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Chemical Characterizaction Sacha Inchi (*Plukenetia Volubilis*) Seed: Oleaginosa Promising From the Colombian Amazon

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ABSTRACT

The physical and chemical characterization of the oil and almond from sacha inchi seed (Plukenetia volubilis), grown in the Guamuez Valley, Department of Putumayo, Colombia was conducted. The whole almond and free fats free from sacha inchi has a high content of oil, protein and essential amino acids, except lysine and tryptophan. The oil extracted from sacha inchi possesses a high degree of unsaturation, rich in essential fatty acids α-linolenic and linoleic, also contains □-carotene and vitamin E. Additionally, the physical properties of density, refractive index, viscosity, iodine index and saponification of sacha inchi oil were measured.

Keywords: Sacha inchi, fatty acids profile, α-linolenic, plukenetia volubilis Linneo, Inca peanut, amino acid profile, cancer chemopreventive agents, chemical composition.

1. Introduction

The polyunsaturated fatty acids α-linolenic (ω-3) and linoleic (ω-6), are essential and necessary for guaranteeing the proper functioning of the human organism, as a prime source of prostaglandins that reduce cardiovascular risk and have great usefulness in the prevention of diverse illnesses associated with the circulatory system (Maurer et al., 2012). Epidemiological studies show that regular consumption of the fatty acids mentioned can reduce cardiovascular risk by 30% preventing the appearance of arrythmias, improve lipid profiles and reduce arterial pressure (Mataix and Gil, 2004). These acids are found in high proportion in the tissue of certain fish and in vegetable sources such as flax seed, nuts, mustard, pumpkin, soy, leafy green vegetables and cereals.

The sacha inchi (Plukenetia volubilis) is a seed-filled plant and perrenial of the euforbiáceas family, which grows to 500 meters above sea level. The oil from sacha inchi seed has the highest polyunsaturated fat composition compared to other oleaginous seeds, at the world level (Gómez, 2005). Linolenic acid (ω-3) is the fatty acid most represented in the seed, accounting for up to 75% of the total oil content. For its part, the seed almond has great food potential providing all the essential amino acids required to comply with the daily requirements of the FAO of all the age groups except babies (Sathe et al., 2002). The seeds of the sacha inchi also contain antioxidants and other substances that combine to sustain its great potential in the cosmetics industry, nutraceutical, food and pharmaceuticals.

In Colombia sacha inchi is still little known at the industrial level, the position of production of derivatives is homemade and investigations of it are reduced to studies on the chemical composition of seeds provided

by the Departments of Caquetá and Tolima (Colombia), results are unknown from other regions important for their biodiversity such as Department of Putumayo, considered the major producer in Colombia.
In consideration of the anterior and to contribute to the knowledge of the biodiversity of the Amazon, the chemical characterization was done of sacha inchi seeds cultivated in the municipality of Guamuez, Department of Putumayo (Colombia). The results obtained provide an incentive for the implementation of agricultural systems cultivation and contribute to the productive diversification and substitution of crops, and the long term industrialization and economic growth of the region (Hamaker et al., 1992).

2. Materials and Methods

2.1. Obtaining prime material

The seeds of sacha inchi (Plukenetia volubilis), cultivated in the municipality of Guamuez Valley, Department of Putumayo (Colombia, Fig. 1), were contributed by a regional business Industrias ECHZ.

