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ORIGINAL ARTICLE

Characterization of commercial Sacha inchi oil according to its composition: tocopherols, fatty acids, sterols, triterpene and aliphatic alcohols

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Abstract Sacha inchi oil (SIO) is one of the largest vegetable oil exports in Peru, used for consumption, in the food industry, cosmetics, and pharmaceuticals; it represents a significant economic income for producers. This study addresses the characterization and quantification of fatty acids, tocopherols, sterols, and alcohols of commercial Sacha inchi oils from Peru. Some of the SIO samples received had a high substance consistency, while others differed in the compounds studied. The results showed that some of the commercialized oils present high levels of γ-tocopherol and δ-tocopherol, while other samples had variable fatty acid compositions; especially in α-linolenic, linoleic, oleic and palmitic acids. Fourteen sterols and eleven alcohols were identified (β-sitosterol, stigmasterol, campesterol, Δ5-avenasterol, triterpene alcohol, lanosterol isomer 1 and cycloartenol) being the major components. Some SIO samples presented the following ratios: The δ-tocopherol/γ-tocopherol ratio was 0.33–0.81, ω-6/ω-3 ratio was 0.77 and a stigmasterol/campesterol ratio of 3.13. The presence of brassicasterol in some commercial oils indicates the addition of rapeseed or canola oil. Tocopherols, fatty acids, sterols and alcohol data provided a classification of SIO samples, by an efficient k-means clustering algorithm analysis. The ANOVA found significant differences between clusters for palmitic acid, oleic acid, γ-tocopherol, δ-tocopherol, campesterol and stigmasterol; these compounds could be used as markers of authenticity in commercial Sacha inchi oils.

Keywords Oil purity · Chromatographic methods · Composition data · Authenticity · Classification

Introduction

Sacha inchi (*Plukenetia volubilis* L.) is a native plant to the Peruvian Amazon and is recognized as a sustainable crop with commercial applications. The oil has been investigated due to its composition and potential health benefits (Wang et al. 2018).

The oil is extracted from the seeds by traditional and industrial methods, cold-pressed methods carry the highest commercial value. The oil is rich in unsaturated fatty acids, 93% of the total. The level of essential fatty acids reported was, α-linolenic acid (C18:3, *cis*, *cis*, *cis*-Δ^9, Δ^{12}, Δ^{15}) about 42% and linoleic acid (C18:2, *cis*, *cis*-Δ^9, Δ^{12}) about 37% (Bondioli et al. 2006; Follegatti-Romero et al. 2009; Fanali et al. 2011; Maurer et al. 2012; Chirinos et al. 2013; Liu et al. 2014; Chasquibol et al. 2016). Triacylglycerols are a very important element when studying authenticity, which has become increasingly important in vegetable oils. Adulteration is generally motivated by the boosting of profits, by replacing a costly component with a cheaper vegetable oil (Abbas and Baeten 2016). In the case of Sacha inchi oil the principal components of the triacylglycerol fraction (> 50%) are, namely, dilinolenoyl-

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linoleoylglycerol (LnLLn), dilinoleoyl-linolenoylglycerol (LLLn) and trilinolenin (LnLnLn), whereas among tocopherols, γ-tocopherol is the most abundant in this oil followed by δ-tocopherol and α-tocopherol. Several aliphatic hydrocarbons were also found containing high concentrations; *n*-tricosane (C23:0), *n*-tetracosane (C24:0) and *n*-pentacosane (C25:0) (Chasquibol et al. 2016). Other compounds such as sterols and alcohols, which comprise a major portion of the unsaponifiable matter, are characteristic of authenticity in vegetable oils. The major plant sterol end products are sitosterol (stigmasta-5-en-3β-ol), stigmasterol [(24E)-stigmasta-5,22-dien-3β-ol], and campesterol (campest-5-en-3β-ol). To our knowledge, there is no published data on aliphatic and triterpene alcohol content. Several polyphenols have been found in Sacha inchi oil such as phenolic alcohols, flavonoids, secoiridoids compounds, lignans and isocoumarin (Fanali et al. 2011).

Recently there has been a growing demand for Sacha inchi oils in domestic and international export markets, this fact suggests the assurance of the quality of oil; authenticity is important to the consumer and to the producers. Characterization and authentication require appropriate analytical methods that provide information about analytical parameters: some of which include; Fingerprinting techniques like near-infrared (NIR), mid-infrared (MIR) and nuclear magnetic resonance (NMR) spectroscopy, proton transfer reaction—time of flight—mass spectrometer (PTR–TOF–MS) (Marone et al. 2017), sensors (e-nose) (Majchrzak et al. 2018) and chromatographic methods like gas chromatography (GC), two-dimensional gas chromatography (GC × GC), with mass selective detectors (Fiori et al. 2016). Inquiries made about the composition of Sacha inchi oil are commonly based on chromatographic techniques. Fatty acids are generally converted to fatty acid methyl esters (FAMEs) and the composition is studied by GC with flame ionization detection (FID) and mass spectrometric (MS) detection. The individual tocopherol contents in Sacha inchi oil are separated by normal-phase liquid chromatography (NPLC) coupled with a fluorescence detector (RF) (Fanali et al. 2011; Chirinos et al. 2013; Chasquibol et al. 2016). The sterol profile is analyzed by GC, and the detection is performed by FID (Chirinos et al. 2013; Chasquibol et al. 2016), where an ionization technique is most frequently used for sterols analysis in Sacha inchi oil.

The aim of this study was to characterize the composition of Sacha inchi oil by analyzing the components: fatty acid methyl esters (FAMEs) by GC–FID, tocopherols by HPLC with fluorescence detection, and sterols, triterpene and aliphatic alcohols by GC–FID/MS method and the assess the quality and authenticity of Sacha inchi labeled as extra virgin.

Materials and methods

Oil samples

Sacha inchi oil samples (n = 27) were obtained from supermarkets in Lima, Peru. One Sacha inchi seed sample obtained from SUDIRGEB (National Germplasm Bank, Genetic Resources and Biotechnology, Lima, Perú) to obtain virgin Sacha inchi oil in keeping with strict quality criteria, to be used as comparison pattern in the analysis of sterols. All samples were filtered and stored until analysis according to sampling protocol at 4 °C, in total darkness, using amber glass bottles.

Determination of tocopherols

Tocopherol compositions were analyzed using methodology identified by the International Union of Pure and Applied Chemistry (IUPAC) (1992). Sacha inchi oil samples (50 mg) were placed in a vial and then dissolved with 1 mL of HPLC-grade hexane and analyzed directly, using a HPLC with fluorescence detection (Hewlett-Packard 1050, CA, USA). The tocopherol separations were performed using a column with (Superspher 60Si 125 mm × 4.0 mm i.d.; 4 µm particle size, Merck). The mobile phase used a mixture of 2-propanol in hexane (0.5:99.5 v/v) at a flow rate of 1.0 mL/min, with an injection volume of 20 µL. The fluorescence detector (Shimadzu RF 535, Japan), operated at an excitation wavelength of λ = 290 nm and an emission wavelength of λ = 330 nm. The identification of the tocopherols was identified in retention times with corresponding standards (α, alpha; β, beta; γ, gamma and δ, delta forms); the results were expressed in mg/kg of oil.

