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Characterization of Glyceridic and Unsaponifiable Compounds of Sacha Inchi (*Plukenetia huayllabambana* L.) Oils

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ABSTRACT: This work deals with the characterization of the main glyceridic and unsaponifiable components of oils obtained from Sacha inchi (*Plukenetia huayllabambana* L.) seed ecotypes collected during two harvests in the Department of Amazonas in Peru. The seed-oil yield was 30.3−41.2%; standing out are the high percentages of the ω3- and ω6-fatty acids series whose ranges lie within those of the present Regulation for Sacha inchi oils. Triacylglycerols with even equivalent carbon number (ECN; 36−42) were the main components. Minor glyceridic polar compounds such as oxidized triglycerides, diglycerides, monoglycerides, and free fatty acids were determined by high-performance size exclusion chromatography. The low campesterol/stigmasterol ratio (1:6), unusual in the majority of vegetable oils, stands out. Regarding aliphatic hydrocarbons, these oils showed a particular profile for the saturated series of odd and even carbon atom numbers. According to our results Sacha inchi *P. huayllabambana* oils can be offered as a good alternative to *P. volubilis*, the species mainly commercialized for this vegetable oil.

KEYWORDS: *oil characterization, polar glyceridic compounds, Sacha inchi P. huayllabambana oil, triglycerides, unsaponifiable components*

INTRODUCTION

Sacha inchi plants from the Peruvian Amazon produce a kind of oil seed with high protein and lipid content.[1] This oil can be consumed virgin (cold-pressing extraction) and is highly valued for its sensory qualities. It can be considered as a gourmet oil as are olive, avocado, wheat germ, rice bran, and argan oils, and it is appreciated for its beneficial health properties and its unique taste and flavor.[2,3]

Nowadays, commercialized Sacha inchi oils come from the *Plukenetia volubilis* L. (*P. volubilis*) species, which grows mainly in the San Martin region, the most productive one in Peru. In September 2004 it was declared to be of "National Genetic Heritage and alternative product in the fight against poverty" by the Congress of the Republic of Peru. Extensive and thorough studies have been undertaken to determine the chemical composition of Sacha inchi (*P. volubilis*) seeds and oils, leading to an exhaustive knowledge of the chemical composition of this species.[4−9] In addition, from 2009 on there is a Regulation (NTP) that specifies the requirements of the analytical parameters that must meet Sacha inchi seed oil from the *Plukenetia* gender.[10]

There are five species of Sacha inchi growing in Peru: *P. brachybotrya*, *P. loretensis*, *P. volubilis*, *P. polyandenia*, and *P. huayllabambana*. Bussmann and co-workers[11] have described them morphologically. *P. huayllabambana* is a novel species which has larger and rougher seeds than *P. volubilis*, the most popular species,[12] and that grows wild in the province of Rodríguez de Mendoza, Amazonas Department. Currently, there are 220 ha of this species in Rodriguez de Mendoza. This is harvested at the beginning of January, ending in August, yielding approximately 1.500 kg·ha^{-1} of seeds. An increasing interest in this species has been raised lately, and thus, Muñoz Jauregui and co-workers[13] have published a broad nutritional study, while Ruiz and co-workers[14] have provided some data about its composition. This new species seems a promising source of high-quality vegetable oil, which gives to these crops a high added value since they can be considered as an alternative cultivar for the local inhabitants of the region.

Our aim was to study the *P. huayllabambana* oils obtained by cold extraction process from seeds collected in the main plantations of the province of Rodríguez de Mendoza, analyzing its major and minor components glyceridic and unsaponifiable matter with the purpose of providing knowledge and supporting the potential of this species for commercialization.

MATERIALS AND METHODS

Materials. Twenty-eight batches of Sacha inchi seeds (*P. huayllabambana*) were harvested between April 2012 and 2013 in the province of Rodriguez de Mendoza, Department of Amazonas. Sampling was conducted at the proper maturation stage, considering the growing altitude (1500−2100 m), the temperature range (18.6−23.1 °C), the pH of the soil (6.2), and the percentage of organic matter (2.3%).

Oil Extraction and Composition of Sacha Inchi Seeds. After shelling and weighing, the oils were extracted by hydraulic pressing and then centrifuged. The process was carried out with

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a prototype designed in the Lima University laboratories, which consists of a cylinder with holes of 1/16 in. in which the seeds were loaded and then pressed by a piston actuated by a hydraulic system assisted by a motor of 1 horsepower (HP). An average amount of 2 kg of shelled seeds was used for each sample in order to obtain enough oil for complete characterization. After the extraction the oil was filtered with a centrifugal filter (30 min, 3600 rpm). Crude oil samples were stored in dark bottles at 4 °C until analysis.

Reagents and Solutions. Acetone, diethyl ether, hexane, propionitrile, and THF were supplied by VWR International (West Chester, PA, USA). The Si-SPE cartridges were from Varian (EA Middelburg, The Netherlands). Standards of 5-α-cholestan-3β-ol and trilinolenin (LnLnLn) were from Sigma-Adrich Co. (St. Louis, MO, USA). Hexamethyldisilazane, pyridine, trimethylchloroxilane, and standards of eicosane and tocopherols were from Merck-España (Merck Group, Darmstadt, Germany). All chemical reagents were analytical grade.

