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Sacha inchi (*Plukenetia volubilis*): A seed source of polyunsaturated fatty acids, tocopherols, phytosterols, phenolic compounds and antioxidant capacity

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Fatty acids (FA), phytosterols, tocopherols, phenolic compounds, total carotenoids and hydrophilic and lipophilic ORAC antioxidant capacities were evaluated in 16 cultivars of Sacha inchi (SI) seeds with the aim to valorise them and offer more information on the functional properties of SI seeds. A high α linolenic (α-Ln) fatty acid content was found in all cultivars (ω3, 12.8–16.0 g/100 g seed), followed by linoleic (L) fatty acid (ω6, 12.4–14.1 g/100 g seed). The ratio ω6/ω3 was within the 0.83–1.09 range. γ- and δ-tocopherols were the most important tocopherols, whereas the most representative phytosterols were β-sitosterol and stigmasterol. Contents of total phenolics, total carotenoids and hydrophilic and lipophilic antioxidant capacities ranged from 64.6 to 80 mg of gallic acid equivalent/100 g seed; from 0.07 to 0.09 mg of β-carotene equivalent/100 g of seed; from 4.3 to 7.3 and, from 1.0 to 2.8 μmol of Trolox equivalent/g of seed, respectively, among the evaluated SI cultivars. Results showed significant differences ($p < 0.05$) among the evaluated SI cultivars in the contents of ω3, ω6, antioxidant capacities and other evaluated phytochemicals. SI seeds should be considered as an important dietary source of health promoting phytochemicals.

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1. Introduction

The Amazonian region is the most important area in terms of biodiversity. It hosts many important crops for the world's agriculture (e.g., cassava, pineapple, cocoa, rubber among others). Still, numerous under exploited Amazonian plant species, with promising economic value, remain little-known and neglected by science (Krivankova et al., 2007). Sacha inchi (*Plukenetia volubilis* Linneo), also known as Inca peanut, wild peanut, sacha peanut or mountain peanut, is an oleaginous plant that belongs to the *Euphorbiaceae* family. It grows in the lowlands of the Peruvian Amazon, having been cultivated for centuries by the indigenous population (Guillén, Ruiz, Cabo, Chirinos, & Pascual, 2003; Gutiérrez, Rosada, & Jiménez, 2011; Hamaker et al., 1992; Krivankova et al., 2007). The Sacha inchi (SI) plant grows in warm climates, at altitudes of up to 1500 m above sea level. SI seeds are a good source of oil (35–60%) and protein (~27%) and contain heat-labile substances with a bitter taste. SI oil is characterised predominantly by high levels of essential fatty acids, namely, C18:3 ω3 (α-Ln, cis,cis,cis-9,12,15-octadecatrienoic acid; α-linolenic) and C18:2 ω6 (L, cis,-cis-9,12-octadecadienoic acid; α-linoleic) fatty acids, representing about 82% of the total oil content. The ratio of ω6/ω3 has been reported to be approximately 0.81 by Hamaker et al. (1992). The presence of other bioactive compounds, such as tocopherols (Follegatti-Romero, Piantino, Grimaldi, & Cabral, 2009), carotenes (Hamaker et al., 1992), polyphenolic compounds (Fanali et al., 2011) and phytosterols, have been previously reported in SI oil (Bondioli, Della Bella, & Rettke, 2006). In addition, the amino-acid profile of the SI protein fraction showed a relatively high level of cysteine, tyrosine, threonine and tryptophan, compared to other oilseed sources (Hamaker et al., 1992). SI seeds are used in different forms by the Amazonian population. The oil is used in the preparation of various meals, the seeds are consumed roasted and the leaves are cooked and consumed (Fanali et al., 2011). SI seeds are also used as a traditional remedy in the Amazon region to treat rheumatic problems and aching muscles.

A wide range consumption of bioactive is important in our diet in terms of health. Thus, α-Ln and L FA are very important for the prevention of coronary heart disease and hypertension, showing a hypocholesterolemic effect (Simopoulos, 2011). In addition, numerous health benefits have been attributed to phytosterols,

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tocopherols, carotenoids and phenolic compounds. Phytosterols have been reported to reduce blood cholesterol and to decrease the risk of certain types of cancer (Lagarda, García-Llatas, & Farré, 2006; Moreau, Whitaker, & Hicks, 2002). Tocopherols have vitamin E properties and display a strong antioxidant activity, conferring protection against lipid peroxidation in biological tissues and foods (Hounsome, Hounsome, Tmos, & Edgard-Jones, 2008). Both carotenoids and phenolic compounds are considered to promote human health, since they are responsible for critical biological functions (Huang, Ou, & Prior, 2005; Krinsky & Johnson, 2005).

SI seeds are an excellent source of macronutrients and bioactive compounds. However, previous reported studies have concentrated on the chemical and functional characterisation of the oil and much less on the entire seed. Rodríguez et al. (2010) showed high variability in morphology, plant production and oil content. The genetic profile of 12 SI accessions was evaluated by Corazon-Guivin et al. (2009), a high diversity was found among the accessions. In general, the reported studies on SI oil and phytochemicals have been carried out with commercial samples (Follegatti-Romero et al., 2009; Gutiérrez et al., 2011; Hamaker et al., 1992). Differences in the chemical and phytochemical composition, among cultivars, have not been established yet. Thus, this study aims to gather more information on the bioactive content present in SI seeds from different cultivars. The objective of this work was to determine the fatty acid (FA) profiles, phytosterols, tocopherols, carotenoids, phenolic compound contents and hydrophilic and lipophilic antioxidant capacities in 16 different cultivars of SI seeds. The results of this work aim to offer more information on the functional properties of SI seeds to favour their promotion and consumption in local and international markets.

2. Materials and methods

2.1. Sample material and chemicals

The different SI seed cultivars were part of the National Collection of Sacha Inchi from Peru. They were kindly provided by Instituto Nacional de Innovación Agraria (INIA) (Lima, Peru). The different cultivars were cultivated in the experimental station 'El Porvenir' of INIA in Tarapoto (Peru) and harvested in March 2011. Table 1 displays the cultivar specific data of collection for the different cultivars. SI seeds (0.5–1 kg/cultivar) were manually cleaned and selected. The skin was removed and the almond kept for further study. The almonds represented, on average, 64% of the total weight of the seed. The almonds were divided in three subsamples of similar weight and were kept in dark polyethylene bags, vacuum sealed and stored at 4 °C. They were independently analysed. The almonds described are treated in this work as SI seeds. SI seeds were further grinded using a coffee mill (~1 mm). The moisture content of the SI seed was determined according to AOAC (1995).

Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid) and 2 N Folin–Ciocalteu reagent, gallic acid and β-carotene were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Randomly methylated β-cyclodextrin (RMDC) was purchased from Cyclolab (Budapest, Hungary). Methyl *tert*-butyl ether (MTE) was purchased from J.T. Baker (New Jersey, USA). Fatty acid methyl ester (FAME) standards were provided by Restek (Bellefonte, PA, USA). Tocopherol standards (α-, β-, γ- and δ-tocopherol) and phytosterol standards (β-cholestanol, β-sitosterol, campesterol and stigmasterol) were purchased from ChromaDex™ (Santa Clara, CA). All solvents and other chemicals of analytical grade were purchased from Merck (Darmstadt, Germany) and Fischer Scientific (Fair Lawn, NJ, USA).

2.2. Quantitative analysis

2.2.1. Fatty acid content

FA SI seed composition required a previous oil extraction. Samples were submitted to a 6 h extraction with petroleum-ether using Soxhlet equipment. A solid/solvent ratio of 1:10 (w/v) was used. After the extraction process, the flask content was filtered and the filtrate was rapidly concentrated, under nitrogen flow, in a 30 °C water bath. The obtained oil was placed in the oven at 30 °C for 1 h, weighed and stored in sealed amber glass vials. The vials were kept at −20 °C until analysis.

The FA composition was determined by gas chromatography (GC) according to the method proposed by Meurens, Baeten, Yan, Mignolet, and Larondelle (2005) with slight modifications. The FA of the oil samples were converted into methyl esters (FAME). FAME were separated by injecting 1 μl of the solution into a Trace GC Thermo Finnigan (Milan, Italy) equipped with a flame ionisation detector and an autoinjector Pal GC from CTC Analytics (Zwingen, Switzerland) and the ChromQuest 3.0 software. The column used was a Restek Rt-2560 (Bellefonte, PA) (0.2 μm, 100 m × 0.25 mm ID). The oven temperature was programmed as follows: initially at 80 °C (for 1 min), increased to 175 °C at 25 °C/min, an isothermal period of 25 min at 175 °C, increased to 205 °C at 10 °C/min, an isothermal period of 205 °C for 4 min, increased from 205 to 225 °C at 10 °C/min and finally an isothermal period of 225 °C for 20 min. The injector and detector temperatures were set at 225 °C. High purity H_2 was used as a carrier gas. FAME were identified and quantified by comparing their retention times to known previously

Table 1
Cultivar specific information of Sacha inchi national collection.

Code/cultivar	Identification	Department	Province	District
PER000394	Shilcayo	San Martín	Maynas	Banda de Shilcayo
PER000395-A	Pinto Recodo	San Martín	Lamas	Pinto recodo
PER000401	Caballococha	Loreto	Mariscal Ramón Castilla	Ramón Castilla
PER000403	Santa Clara Alto	Loreto	Maynas	Alto Nanay
PER000405	Muyuy	Loreto	Requena	Capelo
PER000406-A	Pacaya	Loreto	Requena	Puinahua
PER000406-B	Pacaya	Loreto	Requena	Puinahua
PER000408-B	Río Mamón	Loreto	Maynas	Punchana
PER000416	Chazuta	San Martín	San Martin	Chazuta
PER000417	Saposoa	San Martín	Huallaga	Saposoa
PER000418	Moyobamba	San Martín	Moyobamba	Moyobamba
PER000420	Río Palmira	Loreto	Maynas	Alto Nanay
PER000421	Alto Shamboyacu	San Martín	Lamas	Lamas
PER000422	Alto pucayacu	San Martín	Lamas	Zapatero
PER000597	Chirapa	San Martín	El Dorado	Santa Rosa
PER000598	Shapaja	San Martín	San Martín	Shapaja

injected standards. Standard curves for fatty acids were built with different FAME concentrations within the 6–90 mg/L range. Results were expressed as g of fatty acid per 100 g of seed.

2.2.2. Tocopherol content

The samples were prepared following the methodology reported by Amaral, Alves, Seabra, and Oliveira (2005) with slight modifications. Briefly, 300 mg of SI sample and 100 μl of BHT (10 mg in 1 ml of *n*-hexane) were homogenised for 1 min by vortex mixing, after the addition of each of the following reagents: ethanol (2 ml), extracting solvent (*n*-hexane, 4 ml), and saturated NaCl solution (2 ml). Then, the mixture was centrifuged (4000*g* for 4 min at 1 °C) and the clear upper layer was recovered. The sample was re-extracted twice using 2 ml of *n*-hexane. The combined extracts were taken to dryness under nitrogen, and the residue was reconstituted in a volume of 1.5 ml with *n*-hexane. The extract was dried with anhydrous sodium sulphate (0.5 g), centrifuged (4000*g* for 20 min at 1 °C) and transferred to a dark vial for the subsequently HPLC analysis.

The tocopherol composition was determined by HPLC. The extracts were separated using a normal phase HPLC column on a Waters 2695 Separation Module (Waters, Milford, MA) equipped with an autoinjector, a Waters 2475 multifluorescence detector and the Empower software. A YMC-Pack Silica Col (3 μm, 250 × 4.6 mm column (Kyoto, Japan) and a 4.0 × 2.0 mm guard column were used for tocopherol separation at 35 °C. The mobile phase was composed of *n*-hexane/2-propanol/acetic acid (1000:6:5, v/v/v). Elution was performed at a solvent flow rate of 1.4 ml/min with an isocratic program and 10 μl of sample was injected. The effluent was monitored with the fluorescence detector, programmed at the excitation and emission wavelengths of 290 and 330 nm, respectively. Samples and mobile phases were filtered through a 0.22 μm Millipore filter, type GV (Millipore, Bedford, MA) prior to HPLC injection. Tocopherols were identified and quantified by comparing their retention time to known injected standards. The standard curves for the different tocopherols were built within the range of concentrations of 3–60 mg/L. Results were expressed as μg of tocopherol/g of seed.