Determination of the fatty acid profile

Methyl ester fatty acids (FAMEs) were identified by esterification reaction consistent with methodology specified by Commission Regulation (EU) No 61/2011 (2011). The transesterification reaction was carried out in a 10-mL flask. In summary, around 300 mg of oil samples were dissolved in 0.6 mL of a potassium hydroxide solution in methanol (2 N), FAMEs were extracted using a 5 mL of hexane, and the upper phase was transferred to a sample vial for analysis. The analysis was performed on a Varian 3900 (Walnut Creek, CA, USA) gas chromatograph equipped with a SP^{TM}-2380 fused silica capillary column (60 m × 0.25 mm i.d.: 0.2 µm film thickness, Supelco) and a flame ionization detector (FID). The injector and detector temperature were set at 250 °C, the oven temperature was maintained at 170 °C for 10 min and then programmed to increase from 170 to 200 °C at 1.5 °C/min

intervals, after being kept in an isotherm for 8 min. Hydrogen was used as the carrier gas at a flow rate of 1.0 mL/min. The FAMEs were categorized by the retention time compared to the corresponding benchmarks. The fatty acids were normalized to a sum of 100%, considering the composition (mol%) from fatty acid composition data (area %).

Determination of sterols and alcohols

Preparation of unsaponifiable matter and isolation of sterol and alcohol fractions

Sterol and alcohol fractions were determined after hot saponification of 5.0 g of the oil, 5α-colestan-3β-ol and 1-eicosanol (internal standard) with ethanolic potassium hydroxide. The unsaponifiable matter was then extracted three times with anhydrous diethyl ether and the combined diethyl ether fractions were washed 3–5 times with water. The diethyl ether phase was then dried with anhydrous sodium sulfate and the residue after evaporation was redissolved in 2 mL of chloroform. The sterol and alcohol fractions were separated from the unsaponifiable matter by thin-layer chromatography (ALUGRAM® SIL G/UV_{254}, 0.20 mm, 20 × 20 cm), the plates were developed using a hexane/diethyl ether (65:35, v/v) mixture. Components were visualized with a 0.2% solution of 2,7-dichlorofluorescein, areas of interest were scraped from TLC aluminum sheets into a vial. Diethyl ether was used to extract the sterols and alcohols from the silica and was later evaporated. The sterol and alcohol fractions were derivatized to their trimethylsilyl ethers with 200 µL of a mixture of hexamethyldisilazane and dimethylchlorosilane in pyridine (3:1:9 v/v/v) (Supelco). An aliquot of both fractions was taken from the clear solution and stored at − 10 °C until analysis.

GC–FID and GC–MS analysis

The separation of the silyl derivatives of sterols and alcohols were carried out on a gas chromatograph with a flame ionization detector (Varian 3900), equipped with a HP-5 capillary column (30 m × 0.32 mm i.d.: 0.25 µm film thickness, Agilent J&W). The injection was performed with a split ratio 1:10 and constant flow operating mode at 1.0 mL/min (hydrogen used as carrier gas). Injector and detector temperatures were both set at 290 °C and 320 °C, respectively. The column temperature was held at 263 °C in isothermal mode for 30 min. The peak identification was carried out by calculating the relative retention times to β-sitosterol and the quantification using 5α-cholestan-3β-ol as an internal standard in accordance with Commission Regulation (EU) No 61/2011 (2011). The GC and chromatographic conditions for the alcohols were the same as for the sterols, except for the column temperature which was as follows: 200 °C (hold 8 min), then programmed to 270 °C at a rate of 5 °C/min and kept constant at 270 °C for 15 min.

Sterol and alcohol structures were confirmed by GC–MS analysis performed with a trace GC2000 gas chromatograph coupled with a GCQ/Polaris ion trap mass spectrometer (Thermo Finnigan, Austin, TX, USA); the samples were injected in split mode. Trimethylsilyl ether derivatives were resolved by temperature programming from 180 to 270 °C at 8 °C/min and held in isothermal for 46 min. The GC to MS transfer line and ion source were maintained at 375 °C, with the helium carrier gas head pressure at 15 psi. Electron impact (EI) spectra were recorded at 70 eV, Sterols and alcohols were identified by comparing their mass spectrum. Full spectra (50–1000 amu) were recorded at a scan speed of 2/s decade over the entire elution profile. Data was analyzed by the Xcalibur 1.4 data system from (Thermo Fisher Scientific Inc.).

Statistical analyses

All experiments were carried out in duplicate. The basic statistics were analyzed using Microsoft Excel 2016 software. The fatty acid profile, tocopherols, sterols and triterpene and aliphatic alcohols were used to classify the set of Sacha inchi oil using K-means clustering algorithm and one-way ANOVA with Fisher's Least Significant Difference (LSD), post hoc tests were then applied for comparison among the cluster. The statistical analysis was done using the STATISTICA version 8.0 software package (StatSoft, Inc., Tulsa, Oklahoma, USA).

Results and discussion

Tocopherol contents

Tocopherols are some of the most important natural antioxidants synthesized in most vascular or higher plants, they are also important lipoxidation inhibitors; they play an important role in the oxidative stability of vegetable oils.

The tocopherol contents of Sacha inchi oils analyzed is shown in Table 1. The total content of tocopherols in the oils studied was between 700 and 3300 mg/kg, with an average of 2220 mg/kg, regulations for Sacha inchi oil indicate that the tocopherol content must have a minimum of 1900 mg/kg (γ- and δ-tocopherol) (NTP 2009). 19% of the oil samples presented contained less than the minimum amount required (IP001, IP006, IP007, IP020 and IP024) (Table 2), while most of the samples far exceeded the

Table 1 Contents of different tocopherol isomers (mg/kg) of commercial Sacha inchi oils

Code	α-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total (mg/kg)	Ratio δ/γ
IP001	34.74	900.64	654.36	1589.75	0.73
IP002	5.03	1596.08	1273.04	2874.14	0.79
IP003	3.10	1151.06	1173.70	2327.85	1.02
IP004	4.98	1710.95	1387.29	3103.22	0.81
IP005	5.46	1523.11	1160.12	2688.69	0.76
IP006	134.96	964.48	404.27	1503.70	0.42
IP007	66.50	771.00	358.00	1195.50	0.46
IP008	5.33	1388.25	1055.12	2448.71	0.76
IP009	2.14	1198.79	1228.46	2429.38	1.03
IP010	2.97	1537.16	1152.87	2693.01	0.75
IP011	57.63	1195.45	695.17	1948.26	0.58
IP012	0.59	974.29	1074.91	2049.79	1.10
IP013	3.95	1150.26	911.12	2065.32	0.79
IP014	30.52	1567.88	969.35	2567.75	0.62
IP015	5.10	1358.22	930.00	2293.32	0.68
IP016	19.21	1383.18	979.80	2382.19	0.71
IP-017	5.07	1644.72	1176.32	2826.11	0.72
IP018	7.33	1629.00	1141.96	2778.29	0.70
IP019	82.72	1247.91	681.33	2011.95	0.55
IP020	7.87	786.22	258.89	1052.99	0.33
IP021	5.12	1707.14	1247.62	2959.88	0.73
IP022	193.82	1177.09	598.00	1968.91	0.51
IP023	27.89	1392.18	901.73	2321.80	0.62
IP024	138.53	118.97	453.41	711.00	3.81
IP025	138.59	1152.92	675.20	1966.70	0.59
IP026	7.02	806.17	1154.55	1967.74	1.43
IP027	5.93	1878.52	1347.56	3232.00	0.72
Mean	37.11	1255.99	927.56	2220.66	
SD	53.81	382.40	322.25	624.27	

