plot of inhibition percentage against sample concentration. Tests were carried out in triplicate (Tepe et al., 2005). Samples 1, 1', 2, 2', and DPPH were dissolved in ethanol. Trolox and BHT were used as positive control samples and the results are presented in Table 2.

Statistical analysis

The results were expressed as mean ± S.E.M. One-way analysis of variance (ANOVA) was used. In the antioxidant activity assay, one-way ANOVA test was used follow by Tukey test ($P < 0.001$).

RESULTS AND DISCUSSION

The essential oils obtained by the hydrodistillation process from the two varieties of coconut water, green (sample 1), and yellow (sample 2), showed different chemical compositions. The essential oil from the water of the green coconut variety was characterized by a high level of ketones (42.5%) and esters (29.9%), followed by aldehydes (14.5%) and alcohols (3.2%), and representing 90.1% of the total sample. The major constituents were identified as, 4-hydroxy-4-methylpentan-2-one (30.5%) and *n*-propyl ethanoate (15.3%). The essential oil analysis from the water of the yellow coconut variety was characterized by the presence of thiols (46.2%), carboxylic acids (23.7%), alcohols (12.1%), esters (10.4%), lactones (4.5%), and aldehydes (1.7%), and represented 98.6% of the total sample. The major components of the essential oil of this sample were 3-mercapto-decane (46.2%) and *n*-hexadecanoic acid (18.4%).

The compounds of the coconut water from the green and yellow varieties were analyzed in samples obtained by extraction using petroleum ether as solvent. Eight compounds were identified in sample 1' (green variety), and were characterized as esters (58.3%), ketones (33.5%), and diols (8.0%), representing 99.8% of the total extract. For the specimen of the yellow variety, five components were identified and characterized as diols (74.3%), esters (16.7%), and ketones (6.2%), representing 97.2% of total extract. All of the five constituents identified in the sample 2' (yellow variety) are present in the composition of the green variety, sample 1', but at different concentrations. For the green variety, the major constituents were *n*-propyl ethanoate (53.5%) and 4-methylpentan-2-one (29.0%), whereas for the yellow variety, the major constituents were butane-1,3-diol (67.7%) and *n*-propyl ethanoate (16.7%). The butane-1,3-diol identified in both samples (sample 1' and sample 2') is an important chiral synthon for various optically active compounds, such as azetidinone derivatives leading to penem and carbapenem antibiotics (Matsuyama et al., 2001).

Although the chemical composition of the extracts was different between the hydrodistillation and solvent extraction methods, some similarities were observed. The ester *n*-propyl ethanoate appears

in sample 1 (15.3%) (hydrodistillation process) and in sample 1' (53.5%, petroleum ether extract), and the ketone 4-methylpentan-2-one was also present in sample 1 (12%) and the sample 1' (29%). In addition, by the hydrodistillation process, the aldehyde furfural was shown to be present in both varieties (green and yellow) in the percentage of 14.5% and 0.8%, respectively.

The results of the free radical scavenging effects of the samples 1, 1', 2, and 2' from the hydrodistillation and solvent extraction of both varieties showed concentration-dependant activity. The free radical scavenging effects of samples 1 and 2 were 79.4% and 83.5% at a concentration of 1.0 μg/μL, while it was 76.6%, and 79.3% for 0.50 μg/μL, 64.8% and 67.2% at a concentration 0.25 μg/μL, and 41.2% and 32.5% at a concentration of 0.125 μg/μL, respectively, showing significant antioxidant activity compared with Trolox and BHT (Table 2). The samples obtained from the petroleum ether extractions, sample 1' and sample 2', showed only moderate free radical scavenging activity, with inhibition levels of 31.5 and 34.9% at a concentration of 1.0 μg/μL respectively, and 16.4 and 27.7% inhibition at a concentration of 0.25 μg/μL, respectively.

Alcohols, ketones, lactones, aldehydes, and esters having short carbon chains were detected in the essential oil of the coconut water from the two varieties analyzed, and are probably responsible, in part, for the aroma of coconut water. In addition, the ester *n*-propyl ethanoate was present in both extraction processes, and probably is one of the compounds responsible for the flavor of coconut water.

The free radical scavenging effects of the samples from hydrodistillation and solvent extraction of the water from the two coconut varieties were compared to the positive controls Trolox and BHT in the DPPH free radical system. High scavenger activity was found in the samples obtained from the hydrodistillation process, and moderate activity was observed in the samples from the solvent extraction. The antioxidant activity of these fractions from coconut water is of interest as a potential natural food antioxidant additive, nutraceutical, and requires further evaluation.

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