2.2.3. Phytosterol content

The samples were prepared using the methodology reported by Duchateau et al. (2002) and da Costa, Ballus, Teixeira-Filho, and Teixeira Godoy (2010). Briefly, 100 mg of SI oil was saponified with ethanolic KOH solution (1 ml) at 70 °C for 50 min. The internal standard (1 ml of β-cholestanol 10 mg/L in *n*-heptane) was added to each sample. The unsaponifiable fraction was extracted using liquid–liquid partitioning into 1 ml of distilled water and 5 ml of *n*-heptane. The organic phase was transferred to a test tube containing Na_2SO_4, and the extraction was repeated twice with 5 and 4 ml of *n*-heptane. The *n*-heptane extracts were combined and homogenised before injection into a gas chromatography system.

The phytosterol composition was determined by GC. Phytosterols were separated by injecting 2 μl of the extract to a GC-2010 plus Shimadzu (Kyoto, Japan) equipped with a flame ionisation detector FID-2010, an auto injector AOC-20i. The column used was a Supelco SAC^{TM}-5 (St. Louis, MO, USA) (0.2 μm, 30 m × 0.25 mm ID). The oven temperature was programmed as follows: initially at 250 °C (for 2 min), increased to 285 °C at 25 °C/min, an isothermal period of 285 °C for 32 min and a split ratio of 10. The injector and detector temperatures were set at 300 °C. Helium was used as a carrier gas. Phytosterols were identified and quantified by comparing their retention times to known injected standards. The standard curves for the different phytosterols were built within the 5–100 mg/L concentration range. Results were expressed as mg per 100 g of seed.

2.2.4. Total carotenoid content (TCT)

The method reported by Talcott and Howard (1999) was used for measuring TCT. Half a gram of SI seeds with 5 ml of acetone/ethanol (1:1) solution containing 200 mg/L BHT was homogenised to a uniform consistency and then the mixture was filtered. The filtrate was transferred to a graduated cylinder and solvent was added to a final volume of 5 ml. A 5 ml volume of hexane and 2.5 ml of H_2O were added and shaken vigorously and let to stand for 30 min to allow separation of the phases. Absorbance of the hexane phase was measured at 470 nm. β-carotene was used as a standard for the calibration curve and TCT was expressed as mg of β-carotene equivalent (βCE)/100 g of seed.

2.2.5. Total phenolics content (TPC)

Total phenolics were determined by following the method of Singleton and Rossi (1965). A 0.5 g of SI defatted sample was homogenised with 10 ml of 70% acetone to a uniform consistency and left at 4 °C for 20 h before filtration. Five hundred μl of samples were combined with 1,250 μl of a 7.5% sodium carbonate solution and 250 μl of 1 N Folin–Ciocalteu reagent and allowed to react for 30 min at room temperature. Absorbance of the mixture was measured at 755 nm. Gallic acid was used as a standard. TPC's were expressed as milligram of gallic acid equivalents (GAE)/100 g of seed.

2.2.6. Hydrophilic and lipophilic antioxidant capacity

The method of Arnao, Cano, and Acosta (2001) was used to extract the hydrophilic and lipophilic fractions of the seeds with slight modifications. In brief, 1 g of SI sample was extracted first with 15 ml of 80% methanol (hydrophilic fraction) and then with 10 ml of dichloromethane (lipophilic fraction). All the fractions were centrifuged at 4000*g* for 10 min at 4 °C and stored at −80 °C until analysis. The lipophilic fraction was dried under nitrogen and re-dissolved in acetone containing 7% RMDC. This sample solution was used to measure the lipophilic antioxidant capacity (LAC) meanwhile the hydrophilic fraction was used directly to measure the hydrophilic antioxidant capacity (HAC).

Both hydrophilic and lipophilic antioxidant capacities were determined using the oxygen radical absorbance capacity (ORAC) assay. ORAC analyses were adapted from the procedures described by Ou, Hampsch-Woodill, and Prior (2001) and Huang, Ou, Hampsch-Woodill, Flanagan, and Prior (2002). Antioxidant capacity assays were carried out on a fluorescence plate reader Synergy 2 Biotek (Winooski, VT). AAPH, a water-soluble azo compound, was used as a peroxyl radical generator. Trolox, was used as the standard and fluorescein as a fluorescent probe. The Trolox stock solution was diluted with the phosphate buffer (75 mM, pH 7.4) (for the ORAC HAC) or with 7% RMCD (for the ORAC LAC) to 4, 8, 16, 24 and 32 μM solutions to build up the standard curve. Briefly the procedure to measure the ORAC assay was as follows: 25 μl of a blank (phosphate buffer for the ORAC HAC assay or 7% RMCD solution for the ORAC LAC assay), or Trolox standard, or diluted samples were mixed with 250 μl of fluorescein (55 nM) and incubated for 10 min at 37 °C before automatic injection of 25 μl AAPH solution (153 nM). Fluorescence was measured every minute for 50 min. Fluorescence filters were used at an excitation wavelength of 485 nm and an emission wavelength of 520 nm. The final ORAC HAC and LAC values were calculated using the net area under the decay curves and were expressed as μmol of Trolox Equivalents (TE)/g of seed.

2.3. Statistical analysis

Quantitative data are presented as mean values with the respective standard deviation values corresponding to three replicates. All analyses were processed by the one-way analysis of variance (ANOVA). A Duncan test was used to determine significant

differences. Differences at $p < 0.05$ were considered as significant. The StatGraphics Plus 5 (Statistical Graphics Corp., Herndon, VA, USA) was used for all statistical tests.

3. Results and discussion

Prior to the evaluation of FA, bioactive compounds and antioxidant capacity, moisture and oil content were determined in the seeds of the 16 different SI cultivars (data not shown). Moisture content varied within the 3.9–4.6% range. Similar values were previously reported by Follegatti-Romero et al. (2009) and Gutiérrez et al. (2011). Oil content for the different SI cultivars were within the range 33.4–37.6%. The seeds were not roasted prior to the oil extraction. Gutiérrez et al. (2011) recovered 42% oil using similar extraction method. Higher values of SI oil were reported for roasted seeds followed by solvent extraction (54% oil) as well as from seeds submitted to supercritical CO_2 extraction (54.3% oil) (Follegatti-Romero et al., 2009; Hamaker et al., 1992). The differences in the oil content previously reported for SI seeds, were not only related to the cultivar but to the growing conditions of the seeds and processing (e.g., roasting prior to extraction) and the method of oil extraction (e.g., solvent *vs* supercritical CO_2).

