

Chemical Characterization and Antioxidant Activity of Amazonian (Ecuador) *Caryodendron orinocense* Karst. and *Bactris gasipaes* Kunth Seed Oils

Matteo Radice^{1*}, Derwin Viafara¹, David Neill¹, Mercedes Asanza¹, Gianni Sacchetti², Alessandra Guerrini^{2*} and Silvia Maietti²

¹ Universidad Estatal Amazónica (Km. 2 ½ Vía Napo (paso lateral), Puyo, Pastaza, Ecuador)

² Department of Life Sciences and Biotechnology (SvEB), University of Ferrara, (c.so Ercole I d'Este 32, I-44121 Ferrara, Italy)

Abstract: Nowadays, data concerning the composition of *Caryodendron orinocense* Karst. (Euphorbiaceae) and *Bactris gasipaes* Kunth (Arecaceae) seed oils are lacking. In light of this fact, in this paper fatty acids and unsaponifiable fraction composition have been determined using GC-MS, HPLC-DAD (Diode Array Detector), NMR approaches and possible future applications have been preliminary investigated through estimation of antioxidant activity, performed with DPPH test. For *C. orinocense* linoleic acid (85.59%) was the main component, lauric (33.29%) and myristic (27.76%) acids were instead the most abundant in *B. gasipaes*. *C. orinocense* unsaponifiable fraction (8.06%) evidenced a remarkable content of β -sitosterol, campesterol, stigmasterol, squalene and vitamin E (816 ppm). *B. gasipaes* revealed instead β -sitosterol and squalene as main constituents of unsaponifiable matter (3.01%). Antioxidant capacity evidenced the best performance of *C. orinocense* seed oil. These preliminary results could be interesting to suggest the improvement of the population's incomes from Amazonian basin. In particular the knowledge of chemical composition of *C. orinocense* and *B. gasipaes* oils could be helpful to divulge and valorize these autochthonous plants.

Key words: biodiversity, *Caryodendron orinocense*, *Bactris gasipaes*, seed oils

1 INTRODUCTION

Ecuador is recognized as one of 17 “megadiverse” countries, since significant portion of the country's territory is one of the richer areas in biodiversity of the world¹⁻³.

Native palm trees belonging to the Arecaceae family are among the most useful plant resources in the Amazon. In fact, some species have fruits with high yields in oil both in the mesocarp and in the kernel⁴, representing one of the main sources of oils and fats with several applications in food, pharmaceutical and cosmetic field. Despite their numerous applications, few species have been investigated and more comprehensive studies about composition and biological properties of crude drug derivatives not deeply explored are needed⁵. Among these Arecaceae trees, *Bactris gasipaes* Kunth, commonly known as “chontaduro” in Ecuador, “pejibaye” in Central America, “pupunha” in Brazil or generally “peach palm” in the world is one of the most interesting because its uses and limited knowledge about its phytochemistry and functional properties. It

is a 10-20 m high, spiny stemmed palm with green-yellow or yellow-orange fruits, occurring between November and July⁶. *B. gasipaes* is predominantly cultivated by smallholders in agroforestry systems as cash crop mainly because of starch content of its fruits and, more recently, because of mesocarp fixed oil⁷: the seed (kernel) oil, instead, has never been deeply investigated.

Caryodendron orinocense Karst. (Euphorbiaceae) shares with *B. gasipaes* the same characteristic of being not cultivated for the kernel oil production and poorly investigated for phytochemical and biological properties^{8,9}. It is a tree about 20 m high, commonly known as “inchi” and “mani de arbor” in Ecuador and as “Nuez de Barinas” in Venezuela, that grows wild at the base of the Andes in Venezuela, Ecuador, Perú and Colombia. The fruit, growing in bunches at the ends of the branches hanging over the exterior of the crown between November and June⁹, contains three seeds. The traditional ethnobotanical uses of seed oil concern wound treatment and food preparation¹⁰.

*Correspondence to: Alessandra Guerrini, Department of Life Sciences and Biotechnology, University of Ferrara, C.so Ercole I d'Este, 32, 44121 Italy, Matteo Radice, Universidad Estatal Amazónica, Km. 2 ½ Vía Napo (paso lateral), Puyo, Pastaza, Ecuador
E-mail: grrisn@unife.it (Alessandra Guerrini); mradice@uea.edu.ec (Matteo Radice)

Accepted August 11, 2014 (received for review January 14, 2014)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online

http://www.jstage.jst.go.jp/browse/jos/ http://mc.manuscriptcentral.com/jjocs

The commercial interest of *C. orinocense* derived products is less widespread than that of *B. gasipaes*. However, in light of its current uses, *C. orinocense* kernel oil has interesting perspectives of commercial exploitation, despite the productive chains that nowadays are far to be considered consolidated. In fact, because of the still limited traditional uses of the seeds as food and the oil as wound remedy, small scale cultivations have been established in Pichincha province in Ecuador, on the Pacific slope of the Andes and in Colombia.

In light of all these premises, *B. gasipaes* and *C. orinocense* kernel oils from Amazonian Ecuador have been here studied for the first time for the chemical characterization and the antioxidant activity in order of better investigating these promising tree crops, as possible renewed source for the Amazonian basin economy^{7, 11, 12}. Attention has been focused on fatty acids profile and unsaponifiable fraction as source of functional biomolecules to lead possible exploitation of cultivation of both species towards larger scale production of oil for cosmetic and/or health perspectives.

