Chapter

Sacha Inchi Seed (*Plukenetia volubilis* L.) Oil: Terpenoids

Alexandra Valencia, Frank L. Romero-Orejon, Adriana Viñas-Ospino, Dayana Barriga-Rodriguez, Ana María Muñoz and Fernando Ramos-Escudero

Abstract

Sacha inchi oil is a product obtained from oilseed (*Plukenetia volubilis* L.) and is an excellent source of bioactive compounds, especially in polyunsaturated fatty acids, tocopherols, and sterols. These compounds are causally related to their positive impact on human health. In this study summarizes some monoterpenes, sesquiterpenes, and triterpenes reported in Sacha inchi oil seeds and reviews their sensory properties. The terpenoids that characterize Sacha inchi seed oil are: α-pinene, sabinene, limonene, aristolene, cycloartenol, 24-methylene cycloartenol, lanosterol, β-sitosterol, stigmasterol, campesterol and phytol. The sensory properties of this oil are due to a set of volatile compounds including terpenoids, the odor descriptors of monoterpenes, sesquiterpenes and diterpenes are: flower, pine, turpentine, pepper, wood, lemon, orange, and sweet. These compounds were characterized by gas chromatography with different detectors.

Keywords: sacha inchi seed oil, terpenoids, sensory properties, chromatographic analysis

1. Introduction

The Sacha inchi (*Plukenetia volubilis* L.) plant is a crop that has expanded rapidly in recent decades. This endemic crop of the South American Amazon is found mainly in Peru, Colombia, Ecuador, and Brazil. Other geographical regions of the world where Sacha inchi cultivation has flourished include China, Thailand, Vietnam, and Malaysia [1–4]. Its oleaginous plant has become a crop of economic importance for the food, pharmaceutical and cosmetic industries. Exports in Peru have grown notably for the year 2017, especially for its main products such as oil, roasted seed, and powder, having as main destinations, South Korea, United States, Japan, Canada and France [5].

Kodahl [6] mentioned that Sacha inchi seed has an unusual chemical composition as it contains remarkably high amounts of polyunsaturated fatty acids. According to the NTP [7] indicates that the requirements for the polyunsaturated fatty acids (PUFAs) profile is as follows: α-linolenic acid (ω-3, greater than 42%), linoleic acid (ω-6, greater than 32%) and polyunsaturated fatty acids (greater than 80%) of the total lipid fraction. Other main representatives of the unsaponifiable fraction are tocopherols, which are distributed in the oil as follows: α-tocopherol

(60–70 mg/kg), β-tocopherol (18–29 mg/kg), γ-tocopherol (1108–1367 mg/kg), δ-tocopherol (641–856 mg/kg), and sterols fraction of commercial oils was 1130–3635 mg/kg, and the main sterols were β-sitosterol, stigmasterol, campesterol and Δ5-avenasterol [8, 9]. Other compounds of interest are phenolic compounds (the main classes of phenols found in sacha inchi seed oil (SISO) are phenyl alcohol, isocoumarin, flavonoid, secoiridoid, and lignan) [10], volatile organic compounds (while the classes of VOCs identified in commercial oil were aldehydes, hydrocarbons, alcohols, ketone, furan, and carboxylic acid), and terpenoids [11].

Terpenoids are a large family of chemical compounds which can be found in a large number of plants, many of which have characteristic odors, flavors, and colors, and are main components of essential oils (especially monoterpenes and sesquiterpenes) [12]. Terpenoids can be structurally decomposed into two or more isoprene units or 2-methyl-1,3-butadiene and classified as monoterpenes ($C10H16$), sesquiterpenes ($C15H24$), diterpenes ($C20H32$), triterpenes ($C30H48$), and tetraterpenes or carotenes ($C40H64$) [13]. In vegetable oils, several terpenoids have been identified, these compounds provide aromatic properties (monoterpenoids: myrcene, citral, linalool, thymol, menthol, carvone, eucalyptol, α- and β-pinene, etc.), and are natural fat-soluble pigments (tetraterpenoids: lycopene, γ-carotene, β-carotene, lutein, zeaxanthin, etc.) [14], this last group of chemical species are responsible for transmitting the chromatic characteristics in vegetable oils. A list of oils from conventional and non-conventional plant sources where terpenoids have