Physical−Chemical Determinations. The physical−chemical parameters included in the current legislation for Sacha inchi oils and other regulations for other edible oils were determined in the crude oils.[10]

Free Fatty Acids and Peroxide Values. AOCS Official Methods were followed to determine FFA and peroxide indexes.[15,16]

UV Evaluation. Specific extinction at 270 nm (K270) was evaluated according to the European Union Standard Methods.[17]

Oxidative Stability. The induction time was evaluated using a Rancimat equipment (743 Rancimat Metrohm Co., Basel, Switzerland), following the standard Official Method Cd 12-b-92,[18,19] with an air flow of 20 L·h^{-1} and 100 ± 1 °C.

Other Physical Parameters. Parameters such as viscosity and density were measured using a Stabinger SVM 3000 viscometer (Anton Paar GmbH Graz, Austria). The refractive index was determined with a temperature-controlled Abbe refractometer (Hilger & Watts Ltd., London, U.K.).[3,20]

Major Components. *Triacylglycerol Composition.* The TG composition was obtained as follows: oil samples at a concentration of 50 mg·mL^{-1} in acetone were analyzed by RP-HPLC. The separations were done on a Merck Li-Chrospher 100 RP-18 column (250 mm × 4 mm i.d.; 4 μm particle size) thermostated at 20 °C. The liquid chromatograph (Beckman-Coulter, Fullerton, CA, USA) was equipped with a pumping unit (118 solvent module) and used propionitrile as the mobile phase at a flow rate of 0.6 mL·min^{-1}. Oil solutions in acetone (10 μL) were injected using an autosampler (508 system). Detection was done with a PerkinElmer 200 RI detector.[21]

Polar Glyceridic Compounds. PC were assessed with small modifications of the already published method, by separating the oils in two fractions by Si-SPE using 1 g cartridges.[22,23] Thus, 200 mg of oil samples were weighed in 10 mL volumetric flasks and dissolved in hexane. On each case an aliquot of this solution (2 mL) was introduced into the cartridge and then eluted with 15 mL of the hexane:diethyl ether 87:13 (v/v) admixture. The fraction containing the nonaltered TG was rejected. A second fraction was collected eluting with 15 mL of diethyl ether. The latter fraction was evaporated until dryness, re-dissolved in 1 mL of THF and injected onto the HPLC (Hewlett-Packard 1050 series). The HPSEC system is equipped with an Agilent PL gel of 5 μm, a 100 Å column, and a Merck La Chrom RI Detector L-7490. THF was used as eluent at a flow rate of 0.7 mL·min^{-1}. The injection volume was 20 μL. The compounds eluted from the columns according to their molecular size, so those with the largest molecular sizes were eluted earlier and were quantified as individual percentages of the total eluted compounds and also as absolute quantities using an external standard of pure TG in THF at concentrations of 5−10 mg·mL^{-1}.

Fatty Acid Composition. The FFA composition was determined as the composition of FAME by GC after transesterification of the oils with 2 N KOH in methanol, according to the IUPAC Standard Method.[20,24] The chromatographic analysis was done using an Agilent 5890 GC system (Palo Alto, CA, USA) equipped with a split injector (1:50 split ratio), a polar capillary column (poly(90% biscyanopropyl−10% cyanopropylphenyl)siloxane, 60 m × 0.25 mm i.d.; 0.20 μm film thickness) and a FID. Hydrogen was used as the carrier gas at a flow rate of 1.0 mL·min^{-1}. The detector and injector temperatures were 225 and 250 °C, respectively. The initial oven temperature was 180 °C, and a temperature gradient from 180 to 220 °C at 3 °C min^{-1} was applied. Injections of 1 μL each were performed automatically.

Minor Unsaponifiable Compounds. The percentage of the total unsaponifiable fraction was calculated gravimetrically after subjecting the oil samples to a saponification procedure carried out according to the IUPAC 2401 Official Method.[25]

Tocopherols. Tocopherols were determined according to the IUPAC Standard Method 2432.[26] An oil solution in hexane at a concentration of 10 mg·mL^{-1} was prepared and analyzed by HPLC fitted with a Si-column (250 mm × 4 mm i.d.; 4 μm particle size). The elution solvent was a hexane:2-propanol (99:1, v/v) mixture at a flow rate of 1 mL·min^{-1}. Detection was done by fluorescence (RF-10AXL Shimadzu fluorescence detector), setting excitation and emission at λ = 290 and λ = 330 nm, respectively. For quantitative determinations standards of tocopherols in hexane at concentrations of 4−6 μg·mL^{-1} were prepared and injected.

Sterol Composition. The sterol fraction was isolated from the unsaponifiable matter by silica TLC using plates impregnated with potassium hydroxide with the purpose of retaining the remaining saponified compounds. The plate was developed twice with a mixture of petroleum ether:diethyl ether (87:13, v/v). The isolated fraction was scratched off and extracted with hot chloroform and diethyl ether. The solution was evaporated until dryness, derivatized with 500 μL of the 1:3:9 (v/v/v) trimethylchloroxilane:hexamethyldisilazane:pyridine admixture and analyzed by GC. The gas chromatograph (Agilent 6890N) was equipped with a fused silica low-polarity capillary column (poly(5% diphenyl−95% dimethyl)siloxane, 30 m × 0.25 mm i.d. × 0.25 μm film thickness), and FID. The oven program was isothermal at 260 °C with a split ratio of 1:50. Hydrogen was used as carrier gas at a flow rate of 1 mL·min^{-1}. The temperatures of the injector and detector were 300 °C. The quantitative determination was done using α-cholestanol as internal standard.