This study deals with the FA, sterols, tocopherols characterization and antioxidant activity determination of *B. gasipaes* and *C. orinocense* kernel oils.

2 EXPERIMENTAL PROCEDURES

2.1 Plant material

Ripe fruits of *C. orinocense* and *B. gasipaes* from wild Ecuadorian plants (Napo, Ecuador) were collected during the balsamic period and authenticated in the laboratory of Prof. David Neill (Universidad Estatal Amazonica). The scientific identification and recognition has been processed by examination of botanical parameters based on morphological characteristics of the whole plant, its leaves, flowers and fruits. All the data achieved led to the authentication of the samples. The seeds were removed, cleaned under running tap water, then dried overnight in the oven at 60°C and successively ground in a blade grinder (Model Pulverisette 15, Fritsch GmbH, Idar-Oberstein, Germany) to pass a 0.2 mm mesh. During grinding, provisions were made to ensure that the temperature never exceeded 30°C. After grinding, the flour was stored in the dark at -20°C. Seeds of *C. orinocens* and *B. gasipaes* dried specimens were deposited at Universidad Estatal Amazonica (codes UEA 001/13, UEA002/13, respectively).

2.2 Seed oil extraction

Fifty g of seed flour were put into a cellulose paper cone (Filter Paper Whatman No.1, GE Healthcare Europe GmbH, Freiburg, Germany), transferred into a 500 mL Soxhlet apparatus and extracted with 250 mL of hexane for 2 h⁽⁶⁾. The oil was recovered removing the solvent with a rotary evaporator, transferred into dark glass vials with

Teflon-sealed caps and stored at -20°C until analyses.

2.3 Determination of free fatty acids

About 2 g of oil, exactly weighed, were dissolved in 30 mL of a mixture of ethanol and diethylether (1/1, v/v) and then, under stirring, titrated with a 0.1N KOH solution to the end point of the indicator (5 drops of indicator, 1% phenolphthalein in 95% ethanol) until the pink color persisting for at least 10 s. The acid value was calculated according the following expression:

$$AV = (56.1xNxV)/m$$

where N was the exact normality of KOH solution, V the mL of KOH solution and m the mass in g of sample⁽³⁾.

2.4 Preparation of fatty acid methyl esters (FAME)

The FAME were prepared by transmethylation using sodium methoxide in the presence of methyl acetate⁽⁴⁾. After 5 min at room temperature, the reaction was stopped by adding a saturated solution of oxalic acid in diethyl ether (30 µL) with brief agitation. The mixture was centrifuged at about 1500 rpm for 2 min to precipitate sodium oxalate, and an aliquot of supernatant was directly injected for GC analysis^(15, 16).

2.5 Determination of FAME by GC-MS and GC-FID

GC analysis was performed by a gas chromatograph (Model Varian GC-3800, Agilent Technologies Inc., Santa Clara, California, USA) equipped with a VF-5ms 5% polyand 95% phenyl-dimethyl-siloxane bonded phase column (i.d., 0.25 mm; length, 30 m; film thickness, 0.25 µm, Agilent Technologies Inc., Santa Clara, California, USA), a mass spectrometer (Model Varian MS-4000, Agilent Technologies Inc., Santa Clara, California, USA) using electron impact (EI) and hooked to NIST library. FAME were identified by comparing their GC retention time and the MS fragmentation pattern with those of 37 pure component FAME mix (Sigma-Aldrich Inc., St. Louis, California, USA), methyl esters of C4-C24 saturated and unsaturated fatty acids. Operating conditions were as follows: injector temperature, 300°C; carrier (helium) flow rate, 1 mL/min and split ratio, 1:50. Oven temperature was increased from 100°C to 250°C at a rate of 5°C/min, followed by 10 min at 250°C. The MS conditions were: ionization voltage, 70 eV; emission current, 10 mAmp; scan rate, 1 scan/s; mass range, 29–600 Da; trap temperature, 150°C, transfer line temperature, 300°C. One microliter of each sample was injected. FAME samples were analyzed in GC-FID for quantitative determination through the normalization method, without using correction factors: the relative peak areas for individual constituents were averaged on three different chromatograms of three independent reactions. The relative percentages were determined using a gas-chromatograph (Model GC-Trace, ThermoQuest Corporation, Atlanta, Georgia,

USA) equipped with a FID detector maintained at 300°C; all the others GC conditions were the same of GC-MS method.