SD standard deviation

Table 2 Average composition of tocopherols and fatty acids of some of the commercialized oils do not fulfill the basic requirement established according the Peruvian Technical Regulation (NTP)

	Tocopherols (mg/kg)		
	Total tocopherol	γ-Tocopherol	δ-Tocopherol
IP001, IP006, IP007, IP020 and IP024	< 1900		
IP003, IP009, IP012, IP024 and IP026		850	1017
Equation and correlation coefficient between γ-tocopherol versus δ-tocopherol	δ-toc = 79.2846 + 0.6554298 *γ-toc; r = 0.7272		

	Fatty acids (mol%)				
	Palmitic	Oleic	Linoleic	α-Linolenic	L/Ln
IP001, IP006, IP007, IP011, IP014, IP016, IP019 and IP020	7.84	17.61	42.02	28.16	2.21

According to the NTP (2018): α-tocopherol (60–70 mg/kg), β-tocopherol (18–29 mg/kg), γ-tocopherol (1108–1367 mg/kg), δ-tocopherol (641–856 mg/kg), palmitic acid (between 3.70 and 4.40%), oleic acid (greater than 8.5%), linoleic acid (greater than 32%) and α-linolenic acid (greater than 42%)

minimum value. Fanali et al. (2011) They reported total tocopherol values of 2130 mg/kg, while Follegatti-Romero et al. (2009), showed values of 2.39 g/kg for Sacha inchi oil (by Soxhlet extraction), while the extraction obtained by supercritical CO_2 extraction varied between 2.23 and 3.07 g/kg oil.

On the other hand, when the results are analyzed according to individual tocopherols, it was found that the commercial oils contained an average of 37.11 mg/kg α-tocopherol, 1255.99 mg/kg γ-tocopherol and 927.56 mg/kg δ-tocopherol. The presence of α-tocopherol was detected in all samples, the concentration varied between 0.59 and 193.82 mg/kg. Some reports show small concentrations of α-tocopherol (Fanali et al. 2011; Chirinos et al. 2015), whereas Chasquibol et al. (2016) reported values between 4.2 and 126.5 mg/kg for commercial Sacha inchi oils. The most important tocopherols in this oil are γ- and δ-tocopherol, while the scientific literature reports that γ-tocopherol is found in a higher content than δ-tocopherol (Follegatti-Romero et al. 2009; Fanali et al. 2011; Chirinos et al. 2015, NTP 2018). In this case, five samples of commercial oils (IP003, IP009, IP012, IP024 and IP026) (Table 2) showed higher δ-tocopherol content. It is important to note that the δ-tocopherol/γ-tocopherol ratio (δ/γ) for the mentioned commercial oils was between 1.02 and 3.81, while the rest of the oils showed a 0.33–0.81 ratio. A linear regression analysis was developed with the purpose of observing the multicollinearity between the variables δ- and γ-tocopherol, this analysis showed a correlation coefficient of 0.7272 and the linear regression equation was as follows: δ-toc = 79.2846 + 0.6554298 * γ-toc (Table 2). These results indicate that the behavior of the samples according to these two variables is direct in 87% of the total of the samples, while 13% of samples contradict this association. Sacha inchi oil contains a good source of tocopherols compared to other oils such as flaxseed (377.1 mg/kg), rapeseed (439 mg/kg), corn (886.5 mg/kg), almond (379.8 mg/kg), walnut (209.4) and sunflower oils (535 mg/kg) (Gliszczyńska-Świgło et al. 2007; Yang et al. 2018).

Fatty acid profile

Fatty acids are major components of the saponifiable fraction of vegetable oils. In Sacha inchi oil, the predominant fatty acids are α-linolenic acid (C18:3 ω-3) and linoleic acid (C18:2 ω-6) (Bondioli et al. 2006). According to the NTP (2018) the total content of saturated fatty acids (maximum limit 7.5%), monounsaturated fatty acids (between 8.20 and 13.60%) and polyunsaturated fatty acids (greater than 80%).

Table 3 shows the fatty acid composition and the related ratios and sums of all the samples analyzed. As evidenced, the major compounds are unsaturated fatty acids such as oleic acid (C18:1 ω9), α-linolenic acid (C18:3 ω-3) and linoleic acid (C18:2 ω-6); the saturated fatty acids are palmitic (C16:0) and stearic (C18:0). When all samples were analyzed, palmitic acid varied between 3 and 11%, while stearic acid ranged between 2 and 4%. Palmitic and steric acids observed a wide range of concentrations, in conformity with previous research, these compounds varied between 3 and 5% (Maurer et al. 2012). Unsaturated fatty acid concentrations of oleic acid varied between 9 and 23%, while linoleic acid fluctuated between 21 and 53%, and α-linolenic acid ranged between 10 and 55%, these three fatty acids, also show a wide range of variation. According to results reported by other authors (Follegatti-Romero et al. 2009; Fanali et al. 2011; Maurer et al. 2012), oleic acid varied between 8 and 10%, while linoleic acid fluctuated between 33 and 36% and α-linolenic acid between 44 and 50%.

Table 3 shows the relative levels of five significant parameters: oleic:linoleic acid (O/L) ratio, linoleic:α-linolenic acid (L/Ln) ratio (ω-6/ω-3), sum of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs).