Terpenoids	Class	Effects	Reference
α-Pinene	Monoterpene	Cytogenetic, gastroprotective, anxiolytic, cytoprotective, anticonvulsant, and neuroprotective	[27]
Sabinene	Monoterpene	Antioxidant, antibacterial and antifungal	[29, 30]
Limonene	Monoterpene	Gastroprotective, anti-inflammatory, bradycardic, antiarrhythmic, antitumor, antiviral, and antibacterial	[31–33]
Aristolene	Sesquiterpene	Antifungal, antioxidant, and anticancer	[34, 35]
Cycloartenol	Triterpene	Anticancer, and antidiabetic	[36, 37]
24-Methylene cycloartenol	Triterpene	Antidiabetic	[37]
Lanosterol	Triterpene	Cytotoxic and immunomodulatory	[38, 39]
β-Sitosterol	Sterol	Anticancer, lipid-lowering, anti-inflammatory, and antioxidant	[40–43]
Stigmasterol	Sterol	Lipid-lowering, antiasthmatic, immunomodulatory, antioxidant, and anti-inflammatory	[41, 44]
Campesterol	Sterol	Anti-inflammatory, and cytotoxic	[45]
Phytol	Diterpene	Antitumoral, antimutagenic, antimicrobial, anxiolytic, metabolism-modulating, cytotoxic, antioxidant, autophagy- and apoptosis-inducing, antinociceptive, anti-inflammatory, immune-modulating, antidiabetic, anti-atherogenic, lipid-lowering, antispasmodic, antiepileptic, antidepressant and immunoadjuvant	[46, 47]

Table 1.
Summary of terpenoids of Sacha inchi seed oil and biological effects.

been identified: soybean, olive, rapeseed, sunflowerseed, flaxseed, sesame, pumpkin, pistachio, almond, hazelnut, safflower, hempseed, sacha inchi oils [15–20].

Traditionally, plant-based terpenoids have been used by humans in the food (terpenoids as natural flavorings compounds, preservatives for dairy products, stability of edibles oils flavored with essential oils) [21–23], pharmaceutical (production of pharmaceutical terpenoids for the treatment of human diseases) [24, 25], and chemical industries (natural additives for food or fragrances in perfumery) [26]. Various studies have shown the efficacy of terpenoids due to their biological and medical properties [25, 27, 28]. **Table 1** summarizes most of the effects, however some of heightened interest are mentioned in this section.

This document summarizes some monoterpenes, sesquiterpenes, and triterpenes reported in Sacha inchi oil seeds and reviews their sensory properties.

2. Overview of terpenoids biosynthesis in Sacha inchi seed oil

The biosynthesis of these compounds occurs via the methylerythritol phosphate pathway (MEP) or mevalonate (MVA) pathway involves several reactions to isopentenyl diphosphate production from acetyl CoA. The isopentenyl diphosphate (IPP) combines with dimethyl-allyl diphosphate (DMAPP) to that subsequently converted to geranyl pyrophosphate (GPP) by enzymatic catalysis of isopentenyl diphosphate isomerase. Geranyl pyrophosphate is the substrate to produce monoterpenoids. The enzymatic reaction is mediated by monoterpene synthases [48]. The monoterpenes found in SISO were α-pinene, sabinene and limonene (**Figure 1**). α-Pinene (C10H16) is the main bicyclic monoterpene found in this oil, it is also widely distributed in nature. The sesquiterpenes are formed by the condensation of IPP with GPP to yield farnesyl pyrophosphate (FPP) [50]. The GPP to FPP reaction is mediated by farnesyl pyrophosphate synthase. The only sesquiterpene found in SISO is the aristolene (C15H24) [20]. On the other hand, this biochemical pathway may be used for triterpene (some triterpenes were found in SISO, namely cycloartenol, 24-methylene cycloartenol and lanosterol isomers) and probably sterols (individual sterols found in SISO, namely β-sitosterol, stigmasterol, campesterol, Δ5-avenasterol, Δ5,24- stigmastadienol, Δ7-stigmastenol, Δ7-avenasterol, etc.) [8, 9, 51], and brassinosteroids biosynthesis, whereas geranylgeranyl pyrophosphate (GGPP) is utilized for the biosynthesis of photosynthetic pigments such as carotenoids, chlorophylls and diterpenes (phytol) (**Figure 1**) [9, 52, 53].