2.6 Determination of composition of unsaponifiable fraction

Into a 50 mL flask with a plug, 2 g of oil were exactly weighed and then cold saponified with 20 mL of 1 M methanolic KOH. The flask was placed under constant agitation for 24 h at a temperature of 28°C. After this time had elapsed, the solution was extracted twice with 20 mL of *n*-hexane and 20 mL of water using a separator funnel. The *n*-hexane fractions were then dried with anhydrous sodium sulfate and successively using a rotary evaporator: 5 mg of the unsaponifiable fraction were silanized at room temperature with 2 mL of a silanizing mixture containing pyridine/hexamethyldisilazane/trimethylchlorosilane (5/2/1, v/v/v). After 1 h, the liquid was evaporated under a nitrogen flow in a heat bath at 80°C and then extracted with 0.3 mL of hexane. The conical test tube was placed in ultrasound for 2 min and centrifuged, and the supernatant was withdrawn for injection into the GC. One microliter of the solution was injected in GC-MS and compounds were identified by comparing their GC retention times and the MS fragmentation pattern with those of other oils of known composition, with pure compounds (squalene, campesterol, stigmaterol, β -sitosterol (Sigma-Aldrich)) and by matching the MS fragmentation patterns and retention indices with the above mentioned mass spectra libraries (NIST) and with those in the literature⁶.

Operating conditions were as follows: column Varian VF-5ms 5% poly- and 95% phenyl-dimethyl-siloxane bonded phase (i.d., 0.25 mm; length, 30 m; film thickness, 0.25 μ m Agilent Technologies Inc., Santa Clara, California, USA). Injector temperature, 300°C; carrier (helium) flow rate, 1.2 mL/min; and split ratio, 1:50. Oven temperature was increased from 230°C to 320°C at a rate of 5°C/min, followed by 7 min at 320°C. The MS conditions were: ionization voltage, 70 eV; emission current, 20 mA; scan rate, 1 scan/s; mass range, 29–800 Da; trap temperature, 150°C, transfer line temperature, 320°C.

GC-FID was used for quantitative determination through the normalization method, without using correction factors: the relative peak areas for individual constituents were averaged on three different chromatograms of three independent reactions. The relative percentages were determined using a gas-chromatograph (Model GC-Trace, ThermoQuest Corporation, Atlanta, Georgia, USA) equipped with a FID detector maintained at 350°C; all the others GC conditions were the same of GC-MS method.

2.7 Determination of vitamin E isomers

Normal Phase-HPLC analyses of oils were performed using a modular Jasco HPLC unit (JASCO Corporation,

Tokyo, Japan) which consisted of an injection valve with a 20 μ L sampler loop, a PU-2089Plus pump, a quaternary gradient unit and a photodiode detector MD-2010Plus, according to the methods previously described^{17,18}. A Lichrosorb silica gel Si 60 (5 μ m, 25 \times 0.46 cm; Teknokroma, Barcelona, Spain) column was used and the mobile phase was 0.05% isopropanol/hexane at a flow rate of 1.0 mL/min. Chromatograms were recorded and α -, β -, γ -, and δ -isomer peaks were identified by comparing their retention times and spectra with those of pure tocopherol standards (Sigma-Aldrich). The peak areas were determined by integration using dedicated software (Borwin-PDA ver. 1.50, JMBS Developments, Grenoble, France). For each extract, qualitative and quantitative analysis were performed in triplicate.

2.8 NMR spectroscopy

The ¹³C NMR spectra were recorded on a spectrometer (Model Varian Gemini 400, Agilent Technologies Inc., Santa Clara, California, USA) operating at 100.58 MHz and 303 K. The oil (80 mg/0.8 mL) were dissolved in deuterated chloroform into a 5 mm NMR tube. Spectra were run using a Varian standard pulse sequence "s2pul". The time domain size was 17 K, spectral width 25000 Hz, FID resolution 1.43 Hz. Chemical shifts (ppm) and peak attribution of ¹³C NMR spectra were made according with those of literature¹⁹.

2.9 Antioxidant activity

Radical scavenging activity of the two oils was performed with DPPH radical according to a properly modified method described by Ramadan²⁰ that suggests of taking extravirgin olive oil, as reference standard. DPPH solution was freshly prepared at the concentration of 10⁻⁴ M in hexane, solvent that allowed the complete solubility of lipid fractions and high stability of DPPH radical. Hexane solutions of DPPH radical (3900 μ L) and of each oil (10 mg in 100 μ L di hexane) were mixed and then vortexed for 20 s at room temperature. Against the *n*-hexane as blank, the radical scavenging activity was estimated as residual DPPH percentage of absorbance between DPPH solution (without oil) and sample a 515 nm in a quartz cell with a spectrophotometer (Model Thermo Spectronic Helios γ , Thermo Fisher Scientific Inc., Massachusetts, USA), according to the following equation:

$$\% \text{ residual DPPH} = (1 - (\text{absorbance of control} - \text{absorbance of test sample})) \times 100$$

All experimental measures were performed in triplicate and their mean values were considered.

3 RESULTS AND DISCUSSION

Since the present study has the target to characterise seed oils of *B. gasipaes* and *C. orinocense* for finding possible aspects of exploitation of their current limited uses, we think reasonable to compare our chemical data not only with related literature regarding both the species but also with evidences concerning palm kernel oils or, specifically, a much more known and commercialized oils from *Elaeis guineensis*. This is, in fact, an important palm, cultivated also in South America for its oil, representing a paradigm for evaluating yield, quality and possible uses of those extracted from *B. gasipaes* and *C. orinocense*^{6, 7}. In the same way, since the reference compound more frequently used in literature to estimate the antioxidant capacity of oils is that of extravirgin olive, we have instead choose it as consolidated positive control for comparing our data²⁰.