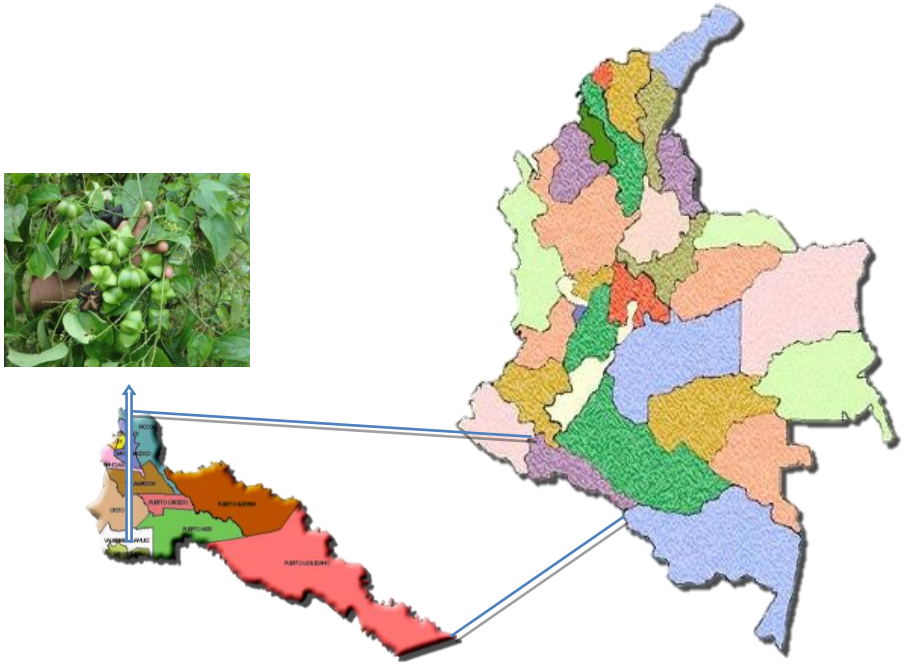

Fig. 1. Geographical location of the region The Guamuez Valley.

2.2. Proximal Analysis

Fort the analysis, the seeds were selected randomly, manually shelled and ground. The proximal composition was determined in the almond and cake (de-fatted almond) of sacha inchi. For this the following methods were used: humidity A.O.A.C. 950.43 (AOAC 2005), ash A.O.A.C. 991.36 (AOAC 2005), ethereal extract A.O.A.C. 920.153 (AOAC 2005), proteín A.O.A.C. 968.06 (factor Kjeldahl: N x 6.25) (AOAC 2005), brute fiber A.O.A.C. 962.09 (AOAC 2005), and in contrast the extract of free nitrogen.

2.3. Amino Acids Profile

The analysis and quantification of amino acids was carried out using the method of Pico-Tag de Waters (Millipore Corp, Milford, MA).

2.4. Physical-Chemical Properties of the oil

The density was determined, index of refraction, iodine index, saponification index and viscosity of sacha inchi oil in accord with the official methods of the A.O.A.C. 920.212, 921.08, 920.159, 920.160 (AOAC, 2005) and the Stokes method, respectively.

2.5. Fatty Acid and Triglycerides Profile

The determination and quantification of fatty acids and triglycerides was done in accord with official methods of the AOCS Ce 1c – 89 and Ce 5-86 (AOACS, 1995), respectively.

2.6. Determination of vitamin E and β-carotene

The analysis and quantification of α-to copherol and β-carotene was done in accord with the method 2432 of the IUPAC (IUPAC, 1987) and official method of the AOAC 941.15 (AOAC, 2005), respectively.

3. Results and Discussion

3.1. Proximal composition of the almond and cake

In table 1 the proximal composition of the almond sacha inchi is shown. The major components are fats and protein, which concurs with other studies conducted and corroborates the importance of this seed from a nutritional viewpoint. Following reports in literature, the oil content in the sacha incha is lower tan that found by Hamaker et.al. (1992) (54%), Follegatti-Romero (2009) (54%), Pascual & Mejía (2000), Hazen & Stowesand (1980), near to that discovered by Gutiérrez (2011), and is within the range reported by Guillén et.al. (2003) (35-60%) and higher than that obtained by Bondiolli & Della Bella (34.42%). The protein count is lower than that found by Hazen & Stowesand (1980) and above that discovered by Pascual & Mejía (2000) and Gutiérrez (2011). Variations in the chemical composite are principally attributed to the differences in place and time of seed harvest. (Follegatti-Romero et al., 2009).

Table 1. Proximal Composition of sacha inchi almond cultivated in the Department of Putumayo, Colombia compared to other studies

Component	Unicauca[a] (2011)	Hazen & Stoewesand (1980)	Pascual & Mejía (2000)	Gutiérrez *et al.* (2011)
Humidity (%)	4,9554 ± 0,0224	4,2	6,37	3,3
Ash (%)	3,0567 ± 0,0109	2,7	2,69	4,0
Fats (%)	42,7523 ± 0,0493	48,7	51,40	42,0
Proteíns (%)	29,85 ± 0,09	33,3	24,21	24,7
Brute Fiber (%)	2,91 ± 0,04	1,6	11,30	30,9
ENN (%)	16,48 ± 0,00	9,5	4,03	

The values represented are the values ± standard deviation (n=3).