In the oils analyzed, the O/L ratio varies between 0.26 and 0.75, other studies in authentic Sacha inchi identified a relationship in the range of 0.23–0.32 (Follegatti-Romero et al. 2009; Maurer et al. 2012; Liu et al. 2014; Triana-Maldonado et al. 2017). The O/L ratio is often used as a stability parameter, and oils with a higher ratio are also those with superior oxidative stability. In olive, rapeseed, peanut and corn oils these values are: 13.89, 2.27, 0.98 and 0.58 respectively (Yang et al. 2013). The remarkably high content of α-linolenic acid makes the oil especially important as an alternative plant-based omega 3 source in any diet (Liu et al. 2014). The L/Ln ratio (ω-6/ω-3) in the oils analyzed is around 0.63–5.32, the data found in the literature for this ratio is between 0.67 and 0.90 (Follegatti-Romero et al. 2009; Maurer et al. 2012; Liu et al. 2014; Triana-Maldonado et al. 2017). Compared to Sacha inchi oil, avocado (20.01), wheat germ (11.30), olive (7.05) and soybean (6.72) oils have higher ω-6/ω-3 ratios (Rueda et al. 2014). Organic dark and white chia seed (0.27, 0.28 respectively) (Alvites-Misajel et al. 2019), flaxseed (0.27) and perilla (0.22) oils have lower ω-6/ω-3 ratios. A high ratio of dietary ω-6/ω-3 polyunsaturated fatty acids is associated with increased risk of cardiovascular diseases, cancer, obesity and inflammatory diseases (Simopoulos 2016), whereas a lowest ω-6/ω-3 ratio (1:1) diet leads to the least atherosclerotic formation. In the case of Sacha inchi oil this proportion is close to 1:1.

Thus, a proportionally higher consumption of n-3 PUFAs may exert suppressive effects on the same (Saini and Keum 2018). The balance of the fatty acid profile is as

Table 3 Fatty acid composition (mol%) of commercial Sacha inchi oils

Code	P	Po	M	S	O	L	Ln	O/L	L/Ln	SFAs	MUFAs	PUFAs
IP001	8.10	0.63	0.08	2.88	23.98	31.66	32.31	0.75	0.98	11.37	24.65	63.96
IP002	4.04	0.07	0.09	3.00	9.86	34.22	48.56	0.28	0.70	7.24	9.97	82.78
IP003	4.06	0.08	0.09	2.91	9.61	34.14	48.95	0.28	0.69	7.18	9.72	83.09
IP004	4.09	0.07	0.10	3.12	10.14	37.58	44.72	0.27	0.84	7.43	10.25	82.30
IP005	3.97	0.07	0.09	3.04	10.87	35.17	46.63	0.30	0.75	7.22	10.97	81.79
IP006	11.02	0.14	0.10	4.09	20.25	53.59	10.06	0.37	5.32	15.90	20.44	63.65
IP007	10.15	0.09	0.10	4.26	20.29	52.16	12.30	0.38	4.24	15.10	20.42	64.46
IP008	4.14	0.07	0.10	3.33	10.28	38.68	43.20	0.26	0.89	7.71	10.40	81.88
IP009	4.42	0.08	0.10	3.19	10.12	35.81	46.11	0.28	0.77	7.83	10.24	81.91
IP010	4.07	0.07	0.10	3.05	10.01	35.92	46.61	0.27	0.77	7.34	10.12	82.52
IP011	8.10	0.09	0.10	3.80	16.69	47.05	23.63	0.35	1.99	12.50	16.81	70.67
IP012	4.13	0.08	0.09	3.05	10.29	34.72	47.54	0.29	0.73	7.33	10.40	82.26
IP013	4.62	0.11	0.11	3.17	11.06	37.68	42.52	0.30	0.88	7.97	11.82	80.20
IP014	5.87	0.08	0.10	3.43	13.13	41.17	35.86	0.31	1.14	9.70	13.25	77.03
IP015	4.45	0.08	0.09	3.32	11.57	31.32	48.99	0.36	0.63	8.01	11.68	80.30
IP016	5.72	0.08	0.10	3.54	12.90	40.64	36.70	0.31	1.10	9.63	13.02	77.34
IP017	3.95	0.07	0.10	2.99	9.70	36.50	46.53	0.26	0.78	7.15	9.81	83.03
IP018	4.08	0.07	0.10	3.08	9.72	36.64	46.14	0.26	0.79	7.38	9.84	82.77
IP019	9.01	0.08	0.10	3.94	18.27	48.61	19.34	0.37	2.51	13.65	18.39	67.95
IP020	4.74	0.07	0.07	3.06	15.44	21.26	55.10	0.72	0.38	8.09	15.54	76.36
IP021	4.58	0.07	0.08	2.63	12.30	35.28	43.41	0.34	0.81	7.53	12.40	78.68
IP022	5.01	0.07	0.09	3.06	12.58	34.67	43.03	0.36	0.80	8.34	12.68	77.70
IP023	4.72	0.07	0.05	2.70	11.36	35.27	44.79	0.32	0.78	7.73	11.47	80.06
IP024	3.70	0.07	0.09	2.87	12.89	33.96	45.05	0.38	0.75	6.83	13.01	79.01
IP025	4.53	0.07	0.09	3.26	12.57	35.74	41.89	0.35	0.85	8.11	12.68	77.63
IP026	4.42	0.08	0.08	3.15	11.51	32.06	48.37	0.35	0.66	7.94	11.62	80.43
IP027	3.74	0.07	0.08	2.92	11.55	36.51	44.44	0.31	0.82	6.90	11.66	80.94
Mean	5.31	0.10	0.09	3.22	12.95	37.33	40.47	0.35	1.20	8.86	13.08	77.80
SD	2.05	0.11	0.00	0.40	3.79	6.67	11.38	0.12	1.12	2.55	3.86	6.12

P, palmitic; Po, palmitoleic; M, margaric; S, stearic; O, oleic; L, linoleic; Ln, α-linolenic; O/L, oleic/linoleic ratio; L/Ln, linoleic/α-linolenic ratio; SFAs, saturated fatty acids; MUFAs, unsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SD, standard deviation

follows: SFAs varied between 6.83 and 15.90%, MUFAs around 9.81–20.44% and PUFAs between 63.65 and 83.09%. These ranges found in Sacha inchi oils show larger margins than those reported by Wang et al. (2018).

The results shown in the Table 2 indicate that fatty acids (palmitic, oleic, linoleic and α-linolenic) show high variability. According to the information obtained from the fatty acids profile (GC–FID), and the information from scientific literature, these oils could be considered as adulterated. The oil samples (IP001, IP006, IP007, IP011, IP014, IP016, IP019 and IP020) are isolated from the rest of the oils. The average values of these oils shows: palmitic acid (8.28 ± 2.00), oleic acid (17.94 ± 4.03), linoleic acid (44.98 ± 7.68), α-linolenic acid (24.31 ± 10.98) and L/Ln ratio (ω-6/ω-3) (2.21 ± 1.70); they have likely been adulterated with other lower quality vegetable oil which could affect effectiveness and quality (Abbas and Baeten 2016). The results show a profile like those found by Maurer et al. (2012) in suspicious Sacha inchi samples. On the other hand, the rest of the commercial Sacha inchi oils show fatty acid contents close to those reported by Wang et al. (2018). These average values are: palmitic acid (4.27 ± 0.35%), stearic acid (3.04 ± 0.18%), oleic acid (11.18 ± 1.46%), linoleic acid (34.66 ± 3.61%), α-linolenic acid (46.13 ± 3.03%) and ω-6/ω-3 ratio was 0.77 ± 0.07.