Aliphatic Saturated Hydrocarbons. HC were isolated by low-pressure column chromatography filled with 15 g of silica gel (Si-60) impregnated with $AgNO_3$.[27] A sample of 0.5 g of oil was introduced into the column, and the fraction containing the aliphatic hydrocarbons was eluted with 80 mL of petroleum ether. This fraction was evaporated until dryness, re-dissolved in 0.5 mL of hexane, and analyzed by GC. The gas chromatograph was equipped with a low-polarity capillary column (poly-5% diphenyl−95% dimethylsiloxane, 12 m × 0.32 mm i.d. × 0.1 μm film thickness) and an on-column injection

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Table 1. Physical−Chemical Parameters of the Sacha Inchi (*P. Huayllabambana* L.) Oils[a]

	harvest 1		harvest 2	
	$M(n=14)$ (%)	range	$M(n=14)$ (%)	range
acidity value	1.8a ± 0.1	0.5−4.7	1.6b ± 0.1	0.5−3.7
peroxide value (meq $O_2 \cdot kg^{-1}$)	8.4a ± 0.2	1.5−19.1	3.3b ± 0.2	2.1−5.6
K_{270}	0.14b ± 0.01	0.10−0.28	0.19a ± 0.01	0.14−0.23
stability (h)	2.8a ± 0.1	2.0−3.4	3.0a ± 1.2	1.8 ± 5.5
density (25 °C) ($g \cdot cm^{-3}$)	0.927a ± 0.001	0.926−0.930	0.925a ± 0.002	0.920−0.930
refractive index	1.480a ± 0.0001	1.480−1.481	1.482a ± 0.0004	1.481−1.482
viscosity (20 °C) ($mm^2 \cdot s^{-1}$)	42.8a ± 0.9	41.2−44.0	41.1a ± 2.2	38.9−43.8

[a]Data are the average of 14 samples and are expressed as the mean ± SD. Different letters (a, b) represent significant differences.

Table 2. Major Compounds of Sacha Inchi (*P. huayllabambana* L.) Oils: Triglyceride, Polar Glyceridic Compounds and Fatty Acid Methyl Esters[a]

	samples			
	harvest 1		harvest 2	
	$M(n=14)$	range	$M(n=14)$	range
	Triglycerides (wt % on total)			
ECN 36 (LnLnLn)	20.0 ± 1.18	17.7−22.5	20.0 ± 1.84	18.0−23.0
ECN 38 (LnLnL)	26.3 ± 0.7	25.3−27.5	26.0 ± 1.21	22.6−27.7
ECN 40 (LnLL + LnLnP + LnLnO)	20.6 ± 0.79	19.2−21.8	20.9 ± 0.86	19.4−23.1
ECN 42 (LLL + OOLn + LLP + POLn)	20.7 ± 1.20	19.4−22.8	22.5 ± 1.10	19.0−24.0
ECN 44 (LLO + OOLn + LLP + POLn)	7.0 ± 0.70	6.0−8.3	7.6 ± 0.94	6.0−10.5
ECN 46 (OOL + LnPP + PLO)	2.5 ± 0.48	1.8−3.4	2.4 ± 0.79	1.39−5.0
ECN 48 (OOO + POO)	1.3 ± 0.75	0.5−2.7	0.5 ± 0.28	0.1−1.0
ECN 50 (SSO)	0.7 ± 0.7	0.0−2.5	<0.01	
	Polar Compounds (wt % on Total)			
oxTG	31.4 ± 3.0	27.7−37.1	21.6 ± 5.9	13.6−29.9
DG	41.6 ± 3.0	37.8−45.4	42.7 ± 5.1	33.5−52.5
MG	2.1 ± 0.7	0.6−3.3	2.5 ± 1.1	1.2−4.5
FA	24.9 ± 3.0	20.8−32.2	33.1 ± 6.2	26.4−47.0
total (% on oil)	3.4a ± 0.7		4.0a ± 1.5	
	Fatty Acids (wt % on Total)			
C16:0	4.6 ± 0.2	4.3−4.9	4.9 ± 0.38	4.4−5.7
C16:1	0.07 ± 0.0	0.06−0.09	0.08 ± 0.02	0.05−0.15
C17:0	0.08 ± 0.0	0.07−0.10	0.08 ± 0.02	0.05−0.21
C18:0	1.6 ± 0.1	1.5−2.0	1.7 ± 0.2	1.3−2.1
ω9-C18:1	8.0 ± 0.6	8.2−10.4	7.8 ± 0.56	6.8−8.8
ω7-C18:1	1.0 ± 0.01	0.9−1.0	0.93 ± 0.06	0.80−1.10
ω6-C18:2	26.2 ± 0.7	25.0−27.3	25.8 ± 0.6	24.5−26.9
C20:0	0.30 ± 0.1	0.20−0.30	0.25 ± 0.05	0.21−0.44
ω3-C18:3	58.1 ± 1.4	55.5−60.4	58.2 ± 1.5	55.9−60.4

[a]Data are the average of 14 samples and are expressed as the mean ± SD. The same letter (a) represents no significant differences.

system. The initial oven temperature was set at 80 °C for 2 min, and the rate was established at 12 $°C \cdot min^{-1}$ up to 280 °C, then at 7 $°C \cdot min^{-1}$, and then up to 340 °C. The injector temperature was 80 °C and was allowed to follow the oven temperature program. The FID temperature was 350 °C. Eicosane (C20:0) at a concentration of 0.05 $mg \cdot mL^{-1}$ was used as internal standard.

