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Minor components in oils obtained from Amazonian palm fruits

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RESUMEN

Componentes minoritarios de aceites obtenidos de frutos de palmeras de la región amazónica

El objetivo de este estudio fue la caracterización de los componentes menores presentes en los aceites obtenidos del mesocarpio de frutos de especies de bacaba (*Oenocarpus bacaba*), buriti (*Mauritia flexuosa*), inajá (*Maximiliana maripa*), pupuña (*Bactris gasipaes*) y tucumá (*Astrocaryum vulgare),* de importante producción en el Estado de Amapá, Brasil.

Se determinaron las dos principales fracciones presentes en los aceites. Por una parte, los compuestos menores derivados de los componentes mayoritarios o triglicéridos (TAG): dímeros de TAG, TAG oxidados y diglicéridos (DAG) relacionados con la calidad de los aceites y, por otra, los principales grupos presentes en la fracción insaponificable (hidrocarburos, alcoholes alifáticos, esteroles y tocoferoles) relacionados con la calidad de los aceites.

Los resultados indicaron que todos los aceites extraídos tenían buena calidad inicial, siendo los DAG los mayoritarios entre los compuestos menores glicerídicos. La concentración de hidrocarburos (50-734 mg·kg^{-1}) y de alcoholes alifáticos (80-490 mg·kg^{-1}) fue muy variable correspondiendo al aceite de inajá el mayor contenido en hidrocarburos y en alcoholes. En el caso de los tocoferoles, las mayores cantidades correspondieron a los aceites de buriti (1567 mg·kg^{-1}) y tucumá (483 mg·kg^{-1}) y la presencia de cantidades significativas de tocotrienoles sólo se detectaron en aceite de inajá. Finalmente, se encontraron concentraciones elevadas de esteroles en todas las muestras, especialmente en los aceites de pupuña (4456 mg·kg^{-1}) y tucumá (2708 mg·kg^{-1}), siendo el β-sitosterol el esterol mayoritario con porcentajes entre 65 y 83%.

PALABRAS CLAVE: Aceites vegetables – Amazonia – Ceras – Diglicéridos – Esteroles – Fracción insaponificable – Frutos de palmeras – Hidrocarburos – Tocoferoles – Triglicéridos oxidados.

SUMMARY

Minor components in oils obtained from Amazonian palm fruits

This study deals with the characterization of minor compounds in oils obtained from the mesocarp of fruits of the main palm species from the State of Amapá, Brazil, i.e. bacaba (*Oenocarpus bacaba*), buriti (*Mauritia flexuosa*), inajá (*Maximiliana maripa*), pupunha (*Bactris gasipaes*) and tucumã (*Astrocaryum vulgare).* The concentration of minor glyceridic compounds, i.e. dimeric triacylglycerols (TAG), the oxidized TAG and diacylglycerols (DAG) related to oil quality, and the compounds of unsaponifiable matter, i.e. hydrocarbons, aliphatic alcohols, sterols and tocopherols have been determined.

The results indicate that the extracted oils had good initial quality, with DAG as the major glyceridic compound. The contents of hydrocarbons (50-734 mg·kg^{-1}) and aliphatic alcohols (80-490 mg·kg^{-1}) were highly variable with inajá oil containing the highest contents. In the case of tocopherols, buriti (1567 mg·kg^{-1}) and tucumã (483 mg·kg^{-1}) oils had the highest contents and the presence of significant amounts of tocotrienols was only detected in inajá oil. Finally, high concentrations of sterols were found in all the samples, particularly in the oils from pupunha (4456 mg·kg^{-1}) and tucumã (2708 mg·kg^{-1}), with β-sitosterol being the major sterol in all the samples with percentages between 65 and 83%.

KEY-WORDS: Amazonian – Diglycerides – Oxidized triacylglycerols– Palm fruits – Sterols – Tocopherols – Unsaponifiable matter – Vegetable oils – Waxes.

1. INTRODUCTION

Oils and fats are complex mixtures containing a wide range of groups of compounds, where each of the groups in turn can contain a very wide range of individual components. Apart from the major compounds, i.e. triacylglycerols, there are two minor fractions of interest in fats and oils. On one hand, the unsaponifiable fraction is constituted by a high number of individual compounds belonging to different groups of components such as hydrocarbons, waxes, sterols, tocopherols, etc. Their importance in the characterization and detection of oil mixtures are of great interest (Aparicio and Aparicio-Ruiz, 2000; Janssen *et al.*, 2009). Also, the nutritional value of edible oils depends on the content and composition of biological active compounds from this fraction such as phytosterols which prevent the intestinal absorption of cholesterol in humans, resulting in a lowering of serum cholesterol (Mackay and Jones, 2011) or tocopherols, vitamin E compounds only synthesized by plants, which are well recognized for their effective inhibition of lipid oxidation in foods and biological systems (Kamal-Eldin and Appleqvist, 1996).