Compared to other food seeds, the oil content in SI seeds was superior to that reported for different soybean cultivars (16.5–17.5%), chia (26.7–35%) and safflower (27.5%) and it is within the range reported for flax seeds (33.6–44.8%), but lower than the range reported for pistacho (50.4–58%) and macadamia kernel nut varieties (63.0–71.8%) (Arena, Campisi, Fallico, & Maccarone, 2007; Bozan & Tenelli, 2008; Ciftci, Przybylski, & Rudzińska, 2012; Ixtaina et al., 2011; Yoshida, Hirakawa, Murakam, Mizushina, & Yamade, 2003; Wall, 2010).

3.1. Fatty acid composition and quantification

The FA profiles for the different 16 SI cultivars is presented in Table 2. Differences in the contents of the different FA are observed among the cultivars. The presence of palmitic, stearic, oleic, vaccenic, linoleic and α-linolenic FA were evidenced. These FA have been previously reported in SI oil (Follegatti-Romero et al., 2009; Guillén et al., 2003; Gutiérrez et al., 2011; Hamaker et al., 1992; Maurer, Hatta-Sakoda, Pascual-Chagman, & Rodriguez-Saona, 2012) except for vaccenic acid which was found in small quantities (∼0.26/100 g of seed). α-Linolenic (37.3–44.2%) and linoleic (35.2–41%) were the most important FA, with ranges of 12.8–16.0 and, 12.4 and 14.1/100 g of seed, respectively. α-Linolenic FA outstood in all SI seed cultivars but high variability was observed among the FA results. Gutiérrez et al. (2011), with SI seeds from Colombia, found quantities of α-linolenic and linoleic FA of 21.3 and 14.0/100 g seeds, respectively. Differences with our results can be attributed to different subspecies, geographical distribution, climate and growing conditions, harvest time, agricultural practices and quantitative method of the analysis.

SI seeds presented values of polyunsaturated fatty acids (PUFA), monounsaturated (MUFA) and saturated (SFA) fatty acids within the 78.0–81.1%; 10.8–13.2% and, 7.9–9.1% ranges, respectively. These characteristics were closely related to the results reported for SI oil by Follegatti-Romero et al. (2009) (84.4%, 8.4% and 6.9%, respectively), by Gutiérrez et al. (2011) (84.2%, 9.1% and 6.8%, respectively) and by Maurer et al. (2012) (77.5%, 10.7% and 8.1%, respectively). In addition, the amounts of PUFA, MUFA and SFA were within the range reported for other seeds rich in polyunsaturated fatty acids, such as chia (*Salvia hispanica*) (80.4%, 10.9% and 8.6%, respectively) and flaxseed (73.6%, 18.5% and 7.8%, respectively) and both seeds also outstood in the content of α-linolenic FA (58.2% and 59.6%, respectively) (Ciftci et al., 2012).

The ratios ω6/ω3 FA found for the 16 different SI seed cultivars were within the 0.83–1.09 range. This range is close to the values of 0.81 and 1.12 reported by Gutiérrez et al. (2011) and Maurer et al. (2012) for SI oil. These ratios were much lower than the values reported for canola (2.22), olive (7.69), soy (6.66) and walnut (5.0) oil (Belitz & Grosch, 1999). Lower values have been reported for flax (0.27) and chia oil (0.26–0.34) by Ixtaina et al. (2011) and Ciftci et al. (2012), respectively.

In addition, the SI seed is consumed as peanuts (as nuts). The most important FA reported in peanuts are oleic (40–50%), linoleic (25–28%) and palmitic (11–16%) acids. Ratios of ω6/ω3 FA for peanuts have been reported to be within the 402–600 range (Rodrigues et al., 2011). Thus, consumption of SI seeds or its oil would be beneficial in terms of health given its high α-linolenic FA contribution and the low ω6/ω3 FA ratio. There are a few vegetable sources in our diet that provide high contents of α-linolenic FA. Several sources of information suggest that that the human diet should evolve into a ratio ω6/ω3 of about one (Simopoulos, 2011). However, today, Western diets have a ratio of 10:1 to 20–25:1, indicating that Western diets are deficient in ω3 FA. ω6 and ω3 FA are not interconvertible in the human body and are important components of practically all cell membranes (Simopoulos, 2011).

Table 2
Fatty acid composition (g/100 g) of 16 cultivars of Sacha inchi seeds.[a,b]