Based on the results in Table 2, the majority component of sacha inchi protein cake, which stands out in applications of agroindustry since its high protein content can be incorporated in foods to impart nutritional value and functional properties. In fact, it has been employed as both animal and human food, in formulating

nutritional mixtures with hard yellow corn, rice, plantain, yucca, flour for noodles, milk and milk derivatives (Pascual y Mejia, 2000; Moure et al., 2006; Reátegui et al., 2006). Besides, it can be used as organic manure, which is a considerable source of Nitrogen for plants and favors the growth and development, including microorganisms which inhabit the ground as the plants which they require. Actually, ECHZ Industries uses this residue as fertilizer together with other vegetable by products for the cultivation of sacha inchi, achieving yields of up to four tons per hectare. However, the interests of the sacha inchi cake must be focused on the food sector position that accomplished to be approved its nutritional value in the best way.

Table 2. Proximal Composition of a sacha inchi cake cultivated in the Department of Putumayo, Colombia compared to other studies

Component	Unicauca[a] (2011)	Pascual & Mejía (2000)	Mondragón (2009)
Humidity (%)	7,1483 ± 0,2390	0,695	5,09
Ash (%)	5,1924 ± 0,0113	8,6599	3,07
Fats (%)	4,8351 ± 0,0184	6,8823	35,44
Proteín (%)	51,23 ± 0,10	58,7197	32,53
Brute Fiber (%)	4,79 ± 0,02	17,1826	3,00
ENN (%)	26,80 ± 0,00	7,8605	20,87

The values represented are the values ± standard deviation (n = 3)..

The results found in the cake of sacha inchi coincides with values obtained by Pascual & Mejía (2000), who found a protein content superior to 50%, a lesser discovery in this case. According to Mondragón (2009), the industrial residue of the sacha inchi oil extraction possesed a content of included protein less tan the fat content. The variations between the results must be in the difference in the extraction process done in each case, he position of this study is extraction with solvent Pascual & Mejía (2000) employed two processes for extraction: hot pressing and solvent, and Mondragón (2009), who discovered a quantity of protein considerably less in respect to the others, who extracted by cold pressing.

3.2. Profile of Amino Acids

Table 3 reports on the amino acid content in the almond of sacha inchi, noting that it posses all the essential amino acids in adequate quantities in respect to requirements established by the Food and Agriculture Organization (FAO), World Health Organization (WHO) and the United Nations (UN), except lysine, an amino acid generally found in dried fruits (Salvadó et al., 2005). A possible underestimation in the value of lysine could be explained by the method employed for its determination, posted during conditions during hydrolysis (HCl 6N, 112 °C, 24 h) which favors the degradation of this amino acid, weakens its capacity to react with carbon groups or with the same protein, diminishing this forms availability for quantification (Mondragón, 2009). We also mention that the tryptophan content not reported was not quantified by the method used weakened by its instability in hydrolysis with HCl.

Table 3. Profile of amino acids in the protein of sacha inchi cultivated in the Department of Putumayo Colombia compared to other studies

AMINOÁCIDS (mg/g protein)	***SACHA INCHI***					**FAO/WHO/ONU Scoring pattern**
	Hamaker (1992)	Sathe (2002)	Gómez (2005)	Mondragón (2009)	Unicauca (2011)	
Essentials						
His	26	10	38	9	28	19
Ile	50	50	20	180	57	28
Leu	64	79	59	69	68	66
Lys	43	72	46	39	52	58
Met	12	14	10	13	12	---
Cys	25	43	10	---	28	---
Met + Cys	37	57	20	13	40	25
Phe	24	9	23	9	21	---
Tyr	55	58	43	22	55	---
Phe + Tyr	79	67	66	31	76	63
Thr	43	57	173	68	88	34
Trp	29	44	89	N.D.	---	11
Val	40	62	42	31	44	35
Non Essentials						
Asp + Asn	111	127	---	132	114	---
Glu + Gln	133	146	---	239	138	---
Ser	64	60	16	58	43	---
Gly	118	48	34	38	63	---
Arg	55	85	21	63	55	---
Ala	36	37	173	35	76	---
Pro	48	45	173	24	51	---
TEAA	411	498	553	440	453	339
TAA	976	1046	970	1029	993	
% TEAA	42,1	47,6	57,0	42,8	45,6	

Levels recommended for pre-school age children (2-5 years old), although recently recommended for evaluation of the quality of proteins in the diet of all age groups, except babies (Joint FAO/WHO Expert Consultation 1990).