Statistical Analysis. Each sample was analyzed in triplicate, and data were presented as the average of the 14 data obtained in each crop. The statistical analyses were run using the GraphPad Prism version 5.01 for Windows software. The crops were compared by ANOVA. When significance was observed, the Tukey test was applied. Significant differences ($p > 0.05$) are specified with different letters.

RESULTS AND DISCUSSION

Oil Yield. Compared to Sacha inchi *P. volubilis*, currently the most widely used cultivar for oil extraction and commercialization, the *P. huayllabambana* species is almost twice its size (1.7−4.1 cm^3 vs 4.1−8.0 cm^3) and weight, having an oil yield after pressing and filtration in the range of 30.3−41.2% with respect to the shelled seeds.

Analytical Indexes and Oxidative Stability. The values of the indexes commonly used to evaluate the initial quality of vegetable oils, i.e., FA, PV, and K_{270}, were found within the expected ranges for crude oils of good quality, with some remarkable exceptions. Regulation for extra virgin and virgin Sacha inchi oils establishes a maximum of 1.0 and 2.0 (expressed as a percent of oleic acid) of FA, respectively. Table 1 shows the results of the quality parameters, and it can be observed that the average value obtained for the 28 oil

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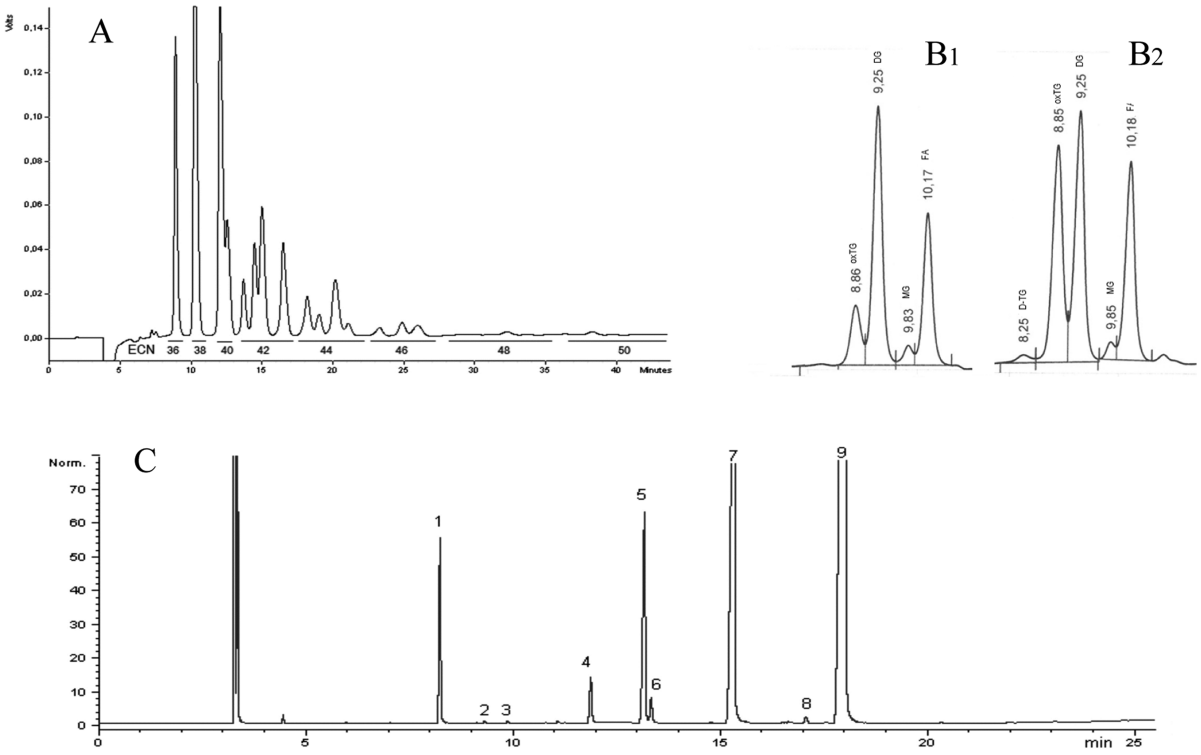

Figure 1. Chromatogram profiles of the major compounds of Sacha inchi *P. huayllabambana* oils, where panels are described as follows: (A) HPLC-RI of triglycerides with ECN 36−50; (B1 and B2) HPSEC-RI of polar compounds and dimers of tryglicerides (D-TG), oxidized triglycerides (oxTG), diglycerides (DG), monoglycerides (MG), free fatty acids (FFA); (C) HRGC of FA for (1) palmitic C16:0, (2) palmitoleic C16:1, (3) margaric C17:0, (4) estearic C18:0; (5) oleic ω9-C18:1, (6) vacenic ω7-C18:1, (7) linoleic ω6-C18:2, (8) arachidic C20:0, and (9) linolenic ω3-C18:3 acids.

samples from the two harvests under study is within the established ranges for virgin oils. Half of the samples can be classified as extra virgin, 29% as virgin, and 21% are neither extra virgin nor virgin oils. These results are indicating that Sacha inchi *P. huayllabambana* seeds are highly susceptible to hydrolysis, yielding oils with acidity over the established limits. The high hydrolyzation level showed is not an exclusive characteristic of *P. huayllabambana* oil samples. Actually some published data on *P. volubilis* also present acidity values over the limits established in the regulations.[28,29]

Regarding the oxidation state of the oils, the mean values were shown to be within the limits included in the regulations, although three of the samples overpassed the 10 meq $O_2 \cdot kg^{-1}$ maximum established in the NTP.