Unsaponifiable matter, sterols and alcohols composition

In general, unsaponifiable matter includes hydrocarbons (squalene), waxes, sterols, alcohols,

tocopherols/tocotrienols, and is present in edible oils at level lower than 2% (Fontanel 2013). The content of the unsaponifiable matter in the analyzed oils showed an average of 0.60 g/100 g oil. Liu et al. (2014) reported a value of 5.03 g/kg in Sacha inchi seed oil from China, whereas in other commercial oils the unsaponifiable matter varied between 0.82 and 2.58% (Chasquibol et al. 2016).

Table 4 shows the sterol profiles corresponding to Sacha inchi oil; fourteen components were evaluated corresponding to 4-desmethyl-sterols. The identification of sterols was carried out by comparison of the mass fragmentation patterns and retention times with those of authentic reference compounds; the identification of confirmed brassicasterol was by GC–MS analysis of a rapeseed oil sample. Two compounds were identified in commercial samples that had not previously been reported in Sacha inchi seed oil; brassicasterol and Δ7-campesterol. Δ5,23-stigmastadienol was not detected in this study, however, Chasquibol et al. (2016) reported the presence of this compound in some samples of commercial Sacha inchi oils with concentrations between 0.1 and 0.4%. The characteristics of campesterol, stigmasterol, chlerosterol, β-sitosterol, Δ5-avenasterol and Δ7-avenasterol match with those reported by Li et al. (2007); Compound 2 (Table 4) was identified as brassicasterol (*m/z* 380, 455 and 470; fragments and molecular ion). MS data concur, that this component is present in rapeseed oils. Compound 7 is assigned as Δ7-campesterol (M^+, *m/z* 472), based on the consensus of MS fragmentation data with those reported by Zhang et al. (2006). Compound 10, sitostanol, is a molecular ion compound *m/z* 488, with characteristic $[M-15]^+$ ions (elimination of one methyl moiety from the derivatization group) as well as *m/z* 383, *m/z* 398 and *m/z* 473. The RR_t and MS fragmentation data concur, that this component is present in sea buckthorn (*Hippophae rhamnoides* L.) seed oil (Li et al. 2007). Compounds 3, 12 and 13 were identified as 24-methylene-cholesterol (M^+, *m/z* 400), Δ5,24-stigmastadienol (M^+, *m/z* 484) and Δ7-stigmastenol (M^+, *m/z* 486) which can also be distinguished by their mass spectra. Sterols, which are found in all fats and oils, constitute a large percentage of the unsaponifiable matter in these oils; they play an important role in the oil stability as they act as polymerization reaction inhibitors at high temperatures. The composition is characteristic to each vegetable oil, they are considered a unique fingerprint of the oil (Li et al. 2016).

The total sterol content ranged from 1623 to 2899 mg/kg (Table 4). The main components in the Sacha inchi oil were β-sitosterol, stigmasterol, campesterol and Δ5-avenasterol. In higher plants, β-sitosterol is usually predominant; this component contributes more than 50% of the total phytosterol contents. Brassicasterol was not detected in authentic Sacha inchi seed oil; however, it's presence was detected in commercial oil samples. The results revealed that this compound was detected in 6 samples (IP019, IP013, IP002, IP021, IP009 and IP015) with a concentration ranging from 7.74 to 47.89%. The most relevant compounds were ranked as follows: β-sitosterol (54.44 ± 10.08%), stigmasterol (20.27 ± 7.03%), campesterol (10.47 ± 4.36%) and Δ5-avenasterol (5.06 ± 2.57%). These results are similar to those reported by Chasquibol et al. (2016) for commercial Sacha inchi oils. The total sterol content in Sacha inchi oils is lower when compared to other edible oils such as corn, sunflower, soybean and rapeseed, however, greater than palm and peanut oils (Hassanien 2013; Xu et al. 2014). Cholesterol percentages, 24-methylene-cholesterol, campestanol, Δ7-campesterol, chlerosterol, sitostanol, Δ5,24-stigmastadienol, Δ7-stigmastenol and Δ7-avenasterol remaine below 1.5%.

The contribution of the stigmasterol/campesterol ratio (SCR) is used as a purity index. This ratio provides additional information about the general processing conditions and, if utilized in combination with other traditional oil quality chemical parameters, it could provide a good way of comparing the overall quality of different oils produced (Chasquibol et al. 2016). The SCR ranged between 0.28 and 3.75 for oils analyzed. The ratio of data based on data from (Bondioli et al. 2006; Chirinos et al. 2015) was between 3.83 and 3.92 in authentic Sacha inchi oil, while commercial oils varied between 2.27 and 4.96 (Chasquibol et al. 2016).

The samples (IP006, IP013 and IP020) show low SCR interaction ratios; this indicates the campesterol concentration is higher than the stigmasterol concentration. These samples reveal values contrary to those reported by Chasquibol et al. (2014) in authentic Sacha inchi seed oil of the *P. volubilis* species. The calculation of the "apparent β-sitosterol", refers to the sum of the concentration of β-sitosterol, Δ5-Avenasterol, Δ-5,23-stigmastadienol, Δ-5,24-stigmastadienol, chlerosterol and sitostanol. This parameter is used as a means to ensure the authenticity of edible oils and fats, particularly in identifying adulteration in vegetable oils, which tend to contain considerably higher levels of desmethylsterols than the original oil. The samples analyzed represented clear sitosterol values between 25.52 and 77.25% with an average of 61.41%. Samples with clear sitosterol values around 51.45–63.76 (except for IP006 and IP011) have an average of 26.01% stigmasterol and 8.30% campesterol, with a 3.13 SCR ratio.

Table 5 provides data on the composition of alcohols in commercial Sacha inchi oil, which was confirmed with the GC–MS compositional analysis. The compounds found were aliphatic alcohols (docosanol, tetracosanol and hexacosanol), diterpene alcohol (phytol) and 4,4-methyl-sterols (24-methylenecycloartanol, cycloartenol and lanosterol

Table 4 Composition of individual sterols and total sterols (mg/kg) of commercial Sacha inchi oils