On the other hand, there is a second fraction of compounds naturally occurring in oils and fats related to the TAG species. They are the mono and diacylglycerols, free fatty acids, oxidized acylglycerols and dimeric triacylglycerols. The determination of these minor components is of great value in establishing oil quality given the relationship between their contents and the incidence of the two main reactions undergone by fats and oils, i.e. hydrolysis and oxidation (Dobarganes *et al*., 1988).

Native palm trees belong to the *Arecaceae* family and are among the most useful plant resources for the Amazonian population. Previous studies starting from different species have shown that their fruits are of great potential interest due to their high lipid content and fatty acid composition (Clement *et al*., 2005). The information on minor compounds is scarce although studies have been carried out on some species (Yuyama *et al*., 2003; Bereau *et al*., 2003; Rodrigues *et al*., 2010; Montúfar *et al*., 2010; Vázquez-Ocmín *et al*., 2010) report the presence of bioactive compounds at considerable levels. Research remains to be done in order to know the specific composition of these sources and even the possibility of them being used as specialty oils (Madawala *et al.,* 2012) in the same way as other oils extracted from fruits.

The aim of this study was to determine the minor compounds present in the oils obtained from the fruits of the main palms from the State of Amapá, Brazil, i.e. bacaba (*Oenocarpus bacaba*), buriti (*Mauritia flexuosa*), inajá (*Maximiliana maripa*), pupunha (*Bactris gasipaes*) and tucumã (*Astrocaryum vulgare)*. A previous detailed study of the triacylglycerol species present in the five oils was carried out by means of HPLC and GC (Santos *et al*., 2013). In this paper, the contents of glyceridic compounds, i.e. dimeric triacylglycerols, oxidized triacylglycerols and diglycerides, as well as the main groups from the unsaponifiable matter, i.e. hydrocarbons, aliphatic alcohols, sterols and tocopherols have been quantified.

2. MATERIALS AND METHODS

2.1. Materials

Five samples of fruits from five palm species, i.e. bacaba (*Oenocarpus bacaba*), buriti (*Mauritia flexuosa*), inajá (*Maximiliana maripa*), pupunha (*Bactris gasipaes*) and tucumã (*Astrocaryum vulgare),* were collected in the State of Amapá, Brazil. The mesocarp of the fruits was separated, moisture was eliminated by freeze-drying before lipid extraction and the lyophilized samples were maintained at –30 °C until extraction and analysis.

2.2. Lipid extraction

The total amount of lipids in the samples was obtained by Soxhlet applying an extraction period of 6 h and diethyl ether as solvent (AENOR, 1991). Then, the solvent was evaporated under vacuum and the extracted oil was dried to constant weight using a stream of nitrogen.

2.3. Analytical determinations

2.3.1. Minor glyceridic compounds

The contents of minor glyceridic compounds were determined gravimetrically according to the IUPAC Standard Method 2.507 (IUPAC, 1992) with slight modifications. Thus, the non-polar and polar fractions were separated from 1 g of oil by silica column chromatography. The non-polar fraction, which contains the non-polar TAG, was eluted with 150 mL of n-hexane/diethyl ether (90:10, v/v). A second fraction, which comprises the total polar compounds, was eluted with 150 mL of diethyl ether. The solvents were evaporated and the contents of the non-polar and polar fractions were determined gravimetrically. The efficiency of the separation was checked by thin layer chromatography using hexane/diethyl ether/acetic acid (80:20:1, v/v/v) for the development of plates and exposure to iodine vapor to reveal the spots. The polar fraction was analyzed by HPSEC to determine the content of dimeric and oxidized monomeric triacylglycerols (TAG), as well as diglycerides (DG) and fatty acids. A chromatograph equipped with a Rheodyne 7725i injector with a 10 µL sample loop, a Knauer 1200 HPLC pump (Knauer, Germany) and a Merck refractive index detector was used. The separation was performed on two 100 and 500Å PL gel columns (30 cm × 0.75 cm I.D.) packed with porous, highly cross-linked polystyrene-divinylbenzene copolymers (film thickness 5 µm) (Agilent Technologies, Santa Clara CA, USA) connected in a series. Tetrahydrofuran (1 mL min^{-1}) was used as the mobile phase and samples were analyzed at concentrations between 15 and 20 $mg{\cdot}mL^{-1}$ in tetrahydrofuran (Dobarganes *et al*., 2000).