3. Terpenoids in Sacha inchi seed oil

In the scientific literature there are few reports on the volatile composition of sacha inchi oil [20, 49]. The terpenoid fractions in the Sacha inchi oil is observed in **Table 2**. The identification of the classes of terpenoids found in Sacha inchi seed oil and commercial Sacha inchi oil were monoterpenes, sesquiterpenes, diterpenes, triterpenes and sterols. The first terpenoids identified in this oil were sterols: β-sitosterol > stigmasterol > campesterol > Δ5-avenasterol [51]. The sterol composition of these main compounds is around ~96%. The sterol content in the Sacha inchi seed oil was reported as 2472 mg/kg. While the sterol contents in commercial oils ranging from 1130 to 3635 mg/kg [8, 9].

The sterol content in Sacha inchi seed oil is represented by the content of β-sitosterol, stigmasterol and campesterol (**Table 2**). The β-sitosterol, followed by stigmasterol or campesterol and other minor sterols (triterpenes) such as

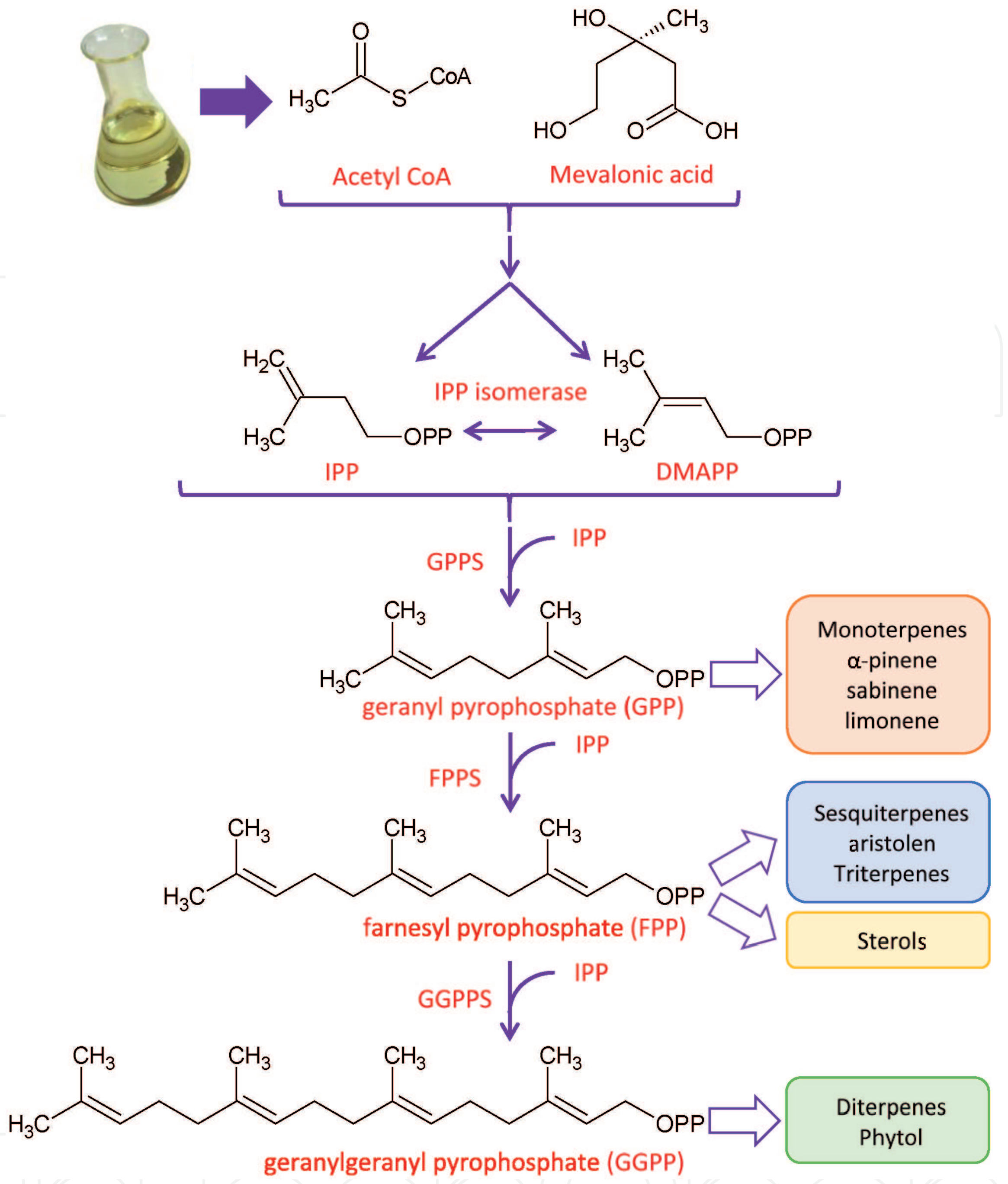


Figure 1.
Biosynthetic pathway of terpenoids and chemical compounds found in Sacha inchi seed oil. The diagram was modified according to Feng et al. [49]. Isopentenyl diphosphate (IPP), dimethyl-allyl diphosphate (DMAPP), geranyl pyrophosphate synthase (GPPS), farnesyl pyrophosphate synthase (FPPS), geranylgeranyl pyrophosphate synthase (GGPPS).