3.1 Kernel oil extraction

C. orinocense oil, extracted by a soxhlet apparatus, evidenced (Table 1) an higher yield value (46.9%) than that obtained by Alfaro⁹ (30%), but comparable to *E. guineen-*

sis kernel oil¹⁸). *B. gasipaes*, instead, reached the value of 11.5%, less than that described in related papers⁶.

3.2 Determination of free fatty acids and unsaponifiable values

Both oils showed lower acid value (Table 1) than that expressed by *E. guineensis* kernel oil (5-22)²¹ and, in particular, *B. gasipaes* which evidenced the lowest data also in comparison with related literature⁶. *B. gasipaes* unsaponifiable matter was good with respect to data previously published for this specie (0.8%) and the values reported for *E. guineensis* kernel oil (1-3%)⁶. *C. orinocense* instead revealed an high yield value with respect to what reported for the seed oil from plants in Venezuela (1.1%)^{9, 22}: further future investigations need to explain this interesting result.

3.3 Fatty acid composition

C. orinocense oil exhibited (Table 2) an high amount of unsaturated linoleic acid (ω6) (85.59%) higher than previously published data (75.13%)⁸ which evidenced an oleic acid content of 11.8%, under the limit of detection in ecua-

Table 1 Kernel oil extraction results, free fatty acids and unsaponifiable values.

	<i>C. orinocense</i>	<i>B. gasipaes</i>	<i>E. guineensis</i> ^{6), 26)}
Oil/kernel (w/w)%	46.9 ± 1.4 (30) ⁹⁾	11.5 ± 0.2 (16.4) ⁶⁾	45-55%
Acid value (mg KOH)	2.4 ± 0.1 (3.0) ⁹⁾	1.7 ± 0.1 (12.2) ⁶⁾	5-22
Unsaponifiable value (%)	8.1 ± 0.2 (1.1) ⁹⁾	3.0 ± 0.1 (0.8) ⁶⁾	1-3

Each value in the table represents the mean ± SD of three replicates.

^{6), 9), 26)} Data of literature (reference 6, 9, 26)

Table 2 Fatty acid composition (%) of Amazonian (Ecuador) kernel oils.

Fatty acid	<i>C. orinocense</i>	<i>B. gasipaes</i>	<i>E. guineensis</i> ²⁶⁾
Caproic (C6:0)	–	–	< 0.4
Caprylic (C8:0)	–	–	3.0 – 6.0
Capric (C10:0)	–	–	3.0 – 5.5
Lauric (C12:0)	–	33.3 ± 0.9 (60.6) ⁶⁾	47.0 – 51.5
Myristic (C14:0)	0.1 ± 0.0	27.8 ± 0.9 (18.9) ⁶⁾	15.5 – 17.0
Palmitoleic (C16:1)	0.1 ± 0.0	–	< 0.3
Palmitic (C16:0)	10.3 ± 0.7 (9.5) ⁹⁾	9.6 ± 0.2 (6.0) ⁶⁾	6.5 – 9.0
Margaric (C17:0)	0.2 ± 0.1	–	–
Linoleic (C18:2)	85.6 ± 1.0 (75.1) ⁹⁾	– (trace) ⁶⁾	1.0 – 3.0
Oleic (C18:1)	– (11.8) ⁹⁾	24.3 ± 0.5 (12.9) ⁶⁾	11.0 – 16.0
Stearic (C18:0)	3.4 ± 0.1 (2.2) ⁹⁾	5.1 ± 0.2 (trace) ⁶⁾	1.5 – 3.0
Arachidic (C20:0)	0.5 ± 0.0	–	< 0.3
Unsaturated fatty acids	85.7 (86.9) ⁹⁾	24.3 (12.9) ⁶⁾	12.0 – 19.3
Saturated fatty acids	14.3 (11.7) ⁹⁾	75.7 (85.5) ⁶⁾	76.5 – 92.7

Each value in the table represents the mean ± SD of three replicates.

^{6), 9), 26)} Data of literature (reference 6, 9, 26)

dorian oil: this is a quite uncommon composition because several seed oils, as those of *Glycine max* and *Helianthus annuus*, evidence about 40-60% content of linoleic acid. However, a lot of kernel oils possess a large amount of MUFA and PUFA²⁰. In good agreement with other kernel palm and copra oils, defined also as “lauric oils”^{6, 22}, *B. gasipaes* highlighted an high content of saturated fatty acids (75.72%), with lauric and myristic acids as main constituents.

In light of these experimental results, *C. orinocense* oil could be considered as an eligible source of $\omega 6$ with possible application as food supplements for the prevention of cardiovascular diseases²³ and as cosmetic ingredient for the role of linoleic acid in the formation and improvement of epidermal barrier²⁴. Since “lauric oils” are widely used in natural foods and in oleochemical industries, *B. gasipaes* oil from Ecuador could be suggested for the same applications²⁵.