Comparing the amino acid profile of the analyzed cake with the with the results obtained by other authors, we can observe some differences and similarities in respect to the recommendations of the FAO / WHO / ONU Hamaker (1992) found that the seed protein of sacha inchi is deficient in leucine and lysine;

Mondragón (2009) found a deficiency of histidine, lysine, valine and all the aromatic amino acids; Sathe (2002) discovered a deficiency of histidine and Gómez (2005) found a deficiency of isoleucine, leucine, lysine, methionine and cysteine. In agreement with the aminogram obtained for the protein of analized sacha inchi, concordance can be established with the results obtained by Mondragón, Gómez and Hamaker in as much a lack of lysine.

Taking into account that in Colombia only Gómez (2005) has analyzed the amino acid profile of sacha inchi, we can say that the protein of sacha inchi cultivated in the Department of Putumayo has better nutritional quality than that cultivated in the Department of Caquetá, although it does not posses a higher content total of essential amino acids, a distinction found by Gómez (2005), only noted deficiency in an essential amino acid (see table 3).

Compared to soy, the actual oilseed species, the most cultivated in the world, is of great nutritional value for its content and quality of protein (Manjaya et al., 2007), we can say that sacha inchi constitutes an alternative or complement to soy protein, which could be converted to an elected ingredient for many different food applications, and consequently a nutritional source of great importance. In fact, the agroindustrial potential of sacha inchi cake is being exploited in the food industry, although in small proportion owing to its limited publicity, in fabrication of products such as: food supplements, nougat, flour, protein cake, and snacks, among others (Huamaní y Flores, 2009; IIAP, 2009).

3.3. Physical and chemical properties of the oil

Table 4 shows the physical and chemical properties of oil extracted from the sacha inchi almond. The density and refraction index of the oil is found to be among the highest values when comparing them with reports by ICONTEC (Instituto Colombiano de Normas Técnicas y Certificación) for some edible oils. The density is between the ranges reported for soybean oil, corn and sesame. The refraction index was found to be above the established range for soybean oil, sunflower, corn and sesame (Bernal, 1993). In agreement with the previous, we can say that sacha inchi oil posses fatty acids of higher molecular weight, besides, a high grade of unsaturation. Said appreciation concurs with the viscosity so sacha inchi oil presents a relatively high value, which corroborates its high unsaturated fatty acid content (Abramovic y Klofutar, 1998; Bailey, 1984).

Table 4. Physical and chemical analysis of sacha inchi oil compared to other authors

Physical/chemical property	Unicauca[a] (2011)	Pascual & Mejía (2000)	Follegatti-Romero (2009)	Gutiérrez (2011)
Density (g/mL) a 25 °C	0,9189 ± 0,0170	0,9227	---	0,9187
Refraction Index at 25 °C	1,4791 ± 0,0008	1,4801	---	1,4791
Viscosity at 20 °C (cP)	35,5 ± 0,3	40,97 (37 °C)	---	35,4
Iodine Index (g de I_2/100 g oil)	198,1 ± 0,7	189	198	193,1
saponification index (mg KOH/g oil)	190,3 ± 0,5	229,6	193	185,2

Los valores presentados son los promedios ± la desviación estándar (n=3).

The iodine index obtained gives an indication of the high grade of sacha inchi oil, that is found to be considerably above the values reported by ICONTEC for edible oils whose iodine index is high, among others, sesame oil, soybean oil and corn. Nonetheless, the results found are between the values reported for marine oils which present an iodine index that exceeds 120 (Bernal, 1993).

The saponification index of sacha inchi oil is found between the range reported by ICONTEC for corn oil, sesame, cotton and peanut, which corroborates that it contains fatty acids of high molecular weight. (Bernal, 1993).

Comparing the physical and chemical properties in this case with results obtained by Pascual & Mejía (2000), Follegatti-Romero (2009) and Gutiérrez (2011), we can observe the existing concordance between them, consequently, to check the high grade of of unsaturated oil of said oilseed.

3.4. Profile of oil fatty acids

Following the obtained results (see table 5), sacha inchi oil posses a high grade of unsaturation (92.7%), which explains the elevated iodine index, with polyunsaturated fatty acids as the principal constituents (83.3%), we find the major proportion of α-linolenic acids (C18:3 n3) and linoleic (C18:2 n6 cis), and the monounsatuarated fatty acids the minority components (9.39%), we encounter principally the acid cis-vaccénico (C18:1 cis iso). Said results concur with other studies conducted, which indicate that sacha inchi oil is constituted essentially of the acids α-linolenic (ω3), linoleic (ω6) and oleic (ω9), whose percentage varies in a range from 42.18 - 57.22%, 27.8 - 38.72% and 8.22 - 10.68%, respectively. Without fail, we must highlight that all the investigations conducted have reported oleica acid content greater than 8%, in the meantime, here we find a very low percentage of this (0.66%) but a value higher in its isomeric position, vaccenic acid, which plays an important role in the inhibition of carcinogenic cells in comparison to oleic acid (Awad et al., 1995). On the other hand, Bondioli (2006), Merino (2009), Follegatti-Romero (2009), Castaño (2010) and Gutiérrez (2011) found a content of ω3 above 50%, while in this case a value lower than this was obtained.