fucosterol, and Δ5-avenasterol are the most representative in vegetable oils. In addition, 50% to 80% of the plant sterols intake comes from oils, spreads, butters, breads, cereals, grains, pastes, and vegetables [55]. On the other hand, other triterpenoids such as cycloartenol, 24-Methylene cycloartenol, and lanosterol were detected in commercial Sacha inchi oil, the contents ranged from 0.10 to 47.44%, 2.59 to 24.15%, 0.80 to 11.79%, respectively. A sole example of diterpene such as phytol were found in the range of 0.10 to 43.51% [9]. The monoterpenoids and sesquiterpene in the sacha inchi oil were α-pinene, sabinene, limonene and aristolene these compounds were also identified by Monroy-Soto et al. [11]. In addition, it has been reported that this class of terpenoids are considered potentiators. In this context, the minimum inhibitory concentration of some monoterpenoids

Terpenoids	Sacha inchi seed oil	Commercial Sacha inchi oil
α-Pinene (μg/kg)		(3.35–1179.24) μg/kg
Sabinene (μg/kg)		(0.87–416.51) μg/kg
Limonene (μg/kg)		(0.93–187.83) μg/kg
Aristolene (μg/kg)		(3.99–34.82) μg/kg
Cycloartenol (%)		(2.59–34.54) %
24-Methylene cycloartenol (%)		(0.80–11.79) %
Lanosterol (%)		(0.10–47.44) %
β-Sitosterol (%)	127.4 mg/100 g	(21.45–68.91) %
Stigmasterol (%)	58.7 mg/100 g	(10.4–27.4) %
Campesterol (%)	15.3 mg/100 g	(5.1–18.9) %
Δ5-Avenasterol (%)		(0.10–7.78) %
Phytol (%)		(0.10–43.51) %

References, for Sacha inchi seed oil: Chirinos et al. [54]. For commercial Sacha inchi oil: Chasquibol et al. [8]; Ramos-Escudero et al. [9].

Table 2.
Summary of terpenoids identified in Sacha inchi oil.