3.4 Composition of unsaponifiable fraction

Both oils evidenced a great amount of β -sitosterol. The oil of *C. orinocense* showed in particular an high content of unsaponifiable matter (8.06%), revealing an high amount of phyosterols as β -sitosterol (55%), campesterol (12%), stigmasterol (11%) accordingly to what previously described⁶ for other kernel oils, as *E. guineensis* oil. The amount of squalene hydrocarbon (12%) was instead not previously determined, while the relative abundance of triterpene alcohols was significantly different (Table 3). In *B. gasipaes* oil, β -sitosterol (51%) was the main component of unsaponifiable matter, followed by squalene (17%) and by

the triterpene cicloartenol (9%) which content was in good agreement with previously reported results⁶. With the exception of squalene, lanosterol and citrostadienol here reported for the first time, and $\Delta 5$ -avenasterol detected in lower amount, *B. gasipaes* composition was comparable with literature data⁶ (Table 3). However, it has to be pointed out that the presence of squalene, suggests the importance of Amazonian plant sources for further or more effective applications related to the properties of this hydrocarbon. Among the different plant sterols, β -sitosterol has been the most intensively investigated with respect to its physiological human effect. Many properties (anti-inflammatory, anticancer, prevention of cardiovascular diseases)²⁷ have been described for β -sitosterol. Squalene, well represented for its abundance in both *C. orinocense* and *B. gasipaes* oils, is a physiological substance in animals and humans known as the precursor of cholesterol biosynthesis. Because of the wide variety of physiological functions such as anticancer and anti-hypercholesterolemia, squalene has a long history of uses as attractive resource for functional foods, supplements or even pharmaceuticals. Antioxidant and oxygen carrier properties predict its potential in preventing cardiovascular diseases²⁵. Moreover, squalene shows some advantages for skin treatments as an emollient and antioxidant, and for hydration and skin antitumor activities because of its role as one of the main component of skin surface polyunsaturated lipids. It is also used as ingredient in topically applied vehicles such as lipid emulsions and nanostructured lipid carriers (NLCs)²⁶⁻²⁹. In light of these considerations, the unsaponifiable matter of *C. orinocense* and *B. gasipaes*

Table 3 Composition (%) in unsaturated hydrocarbons, sterols, triterpene alcohols.

Compound	<i>C. orinocense</i>	<i>B. gasipaes</i>	<i>B. gasipaes</i> ⁶ / <i>E. guineensis</i> ^{14, 26}
Squalene	11.7 ± 1.1	16.9 ± 1.5	-/-
Cholesterol	0.8 ± 0.1 (0.9) ^a	–	0.3/3.0
Campesterol	12.2 ± 0.9 (14.6) ^a	6.3 ± 0.5 (9.5) ^a	3.0/8.9
Stigmasterol	11.0 ± 1.0 (13.2) ^a	2.9 ± 0.1 (4.4) ^a	1.8/11.0
β -Sitosterol	55.0 ± 2.5 (65.9) ^a	51.3 ± 1.8 (77.4) ^a	73.2/70.1
$\Delta 5$ -Avenasterol	3.3 ± 0.1 (4.0) ^a	4.8 ± 0.1 (7.2) ^a	21.7/6.0
Lanosterol	1.2 ± 0.1 (1.4) ^a	1.0 ± 0.1 (1.5) ^a	-/-
Cicloartenol	1.3 ± 0.1 (61.9) ^b	8.7 ± 0.3 (55.4) ^b	65.3/28.0
24-Methylenecicloartenol	–	5.0 ± 0.4 (31.8) ^b	34.7/6.4
Citrostadienol	0.8 ± 0.2 (38.1) ^b	2.0 ± 0.2 (12.8) ^b	-/65.6

Each value in the table represents the mean ± SD of three replicates.

^a) Relative percentage calculated on total amount of sterols

^b) Relative percentage calculated on total amount of triterpene alcohols

^{6, 14, 26}) Data of literature (reference 6, 14, 26): relative percentage calculated on total amounts of sterols and triterpene alcohols separately

possesses an interesting composition that could be linked to the antioxidant capacity detected.

3.5 Vitamin E isomers content

It is known that the antioxidant activity of plant lipid derivatives could be related to vitamin E content. In this regard, the results reported in **Table 4** for *C. orinocense* oil shows the γ -tocopherol as the most abundant isomer (575 mg/kg oil). Even if some differences between α - and δ -tocopherol amounts have been detected with previously published data⁸⁾ for Nuez de Barinas of Venezuela, the total content of vitamin E was in good agreement, corresponding to the 1.0% of unsaponifiable matter. In *B. gasipaes*, Vitamin E quantity was 1.9% of unsaponifiable matter, with the predominance of α -tocopherol, known to have the most effective antioxidant capacity among the isomers³⁰⁾. These quantitative data are considerably higher than that reported in literature (59 ppm vs 12-18 ppm)⁶⁾. However the results were for both species in good agreement with other kernel oils which are reported to have total tocopherol content as 0.1-1.6% of the unsaponifiable matter. Considering the whole oils, it could be concluded that *C. orinocense* is a more promising source of vitamin E than *B. gasipaes*, since the amount of unsaponifiable fraction is lower in the latter amazonian species (816 mg/kg vs 59 mg/kg).

Table 4 Determination of vitamin E isomers.

