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Sacha inchi as potential source of essential fatty acids and tocopherols: multivariate study of nut and shell

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ABSTRACT. The present study investigated the fatty acid composition, tocopherols and nutritional factors in the nut and shell of Sacha inchi (*Plukenetia volubilis*) through multivariate data analysis. The nut showed a high lipid content (48.5%), while the shell showed a low content (1.2%), although both parts of the plant had similar fatty acid composition. Low contents of saturated fatty acids were found in both parts, indicating anti-atherogenic, anti-thrombogenic and hypercholesterolemic effects. The content of n-3 fatty acids (438.7 mg g^{-1} of total lipids) found in the nut corroborates with the literature, while the content found in shell (329.4 mg g^{-1}) is not previously described. The total tocopherol content was higher than other oilseeds. The great amount of α-tocopherol present in the shell is highlighted since this is considered primarily responsible for the metabolic activity of vitamin E. Dietary Reference Intakes proved that both parts of Sacha inchi have a good nutritional supply. The use of multivariate analysis allowed nuts and shells to be distinguished and their constituents to be checked. The incorporation of Sacha inchi in the human diet is promising due to its intrinsic characteristics, as well as the use of the shell in food processing.

Keywords: *Plukenetia volubilis* L., alfa-linolenic, linoleic, vitamin E, antioxidants, chemometric.

Sacha inchi como fonte potencial de ácidos graxos essenciais e tocoferóis: estudo multivariado da castanha e casca

RESUMO. O presente estudo investigou a composição em ácidos graxos, tocoferóis e seus fatores nutricionais na castanha e casca de Sacha inchi (*Plukenetia volubilis*) com o uso da análise multivariada dos dados. A castanha apresentou um elevado teor de lipídios (48.5%), quando comparado com a casca (1.2%). Com relação à composição em ácidos graxos, ambas as partes foram semelhantes, sendo verificados baixos percentuais de ácidos graxos saturados e possivelmente efeitos antiaterogênico, antitrombogênico e hipercolesterolêmico. O conteúdo de n-3 (438,7 mg g^{-1} lipídios totais) encontrado na castanha foi semelhante ao relatado na literatura, enquanto o teor analisado na casca (329,4 mg g^{-1}), não foi mencionado em outros trabalhos. O conteúdo de tocoferóis foi elevado quando comparado com outras oleaginosas. Destaca-se o conteúdo de α-tocopherol presente na casca, que é considerado a principal responsável pela atividade metabólica da vitamina E. Através da DRI foi verificado um bom aporte nutricional nas partes da Sacha inchi. O uso da análise multivariada permitiu distinguir os lotes da castanha e casca, bem como verificar os seus constituintes. A incorporação da Sacha inchi na dieta apresenta-se promissora devido as suas características intrínsecas, sendo ressaltado o aproveitamento da casca e a sua aplicação no processamento de alimentos.

Palavras-chave: *Plukenetia volubilis* L., alfa-linolênico, linoleico, vitamina E, antioxidante, quimiometria.

Introduction

Sacha inchi (*Plukenetia volubilis* L.), commonly called 'Inca Inchi', 'Inca Peanut', 'Mountain Peanut', 'Sacha Peanut', 'Supua', 'Ticazo', 'Sacha Maní', 'Maní del Inca', 'Maní Del Monte' or 'Maní Jibaro', is a plant which belongs to the Euphorbiaceae family and is found in the Amazon forest of Peru at altitudes of 200-1500 meters (HAMAKER et al., 1992). The nut can be considered an excellent source of proteins and lipids, with levels of 27-30 and 40-60%, respectively (CAI, 2011).

The lipid fraction of the Sacha inchi nut is primarily composed of unsaturated fatty acids, which comprise about 90% of the total lipids (ZULETA et al., 2012). According to the Institute of Medicine (2002/2005), the consumption of saturated fatty acids should be avoided in a balanced diet. Follegatti-Romero et al. (2009), Fanali et al. (2011)

and Gutiérrez et al. (2011) reported contents of 33.4-36.2% of linoleic fatty acids (18:2n-6) and 46.8-50.8% of alfa-linolenic acid (18:3n-3). According to Ratnayake and Galli (2009), these fatty acids are considered essential because they cannot be metabolized in the human body and must be consumed through diet.