(α-pinene and limonene) on bacteria such as *Escherichia coli*, *Salmonella enterica* and *Staphylococcus aureus* have been reported previously [56]. Furthermore, these monoterpenoids have shown a potent antioxidant activity, especially α-pinene followed by limonene, both presented a 50% inhibitory concentration values (IC_{50}) equal to 12.57 and 13.35 mg/mL, respectively. In this regard, terpenoids have huge potential as natural food preservatives for use in the food industry [57].

The storage food products are subject to changes in the chemical composition and as a result the formation of undesirable volatile compounds. Therefore, terpenoids as natural preservatives can be used to slow down food spoilage. Some monoterpenoids such as limonene can be used as substitutes for synthetic antioxidants (TBHQ, BHA, BHT) and improves oxidative stability in edible oils [58]. Wang et al. [58] have mentioned that monoterpenoids can be used as a reference for the food manufacturing, lifestyle, and nutrition in the future.

4. Terpenoids and sensory properties in Sacha inchi seed oil

Terpenoids are compounds responsible for the smell of most plants. Phytol, α-pinene, sabinene, limonene, and aristolene have been found in Sacha inchi oil (**Table 3**). These compounds provide some odor notes such as flower, pine, turpentine, pepper, wood, lemon, orange, and sweet. The content of monoterpenoids and sesquiterpenoids in Sacha inchi oil, fraction constituted about 9.0% of total volatile fraction. Ramos-Escudero et al. [20] have mentioned that these compounds are responsible for the floral aroma in this oil. However, the sensory characteristics of Sacha inchi oil not only correspond to the sensory notes of the terpenoids, but to a combination of sensory attributes such as herbal, green, nutty, seeds, butter, rancid, fruity, floral, and woody [20, 59]. Different volatile compounds including terpenoids have been identified in vegetable oils and each compound has different characteristics of key odorants. For example, in virgin sunflower oil the most preferred attributes were sweet and wood/vegetable resin, the latter possibly due

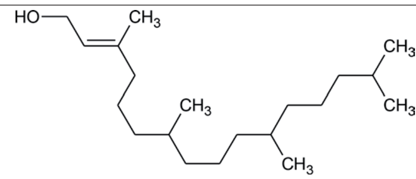
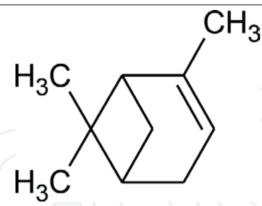
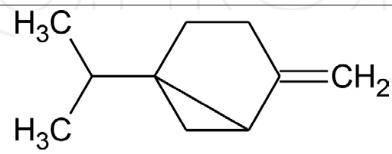
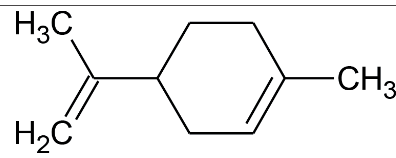
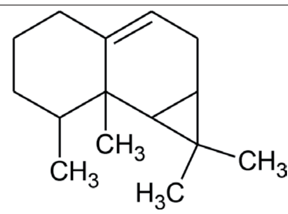
Terpenoids	MF/MW	Structure	Percepts
Phytol	$C_{20}H_{40}O$ 296.54 g/mol		flower
α-Pinene	$C_{10}H_{16}$ 136.23 g/mol		pine, turpentine
Sabinene	$C_{10}H_{16}$ 136.23 g/mol		pepper, turpentine, wood
Limonene	$C_{10}H_{16}$ 136.23 g/mol		lemon, orange
Aristolene	$C_{15}H_{24}$ 204.35 g/mol		flower, sweet

Table 3.
Terpenoids, structures, and percepts of in Sacha inchi seed oil.

to the presence of terpenes such as linalool and α- and β-pinene. Furthermore, the sensory profile of Niger seed oil showed positive attributes such as dried fruit, spicy and bitter, which could be related to the presence of some terpenes, specifically limonene and phellandrene. On the other hand, the sensory notes of pine perceived in the pine nut (*Pinus pinea*) oil were described under the wood/plant resin attribute, which could be attributable to the high contents of α-pinene as well as other terpenes such as β-pinene, β-myrcene and α- and γ-terpinene present in its volatile composition [15].