Code	Composition (mol%)														USM (%)	TS (mg/kg)	AS (%)	SCR (%)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14				
IP001	0.49	ND	0.56	7.46	0.17	14.61	0.65	0.97	68.91	0.33	4.43	0.64	0.41	0.46	0.48	2350.96	75.18	1.96
IP002	0.75	10.08	0.26	7.05	0.07	25.56	2.14	0.67	46.40	0.35	5.32	0.53	0.62	0.18	0.52	1173.43	53.27	3.63
IP003	0.62	ND	0.30	8.95	0.12	24.73	0.41	1.03	54.56	0.35	6.70	0.28	1.72	0.24	0.78	1130.26	62.91	2.76
IP004	0.35	ND	ND	7.34	ND	27.50	1.71	0.48	53.96	0.38	7.76	0.31	0.22	0.00	0.57	1445.32	62.88	3.75
IP005	0.68	ND	0.13	7.19	ND	24.86	0.96	0.56	49.91	0.59	3.03	2.15	2.15	7.78	0.19	1611.07	56.25	3.46
IP006	0.35	ND	0.06	19.19	0.43	18.26	1.25	0.57	56.11	0.85	1.18	1.13	0.30	0.32	0.47	2795.23	59.85	0.95
IP007	0.27	ND	0.18	9.53	0.29	15.93	0.85	0.70	68.46	0.56	1.24	0.98	0.88	0.12	0.79	2332.58	71.95	1.67
IP008	0.27	ND	ND	8.20	ND	27.23	1.10	0.61	53.95	0.34	6.77	0.32	0.23	1.00	0.52	2856.33	61.98	3.32
IP009	0.28	12.08	0.31	6.86	ND	22.44	4.56	0.70	44.75	0.45	5.83	1.43	0.10	0.21	0.61	2625.54	53.15	3.27
IP010	0.23	ND	ND	8.06	ND	27.09	0.64	0.67	54.96	0.29	7.39	0.46	0.16	0.06	0.77	2657.54	63.76	3.36
IP011	0.36	ND	0.51	13.81	0.32	18.23	2.41	0.61	42.61	1.18	1.05	7.24	3.01	8.67	0.75	2629.32	52.69	1.32
IP012	0.31	ND	ND	8.14	ND	25.33	1.09	0.61	57.29	0.38	6.66	0.20	ND	ND	0.90	2504.86	65.14	3.11
IP013	0.45	8.04	0.10	11.45	ND	3.16	ND	ND	65.22	1.00	ND	1.44	3.27	5.87	0.64	2048.45	67.67	0.28
IP014	0.34	ND	ND	9.40	ND	11.40	0.86	0.98	66.55	0.41	8.53	0.74	0.58	0.21	0.50	2651.35	77.21	1.21
IP015	0.30	47.89	0.78	6.04	0.19	8.22	9.54	0.66	21.45	0.53	1.05	1.83	1.30	0.22	0.76	2401.50	25.52	1.36
IP016	0.30	ND	ND	12.35	0.24	25.76	0.69	0.52	52.96	0.35	5.83	0.45	0.40	0.13	0.50	2375.63	60.12	2.09
IP017	0.40	ND	ND	8.13	ND	27.20	1.48	0.60	54.92	0.48	6.61	ND	ND	0.17	0.76	2723.94	62.61	3.35
IP018	0.50	ND	ND	7.92	0.31	29.18	0.45	0.62	53.21	0.35	6.88	0.33	0.02	0.23	0.58	2076.32	61.40	3.68
IP019	0.22	7.74	0.45	17.01	0.28	18.32	2.13	0.63	44.83	0.93	0.74	2.12	2.92	1.68	0.48	2614.80	49.24	1.08
IP020	0.20	ND	0.95	23.95	0.13	7.48	1.80	0.83	52.07	0.19	8.02	2.49	1.05	0.86	0.69	3635.58	63.59	0.31
IP021	0.63	11.69	ND	8.49	0.21	24.60	2.47	0.73	44.75	0.45	4.91	0.60	0.27	0.19	0.55	1565.25	51.45	2.90
IP022	0.47	ND	0.13	15.93	0.42	17.07	0.84	0.81	58.91	0.64	1.99	1.23	1.25	0.32	0.67	2255.65	63.57	1.07
IP023	0.57	ND	ND	6.41	0.15	15.67	1.36	0.54	67.26	0.51	6.94	ND	0.32	0.28	0.59	1692.35	75.25	2.44
IP024	0.45	ND	ND	13.51	ND	17.22	ND	ND	65.89	0.55	ND	1.66	0.36	0.37	0.51	1687.43	68.09	1.28
IP025	0.30	ND	ND	12.72	0.28	17.68	0.60	0.84	61.12	0.44	3.44	0.95	1.14	0.49	0.53	2213.03	66.79	1.39
IP026	0.43	ND	ND	8.08	0.20	26.57	0.77	0.80	54.08	0.31	7.69	0.62	0.25	0.20	0.76	2086.91	63.51	3.29
IP027	0.40	ND	0.41	9.44	0.13	26.05	0.10	0.82	54.83	0.36	6.47	0.49	0.39	0.11	0.39	2037.20	62.97	2.76
Mean	0.40	16.25	0.37	10.47	0.23	20.27	1.57	0.70	54.44	0.50	5.06	1.23	0.93	1.21	0.60	2229.92	61.41	2.26
SD	0.15	15.60	0.23	4.36	0.10	7.03	1.88	0.15	10.08	0.24	2.57	1.42	0.97	2.41	0.15	570.34	10.14	1.11

1, Cholesterol; 2, brassicasterol; 3, 24-methylene-cholesterol; 4, campesterol; 5, campestanol; 6, stigmasterol; 7, Δ7-campesterol; 8, chlerosterol: 9, β-sitosterol; 10, sitostanol; 11, Δ5-avenasterol; 12, Δ5,24-stigmastadienol; 13, Δ7-stigmastenol and 14, Δ7-avenasterol

USM, unsaponifiable matter; TS, total sterol, AS, apparent sitosterol (sum: chlerosterol, β-sitosterol, sitostanol, Δ5-avenasterol and Δ5,24-stigmastadienol); SCR, stigmasterol/campesterol ratio; ND, not detected; SD, standard deviation

Table 5 Composition of alcohol fractions and total alcohols (mg/kg) of commercial Sacha inchi oils