Cultivar	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1 *n*-9)	Vaccenic (C18:1 *n*-11)	Linoleic (C18:2)	α-Linolenic (C18:3)	Saturated fatty acids	Monounsaturated fatty acids	Polyinsaturated fatty acids	Total fatty acids
PER000394	1.7 ± 0.0^{f}	1.2 ± 0.0^{fg}	3.9 ± 0.0^{h}	0.24 ± 0.02^{bc}	13.4 ± 0.0^{d}	15.0 ± 0.1^{bc}	2.8 ± 0.0^{g}	4.1 ± 0.0^{h}	28.3 ± 0.1^{b}	35.2 ± 0.1^{fg}
PER000395A	1.8 ± 0.0^{cd}	1.3 ± 0.0^{a}	4.7 ± 0.0^{a}	0.25 ± 0.01^{abc}	13.8 ± 0.0^{c}	15.6 ± 0.1^{a}	3.2 ± 0.0^{bc}	5.0 ± 0.0^{a}	29.3 ± 0.1^{a}	37.4 ± 0.1^{a}
PER000401	1.9 ± 0.0^{c}	1.2 ± 0.0^{b}	4.0 ± 0.1^{g}	0.26 ± 0.01^{abc}	13.0 ± 0.0^{f}	16.0 ± 0.6^{bc}	3.1 ± 0.0^{bc}	4.2 ± 0.0^{g}	28.0 ± 0.5^{c}	35.4 ± 0.4^{ef}
PER000403	2.0 ± 0.0^{b}	1.2 ± 0.0^{ef}	4.1 ± 0.0^{fg}	0.28 ± 0.02^{abc}	14.1 ± 0.1^{a}	12.8 ± 0.0^{i}	3.1 ± 0.0^{bc}	4.3 ± 0.0^{fg}	27.0 ± 0.1^{fg}	34.9 ± 0.2^{kl}
PER000405	1.8 ± 0.0^{cd}	1.1 ± 0.0^{fg}	4.0 ± 0.1^{fg}	0.28 ± 0.03^{ab}	13.2 ± 0.0^{e}	14.8 ± 0.1^{cd}	3.0 ± 0.0^{ef}	4.3 ± 0.0^{fg}	27.9 ± 0.1^{c}	35.9 ± 0.1^{fg}
PER000406A	2.0 ± 0.0^{b}	1.2 ± 0.0^{bcd}	4.4 ± 0.0^{bc}	0.28 ± 0.02^{ab}	13.9 ± 0.0^{b}	13.7 ± 0.1^{h}	3.2 ± 0.0^{b}	4.6 ± 0.0^{bc}	27.6 ± 0.1^{d}	35.4 ± 0.1^{ef}
PER000406B	1.9 ± 0.0^{b}	1.2 ± 0.0^{fg}	4.1 ± 0.1^{ef}	0.26 ± 0.01^{abc}	12.8 ± 0.0^{h}	14.4 ± 0.1^{efg}	3.1 ± 0.1^{cd}	4.4 ± 0.1^{ef}	27.2 ± 0.1^{fg}	34.6 ± 0.1^{jk}
PER000408B	1.8 ± 0.0^{e}	1.1 ± 0.0^{h}	3.5 ± 0.1^{j}	0.26 ± 0.01^{abc}	12.9 ± 0.0^{g}	15.7 ± 0.1^{a}	2.8 ± 0.1^{g}	3.8 ± 0.1^{j}	28.4 ± 0.1^{b}	35.0 ± 0.0^{hi}
PER000416	1.8 ± 0.0^{de}	1.2 ± 0.0^{def}	4.2 ± 0.0^{d}	0.26 ± 0.02^{abc}	12.6 ± 0.1^{i}	14.6 ± 0.1^{de}	3.0 ± 0.1^{ef}	4.4 ± 0.1^{d}	27.3 ± 0.1^{ef}	34.8 ± 0.1^{ij}
PER000417	1.8 ± 0.0^{cd}	1.2 ± 0.0^{bcde}	4.3 ± 0.0^{c}	0.24 ± 0.02^{c}	13.2 ± 0.0^{e}	14.2 ± 0.0^{fg}	3.1 ± 0.0^{de}	4.6 ± 0.0^{c}	27.5 ± 0.1^{de}	35.1 ± 0.0^{gh}
PER000418	1.8 ± 0.0^{cde}	1.2 ± 0.0^{cde}	4.2 ± 0.0^{de}	0.25 ± 0.02^{abc}	13.7 ± 0.0^{c}	14.6 ± 0.0^{de}	3.0 ± 0.0^{ef}	4.4 ± 0.0^{de}	28.3 ± 0.1^{b}	35.8 ± 0.1^{d}
PER000420	1.9 ± 0.0^{c}	1.1 ± 0.0^{g}	4.2 ± 0.0^{de}	0.26 ± 0.02^{abc}	12.4 ± 0.0^{j}	14.4 ± 0.1^{def}	3.0 ± 0.0^{f}	4.4 ± 0.0^{de}	26.9 ± 0.0^{gh}	34.3 ± 0.0^{l}
PER000421	2.1 ± 0.0^{a}	1.2 ± 0.0^{bcde}	4.4 ± 0.0^{b}	0.29 ± 0.0^{a}	14.0 ± 0.0^{a}	15.0 ± 0.1^{bc}	3.2 ± 0.1^{a}	4.7 ± 0.1^{b}	29.1 ± 0.1^{a}	37.1 ± 0.0^{b}
PER000422	1.6 ± 0.0^{g}	1.1 ± 0.0^{h}	3.8 ± 0.0^{i}	0.23 ± 0.02^{c}	12.6 ± 0.0^{i}	14.2 ± 0.0^{g}	2.6 ± 0.0^{h}	4.0 ± 0.0^{i}	26.8 ± 0.1^{h}	33.4 ± 0.0^{m}
PER017597	1.8 ± 0.0^{c}	1.2 ± 0.0^{bc}	4.2 ± 0.0^{de}	0.28 ± 0.02^{ab}	14.1 ± 0.0^{a}	15.2 ± 0.0^{b}	3.1 ± 0.0^{bc}	4.4 ± 0.0^{de}	29.2 ± 0.1^{a}	36.8 ± 0.1^{c}
PER017598	1.9 ± 0.0^{c}	1.3 ± 0.0^{a}	3.8 ± 0.1^{i}	0.26 ± 0.03^{abc}	13.4 ± 0.0^{d}	15.0 ± 0.1^{b}	3.2 ± 0.0^{bc}	4.0 ± 0.0^{i}	28.4 ± 0.1^{b}	35.6 ± 0.1^{de}

[a] Values are means ± standard deviation ($n = 3$).
[b] Values in the same column with the same superscript letter are not significantly different ($p > 0.05$).

Table 3
Tocopherol composition (mg/100 g) of 16 cultivars of Sacha inchi seeds.[a,b]