5. Comparison of terpenoid contents in other vegetable oils

Information about the volatile composition, including some terpenoids in vegetable oils can be found in published reports. Aguilar-Hernández et al. [60] reported the profile of terpenoids including monoterpenes and sesquiterpenes in lemon peel oil. In this oil around 23 terpenoids have been found, the most relevant being limonene, γ-terpinene, sabinene, α-pinene, β-pinene, α-thujene, terpinolene, α-terpineol, neral, geranial, and trans- α- bergamotene. Ivanova-Petropulos et al. [17] reported a higher content of terpenoids in sunflower seed oil and pumpkin seed oil. The most common monoterpenoids and sesquiterpenoids in both oils were: α-thujene, α-pinene, α-fenchene, camphene, verbenene, sabinene, 2-β-pinene, α-phellandrene, α-terpinene, DL-limonene, β-phellandrene, 1,8-cineole, *o*-cymene, *p*-cymene, γ-terpinene, α-terpinolene, α-campholenal,

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	Compounds	Flaxseed oil	Rapeseed oil	Sesame seed oil	Sunflower seed oil	Pumpkin oil	Sacha inchi oil	Pistachio oils	Almond oil	Hazelnut oil
1	α-thujene	X			X	X		X		
2	α-pinene	X	X	X	X	X	X	X		
3	β-pinene							X		
4	α-fenchene	X			X	X				
5	camphene	X	X		X	X		X		
6	verbenene	X	X		X	X				
7	sabinene				X	X	X			
8	2-β-pinene	X	X		X	X				
9	α-phellandrene	X		X	X	X				
10	3-carene	X						X		
11	4-carene							X		
12	α-terpinene	X	X	X	X	X		X		
13	β-ocimene			X						
14	limonene						X	X		
15	β-phellandrene	X			X	X				
16	1,8-cineole	X	X		X	X				
17	o-cymene				X	X		X		
18	p-cymene	X	X	X	X	X				
19	γ-terpinene	X	X		X	X				
20	α-terpinolene	X			X	X		X		
21	α-campholenal	X			X	X				

	Compounds	Flaxseed oil	Rapeseed oil	Sesame seed oil	Sunflower seed oil	Pumpkin oil	Sacha inchi oil	Pistachio oils	Almond oil	Hazelnut oil
22	trans-pinocarveol				X	X				
23	α-phellandren-8-ol				X					
24	borneol				X	X				
25	4-terpineol				X	X				
26	3-pinanone				X	X				
27	2-pinen-10-ol				X	X				
28	myrtenal				X	X				
29	verbenone				X	X				
30	α-cubebene				X	X				
31	camphor	X			X	X				
32	α-copaene				X	X				
33	β-elemene				X	X				
34	β-bourbonene				X	X				
35	β-selinene				X	X				
36	β-myrcene							X		
37	2-norpinene			X	X	X				
38	aristolen				X	X	X			
39	γ-cadinene				X	X				
40	calarene				X	X				
41	α-amorphene				X	X				

	Compounds	Flaxseed oil	Rapeseed oil	Sesame seed oil	Sunflower seed oil	Pumpkin oil	Sacha inchi oil	Pistachio oils	Almond oil	Hazelnut oil
42	β-bisabolene				X	X				
43	δ-cadinene				X	X				
44	longifolene							X	X	X
45	*α*-terpineol							X	X	X

Table 4.
Chemical characterization of terpenoids detected in vegetable oils.

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trans-pinocarveol, borneol, 4-terpineol, 3-pinanone, 2-pinen-10-ol, myrtenal, verbenone, α-cubebene, camphor, α-copaene, β-elemene, β-bourbonene, β-selinene, 2-norpinene, aristolen, γ-cadinene, calarene, α-amorphene, β-bisabolene, δ-cadinene. While the main terpenoids in the following oils were: flaxseed (α-pinene, camphene, verbenene, 2-β-pinene, 3-carene, α-terpinene, DL-limonene, 1,8-cineole, γ-terpinene, and α-terpinolene), rapeseed (*p*-cymene), and sesame seed (2-norpinene). Other vegetable oils have different terpene profiles [18, 19] (**Table 4**).