Concerning the absorbance at 270 nm, there is not a limit established in the NTP Regulation. Olive oil regulation includes a limit of 0.22 for extra virgin and 0.25 for virgin olive oils. The results on the analyzed Sacha inchi oils drove us to categorize a high number of samples (24) as extra virgin, and 3 as virgin, whereas only one dropped out of both categories.

Most of the PV data reported for *P. huayllabambana* were within the limits established by the regulations. It can be observed that some of the samples from the first harvest yielded oils with PV over the limit, but that the oils obtained from the second harvest showed a significantly lower value, which means lower oxidation level, undoubtedly due to a more suitable storage period in the second repetition of the study. Nevertheless their absorbance at 270 nm is higher, although with no meaningful differences.

The oxidative stability determined by Rancimat gave IP of 2.0−3.4 h, at 100 °C. These values are rather low compared to those of other edible oils such as olive or sunflower oils, but they are similar or even higher than those of oils with similar unsaturation level, such as safflower or flax oils.[30] No significant differences are found between the two harvests under study.

We have to consider that these oils were obtained from raw seeds. In the case of carrying out a previous seed roasted process, an increase of the oil stability is to be expected, as occurred with argan seeds.[2,3] These kinds of processes have been recently carried out with Sacha inchi (*P. volubilis*).[31]

Density, refractive index, and viscosity are physical parameters determined to differentiate and to characterize the oils and mainly are used for commercial purposes. The density values at 20 °C were in the 0.920−0.930 $g \cdot cm^{-3}$ range and were within the requirements of the NTP. These values are slightly higher than those reported at the same temperature for other vegetable oils such as sunflower or olive (0.898−0.906).[3]

The refractive index at 20 °C for the first harvest ranged from 1.480 to 1.482 and is within the ranges set in the NTP. For the second harvest the average of the 14 samples under study was over the limit established in the NTP. These results were also higher than those reported for other vegetable oils (1.461 to 1.474).[32]

The kinematic viscosity data at 20 °C were in the 38.9−44.0 $mm^2 \cdot s^{-1}$ range, the averages being 42.8 and 41.1 $mm^2 \cdot s^{-1}$ for the first and second harvests, respectively. These values are higher than those reported at the same temperature for *P. volubilis*.[8]

Major Saponifiable Compounds. *Triglyceride Molecular Species.* A detailed study of the TG was carried out by means of RP-HPLC-RI, and it is summarized in Table 2. The eluting order of each TG molecular species can be determined using

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Table 3. Main Unsaponifiable Components, Sterols, Tocopherols, and Aliphatic Hydrocarbons, Determined by Split-GC, FL-HPLC, and on-Column-GC, Respectively, of Sacha Inchi (*P. huayllabambana* L.) Seed Oils[a]

	Samples			
	harvest 1		harvest 2	
	M (n=14)	Range	M (n=14)	Range
UNSAPONIFIABLE MATTER (%)	$1.30^{a(*)}$ ± 0.50	0.70 - 2.20	$0.83^{b(*)}$ ± 0.24	0.70 – 1.35
TOCOPHEROLS (wt% on total)				
α	n.c.	-	n.c.	-
β	n.d.	-	n.d.	-
γ	68.9	65.2-70.0	63.5	53.6-67.1
δ	31.1	30.0-34.8	36.5	32.9-46.4
Total ($mg \cdot kg^{-1}$)	2820.6^{a}±379.4	1810.8-2977.9	2329.0^{b}±361. 5	1973.1-2692.8
STEROLS (wt% on total)				
Cholesterol	0.3 ± 0.06	0.2 – 0.4	0.3 ± 0.04	0.2 – 0.4
Campesterol	5.0 ± 0.30	4.5 – 5.6	4.8 ± 0.18	4.6 – 5.1
Stigmasterol	29.7 ± 1.60	26.5 – 32.3	30.5 ± 1.14	29.2 – 32.3
Δ7-Campesterol	0.5 ± 0.39	0.1 – 1.0	0.5 ± 0.10	0.1 – 1.1
Clerosterol	0.7 ± 0.09	0.6 – 0.8	0.8 ± 0.04	0.8 – 0.9
β-sitosterol	60.1 ± 1.57	57.9 – 63.1	59.2 ± 1.52	57.1 – 61.5
Sitostanol	0.6 ± 0.09	0.5 – 0.8	0.6 ± 0.06	0.5 – 0.7
Δ5-Avenasterol	1.6 ± 0.18	1.2 – 2.0	1.7 ± 0.07	1.6 – 1.8
Δ5,24-Stigmastadienol	0.3 ± 0.09	0.2 – 0.4	0.4 ± 0.04	0.3 – 0.4
Δ7-Stigmastenol	0.6 ± 0.09	0.5 – 0.8	0.7 ± 0.13	0.6 – 0.9
Δ7-Avenasterol	2.3 ± 1.57	0.2 – 3.4	0.3 ± 0.04	0.3 – 0.3
Total ($mg \cdot kg^{-1}$)	1931.2^{a} ± 143.2	1652.9 – 2236.0	1877.7^{a} ± 92.2	1788.4 – 2013.3
ALIPHATIC HYDROCARBONS (wt% on total)				
$H_{21:0}$	10.6± 1.55	10.2-14.0	10.4± 0.44	10.2-10.7
$H_{22:0}$	10.3± 1.07	10.0-12.6	11.0± 0.10	10.9-11.1
$H_{23:0}$	38.5± 1.16	37.8-41.8	37.3± 1.00	36.4-38.6
$H_{24:0}$	18.8± 1.00	17.9-19.7	18.7± 0.55	17.9-19.3
$H_{25:0}$	14.0± 1.57	13.7-16.3	14.2± 0.48	13.8-14.7
$H_{26:0}$	2.6± 0.15	2.3-2.8	2.5± 0.12	2.4-2.6
$H_{27:0}$	1.8± 0.10	1.5-2.0	2.0± 0.15	1.1-2.6
$H_{28:0}$	1.9± 0.38	1.4-2.2	2.1± 0.22	1.5-2.7
$H_{29:0}$	1.5± 0.78	0.5-2.0	1.8± 0.98	0.5-2.7
Total ($mg \cdot kg^{-1}$)	137.5^{a} ± 16.7	114.8-170.7	139.6^{a} ± 4.89	134.8-144.6
Odd/Even Ratio	2.0±0.2		1.9±0.2	