Cultivar	α-Tocopherol	β-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total tocopherol
PER000394	1.17 ± 0.02^{defgh}	0.81 ± 0.06^{efg}	65.1 ± 1.6^{cde}	34.2 ± 0.8^{f}	100 ± 2.3^{de}
PER000395A	1.17 ± 0.00^{defgh}	0.77 ± 0.02^{efg}	65.4 ± 1.3^{cde}	38.2 ± 0.7^{e}	105 ± 2.0^{cd}
PER000401	1.17 ± 0.03^{cdefgh}	0.77 ± 0.02^{fg}	60.6 ± 3.2^{ef}	39.0 ± 1.9^{de}	100 ± 5.1^{de}
PER000403	1.19 ± 0.03^{bcdefgh}	0.91 ± 0.04^{bc}	66.6 ± 3.2^{cd}	41.0 ± 2.1^{cde}	109 ± 5.3^{cd}
PER000405	1.26 ± 0.03^{ab}	0.82 ± 0.02^{de}	68.5 ± 1.8^{c}	32.7 ± 0.8^{fg}	102 ± 2.6^{de}
PER000406A	1.27 ± 0.02^{a}	0.77 ± 0.02^{efg}	64.3 ± 3.1^{cde}	29.2 ± 1.4^{h}	94.3 ± 4.5^{ef}
PER000406B	1.20 ± 0.03^{abcdef}	0.75 ± 0.03^{g}	60.8 ± 3.2^{def}	38.1 ± 1.8^{e}	99.8 ± 5.1^{de}
PER000408B	1.19 ± 0.02^{bcdefg}	0.67 ± 0.02^{h}	56.8 ± 1.8^{f}	30.2 ± 1.1^{gh}	87.8 ± 3.0^{f}
PER000416	1.25 ± 0.07^{abc}	0.78 ± 0.01^{efg}	60.5 ± 1.7^{ef}	33.4 ± 1.0^{fg}	94.8 ± 2.8^{ef}
PER000417	1.13 ± 0.02^{gh}	0.79 ± 0.02^{efg}	49.0 ± 4.8^{g}	28.7 ± 2.7^{h}	78.6 ± 7.5^{g}
PER000418	1.16 ± 0.02^{efgh}	0.79 ± 0.01^{efg}	57.8 ± 2.1^{f}	29.2 ± 1.9^{h}	87.8 ± 4.1^{f}
PER000420	1.20 ± 0.05^{abcdef}	0.92 ± 0.03^{ab}	81.4 ± 2.1^{a}	54.6 ± 1.3^{a}	137 ± 3.5^{a}
PER000421	1.22 ± 0.06^{abcde}	0.87 ± 0.02^{cd}	69.6 ± 1.2^{bc}	47.6 ± 2.2^{b}	118 ± 3.5^{b}
PER000422	1.14 ± 0.01^{fgh}	0.86 ± 0.05^{cd}	61.0 ± 4.0^{def}	44.3 ± 2.4^{c}	106 ± 6.5^{cd}
PER017597	1.24 ± 0.06^{abcd}	0.82 ± 0.03^{def}	74.8 ± 7.2^{b}	37.8 ± 4.2^{e}	114 ± 11.4^{bc}
PER017598	1.13 ± 0.01^{h}	0.95 ± 0.01^{a}	70.1 ± 0.9^{bc}	42.2 ± 0.4^{cd}	113 ± 1.4^{bc}

[a] Values are means ± standard deviation ($n = 3$).
[b] Values in the same column with the same superscript letter are not significantly different ($p > 0.05$).

3.2. Tocopherol composition and quantification

Tocopherols α, β, γ and δ were detected in all the SI seed cultivars in different amounts (Table 3, Fig. 1). The total tocopherol content fluctuated within the range 78.6–137.0 mg/100 g seed, respectively. The presence of α-, γ- and δ-tocopherol has been previously reported in SI oil by Fanali et al. (2011). Follegatti-Romero et al. (2009), however, only reported γ- and δ-tocopherol. γ- and δ-tocopherol were the most important isomers in all cultivars representing 57.4–68.2% (56.8–81.4 mg/100 g of seed) and 30.9–40.3% (29.2–47.6 mg/100 g of seed) of the total tocopherol content, respectively. α- and β-tocoperols were within the range 1.13–1.25 and, 0.75–0.95 mg/100 g seed, respectively, representing less than 3% of total tocopherols. Follegatti-Romero et al. (2009) reported γ- and δ-tocopherol contents of 1.14 and 1.25 g per kg of SI oil extracted by supercritical CO_2. These values reported per seed weight represent 61.9 and 67.8 mg/100 g seed, respectively. δ-tocopherol contents were higher than in the 16 SI seed cultivars analysed. The encountered differences might be related to different factors, such as oil extraction technology, growing conditions of the crop, genetic factors, etc. The 16 SI seed cultivars presented total tocopherol contents higher than those reported for flaxseed (9.3 and 14.3 mg/100 g seed, Oomah, Kenaschuk, & Mazza, 1997 and Bozan & Tenelli, 2008, respectively), also higher contents of α and δ tocopherol in SI seeds were found. Lower total tocopherol, γ and δ tocopherol contents have also been reported for the Brazil nut (15.2, 7.4 and 0.59 mg/100 g, respectively; da Costa et al., 2010). In comparison to other highly consumed nuts (cashews, hazelnuts, peanuts, pecans and pistachios), SI seeds displayed higher contents of total tocopherols, beta, delta and gamma tocopherols (Kornsteiner, Wagner, & Elmadfa, 2006).

Tocopherols are recognised as potent lipophilic antioxidant compounds. The antioxidant activity of tocopherols in lipids follows this order: γ > δ > α > β (Schmidt & Pokorný, 2005). The significant quantities of γ- and δ-tocopherols found in SI seeds constitute an antioxidant protection factor for the seed and oil rich in PUFAs, if proper extraction techniques are used as to maximise tocopherol recovery. The presence of important quantities of PUFA, in particular α-linolenic FA in SI seeds could be associated to an important tocopherol synthesis (mainly the γ- and δ isomers) as a defense mechanism against oxidative processes. For flaxseed oil rich in α-linolenic FA, Bozan and Tenelli (2008) reported that the high degree of insaturation of the oil was responsible for the higher tocopherol content (especially γ-tocopherols) compared to safflower seed oil which presented a lower tocopherol content and similar PUFA content (mainly linoleic FA). High amounts of

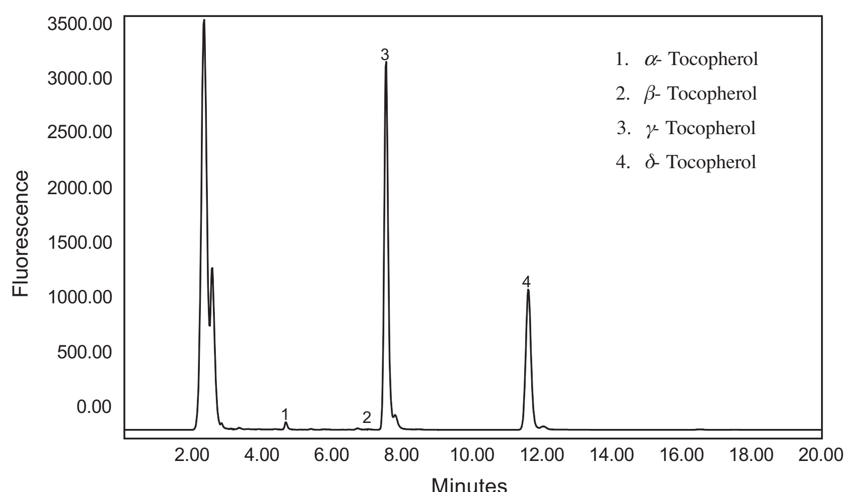

Fig. 1. Tocopherol profile for the Sacha inchi cultivar SI PER000420.