2.3.2. Unsaponifiable matter

Unsaponifiable matter was determined according to the AOCS Official Method Ca 6b-53 (AOCS, 2001).

2.3.3. Hydrocarbons

Hydrocarbons were obtained by adsorption chromatography starting from 1 g of oil according to the IUPAC Standard Method 2.507 (IUPAC, 1992) for silica column preparation except that the glass column was filled with a slurry of silica gel and hexane, the elution solvent for hydrocarbons. The elution of hydrocarbons is carried out with 150 mL of hexane. One mL of a solution of squalane (1,025 $mg.mL^{-1}$) was initially added to the samples as internal standard for quantitative purposes. GC was performed using an Agilent 7890A chromatograph

equipped with a cold on-column injector with an oven-track system and a flame-ionization detector. An HP-5 column (5% diphenyl/95% dimethyl polysiloxane, length 15 m, 0.32 mm i.d. and 0.1 μm film thickness; Agilent Tech.) was used. Hydrogen (140 kPa inlet pressure) was used as carrier gas and nitrogen as makeup gas. The oven temperature was held at 80 °C for 5 min and then increased at 45 °C·min^{-1} to 120 °C and then at 5 °C·min^{-1} to 310 °C where it was held for 7 min. The detector temperature was 350 °C. The concentration of hydrocarbons was obtained from the total area and the internal standard area.

2.3.4. Phytosterols and aliphatic alcohols

The composition of free sterols in the oils was determined in the unsaponifiable fractions obtained from 2 g of samples according to the IUPAC Standard Method 2.404 (IUPAC, 1992). Previously, for quantification purposes, 1 mg of α-cholestanol and 0.5 mg of heneicosanol were added to the samples as internal standard for free sterols and aliphatic alcohols, respectively. Both groups of compounds were isolated by thin layer chromatography before silylation and analysis. Separation by GC was performed using an Agilent 7890A chromatograph equipped with a flame ionization detector, a PTV injector and a HP-5 fused silica capillary column (30 m length, 0.32 mm i.d., 0.20 μm film thickness). Hydrogen was used as carrier gas. The detector temperature was 325 °C. The initial oven temperature was 75 °C (1 min) and a ballistic temperature gradient of 40 °C min^{-1} to 250 °C and then maintained for 25 min for free sterols. For aliphatic alcohols, the initial oven temperature was 75 °C (1 min), a first ballistic temperature gradient of 40 °C min^{-1} to 180 °C and then a second ramp to 300 °C at 5 °C·min^{-1}. The maximum temperature was maintained for 30 minutes.

2.3.5. Tocopherols

Tocopherols were determined by HPLC with fluorescence detection (excitation at 290 nm and emission at 330 nm), following the IUPAC Standard Method 2.432 (IUPAC, 1992). The column was a LiChrosorb Si 60 packed with silica (5 μm particle size) (Merck, Darmstadt, Germany). Sample solutions of 50 mL·min^{-1} were used and the mobile phase was *n*-hexane/isopropanol (99:1, v/v), with a flow rate of 1 mL·min^{-1}.

3. RESULTS AND DISCUSSION

Table 1 summarizes the fatty acid composition as a reference of the major compounds for these palm species. Triacylglycerols and fatty acid compositions have been detailed previously (Santos *et al*., 2013). As can be observed, monounsaturated fatty acids were the most abundant in all the species and, consequently, they could be considered healthy oils which give protection against cardiovascular diseases.

Table 2 shows the quantitative results for the minor glyceridic compounds other than TAG, i.e. dimeric TAG, oxidized monomeric TAG and diacylglycerols (DAG). Their concentration is indicative of oil quality as they are compounds originated by thermal polymerization, oxidation and hydrolysis, respectively. And consequently, the

Table 1
Fatty acid composition of oils from the mesocarp of Amazonian palm fruits

Fatty acid methyl esters (FAME)	Palm Species				
	Bacaba	Buriti	Inajá	Pupunha	Tucumã
Saturated FAME	30.6	22.4	42.0	41.3	26.0
Monounsaturated FAME	47.3	72.3	40.8	51.4	64.7
Polyunsaturated FAME	20.6	3.9	14.4	5.3	7.2

Table 2
Concentration of minor glyceridic compounds (%wt) in oils from the mesocarp of Amazonian palm fruits determined by HPLC