[a]Data are the average of 14 samples and are expressed as the mean ± SD. Different letters (a, b) represent significant differences. n.c., not quantified, <1%; n.d.: not detected.

the standard of LnLnLn, which elutes as the first TG peak. One should take into account that under the isocratic conditions used in this work the retention times for TG are directly proportional to the number of carbon atoms and inversely proportional to the total number of double bonds in the three fatty acyl chains. TG molecular species can be grouped according to their ECN, defined as the total acyl-carbon number in the molecule minus twice the total number of double bonds of the fatty acids comprising the TG.[21]

As one can observe in Figure 1A the ECN of the main TG identified are within the range from 36 to 42, normally with combinations of Ln and L. Thus, the four main TG are LnLnLn, LnLnL, LnLL, and LLL (ECN 36, 38, 40, and 42, respectively). Also TG with ECN 44, 46, 48, and 50 are found, but in minor proportions. Fanali and co-workers[6] reported the TG composition of Sacha inchi *P. volubilis* oils from Peruvian producers for the first time. Remarkable differences can be found with the *P. huayllabambana* species presently studied. Thus, although in both species LnLnL (ECN 38) is the major TG, for *P. huayllabambana* this TG is higher than the whole group of TG with ECN 40 (26% vs 21%), which is the main TG group reported for *P. volubilis* (28.7%).[6]

Glyceridic Polar Compounds. The quantitative determination of glyceridic polar compounds gives a direct evaluation of all the compounds having polarity higher than that of the TG, the major compounds in edible fats and oils. The composition and profile of the PC is the most valuable and common methodology utilized for evaluating the alteration in heated oils. The fraction isolated by Si-SPE was quantified using HPSEC, separating the compounds according to their molecular size: D-TG, oxTG, DG, MG, and FFA (Figure 1B1 and 1B2). Quantitative PC data (Table 2), which include values between 2.3 and 7.5% for Sacha inchi *P. huayllabambana* oils, show percentages below 5−6% in most of the samples studied, which is indicative of good quality oils.[34] No comparison can be done with other Sacha inchi species since no PC data have been reported. As can be observed, the PC fraction is mainly composed of DG and FFA in a ratio of 2:1 approximately. It is noteworthy the presence of MG in proportions around 0.6−3.3% in all analyzed samples, confirming that the susceptibility of the seed to hydrolization goes beyond the DG formation. Some of the evaluated samples exhibited a high level of compounds related to hydrolization such as DG and FA, and also important quantities of oxTG and D-TG (Figure 1B2).