Table 4
Phytosterol composition (mg/100 g) of 16 cultivars of Sacha inchi seeds.[a,b]

Cultivar	Campesterol	Stigmasterol	β-sitosterol
PER000394	7.4 ± 0.3[cde]	21.9 ± 0.5[ef]	46.6 ± 0.4[def]
PER000395A	8.5 ± 0.5[ab]	25.4 ± 1.1[abc]	49.2 ± 1.2[bcde]
PER000401	7.1 ± 0.4[e]	23.4 ± 0.4[bcdef]	45.4 ± 1.0[f]
PER000403	7.8 ± 0.3[abcde]	25.5 ± 3.6[ab]	49.8 ± 1.7[bcd]
PER000405	8.3 ± 0.7[abc]	25.2 ± 0.5[abc]	50.1 ± 2.1[abc]
PER000406A	8.8 ± 0.7 [a]	26.9 ± 0.7[a]	45.9 ± 1.6[ef]
PER000406B	8.0 ± 1.2[abcde]	21.2 ± 1.3[f]	48.2 ± 1.9[cdef]
PER000408B	8.2 ± 0.5[abcd]	24.8 ± 1.1[abcd]	53.2 ± 2.0[a]
PER000416	7.6 ± 0.2[bcde]	24.9 ± 0.4[abcd]	48.0 ± 0.2[cdef]
PER000417	7.4 ± 0.4[cde]	23.5 ± 0.4[bcdef]	46.7 ± 0.7[def]
PER000418	7.2 ± 0.6[de]	22.9 ± 0.8[cdef]	49.4 ± 0.5[bcd]
PER000420	7.6 ± 0.4[bcde]	23.7 ± 0.7[bcdef]	48.9 ± 0.7[bcde]
PER000421	7.7 ± 0.5[bcde]	24.2 ± 1.1[bcde]	50.8 ± 2.0[abc]
PER000422	7.9 ± 0.3[abcde]	25.8 ± 2.2[ab]	50.7 ± 3.9[abc]
PER017597	7.4 ± 0.5[cde]	25.5 ± 1.4[abc]	45.2 ± 3.1[f]
PER017598	8.4 ± 0.4[abc]	22.5 ± 0.6[def]	52.2 ± 0.3[ab]

[a] Values are means ± standard deviation ($n = 3$).
[b] Values in the same column with the same superscript letter are not significantly different ($p > 0.05$).

tocopherols are usually associated to PUFA content (Shahidi, 2004). No correlations were found between the total tocopherols, γ-, δ-, α- and β-tocopherol and the PUFA and MUFA contents for the different SI cultivars as previously reported by Bozan and Tenelli (2008) and Ciftci et al. (2012).

3.3. Phytosterol composition

Significant differences ($p < 0.05$) were observed in the contents of campesterol, stigmasterol and β-sitosterol for the 16 evaluated SI seeds (Table 4). The sum of these three phytosterols was within the 75.7–86.2 mg/100 g seed range. Campesterol, stigmasterol and β-sitosterol were within 7.1–8.8, 21.2–26.9 and 45.2–50.8 mg/100 g seed ranges, respectively, outstanding the content of β-sitosterol. These results are in agreement to those reported by Bondioli et al. (2006) for SI oil where β-sitosterol was the most important phytosterol followed by stigmasterol and campesterol, representing 92% of total phytosterols. These same phytosterols have been reported as the most important in flax and chia seed lipids (Ciftci et al., 2012). The most abundant sterols in plants are 4-demethylsterols (sitosterol, campesterol, and stigmasterol), Δ^5-avenasterol, and Δ^7-avenasterol. Sitosterol is the most predominant (90%) phytosterol (Lagarda et al., 2006).

In general nuts, seeds and cereals are important sources of phytosterols. Average values of phytosterols of 95 and 270 mg/100 g of product have been reported by Phillips, Ruggio, and Ashraf-Khorassani (2005) for whole and ground flaxseed, almond, Brazil nut, cashew, hazelnut, macadamia nut, pecan, pistachio and black walnut. Lower values were found for SI seeds in this study. However, for different cultivars of barley, oat, rye and wheat from Finland average values of total phytosterols of ∼39.4, 39.4, 83 and 60.2 mg/100 g of product, respectively, have been reported by Piironen, Toivo, and Lampi (2002). These values are lower than those found in this study for SI seeds. Our results indicate that SI seeds could be considered as an important source of phytosterols. Consumption of SI seeds could contribute to increase the ingested quantities of this class of compounds.

3.4. Carotenoid and phenolic compound contents

Total carotenoids for the different SI cultivars fluctuated within the 0.07–0.09 mg βCE/100 g of seed range (data not shown). These compounds were present in low quantities, compared to the other evaluated bioactive compounds in this work. Carotenoids have been reported in SI oil in quantities of 0.08 mg /100 g (Hamaker et al., 1992). Fujisawa et al. (2008) reported in flaxseed enriched in carotenoids through metabolic engineering, contents within the range 6.54–15.6 mg/100 g, which corresponded to 7.8- to 18.6-fold increase, compared with those of the untransformed controls. These reported values are much higher than those found in SI seeds. No carotenoids were detected by Kornsteiner et al. (2006) in almonds, Brazil nuts, cashews, hazelnuts, macadamias, peanuts, pecans and walnuts. However, β-carotene and lutein were present in pistachos with values of 0.20 and 2.25 mg/100 g, respectively.