Tocopherol	<i>C. orinocense</i>	<i>B. gasipaes</i>
	mg tocopherol/kg oil	
α -tocopherol	175 \pm 2 (28) ⁸⁾	47 \pm 1 (2-3) ⁶⁾
β -tocopherol	9 \pm 1 (-)	7 \pm 1 (0-1) ⁶⁾
γ -tocopherol	575 \pm 4 (520) ⁸⁾	4 \pm 1 (-) ⁶⁾
δ -tocopherol	57 \pm 2 (148) ⁸⁾	- (-) ⁶⁾
Total	816 (688) ⁸⁾	59 (12-18) ⁶⁾

Each value in the table represents the mean \pm SD of three replicates.

^{6), 8)} Data of literature (reference 6, 8)

Tocotrienols are instead under the limit of instrument detection for both oils.

3.6 NMR fingerprinting of seed oils

The NMR fingerprinting of the two oils confirmed the results described in previous paragraphs (**Fig. 1**). The acidity, firstly determined for both oils by titration (**Table 1**), was not revealed (typical signals are in the range 176-178 ppm) because of the signal levels under the detection limit (about 5%). This data is representative of the fact that only the two chemical shifts at 172.8 and 173.3

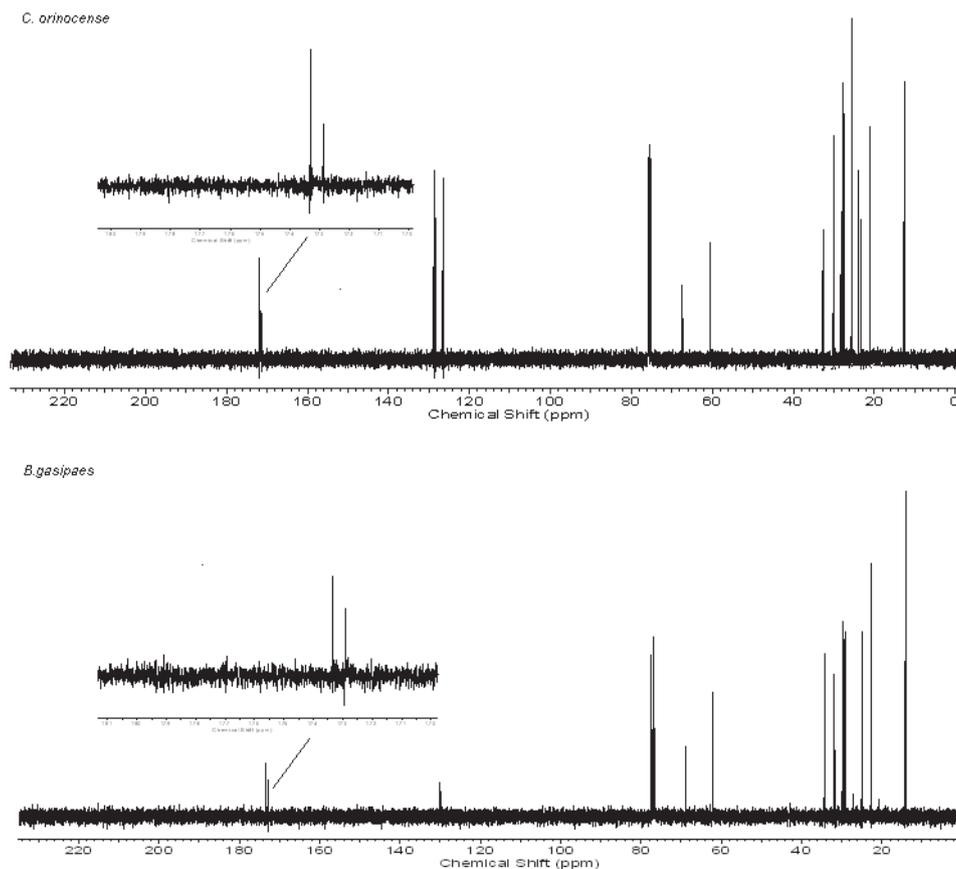


Fig. 1 ¹³C NMR spectra of oils.

ppm were detected, due to C1 linked to glycerol. Characteristic signals of methylenic groups of linoleic acid were detected at 127.8(C12) and 128.2(C10) ppm for *C. orinocense*. *B. gasipaes* oil, that highlighted only the unsaturated oleic acid, evidenced the presence of its double bond at 130.0(C9) and 129.7(C10) ppm¹⁷⁾.

3.7 Antioxidant activity

Since related literature reports extravirgin olive oil as main reference for comparing the antioxidant capacity of oils, for *C. orinocense* and *B. gasipaes* has been also adopted this strategy to point out, for the first time, possible functional applications²⁰⁾. *B. gasipaes*, revealed poor antioxidant activity with values not comparable to that of the reference, while *C. orinocense* showed instead better results than that expressed extravirgin olive oil, suggesting possible exploitation of the oil towards cosmetic field. In fact, it is noteworthy that *C. orinocense* presented an antioxidant profile better than that expressed by olive oil, reaching the 50% of radical inhibition after 2 h (Fig. 2). The significant strongest antiradical action of *C. orinocense* oil, as reported in literature for other vegetable oils³⁰⁾ may be due to different factors as a) the higher level of α -tocopherol, which possesses *in vitro* the most antioxidant effectiveness among all tocopherols, together with the total amounts of vitamin E that are both considerably higher than *B. gasipaes* oil and slightly lower than extravirgin oil, b) the highest unsaponifiable value³⁰⁾, c) the highest content of PUFA (polyunsaturated fatty acids), specifically linoleic acid with respect to other two oils. In fact, the susceptibility of fatty acids to oxidation (that is their antioxidant properties) is thought to be directly dependent on their degree of unsaturation. However, some *in vitro* and *in vivo* studies suggest that the relation between chemical struc-

ture and susceptibility to oxidation is not as straightforward as hypothesized from theoretical viewpoints³¹⁾.