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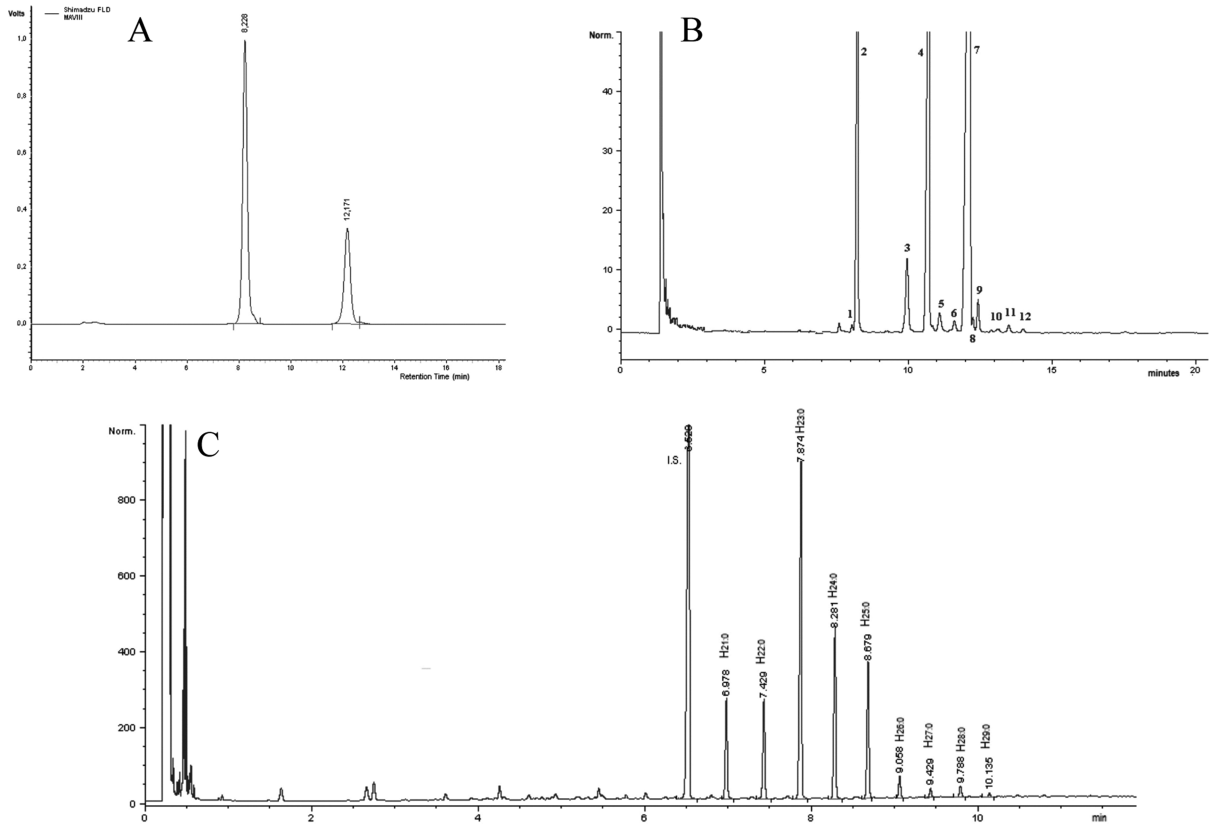

Figure 2. Chromatogram profiles of the main unsaponifiable compounds of the Sacha inchi *P. huayllabambana* oils: (A) tocopherols HPLC-FL profile (Rt 8.22, γ-tocopherol; Rt 12.17, δ-tocopherol); (B) sterol GC profile for (1) cholesterol, (2) I.S (Internal Standard: α-cholestanol), (3) campesterol, (4) stigmasterol, (5) Δ7-campesterol, (6) clerosterol, (7) β-sitosterol, (8) sitostanol, (9) Δ5-avenasterol, (10) Δ5,24-stigmastadienol, (11) Δ7-stigmastenol, and (12) Δ7-avenasterol; (C) aliphatic saturated hydrocarbon on-column GC profile. I.S: Internal Standard, H20.

The PC method, designed in the beginning for oils subjected to frying processes, gives us valuable information on the alteration of the Sacha inchi seeds due to hydrolysis. Moreover, all oil samples contain oxTG as has been seen in other edible oils but an important number of them also have high quantities of minor glyceridic components, which indicates the facility of Sacha inchi oils to oxidative alteration, which is expected due to their high unsaturation degree.

Fatty Acid Composition. A representative chromatogram of the FAME of Sacha inchi *P. huayllabambana* oil is presented in Figure 1C. There we can observe the high percentage of Ln, as well as the presence of ω7-C18:1 and the absence of C20:1. The results (Table 2) indicate no significant differences between the two studied harvests. The high contents of Ln (55.5−60.7%) and L (25.0−27.3%) stand out. These values are slightly different from those reported for Sacha inchi *P. volubilis*.[28,35] The ω6:ω3 ratio determined in this work for Sacha inchi *P. huayllabambana* is 0.45:1, which is lower than those reported for Sacha inchi *P. volubilis* (0.75:1), since for the commercial species the Ln content is low (47−50%).

Main Unsaponifiable Components. We have studied the total quantity of the unsaponifiable matter as well as the sterol, tocopherols, and aliphatic hydrocarbons profiles and contents.

The total unsaponifiable matter (Table 3) differs for both harvests and in some samples is out of the limits established in the regulations (0.36%). At this point it is important to highlight that the limit established in the regulation is not really reasonable since just the addition of the values regarding valuable unsaponifiable components such as sterols and tocopherols exceeds it.

Figure 2 represents the chromatograms of the main unsaponifiable compounds of Sacha inchi *P. huayllabambana* oils studied here.

Tocopherols. γ-Tocopherol and δ-tocopherol are the main species that can be directly separated and quantified by HPLC using fluorescence detector (Figure 2A). In a few number of samples also α-tocopherol is present, but with percentages lower than 0.1. γ-Tocopherol was the major tocopherol with percentages above 65% in most of the samples. High variability is found in their total quantities not only between harvests but also among samples within the same harvest (Table 3). This fact can be explained by taking into account that these compounds, specially δ-tocopherol, are powerful antioxidants and protect the highly unsaturated TG of Sacha inchi oils against oxidation, resulting in their degradation. The results obtained regarding the tocopherol content of the Sacha inchi *P. huayllabambana* are slightly higher than data published for *P. volubilis* species.[6,28] However, they are above the maximum limit established in the most recent Peruvian Regulations for Sacha inchi *P. volubilis* oils.[36] This recent regulation concerning only *P. volubilis* species establishes a maximum limit of 0.7, 0.29, 136.7, and 85.6 mg·kg^{-1} for α, β, γ, and δ, respectively, which is at the least strange.