Code	Composition (mol%)											TAA (%)	TDA (%)	TTA (%)	TA (mg/kg)
	1	2	3	4	5	6	7	8	9	10	11				
IP001	5.36	0.25	0.30	0.60	5.15	51.05	19.91	1.50	5.50	3.52	6.86	1.14	5.36	86.63	6032.65
IP002	0.69	0.69	0.93	1.38	8.84	34.63	9.09	3.16	22.48	7.23	10.89	3.00	0.69	85.42	1476.26
IP003	1.13	0.18	0.32	0.91	1.46	54.12	23.99	2.59	7.60	2.29	5.40	1.41	1.13	92.06	2233.31
IP004	10.36	0.29	0.35	0.75	1.67	38.62	37.08	2.73	3.69	1.59	2.86	1.39	10.36	85.39	5934.27
IP005	0.50	0.38	0.46	0.69	5.37	52.90	5.30	1.42	16.76	5.90	10.32	1.53	0.50	87.64	1699.92
IP006	7.03	0.46	0.14	0.42	2.94	38.58	33.79	2.16	6.42	2.33	5.73	1.01	7.03	86.23	4063.93
IP007	15.27	0.35	0.20	1.07	2.41	45.49	8.72	1.09	8.73	5.17	11.52	1.61	15.27	71.60	4976.65
IP008	0.10	0.03	0.14	0.16	2.22	46.26	36.93	2.15	3.02	2.97	6.03	0.32	0.10	93.55	10,562.80
IP009	0.33	0.21	0.24	0.45	8.65	63.56	2.62	0.25	7.33	5.47	10.89	0.89	0.33	87.89	6131.02
IP010	0.40	0.43	0.23	0.65	9.60	15.68	17.75	1.53	22.95	5.06	25.71	1.31	0.40	72.58	4369.15
IP011	18.13	2.65	0.36	1.47	9.65	10.88	0.66	1.60	20.34	10.01	24.26	4.48	18.13	53.13	2051.76
IP012	14.85	0.30	0.14	1.43	2.69	42.85	9.95	1.35	8.33	5.58	12.53	1.87	14.85	70.75	7292.69
IP013	15.02	0.32	0.16	1.28	2.58	43.92	9.45	1.24	8.49	5.42	12.12	1.77	15.02	71.09	6134.67
IP014	8.16	0.34	0.37	0.48	4.63	36.99	23.95	2.23	10.58	3.55	8.72	1.19	8.16	81.93	3424.00
IP015	34.99	0.61	0.52	1.17	13.29	6.84	0.74	4.61	24.13	3.36	9.74	2.30	34.99	52.97	3665.66
IP016	0.20	0.08	0.11	0.43	1.61	42.15	47.44	1.94	2.59	1.07	2.37	0.62	0.20	96.81	3150.07
IP017	0.19	0.11	0.08	0.19	1.60	44.72	43.21	2.40	3.27	1.42	2.80	0.38	0.19	96.63	6740.60
IP018	2.49	0.13	0.09	0.27	1.52	49.53	31.81	1.45	5.90	2.49	4.32	0.49	2.49	92.70	3041.54
IP019	2.56	3.12	3.18	1.32	8.24	3.47	1.14	2.25	32.85	11.79	30.06	7.62	2.56	59.75	1033.99
IP020	0.52	0.63	0.30	0.78	14.15	0.62	0.86	1.29	34.54	7.33	38.99	1.71	0.52	58.79	5588.23
IP021	0.40	0.06	0.08	0.23	2.17	43.47	42.73	2.59	4.96	0.80	2.52	0.37	0.40	96.71	6063.37
IP022	43.51	0.40	0.27	0.63	2.28	32.71	7.52	0.39	6.12	1.42	4.76	1.30	43.51	50.43	1400.01
IP023	1.64	0.33	0.08	0.60	6.65	45.45	0.15	0.10	22.49	6.33	16.17	1.02	1.64	81.17	2186.58
IP024	0.48	0.52	0.55	0.50	15.18	30.10	0.10	0.37	21.27	7.57	23.37	1.57	0.48	74.58	881.95
IP025	0.95	0.04	0.05	0.41	1.86	32.03	45.05	1.14	10.11	1.80	6.56	0.50	0.95	91.99	2810.14
IP026	0.37	0.14	0.11	0.17	2.73	66.79	2.42	0.10	12.41	4.88	9.88	0.42	0.37	89.33	1923.59
IP027	15.23	0.41	0.29	0.11	1.59	52.16	5.74	0.45	10.00	4.42	9.59	0.82	15.23	74.37	2780.18
Mean	7.44	0.50	0.37	0.69	5.21	37.98	17.34	1.63	12.70	4.47	11.67	1.56	7.44	79.34	3987.00
SD	10.98	0.71	0.59	0.43	4.25	17.24	16.51	1.04	9.18	2.75	9.21	1.51	10.98	14.35	2342.81

1, Phytol; 2, docosanol; 3, tetracosanol; 4, hexacosanol; 5, triterpene alcohol *m/z* 534; 6, triterpene alcohol *m/z* 484; 7, lanosterol isomer 1; 8, lanosterol isomer 2; 9, cycloartenol; 10, 24-methylene cycloartenol; 11, unidentified *m/z* 484

TAA total aliphatic alcohol, *TDA* total diterpene alcohol, *TTA* total triterpene alcohol, *TA* total alcohol, *SD* standard deviation

isomers) and triterpene alcohols. The identification of alcohols is based on the comparison of retention times and the mass spectrum. The alcohols identified coincide with those found in the literature that have characteristic mass spectra with fragmented ions [M^+-15], due to the loss of a methyl group (Lerma-García et al. 2009), as well as with their respective fragmentation patterns. Component 5 corresponds to a triterpene alcohol of *m/z* 534, with abundant ions at *m/z* 149, 282, 353 and 467. Component 6 at *m/z* 484, corresponds to a triterpene alcohol and main ion *m/z* 379 [M^+-105]. Component 11 was not identified; this compound has *m/z* 484 and the most prominent ion was *m/z* 357. Compounds 7 and 8 are lanosterol isomers at *m/z* 498 and with fragments of isomer 1 *m/z* 483(63) [M^+-15] and 393(100) [M^+-105]; and isomer 2 *m/z* 483(35) [M^+-15] and 93(100) [M^+-105]. These compounds are widely distributed in the plant kingdom, however there is little information available on Sacha inchi oil. In several studies these minor components have been used to establish the authenticity and quality of oils (Lerma-García et al. 2009).

The alcohol compositions of the commercial Sacha inchi oils studied are listed in Table 5. The total alcohol content ranged from 881.95 to 10,562.80 mg/kg, with an average of 3987.00 mg/kg. Aliphatic alcohol content was 1.56%, of which hexacosanol and docosanol were the most abundant, each about 0.69 and 0.50% respectively. The commercial

Table 6 K-means clustering algorithm analysis on the tocopherols, fatty acids, sterols and alcohols composition of commercial Sacha inchi oils