4 CONCLUSION

The amazonian Ecuador *C. orinocense* and *B. gasipaes* oils were for first time studied for their chemical composition and the antioxidant activity. On the basis of the results achieved mainly concerning the high level of linoleic acid and of unsaponifiable matter characterized by β -sitosterol, squalene and vitamin E, with good and promising radical scavenging activity, the *C. orinocense* oil could be suggested as food supplement and/or cosmetic ingredient, similarly to what reported for the same plant source from different geographical area⁸⁾. *B. gasipaes*, that can be considered for the fatty acid composition as a "lauric oil", evidenced a lower antioxidant activity if compared to extravirgin olive oil, chosen as reference. However, since *B. gasipaes* presented chemical evidences comparable to that of kernel palm oil, similar uses cannot be excluded even if further investigations are needed. In light of these preliminary evidences, further researches will be performed about the efficacy and safety of both oils to better define possible applications mainly in cosmetic and dietary fields. The characterization of these oils for new functional uses, also different from those suggested for the same species of different geographical origin²⁰⁾, will contribute to better valorize the amazonian Ecuador plants for commercial exploitation.

ACKNOWLEDGMENTS

The Authors wish to thank Miss Deysi Daniela Ortega Álvarez, Dr. Immacolata Maresca, Dr. Luis Morales Naranjo for their skilful technical assistance. This scientific work was financially supported by the Prometeo Project of the Ministry of Higher Education, Science and Technology (SENESCYT) of the Republic of Ecuador.

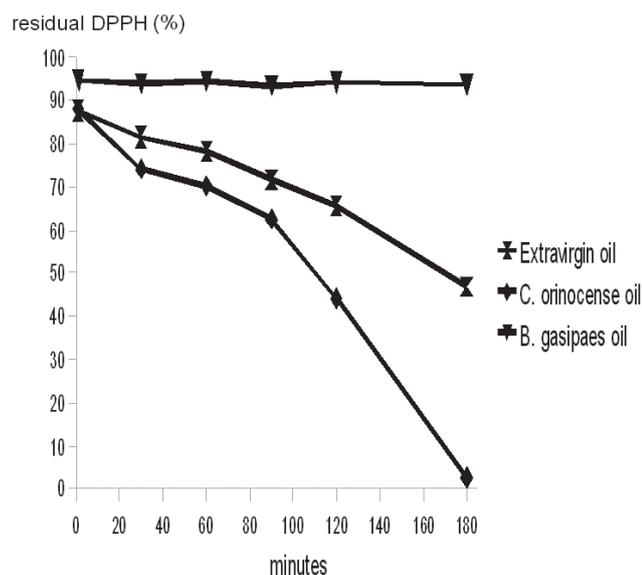


Fig. 2 DPPH radical scavenging activity of oils.

References

- 1) Mittermeier, R. A., Gil P. R. and Mittermeier, C. G. *Megadiversity: Earth's Biologically Wealthiest Nations*. Conservation International, Cemex (1997).
- 2) Tuomisto, H. A consistent terminology for quantifying species diversity? Yes, it does exist. *Oecologia* **164**, 853-860 (2010).
- 3) Dangles, O.; Nowicki, F.; Mena, B. *Biota Maxima: Ecuador biodiverso*. PUCE-IRD Ed., Quito, pp.252 (2009).
- 4) Clement, C. R.; Lleras Pérez, E.; Van Leeuwen, J. O