Total phenolic compounds for the 16 different SI cultivars are displayed in Table 5. The range value of TPC for the sixteen SI cultivars was within the 64.6–80.0 mg GAE/100 g seed range. Significant differences ($p < 0.05$) in TPC content were observed for the different evaluated cultivars. Lower values of TPC were reported for almonds, macadamias and pine nuts (32–47 mg GAE/100 g) compared to SI seeds but higher values were reported for Brazilian nuts, cashews, hazelnuts, peanuts, pecans, pistachos and walnuts (from 112 to 1625 mg GAE/100 g) by Kornsteiner et al. (2006) and John and Shahidi (2010) as well as for flax and safflower seeds (383 and 559 mg GAE/100 g; Bozan & Tenelli, 2008). Fanali et al. (2011) identified the phenolic compounds from SI oil obtained by direct pressure of the seeds. HPLC-PDA and ESI-MS analysis

Table 5
Total phenolic compounds and hydrophilic, lipophilic and total ORAC antioxidant capacity of 16 cultivars of Sacha inchi seeds.[a,b]

Cultivar	Total phenolic compounds (mg GAE/100 g)	Antioxidant capacity (μmol TE/g)		
		Hydrophilic	Lipophilic	Total
PER000394	66.7 ± 0.7[gh]	5.8 ± 0.4[cd]	1.6 ± 0.1[i]	7.4 ± 0.5[ghi]
PER000395A	68.4 ± 0.2[fg]	5.7 ± 0.5[cd]	1.8 ± 0.0[fg]	7.4 ± 0.4[fgh]
PER000401	73.8 ± 0.6[bcd]	5.8 ± 0.3[cd]	1.8 ± 0.1[f]	7.6 ± 0.2[fgh]
PER000403	72.2 ± 1.6[cd]	6.1 ± 0.5[bc]	1.6 ± 0.1[hi]	7.7 ± 0.6[efg]
PER000405	66.9 ± 0.2[gh]	5.5 ± 0.0[d]	1.0 ± 0.0[j]	6.5 ± 0.0[j]
PER000406A	65.9 ± 0.3[gh]	5.6 ± 0.2[d]	1.6 ± 0.0[hi]	7.2 ± 0.2[hi]
PER000406B	64.6 ± 0.5[h]	4.9 ± 0.4[e]	1.7 ± 0.0[gh]	6.6 ± 0.4[j]
PER000408B	66.9 ± 0.5[fgh]	6.4 ± 0.0[b]	2.8 ± 0.0[a]	9.2 ± 0.0[b]
PER000416	67.0 ± 0.0[fgh]	6.3 ± 0.1[b]	2.2 ± 0.1[d]	8.5 ± 0.1[cd]
PER000417	69.6 ± 0.1[ef]	7.3 ± 0.3[a]	1.7 ± 0.2[fg]	9.0 ± 0.1[b]
PER000418	67.5 ± 1.0[fg]	6.4 ± 0.1[b]	2.5 ± 0.0[b]	8.9 ± 0.1[bc]
PER000420	68.1 ± 0.2[fg]	6.9 ± 0.1[a]	2.4 ± 0.0[c]	9.3 ± 0.1[b]
PER000421	75.2 ± 2.3[b]	5.8 ± 0.1[cd]	2.1 ± 0.0[d]	7.9 ± 0.1[ef]
PER000422	80.0 ± 1.8[a]	7.2 ± 0.1[a]	2.6 ± 0.0[b]	9.8 ± 0.1[a]
PER017597	74.6 ± 4.4[bc]	6.1 ± 0.0[bc]	2.0 ± 0.1[e]	8.1 ± 0.1[de]
PER017598	71.3 ± 0.7[de]	4.3 ± 0.2[f]	2.6 ± 0.0[b]	6.9 ± 0.3[ij]

[a] Values are means ± standard deviation ($n = 3$).
[b] Values in the same column with the same superscript letter are not significantly different ($p > 0.05$).

revealed phenyl alcohol, flavonoid, secoridoid, and lignan type of phenolic compounds. Our work was limited to TPC quantification.

3.5. Hydrophilic, lipophilic and total ORAC antioxidant capacity

Significant differences in the hydrophilic, lipophilic and total antioxidant capacity were observed among the 16 SI cultivars (Table 5). The hydrophilic (HAC), lipophilic (LAC) and total antioxidant capacity (TAC) were within the ranges of 4.3–7.3, 1.0–2.8 and 6.5–9.8 μmol TE/g of seed, respectively. No studies in the literature have reported the ORAC antioxidant capacity in SI seeds and oil. Wu et al. (2004) reported ORAC TAC values of 14.1 for Brazilian nut, 179.4 for pecans and 7.19 μmol TE/g for pine nuts; this last value was comparable to SI seed. LAC represented ~15–38% of TAC for SI seeds. A similar value of 14.9–39.3% was reported by Wu et al. (2004) for Brazilian nuts, cashews, macadamias and pine nuts; but lower (~2.4–8.6%) for almonds, hazelnuts, peanuts, pecans, pistachios and walnuts.

Finally, low correlations between LAC and α-, β-, γ-, δ- and total tocopherols; between LAC and total carotenoids and between HAC and total phenolic compounds were found. It might be possible that the different values of antioxidant capacity are due to individual, additive, synergistic and antagonist effects of the pool of antioxidants (either of hydrophilic and lipophilic nature) present in the samples.

4. Conclusions

Differences in the composition of fatty acid profiles, different bioactive compounds and antioxidant capacities were observed for 16 different SI seeds. Important contents of α-linolenic FA and low ω6/ω3 ratio were observed for the 16 SI seeds evaluated. In addition, considerable amounts of tocopherols (γ- and δ-tocopherols), phytosterols (β-sitosterol and stigmasterol) and total phenolic compounds were observed in all SI seed cultivars. No correlations were found between the different hydrophilic and lipophilic bioactive compounds and the antioxidant capacity, suggesting a complex interaction of the different compounds in the mode of action of the different antioxidants present in the samples.

SI seed sparks attention as an important and alternative source of omega-3 and other bioactive compounds. To our knowledge, this is the first study focused on the valorisation of 16 different cultivars of SI seeds and based on their phytochemical composition besides its use as oil source. Thus, current studies of our group are focused on the effect of 'roasting' on the phytochemical composition.

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