Fanali et al. (2011) highlight the presence of antioxidant compounds such as flavonoids and tocopherols in nut; the latter may reduce the risk of heart disease, type 2 diabetes and cancer (KÖKSAL et al., 2006; YANG, 2009). Tocopherols and tocotrienols are fat-soluble compounds and fractions of vitamin E, which are identified by the prefixes α, β, γ and δ. They are compounds with different activities of vitamin E and the isomer α-tocopherol is the most biologically active. These compounds are found only in plants (YADA et al., 2011).

Sacha inchi oil is obtained after the process of cold pressing and peeling nuts. The co-products generated, especially the shell, can be exploited in food processing and/or as cementitious materials due to their physico-chemical composition, as reported by Ferreira et al. (2006) for Brazil nuts and Lima and Rossinolo (2010) for the co-products of cashew nuts, which are oilseeds with characteristics similar to the Sacha inchi.

Multivariate analysis enables the extraction of additional information when compared to univariate analysis. This chemometric tool allows pattern recognition, gathering of information, reduction of data dimensionality and organization of data in a simpler structure, which is easier to understand. The principal component analysis (PCA) is based on performing linear comparisons of the original variables. The principal components (PC) are mutually orthogonal and the explained variance decreases with an increase in PC number (CORREIA; FERREIRA, 2007).

There are few available studies about the Sacha inchi nut and the potential use of its shells, which are obtained as co-products from the extraction of crude oil. Therefore, the present study investigated the fatty acid composition, tocopherols and their nutritional factors in nuts and the shell of Sacha inchi.

Material and methods

Sampling

Sacha inchi grains (*Plukenetia volubilis*) were purchased from the local market in Lima, Peru. This plant is obtained from the plant extractivism performed in the Peruvian Amazon. Sampling consisted of 3 batches of 5 kg, collected at intervals of 30 days.

The Sacha inchi nut was separated from the shell using a manual breaker, then it was ground using a food processor (Philips - Walita) to form a homogeneous paste; the shell was ground in a hammer mill to obtain a flour which was sieved using a 14 mesh sieve. The two fractions (nut paste and shell flour) were vacuum-packed and protected from the light and were stored frozen until analysis.

Lipid extraction

The total lipids were extracted with methanol, chloroform and water (2:2:1) according to Bligh and Dyer (1959), using 3.50 g of sample, and adding 12.0 mL of water to correct the moisture.

Fatty acid composition

To determine the fatty acid composition, the lipids were converted into fatty acid methyl esters (FAME) and were methylated according to Hartman and Lago (1973). The FAME were separated using a gas chromatograph CP-3380 (Varian, USA) fitted with a flame ionization detector and a CP 7420-select Fame fused-silica capillary column (100 m x 0.25 mm x 0.25 μm cyanopropyl). The gas flows were carrier gas hydrogen 1.4 mL $min.^{-1}$, make-up gas nitrogen 30 mL $min.^{-1}$, synthetic air 300 mL $min.^{-1}$ and flame gas hydrogen 30 mL $min.^{-1}$; the sample was injected in a split ratio of 1:100. The injector and detector temperatures were 235°C. The column temperature was maintained at 165°C for 4 min., increased by 4°C $min.^{-1}$ to 185°C and maintained for 5 min., and then raised from 185°C by 10°C $min.^{-1}$ to 225ºC and maintained for 10 min.

The retention times were compared to those of standard methyl esters (Sigma, USA). The fatty acids were quantified using tricosanoic acid methyl ester (Sigma, USA) as an internal standard, following Joseph and Ackman (1992). The peak areas were determined with software Star 5.0 (Varian, USA) and the concentrations were expressed in mg g^{-1} of total lipid.

Tocopherols determination

Samples were saponified and the isomers of vitamin E were extracted according to the methodology described by Delgado-Zamarreño et al. (2004), only changing the extraction time to four hours. Under stirring and protected from light, 50.0 mL of ethanol, 5.0 mL of aqueous solution of ascorbic acid 10% (w v^{-1}), 10 mL of aqueous solution of potassium hydroxide 80% (w v^{-1}) and 25 mL of water were added to 2.00 g of ground sample. The unsaponifiable material extraction was performed

with hexane and water. The hexane phase, which contained the tocopherol fraction, was collected and evaporated using evaporator en route under vacuum at 50°C and the residue was dissolved in methanol.