6. Application of chromatographic techniques in Sacha inchi seed oil

There are few reports about the chemical characterization of the terpenoids in the Sacha inchi oil (**Table 5**). The separation of the different analytes from the sterol fraction was conducted using the following columns: SAC™-5/Merck (Phase: 5% diphenyl/95% dimethyl polysiloxane), HP-5/Agilent J&W (Phase: 5% phenyl-methylpolysiloxane), and SPB-5/Merck (5% diphenyl/95% dimethyl polysiloxane). While the separation of volatile compounds was carried out using columns with high polar (DB-WAX/Agilent J&W, and TRB-WAX/Teknokroma/ 100% polyethylene glycol) and nonpolar (DB-5/Agilent J&W/5% phenyl-methylpolysiloxane) stationary phases.

Monroy-Soto et al. [11] evaluated the volatile composition of Colombian commercial Sacha inchi oil using headspace-solid phase microextraction coupled GC–MS-O. Ramos-Escudero et al. [20] analyzed the Peruvian commercial Sacha inchi by HS-SPME/GC–MS, through which 16 volatile compounds (among them limonene, α-pinene, and sabinene) may have a significant influence upon perceived flavor and odor.

Analytes	Column	Technique	Methods	Extraction	Reference
Sterol	SAC™-5 (30 m x 0.25 mm ID)	GC-FID	C	S	[61, 62]
Sterol	HP-5 (30 m x 0.32 mm ID)	GC-FID/MS	C, A	P	[9]
Sterol	SPB-5 (30 m x 0.32 mm ID)	GC-FID	C	P	[8, 63]
Terpenes	DB-WAX (30 m x 0.25 mm ID) DB5 (30 m x 0.25 mm ID)	HS-SPME-GC–MS-O	C	P	[11]
Terpenes	ATR-WAX (60 m x 0.25 mm ID)	HS-SPME/GC–MS	C, A	P	[20]

List of abbreviations: GC-FID, Gas chromatography-flame ionization detector; GC–MS, Gas chromatography–mass spectrometry; HS-SPME, Headspace-solid phase microextraction; GC–MS-O, Gas Chromatography–Mass Spectrometry-Olfactometry. A, authentication; C, characterization; P, cold pressed; S, solvent.

Table 5.
Characterization and authentication of Sacha inchi oil.

7. Conclusions

Sacha inchi oil is a product of economic importance that has been characterized according to its chemical composition. At present several classes of chemical compounds have been identified and quantified, and more recently the volatile

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composition. The volatile organic compounds correspond to notes generated by alcohols, aldehydes, ketones, and terpenoids. The classes of terpenoids found in Sacha inchi oil were monoterpenes, sesquiterpenes, diterpenes, and triterpenes. These compounds provide different sensory properties in the oil. Furthermore, the characterization is conducted mainly by gas chromatography (GC) coupled to flame ionization detector (FID) and mass spectrometry (MS) detection.

Conflict of interest

The authors declare no conflict of interest.

Author details

Alexandra Valencia[1], Frank L. Romero-Orejon[1], Adriana Viñas-Ospino[1,2], Dayana Barriga-Rodriguez[3], Ana María Muñoz[1,4] and Fernando Ramos-Escudero[1,4]*

1 Unidad de Investigación en Nutrición, Salud, Alimentos Funcionales y Nutraceúticos, Universidad San Ignacio de Loyola (UNUSAN-USIL), Lima, Perú

2 Nutrition and Food Chemistry, University of Valencia, Burjassot, Spain

3 Facultad de Ciencias de la Salud, Universidad San Ignacio de Loyola, Lima, Perú

4 Instituto de Ciencias de los Alimentos y Nutrición, Universidad San Ignacio de Loyola (ICAN-USIL), Campus Pachacamac, Sección B, Lima, Perú

*Address all correspondence to: diomedes.fernando@gmail.com

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