Group of compounds	Palm Species				
	Bacaba	Buriti	Inajá	Pupunha	Tucumã
Dimeric TAG	0.2 ± 0.0	0.1 ± 0.0	–	–	–
Oxidized monomeric TAG	1.1 ± 0.2	0.7 ± 0.1	1.2 ± 0.1	0.8 ± 0.2	1.2 ± 0.1
Diacylglycerols	2.9 ± 0.1	1.5 ± 0.1	2.0 ± 0.0	1.6 ± 0.2	2.8 ± 0.0
Fatty acids*	1.0± 0.1	0.9 ± 0.0	0.5 ± 0.0	1.3 ± 0.1	1.2 ± 0.0
Total (mg.kg^{-1})	**5.0 ± 0.1**	**3.3 ± 0.1**	**3.7 ± 0.1**	**3.6 ± 0.5**	**5.2 ± 0.1**

mean ± standard deviation (n = 3). TAG, triacylglycerols. *also including polar unsaponifiable matter.

content of these groups of compounds should be as low as possible. As can be observed, the major compounds in all the samples were DAG. They were present in the range of 1 to 3%, similar to those found in virgin olive oils and they are not only attributed to the enzymatic or chemical hydrolysis of TAG occurring before or during the oil extraction process but also to the incomplete biosynthesis of TAG (Vázquez-Roncero *et al*., 1965; Castellani *et al*., 2008). Oxidized monomeric TAG were around 1% in all the samples, which are normal levels in good quality oils, while the level of dimeric TAG was very low, as expected for crude oils due to the absence of high temperatures in their extraction (Ruíz-Méndez *et al*., 1997). Finally, the total values found, between 3.3 and 5.2%, were low and denote good quality oils (Lumley, 1988).

Table 3 shows unsaponifiable matter and the total concentrations of hydrocarbons and aliphatic alcohols. To our knowledge, there is no previous information on the content of these two groups of compounds, probably because the composition is very complex. As can be observed, the concentrations were highly variable for both groups. Thus, hydrocarbons ranged from 50 to 734 mg·kg^{-1} and alcohols from 80 to 490 mg·kg^{-1}. The high concentration of hydrocarbons and alcohols in inajá oil stands out where octacosanol and triacontanol were the most abundant aliphatic alcohols in the samples. As for the unsaponifiable fraction, the contents vary from 0.8 to 1.8% and were similar to the values found in most edible vegetable oils.

The tocopherol composition is given in Table 4. As can be observed, the content is also highly variable, with the mean value ranging from 85 mg·kg^{-1} for inajá oil to 1567 mg·kg^{-1} for buriti oil, which seems to indicate that this oil has an promising potential as a dietary source due to its high vitamin E content. α-tocopherol was the major compound in all the oils and the presence of tocotrienols in significant amounts was only detected in inajá oil. Figure 1 shows the chromatographic profile of

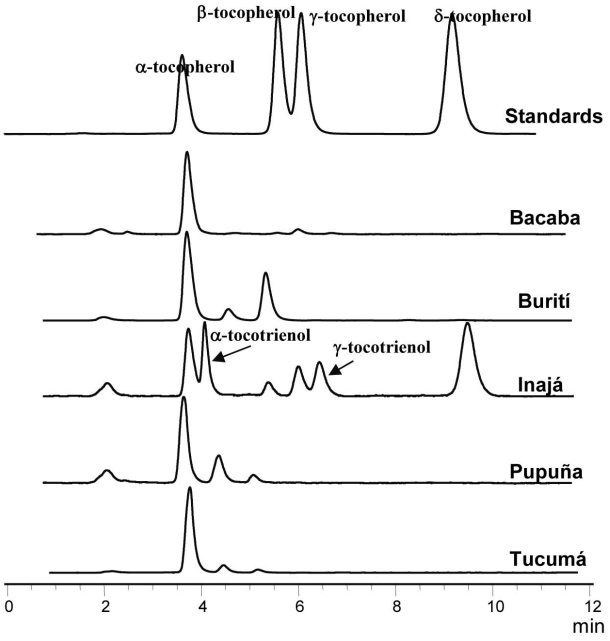

Figure 1
Gas chromatographic profiles of the tocopherols of oils from the mesocarp of fruits from Amazonian palm trees.