Vitamin E was determined using High-Efficiency Liquid Chromatography (Varian), with a C18 column (microsorb, 250 × 4.6 mm, with 5 μm particles) fitted with a scanning UV/Vis detector. The mobile phase used was methanol/dichloromethane in the ratio 85:15 (v v^{-1}), and the flow rate was 0.8 mL min^{-1} (KORNSTEINER et al., 2006). The tocopherols were quantified using the external standard method of δ-tocopherol, (β+γ)-tocopherol and α-tocopherol (Sigma, USA), according to Instituto Adolfo Lutz (IAL, 2005). This involved the sum of the β-tocopherol and γ-tocopherol isomers, since the separation of these is not possible by this methodology (KORNSTEINER et al., 2006).

Vitamin E activity

The activity of vitamin E in the samples was represented according to Kornsteiner et al. (2006); the value found for each isomer, in milligrams, was multiplied by the equivalent factor for α-tocopherol (α-TE). For α-tocopherol, α-TE = mg x 1.0; for (β + γ)-tocopherol, α-TE = mg x 0.25; and for δ-tocopherol, α-TE = mg x 0.01.

Calculation of the dietary reference intake

The Dietary Reference Intake (DRI) is a percentage estimate of the daily nutrient requirements per age and gender, established by the Institute of Medicine (2001, 2011) for individuals aged over 12 months. The DRI of vitamin E, n-3 and n-6 were determined as the mean amounts in 10-g portions, as proposed by Brasil (2003) for the Brazil nut.

Indices of the nutritional quality of lipids

A better approach to the nutritional evaluation of fat is the utilization of indices based on the functional effects of fatty acid composition. These indices were available for atherogenicity (IA) = [(12:0 + (4 x 14:0) + 16:0)] / (MUFA + n-6 + n-3), and thrombogenicity (IT) = (14:0 + 16:0 + 18:0) / [(0.5 x MUFA) + (0.5 x n-6) + (3 x n-3) + (n-3:n-6)], by Ulbricht and Southgate (1991); and the hypocholesterolemic/hypercholesterolemic fatty acid ratio (HH) = (18:1n-9 + 18:2n-6 + 20:4n-6 + 18:3n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3) / (14:0 +16:0), according to Santos-Silva et al. (2002).

Statistical analysis

Lipid extraction, fatty acid composition, tocopherols determination, vitamin E activity and indices of the nutritional quality of lipids were carried out in triplicate on the three different batches. The results were compared using the Student's t-test with a 5% ($p < 0.05$) significance level for rejection of the null hypothesis. The Principal Component Analysis (PCA) tool was used for multivariate analysis. The individual average of the three batches was used for the PCA of the main fatty acids (18:1n-9, 18:2n-6, 18:3n-3, n-6:n3 and PUFA:SFA) and another for the tocopherols. Means were auto-scaled for all of the variables to present the same weight and two-dimensional graphs of PCA. All statistical analyses were conducted using Statistica, version 7.0 (Statsoft, USA).

Results and discussion

Table 1 shows the total lipid content for nuts and shells of Sacha inchi, which presented a significant difference ($p < 0.05$). Studies performed by Follegatti-Romero et al. (2009) and Gutiérrez et al. (2011) showed levels of 54 and 42% of oil in Sacha inchi nut, through extraction by supercritical carbon dioxide and chloroform:methanol (v v^{-1}, 1:1), respectively. There are no reports in the literature about the composition of the shell of Sacha inchi.

Table 1. Total lipids and indices of the nutritional quality of the lipid fraction in Sacha inchi peanut and shell.

Fraction	Total lipids	Indices		
		IA[1]	IT[2]	HH[3]
Nut	48.52[a] ± 1.05	0.05[b] ± 0.01	0.05[b] ± 0.01	20.48[a] ± 0.12
Shell	1.24[b] ± 0.17	0.12[a] ± 0.01	0.12[a] ± 0.01	7.94[b] ± 0.54

Means followed by the same letters in columns do not differ by the Student's t-test ($p < 0.05$). [1]IA: Index of atherogenicity. [2]IT: Index of thrombogenicity. [3]HH: Hypocholesterolemic/hypercholesterolemic fatty acid ratio. n = 9 replicates.

The fatty acid compositions of the parts of Sacha inchi were similar, as shown in Table 2. The levels of saturated fatty acids were 7.7 and 15.8%, respectively, in the lipid fraction of the nut and the shell. Maurer et al. (2012) showed that the following vegetable oils exhibit increasing levels of saturated fatty acids: canola < sunflower < flaxseed < corn < olive < cotton, ranging from 8.5 to 25.2%; these values are higher than those presented by Sacha inchi oil analyses.