- potencial das palmeiras tropicais no Brasil: acertoss e fracassos das últimas décadas. *Agrociência* **9**, 67-71 (2005).
- 5) Santos, M. F. G.; Marmesat, S.; Brito, E. S.; Alves, R. E.; Dobarganes, M. C. Major components in oils obtained from Amazonian palm fruits. *Grasas Aceites* **64**, 328-334 (2013).
 - 6) Bereau, D.; Benjelloun-Mlayah, B.; Banoub, J.; Bravo, R. FA and unsaponifiable composition of five Amazonian palm kernel oils. *J. Am. Oil Chem. Soc.* **80**, 49-53 (2003).
 - 7) Graefe, S.; Dufour, D.; van Zonneveld, M.; Rodriguez, F.; Gonzalez, A. Peach palm (*Bactris gasipaes*) in tropical Latin America: implications for biodiversity conservation, natural resource management and human nutrition. *Biodiver. Conserv.* **22**, 269-300 (2013).
 - 8) Alfaro, M. J.; Padilla, F. C.; Perez, R. M. *Caryodendron orinocense* ("nuez de Barinas") oil: tocopherol content and use in cosmetic. *Int. J. Cosmetic Sci.* **22**, 335-340 (2000).
 - 9) Perez, R. M.; Alfaro, M. J.; Padilla, F. C. Evaluation of "nuez de Barinas" (*Caryodendron orinocense*) oil for possible use in cosmetic. *Int. J. Cosmetic Sci.* **21**, 151-158 (1999).
 - 10) Pabon, E. M. A. Oleaginosas de la Amazonía "El Inchi". *Colombia Amazónica* **1**, 70-79 (1982).
 - 11) Reckin, J. The orinocut-a promising tree crop for the tropics. *International Tree Crops Journal* **2**, 105-119 (1983).
 - 12) Schultes, R. E. The Amazonia as a source of new economic plants. *Economic Botany* **33**, 259-266 (1979).
 - 13) ISO 660:2009. Animal and vegetable fats and oils. Determination of acid value and acidity. (2009).
 - 14) Bruni, R.; Medici, A.; Guerrini, A.; Scalia, S.; Poli, F.; Muzzoli, M.; Sacchetti, G. Wild *Amaranthus caudatus* seed oil, a nutraceutical resource from Ecuadorian flora. *J. Agric. Food Chem.* 5455-5460 (2001).
 - 15) Zeng, A. X.; Chin, S. T.; Nolvachai, Y.; Kulsing, C.; Sidsky, L. M.; Marriott, P. J. Characterisation of capillary ionic liquid columns for gas chromatography-mass spectrometry analysis of fatty acid methyl esters. *Anal. Chim. Acta* **803**, 166-173 (2013).
 - 16) Kloos, D. P.; Gayl, E.; Lingeman, H.; Bracher, F.; Müller, C.; Mayboroda, O. A.; Deelder, A. M.; Niessen, W. M. A.; Gieral, M. Comprehensive gas chromatography-electron ionisation mass spectrometric analysis of fatty acids and sterols using sequential one-pot silylation: quantification and isotopologue analysis. *Rapid Commun. Mass Spectrom.* **28**, 1507-1514 (2014).
 - 17) Guerrini, A.; Bruni, R.; Maietti, S.; Poli, F.; Rossi, D.; Paganetto, G.; Muzzoli M.; Scalvenzi L.; Sacchetti G. Ecuadorian stingless bee (Meliponinae) honey: A chemical and functional profile of an ancient health product. *Food Chem.* **114**, 1413-1420 (2009).
 - 18) Han, N. M.; May, C.Y. Chromatographic Analyses of Tocopherols and Tocotrienols in Palm Oil. *J. Chromatogr. Sci.* **50**, 283-286 (2012).
 - 19) Sacchi, F.; Addeo, F.; Paolillo L. ¹H and ¹³C NMR of virgin olive oil. An overview. *Magn. Reson. Chem.* **35**, S133-S145 (1997).
 - 20) Ramadan, M. F.; Morsel, J.-T. Screening of the antiradical action of vegetable oils. *J. Food Comp. Anal.* **19**, 838-842 (2006).
 - 21) Proserpio, G. *Chimica e Tecnica Cosmetica 2000. Le sostanze di base*. Sinerga, Milano (2000).
 - 22) Fontanel, D. *Unsaponifiable matter in plant seed oils*. Springer, London (2013).
 - 23) Jakobsen, M. U.; O'Reilly, E. J.; Heitmann, B. L.; Pereira, M. A.; Bälter, K.; Fraser, G. E.; Goldbourt, U.; Hallmans, G.; Knekt, P.; Liu, S.; Pietinen, P.; Spiegelman, D.; Stevens, J.; Virtamo, J.; Willett, W. C.; Ascherio, A. Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. *Am. J. Clin. Nutr.* **89**, 1425-1432 (2009).
 - 24) Khnykin, D.; Minerand, J. H.; Jahnsen, F. Role of fatty acid transporters in epidermis. Implications for health and disease. *Dermatoendocrinol.* **3**, 53-61 (2011).
 - 25) Singh, S. P.; Singh, D. Biodiesel production through the use of different sources and characterization of oils and their esters as the substitute of diesel: A review. *Renew. Sust. Energ. Rev.* **14**, 200-216 (2010).
 - 26) Capella, P.; Fedeli, E.; Bonaga, G.; Lercker, G. *Manuale degli oli e dei grassi*. Tecniche Nuove, Italia (1997).
 - 27) Grattan, B. J. Plant sterol as anticancer nutrients: evidence for their role in breast cancer. *Nutrients* **31**, 359-387 (2013).
 - 28) Bhilwade, H. N.; Tatewaki, N.; Nishida, H.; Konishi, T. Squalene as novel food factor. *Curr. Pharm. Biotechnol.* **11**, 875-880 (2010).
 - 29) Huang, Z. R.; Lin, Y.-K.; Fang, J.-Y. Biological and pharmacological activities of squalene and related compounds: potential uses in cosmetic dermatology. *Molecules* **14**, 540-554 (2009).
 - 30) Ramadan, M. F.; Kinni, S. G.; Mörsel, J. T. Biocative lipids, fatty acids and radical scavenging activity of Indian *Celastrus paniculatus* oil. *J. Appl. Bot. Food Qual.* **83**, 157-162 (2010).
 - 31) Richard, D.; Kefi, K.; Barbe, U.; Bausero, P.; Visioli, F. Polyunsaturated fatty acids as antioxidants. *Pharmacol. Res.* **57**, 451-455 (2008).