Table 3
Quantification of the unsaponifiable fraction (%), hydrocarbons and fatty alcohols (mg.kg^{-1}) in oils from the mesocarp of Amazonian palm fruits

Group of compounds	Palm Species				
	Bacaba	**Buriti**	**Inajá**	**Pupunha**	**Tucumã**
Unsaponifiable matter	1.0 ± 0.1	1.3 ± 0.3	0.8 ± 0.1	1.3 ± 0.1	1.8 ± 0.2
Hydrocarbons	50 ± 0.5	145 ± 1.3	734 ± 126	44 ± 3.0	110 ± 0.4
Fatty alcohols	80 ± 14.7	149 ± 8.3	490 ± 54.4	202 ± 29.9	428 ± 75.1

mean ± standard deviation (n = 3).

Table 4
Concentration of tocopherols (mg.kg^{-1}) in oils from the mesocarp of Amazonian palm fruits determined by HPLC

Compound	Palm Species				
	Bacaba	**Buriti**	**Inajá***	**Pupunha**	**Tucumã**
α-tocopherol	148 ± 41	1100 ± 198	26 ± 16	117 ± 18	480 ± 40
β-tocopherol	tr	466 ± 26	3 ± 1	tr	3 ± 2
γ-tocopherol	tr	ND	18 ± 1	ND	ND
δ-tocopherol	ND	ND	37 ± 1	ND	ND
Total (mg.kg^{-1})	**148 ± 41**	**1567 ± 205**	**85 ± 16**	**117 ± 18**	**483 ± 40**

mean ± standard deviation (n = 3); tr, traces; ND, not detected; * also containing 26 and 24 mg·kg^{-1} of α- and γ-tocotrienols, respectively.

Table 5
Sterol composition (%) and total sterols (mg.kg^{-1}) of oils from the mesocarp of Amazonian palm fruits by GC-FID

Sterol	Palm Species				
	Bacaba	**Buriti**	**Inajá**	**Pupunha**	**Tucumã**
Campesterol	11.0 ± 0.2	6.6 ± 0.3	18.8 ± 1.8	10.9 ± 0.7	13.9 ± 0.5
Stigmasterol	12.6 ± 0.2	16.8 ± 0.9	5.4 ± 0.3	4.2 ± 0.4	8.1 ± 1.3
Δ5,23-Stigmastadienol	ND	ND	4.1 ± 2.4	ND	ND
β-Sitosterol	76.4 ± 0.3	76.6 ± 0,6	65.4 ± 3.1	82.2 ± 1.5	76.6 ± 0.9
Δ5-Avenasterol	tr	tr	2.4 ± 0.5	2.7 ± 0.4	1.4 ± 0.5
Δ 5,24-Stigmastadienol	ND	ND	2.3 ± 1.0	ND	ND
Δ 7-Stigmastenol	ND	ND	0.6 ± 0.9	ND	ND
Total (mg.kg^{-1})	**981 ± 49**	**2332 ± 231**	**1463 ± 244**	**4456 ± 372**	**2708 ± 120**

mean ± standard deviation (n = 3); tr, traces; ND, not detected.

tocopherols and tocotrienols for the five samples. Previous results reported concentrations of a similar order for buriti oil (Silva *et al*., 2009; Costa *et al*., 2010) and pupunha (Bereau *et al*. 2003), lower concentrations for buriti, inajá and tucumã oils (Rodrigues *et al*., 2010) and higher concentrations for bacaba oil (Montúfar *et al*., 2010).

Table 5 shows the percentages of phytosterol species and the total concentrations in the oils. As can be observed, β- sitosterol was the major compound in the group in all the oils ranging from 65 and 83% followed by campesterol (6.6-18.8%) and stigmasterol (4.2-16.8%). The total sterol concentrations were variable, ranging from around 1000 mg·kg^{-1} in bacaba oil to more than 4000 mg.kg^{-1} in pupunha oil. Similar contents have been reported for buritί (Costa *et al*., 2010), tucumã (Bereau *et al*. 2003) and inajá oils (Bereau *et al*., 2003; Costa *et al*., 2010) and lower for pupunha oil (Bereau *et al*., 2003). Given the interest of phytosterols as functional ingredients, buriti, pupunha and tucumã oils would be good edible oils from a nutritional point of view.

In summary, from the analysis of the oils from the Amazonian fruits it can be deduced that they have a great potential as edible vegetable oils. Apart from the high content of monounsaturated fatty acid, bioactive compounds may give them an added value as healthy oils. In particular, concerning the two groups of bioactive compounds analyzed, i.e. tocopherols and sterols, pupunha and buriti oils stand out. Pupunha oil is an excellent oil due to its high content of phytosterols while buritί oil has also a high vitamin E content.

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