Stroher et al. (2012) reported that some manufacturers have increased the amount of saturated fatty acids in order to reduce 'trans' fatty acids, but according to the Institute of Medicine (2002/2005), the intake of saturated fatty acids should be avoided in a balanced diet. Therefore, Sacha inchi could be a good choice for food due to its low saturated fatty acids content.

The classes of fatty acids and their relationship to the proper functioning of the body may be

attested by the use of nutritional indices (SANTOS-SILVA et al., 2002; ULBRICHT; SOUTHGATE, 1991) and their ratios (HARWOOD et al., 2007; SIMOPOULOS, 2011). Table 1 shows the atherogenicity and thrombogenicity indexes which are associated with the presence of lauric (12:0), myristic (14:0), palmitic (16:0) and stearic (18:0) fatty acids, and the increased incidence of coronary diseases when compared to monounsaturated fatty acids, especially oleic (18:1 n-9) and the series omega 3 and 6. Ulbricht and Southgate (1991) found values of IA and IT for sunflower oil that were similar to this study and emphasized the direct relationship between the lowest ratio and an attenuated risk of coronary disease.

The major ratios HH (Table 1) and PUFA: SFA (Table 2) are important due to the hypocholesterolemic effect and the prevalence of polyunsaturated fatty acids that are involved with the lowest risk of cardiovascular disease (RATNAYAKE; GALLI, 2009). According to Simopoulos (2011), the excessive consumption of lipids, 'trans' fatty acids and an unbalanced n-6:n-3 ratio are related to a higher frequency of myocardial infarction cases, hypercholesterolemia, increased low density lipoprotein (LDL) cholesterol and blood pressure, atheroma, lipid disorders and other disorders. The n-6:n-3 ratio of the Sacha inchi is near the ideal value of 1:1 (SIMOPOULOS, 2011).

Table 2. Fatty acid absolute quantification of Sacha inchi peanut and shell.

Fatty acid (mg g^{-1} TL)	Nut	Shell
16:0	$42.11^{b} \pm 0.76$	$96.75^{a} \pm 3.68$
18:0	$29.86^{b} \pm 0.55$	$47.40^{a} \pm 0.62$
18:1n-9	$85.24^{b} \pm 1.72$	$112.95^{a} \pm 2.03$
18:2n-6	$338.51^{a} \pm 7.42$	$324.54^{a} \pm 9.75$
18:3n-3	$438.77^{a} \pm 9.84$	$329.48^{b} \pm 15.79$
Sums and ratios of fatty acid		
SFA	$71.98^{b} \pm 1.06$	$144.15^{a} \pm 4.07$
MUFA	$85.24^{b} \pm 1.72$	$112.95^{a} \pm 2.03$
PUFA	$777.28^{a} \pm 12.32$	$654.02^{b} \pm 18.56$
n-6	$338.51^{a} \pm 7.42$	$324.54^{a} \pm 9.75$
n-3	$438.77^{a} \pm 9.84$	$329.48^{b} \pm 15.79$
PUFA:SFA	$10.80^{a} \pm 0.13$	$4.54^{b} \pm 0.04$
n-6:n-3	$0.77^{b} \pm 0.03$	$0.99^{a} \pm 0.06$

Means followed by the same letters in rows do not differ by the Student's t-test ($P < 0.05$). TL: total lipids, SFA: total saturated fatty acids, MUFA: total monounsaturated fatty acids, PUFA: total polyunsaturated fatty acids, n-6: total omega-6 fatty acids and n-3: total omega-3 fatty acids. n = 9 replicates.

Figure 1 shows the chromatograms of the tocopherols analysis. The contents of tocopherols isomers found for the Sacha inchi nut and shell are presented in Table 3. The isomer α-tocopherol was not detected in Sacha inchi oil by Follegatti-Romero et al. (2009), but Fanali et al. (2011) found this isomer; they also found high levels of γ-tocopherol. Costa et al. (2010) showed higher activity of vitamin E for Brazil nuts. However, studies that analyzed linseed and other oilseeds (RYAN et al., 2007) and soybean (BOSCHIN, ARNOLDI, 2011) showed lower contents of total tocopherols and lower activity of vitamin E than for Sacha inchi. The isomer α-tocopherol is the most biologically active form of vitamin E (YADA et al., 2011) and is related to the protection of unsaturated lipids present in biological systems, since it is a lipophilic substance (TAIPINA et al., 2009).