Sterols. Related to the sterols GC profile (Figure 2B), 12 peaks can be separated including α-cholestanol, which is added as an internal standard for quantitation purposes. The major peak corresponds to β-sitosterol (peak 7), as in the majority of

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vegetable oils, with percentages above 60%. Stigmasterol (peak 4) is the second most important sterol in Sacha inchi oils, which is in agreement with recent data reported by Muñoz Jáuregui and co-workers[13] where sterols were determined by HPLC. In rare cases, as occur in Sacha inchi oils, including the *P. volubilis* species, the stigmasterol content is higher than the campesterol concentration.[5] For most edible oils, the ratio campesterol/stigmasterol is higher than 1.[37] This is the case of olive oil, where its regulation establishes explicitly that the stigmasterol content must be lower than that of campesterol.[17] The campesterol/stigmasterol ratios calculated in this work are within the 0.14−0.21 range, the stigmasterol concentration being six times that of campesterol. This high stigmasterol content can provide interesting properties to Sacha inchi oils since this phytosterol plays an important role in reducing the inflammatory processes.

Aliphatic Saturated Hydrocarbons. HC form a remarkable group of minor unsaponifiable compounds present in edible fats and oils. At present, they are considered as intermediate metabolites in the formation of other minor compounds, such as aliphatic alcohols, with specific roles. Figure 2C shows an on-column gas chromatogram corresponding to the aliphatic saturated hydrocabons of Sacha inchi *P. huayllabambana* oil. The main peaks correspond to H21 heneicosane; H22 docosane; H23 tricosane; H24 tetracosane, and H25 pentacosane. A minor amount of H19 nonadecane; H26 hexacosane, H27 heptacosane, H28 octacosane, and H29 nonacosane are also present. Surprisingly both the odd and even series were present. The procedure was applied using impregnated silver nitrate silica to ensure that only saturated hydrocarbons eluted from the silica column. Results of the mean concentrations of the main aliphatic saturated hydrocarbons and also their total quantities evaluated using as standard a solution of heneicosane (*n*-H20:0) at concentration of 0.05 $mg \cdot mL^{-1}$ are included in Table 3. The high concentrations of the H23:0 hydrocarbon with values of 36−38%, followed by those of H24 (17−19%) and H25 (13−15%), are remarkable. As can be observed, the area relation between hydrocarbons with even and odd carbon-atom number is around 2, although the odd series is the most abundant one, as in the main edible vegetable oils. However, the even carbon number series is present in important concentrations. This fact must be pointed out as distinctive of Sacha inchi oils when compared with other vegetable oils.[37] Data on hydrocarbons and their relative concentrations in edible oils have been scarcely studied with regard to their usefulness. In this sense it has been proposed as a good parameter for beeswaxes authentication, allowing one to discriminate in the adulterations with paraffin[33] and also to distinguish among olive oil varieties.[38]

To summarize, we will conclude as follows: The concentration ranges of minor and major components of cold-pressing Sacha inchi *P. huayllabambana* oils studied can serve as a valuable guide in forthcoming enlargement of the regulations. Changes and extension in the normative are necessary as it would ensure better control of these oils avoiding the presence of adulteration with other seeds of lower prices. It also serves to encompass analytical parameters of other Sacha inchi species since there are almost no studies concerning this. It should be pointed out that some objective analytical determinations can help to characterize Sacha inchi oils both *P. volubilis* and *P. huayllabambana*. Such is the case of their characteristic sterol profile with its particular campesterol/stigmasterol ratio. Determinations of FAME and TG compositions indicate that *P. huayllabambana* oils are more unsaturated than *P. volubilis*, which affects to the presence of TG molecular species with lower ECN for the first of the species. Results demonstrate that the studied species have a seasonal stable composition, not influenced by the harvest year significantly.

Finally, their high susceptibility to oxidative and hydrolytic alterations is another common characteristic of these valuable oils. At this point it is convenient to emphasize the need to protect the product from the time of seed harvest, passing by the oil extraction up to their marketing, in order to guarantee their unique and valuable properties.

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ABBREVIATIONS USED

DG, diglycerides; ECN, equivalent carbon number; FA, free acidity; FAME, fatty acid methyl esters; FFA, free fatty acids; FID, flame ionization detector; GC, gas chromatography; HC, hydrocarbons; RP-HPLC, reverse phase−high-performance liquid chromatography; HPSEC, high performance size exclusion chromatography; i.d., internal diameter; IP, induction periods; I.S., internal standard; L, linoleic acid; Ln, linolenic acid; MG, monoglycerides; oxTG, oxidized triglycerides; PC, glyceridic polar compounds; PV, peroxide value; RI, refractive index; Rt, retention time; Si-SPE, silica solid phase extraction; TG, triglycerides; THF, tetrahydrofuran; TLC, thin layer chromatography; UC, unsaponifiable compounds

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