Table 5. Profile of sacha inchi oil fatty acids

FATTY ACIDS	Unicauca (2011)	Gutiérrez (2011)	Castaño (2010)	Hamaker *et al.* (1992)	Follegatti-Romero *et al.* (2009)
Saturated (%)					
$C_{14:0}$, Mirístico	0.0	---	---	0.0	---
$C_{16:0}$, Palmítico	4.20	4.4	3.60	4.5	4.24
$C_{17:0}$, Margárico	0.091	---	---	---	---
$C_{18:0}$, Esteárico	2.89	2.4	2.90	3.2	2.50
$C_{20:0}$, Araquídico	0.080	---	0.09	---	---
$C_{22:0}$, Behénico	0.042	---	1.20	---	---
Unsaturated (%)					
$C_{16:1}$, Palmitoleic	0.046	---	0.07	0.0	---
$C_{18:1}$, *Cis*-Vaccenic	8.45	---	---	---	---

$C_{18:1}$, Oleic (ω9)	0.66	9.1	8.50	9.6	8.41
$C_{18:2}$, Linoleic (ω6)	35.3	33.4	33.90	36.8	34.08
$C_{18:2}$ *ct*	0.061	---	---	---	---
$C_{18:3}$, Linolenic (ω3)	47.7	50.8	50.20	45.2	50.41
$C_{18:3}$ *ctt-cct*	0.22	---	---	---	---
$C_{18:3}$ *ctc*	0.062	---	---	---	---
$C_{20:1}$, Gadoleic	0.23	---	0.32	0.0	0.16

Comparing the composition of sacha inchi fatty acid with other oilseeds sedes such as soy, canola, sunflower, and flax; which are among the principal cultivated oilseeds with a global production base, except the last (Vollmann and Rajcan, 2010); we can confirm mentioned by other authors, who signal that said oil posses the highest polyunsaturated fatty acid content in the world in respect to othernvegetable oils. Only flaxseed oil can compare with the position of sacha inchi whose major constituent is ω3 (58% vs. 47,7%), although its content of ω6 is significantly less (14% vs. 35,3%) (Belitz and Grosch, 1997). Considering the anterior mention, it is necessary to highlight the importance of sacha inchi oil in health and nutrition, a position that essential fatty acids (ω3 and ω9) present in participation in a wide variety de fundamental metabolic functions: reducing the risk of cardiovascular diseases (the principal cause of mortality in the world), has chemopreventive activity, among others (Huamán, et al., 2008; Rose and Connolly, 1999; Sangiovanni and Chew, 2005; Wesley, 1998).

On the other hand, we must mention that sacha inchi oil contains other fatty acids in lesser proportions. Among these the positional isomers and geometrics of the linoleic and α-linolenic (C18:2 ct, C18:3 cct-ctt y C18:3 ctc) acids stand out, considering that they have potentially benefitial biological effects, such as anti-tumoral, anti-aterogenic, anti-diabétic and anti-obesity; likewise, they have demonstrated that they prevent the appearance of hypertension. Each isomer completes a different function: rumenic acid (C18:2 cis 9-trans 11) has an anti-cancer effect; α-eleostérico acid (C18:3 cis 9-trans 11-trans 13) and punicic acid (cis 9-trans 11-cis 13), are potential tumor suppresants and effectively act as antioxidants and anti-inflamatory agents, as the first best antioxidant and the second best anti-inflamatory agent owing to their high content of double bonds in trans y cis positions, respectively; the acid C18:3 cis 9-trans 12-trans 15 reverts modifications of the fatty acids induced in cardiolipin (a fundamental lípid in bioenergetic mitochondrial function) in a diet lacking in essential fatty acids (Chen et al., 2007; Nagao and Yanagita, 2005; Saha and Ghosh, 2011; Wolff et al., 1993).