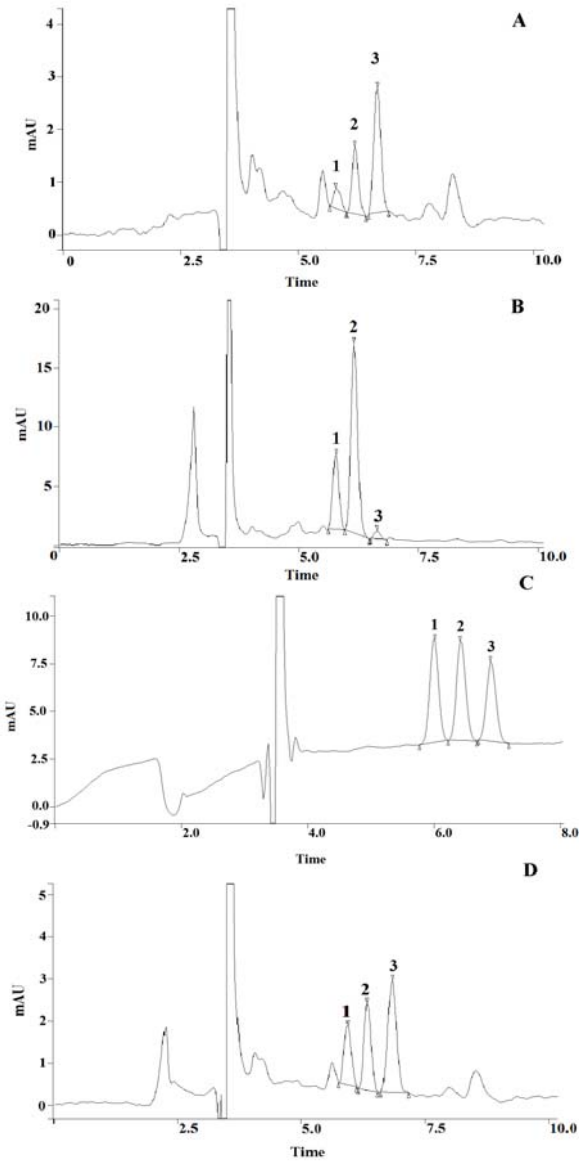

Figure 1. Representative chromatograms with identification of the isomers of tocopherols. 1. δ-Tocopherol; 2. β+γ-Tocopherol; 3. α-Tocopherol. A – Sacha inchi shell: 1. 5.700 min.; 2. 6.200 min.; 3. 6.680 min. B – Sacha inchi nut: 1. 5.700 min.; 2. 6.147 min.; 3. 6.627 min. C –Standards of tocopherol isomers: 1. 6.013 min.; 2. 6.413 min.; 3. 6.893 min. D – Sacha inchi nut with isomers standards: 1. 5.933 min.; 2. 6.333 min.; 6.840 min.

Table 3. Tocopherol composition (mg $100g^{-1}$) of Sacha inchi peanut and shell analyzed.

Fraction	δ -Tocopherol	β+γ -Tocopherol	α -Tocopherol	Total tocopherol content	α -TE[1]
Nut	$2.95^{a} \pm 0.01$	$5.05^{a} \pm 0.15$	$0.99^{b} \pm 0.06$	$8.99^{a} \pm 0.16$	$2.29^{a} \pm 0.07$
Shell	$0.57^{b} \pm 0.01$	$0.65^{b} \pm 0.01$	$1.84^{a} \pm 0.02$	$3.06^{b} \pm 0.02$	$2.01^{b} \pm 0.01$

Means followed by the same letters in columns do not differ by the Student's t-test ($p < 0.05$). [1]α-TE: α-tocopherol equivalents. n = 9 replicates.

Table 4 presents the nutritional contribution (INSTITUTE OF MEDICINE, 2001, 2011) of the nut and shell of Sacha inchi for different age groups, based on the value per portion set forth by Brasil (2003). The Dietary Reference Intake showed that the Sacha inchi nut presents a high contribution of n-3 fatty acids for all of the analyzed populations. Despite a lower contribution compared to the nut, the shell may be a promising source of n-3 and tocopherols (Table 4), mainly due to their metabolic activity (Table 3). Therefore, both fractions of Sacha inchi could be greatly applied and consumed by humans.