Variables	Cluster number (number of oils)			MGD	SDGD	*p*
	Cluster 1 (n = 5)	Cluster 2 (n = 13)	Cluster 3 (n = 9)			
α-Tocopherol	76.52a	42.08a	8.05a	42.22	34.24	0.061
γ-Tocopherol	708.26c	1198.14b	1643.84a	1183.41	467.96	0.000
δ-Tocopherol	425.81c	927.62b	1206.24a	853.22	395.50	0.000
Palmitic acid	7.54a	5.18b	4.27b	5.66	1.69	0.010
Palmitoleic acid	0.20a	0.08a	0.07a	0.12	0.07	0.058
Margaric acid	0.09a	0.09a	0.09a	0.09	0.00	0.654
Stearic acid	3.43a	3.26a	3.03a	3.24	0.20	0.169
Oleic acid	18.57a	12.26b	10.81b	13.88	4.13	0.000
Linoleic acid	38.53a	37.42a	36.55a	37.50	0.99	0.876
α-Linolenic acid	30.96a	41.16a	44.77a	38.96	7.16	0.085
Cholesterol	0.33a	0.38a	0.40a	0.37	0.03	0.699
Brassicasterol	1.99a	6.78a	3.53a	4.10	2.45	0.555
24-Methylene-cholesterol	0.29a	0.17a	0.08a	0.18	0.10	0.236
Campesterol	14.12a	10.13a	7.82b	10.69	3.18	0.021
Campestanol	0.20a	0.17a	0.08a	0.15	0.06	0.222
Stigmasterol	14.43b	19.05b	24.60a	19.36	5.10	0.018
Δ7-Campesterol	0.88a	1.92a	1.16a	1.32	0.54	0.483
Chlerosterol	0.61a	0.64a	0.68a	0.64	0.03	0.867
β-Sitosterol	61.39a	51.98a	52.86a	55.41	5.20	0.171
sitostanol	0.49a	0.57a	0.41a	0.49	0.08	0.287
Δ5-Avenasterol	2.93a	4.15a	6.24a	4.44	1.67	0.062
Δ5,24-Stigmastadienol	1.36a	1.39a	0.62a	1.12	0.44	0.423
Δ7-Stigmastenol	0.59a	1.22a	0.49a	0.77	0.40	0.170
Δ7-Avenasterol	0.42a	1.48a	0.99a	0.96	0.53	0.692
Phytol	5.73a	10.29a	4.27a	6.76	3.14	0.434
Docosanol	0.44a	0.65a	0.31a	0.47	0.17	0.571
Tetracosanol	0.30a	0.44a	0.32a	0.35	0.08	0.865
Hexacosanol	0.67a	0.80a	0.53a	0.67	0.14	0.346
Triterpene alcohol *m/z* 534	7.96a	4.92a	4.11a	5.66	2.03	0.260
Triterpene alcohol *m/z* 484	33.17a	37.77a	40.97a	37.30	3.92	0.734
Lanosterol isomer 1	12.68a	14.47a	24.07a	17.07	6.13	0.331
Lanosterol isomer 2	1.28a	1.52a	2.00a	1.60	0.36	0.420
Cycloartenol	15.29a	12.75a	11.18a	13.07	2.08	0.739
24-Methylene cycloartanol	5.19a	4.80a	3.61a	4.53	0.82	0.512
Unidentified *m/z* 484	17.29a	11.60a	8.64a	12.51	4.40	0.249
Samples in each cluster						
Cluster 1	IP001, IP006, IP007, IP020, IP024					
Cluster 2	IP003, IP008, IP009, IP011, IP012, IP013, IP015, IP016, IP019, IP022, IP023, IP025, IP026					

Table 6 continued

Variables	Cluster number (number of oils)			MGD	SDGD	*p*
	Cluster 1 (n = 5)	Cluster 2 (n = 13)	Cluster 3 (n = 9)			
Cluster 3	IP002, IP004, IP005, IP010, IP014, IP017, IP018, IP021, IP027					

The data are represented as the mean (one-way ANOVA followed by Fisher's Least Significant Difference post hoc test)

MGD mean for grouped data, *SDGD* standard deviation from grouped data

Statistically significant difference between clusters; $p < 0.05$

oils (IP011 and IP019) showed quantities above 4.0%. In other studies (*Amaranthus cruentus* L.) docosanol in amaranth oil and hexacosanol in olive oil (Boulkroune et al. 2017) were shown as the most important aliphatic alcohols, diterpene alcohol (phytol) showed an average of 7.44%. Phytol concentrations ranged between 0.10 and 43.51%. The two samples (IP015 and IP022) showed phytol content in the range of 34.99–43.51%, respectively, while six samples (IP011, IP007, IP027, IP013, IP012 and IP004) showed a range between 10–36 and 18.13%, while the rest of the samples remained below 8.16%. Phytol is naturally occurring in nature, released during chlorophyll breakdown and is present in the unsaponifiable matter of the oil (Vetter et al. 2012), cold-pressed oils showed a higher content of phytol than in refined oils. Triterpene alcohol *m/z* 484, was the predominant compound, with an average of 37.98%, followed by the lanosterol isomer 1, with a 17.34% average. Cycloartenol and 24-methylenecycloartanol were also detected with an average of 12.70 and 4.47%, respectively.

Multivariate data analysis

K-means clustering algorithm was used to classify commercial Sacha inchi oils, this multivariate analysis used 35 variables: tocopherols (3 variables), fatty acids (7 variables), sterols (14 variables) and triterpene and aliphatic alcohols (11 variables).

Table 6 represents the three groups formed by the K-means clustering algorithm for commercial Sacha inchi oils. Cluster 1 contains 19% of the samples (n = 5), Cluster 2 consists of 48% of the samples (n = 13), while Cluster 3 consists of 33% of the samples (n = 9). The mean values between the groups are significantly different ($p < 0.005$, obtained by one-way ANOVA) for the variables (γ-tocopherol, δ-tocopherol, palmitic acid, oleic acid, campesterol and stigmasterol). The oils in Cluster 1 contained samples with the highest levels of palmitic acid and oleic acid, while the samples included in Cluster 3 represent the highest γ-tocopherol, δ-tocopherol and stigmasterol content. Cluster 2 represents the samples with a moderate campesterol content (10.13%) that is, between cluster 1 and 2 there is no significant differences. The rest of the variables show no significant differences between the clusters. The oils belonging to cluster 1 have a higher mean value of palmitic acid, oleic acid and campesterol and a lower mean value of γ-tocopherol, δ-tocopherol, and stigmasterol than the oils belonging to cluster 3. The variables with statistical significance ($p < 0.05$) could be used as markers of authenticity in Sacha inchi oils, namely, γ-tocopherol, δ-tocopherol, palmitic acid, oleic acid, campesterol and stigmasterol. Many major and minor compounds have been used as markers of authenticity in vegetable oils (Chasquibol et al. 2016; Li et al. 2016). Although, the chemical composition of vegetable oils can dramatically change due to environmental and processing conditions. The clustering of the Sacha inchi oils may have different characteristics due to high genetic morphological and phytochemical variability that may be confused with genus *Plukenetia*. Five species based on morphological characteristics were described in the Peruvian Amazon: *Plukenetia volubilis* L., *Plukenetia brachybotrya* Müll. Arg, *Plukenetia loretensis* Ule, and *Plukenetia polyadenia* Müll. Arg (Tropicos.org. Missouri Botanical Garden), and most recently, *Plukenetia huayllabambana* (Bussmann et al. 2009). Each one of these species represents a considerable variation in seed size.

Conclusion

The demand for Sacha inchi oil consumption has experienced an increase as awareness of its health benefits. In addition to be a new vegetable oil resource and functional ingredient. The classification of "extra virgin" for Sacha inchi oil is a requirement for local producers, since the oil obtained is associated with the price and a higher quality product by consumers. In this context, 100% of the Sacha

inchi oil sold in the Peruvian market is labeled as "extra virgin" or premium extra virgin. To emphasize the uniqueness of this product, it is necessary and important the characterization and authentication using chromatographic analytical methods. Our results of the profile of tocopherols and fatty acid composition warn us about the existence of thirteen samples that do not comply with concentrations established for these compounds. Only three samples showed a higher content of campesterol than stigmasterol, in some samples brassicasterol was detected that was not detected in authentic oil. Additionally, K-means clustering algorithm analysis was useful to visualize the projection of the different clusters formed for each oil analyzed. In this study, at least six variables could be used as authenticity guidelines in commercial Sacha Inchi oils.

It would be interesting to determine the chemical composition of the seeds and oils based on geographical origin in a future study.

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