3.5. Profile of oil triglycerides

Table 6 shows the distribution of triglycerides (TAG) in sacha inchi oil, observing that it contains 12 species, of which 4 were not identified because they were not included with the standards necessary for their identification. In agreement with the fatty acid profile we can say among the fatty acids which form part of the TAG α-linolénico acid is not distinguished, the principal constituent of sacha inchi oil, none of the TAG's determiners are found, at the same time, the TAG´s not identified are the major oil components. Nonetheless, it is not possible to know in an accurate manner the position of said fatty acid (sn-1, sn-2, o sn-3) nor its proportion, in respect to the other fatty acids, in the unknown TAG´s. The results obtained reflect

the close relation between fatty acid content and triglycerides in the oil, and consequently, with the physical and chemical properties of the same (Nergiz and Dönmez, 2004). It is important to highlight that in all the studies conducted on sacha inchi seed, in Peru as well as in Colombia, an analysis of the triglyceride composition of extracted oil has not been conducted until this one.

Table 6. Profile of triglycerides in sacha inchi oil a

TAG	%
PPP	2.27
POP	7.29
PLP	4.80
POO	2.85
PLO	2.02
PLL	6.08
SSS	8.97
SOO	5.29
TAG NI	10.6
TAG NI	14.6
TAG NI	21.1
TAG NI	14.1

3.6. Determination of vitamins E and β-carotene

The content of α-tocopherol and β-carotene in sacha inchi oil are shown in table 7, and you can observe the concentration of α-tocopherol differs from the results obtained by other authors, who determined, besides this isomer, γ and δ-tocopherol, as the first antioxidant most active in the organism, and the two last, the antioxidants most active in food lipids (Schmidt y Pokorný, 2005). For this reason, the presence of tocopherols in sacha inchi oil is of vital importance, owing to its antioxidant activity offers the same stability, on the contrary, it oxidizes easily so an oil with a high content of polyunsaturated fatty acids is more susceptible to rancid oxidation. Jointly, the consumption of this vitamin helps to prevent the risk of cardiovascular diseases and various types of cancer (Singh et al., 2007).

Table 7. Content of tocopherols and β-carotene in sacha inchi oil compared to other studies

	Unicauca (2011)	**Hamaker *et al.* (1992)**	**Bondioli & Della Bella (2006)**	**Follegatti-Romero *et al.* (2009)**
Tocoferoles (mg/100 g)				
α– – tocoferol	84	3.8 – 6.3	143	0.00
γ – tocoferol	---	---	83 (*β*+*γ*)	114
δ– tocoferol	---	---	Trazas	125
Vitamina A (mg/100 g)				
Carotene	0.153	0.08	---	---

On the other hand, we should mention that sacha inchi oil must be consumed en soft-gels or salads, during a lengthy period of food preparation considerable loss of vitamin E content occurs that causes its instability, processes in fried foods, grilling and slow fire cooking produce the greatest losses of this vitamin, as when exists major contact with heat and oxygen (Mataix y Ochoa, 2002).

As for the content of □-carotene, you can see that the value obtained is greater than that found by Hamaker (1992), however, it continues lower in respect to the concentration of this pigment in other foods (Aranda, 2009; Dauqan et al., 2010).

Finally, it's posible to mention that variations among the results obtained for sacha inchi sedes cultivated in the Department of Putumayo (Colombia) and those reported by other authors, are attributed principally to the different subspecies, and in some cases, to the methods used in the process of extraction of seed oil, to the differences in place and time of harvesting of the seeds, as the majority of studies have been carried out with seeds provided from Peru, in Colombia Castaño (2010), Gómez (2005) and Gutiérrez (2011) have effected analyses with seeds proceeding from the Departments of Caquetá and Tolima (Aranda, 2009; Gutiérrez et al., 2011).

4. Conclusion

Sacha inchi sedes cultivated in the Department of Putumayo (Colombia) present s high content of oil (42.75%) and proteín (29.85%). The oil contains a high content of polyunsaturated fatty acids (83.3%), including the essential fatty acids α-linolenic (47.7%) and linoleic (35.3%) the principal constituents. A diference found in other studies, in which have been reported oleico acid as a major component of monounsaturated fatty acids, the acid cis-vaccenic is the monounsaturated fatty acid which is most abundant. The cake, the residue obtained in the extraction of oil, is principally composed of protein (51.23%), which has great nutritional value owing to possessing 9 of the 11 essential amino acids in adequate quantities, following the requirements established by the FAO/WHO/ONU for adults. Due to this, we must highlight the importance of sacha inchi seed now that for its chemical composition posses great potential for industrial level use in the pharmaceutical and food sectors in the elaboration of functional foods and nutraceutical products.

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