Figure 2 shows the principal component analysis (PCA) of fatty acids selected from the loadings (Figure 2A) to evaluate the distribution of the batches (Figure 2B). Through the decomposition into principal components (PC1 and PC2), it is possible to explain 99.88% of data variance. PC1 (Figures 2A and 2B) can distinguish batches of nuts and shells. This is due to the higher concentration of leic fatty acids (18:1 n-9) and the PUFA:SFA ratio for batches of the shell.

Table 4. Vitamin E, n-3 and n-6 contents in 10-g of Sacha inchi peanut and shell portions as percentages of Dietary Reference Intake (DRI) per age and gender.

Age group	Vitamin E		n-3		n-6	
(years)	Nut	Shell	Nut	Shell	Nut	Shell
Children						
1-3	14.98	3.32	304.14	5.83	70.39	0.57
4-8	12.84	2.84	236.55	4.53	49.27	0.40
Men						
9-13	8.17	1.81	177.41	3.40	41.06	0.33
14-18	5.99	1.33	133.06	2.55	30.80	0.25
19-50	5.99	1.33	133.06	2.55	28.98	0.24
>51	5.99	1.33	133.06	2.55	35.20	0.29
Women						
9-13	8.17	1.81	212.90	4.08	49.27	0.40
14-18	5.99	1.33	193.54	3.71	44.79	0.37
19-50	5.99	1.33	193.54	3.71	41.06	0.33
> 51	5.99	1.33	193.54	3.71	44.79	0.37
Pregnant						
14-50	5.99	1.33	152.07	2.92	37.90	0.31
Lactating						
14-50	4.73	1.05	163.77	3.14	37.90	0.31

Analyzing the PCA of tocopherols in Figure 3, PC1 was plotted versus PC2. PC1 separated the batches of Sacha inchi fractions (Figure 3B) and explained 97.49% of the variance. α-tocopherol contributed positively to the separation of batches of shell when compared with the Sacha inchi nut (Figure 3A). Table 3 confirms the higher amount of this isomer of vitamin E in the shell.

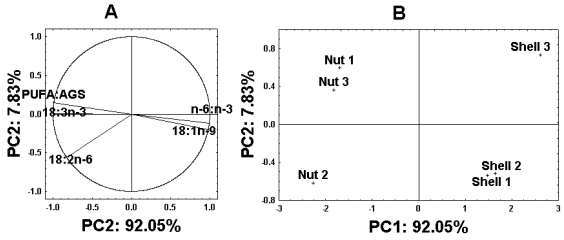

Figure 2. Principal components analysis of fatty acids in Sacha inchi. A: Loadings; B: Scores.
SFA: total saturated fatty acids, MUFA: total monounsaturated fatty acids, PUFA: total polyunsaturated fatty acids, n-6: total omega-6 fatty acids and n-3: total omega-3 fatty acids.

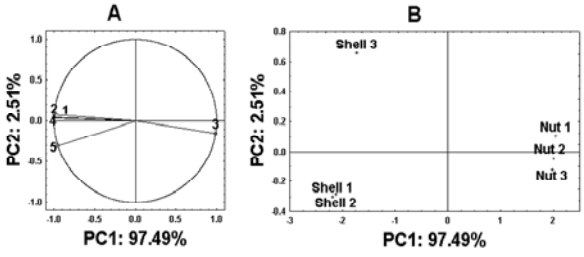

Figure 3. Principal components analysis of tocopherols in Sacha inchi.
A: Loadings; B: Scores. 1: δ-Tocopherol; 2: β+γ-Tocopherol; 3: α-Tocopherol; 4: Total tocopherol content; 5: vitamin E activity (α-TE).

Conclusion

The present study showed that the Sacha inchi nut is an excellent source of essential fatty acids, high contents of tocopherols and presents anti-atherogenic, anti-thrombogenic and hypocholesterolemic effects. The values of DRI enable the evaluation of the nutritional potential of Sacha inchi fractions. The multivariate analysis allowed the distinction of batches of nuts and shells, as well as evaluating the weights of its constituents. The incorporation of Sacha inchi in the human diet is promising due to its intrinsic characteristics, as well as the use of the shell in food processing.

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