



CHARACTERIZATION OF SACHA INCHI SEED OIL (*Plukenetia Volubilis*) FROM 'CANTON SAN VICENTE, MANABÍ, ECUADOR', OBTAINED BY NON-THERMAL EXTRUSION PROCESSES

CARACTERIZACIÓN DEL ACEITE DE LA SEMILLA DE SACHA INCHI (*Plukenetia Volubilis*) DEL CANTÓN SAN VICENTE, MANABÍ, ECUADOR, OBTENIDA MEDIANTE PROCESOS NO TÉRMICOS DE EXTRUSIÓN

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Resumen

Sacha Inchi (*plukenetia Volubilis*) es una planta originaria de Perú. El fruto es una semilla oleaginosa la cual posee un alto contenido de ácidos grasos polinsaturados, en la cual se destaca el omega 3 y 6. Para la extracción de este aceite se utilizó el método de compresión por extrusión, utilizando un tornillo sinfín, que aumenta la presión de la masa, separando así el aceite contenido dentro de la semilla. Para esto, se utilizó un extractor experimental el cual fue adaptado y puesto en marcha para la obtención de este aceite, extrayéndolo a temperatura ambiente. Con la finalidad de establecer las características fisicoquímicas de la semilla se realizaron análisis de humedad, fibra, ceniza, grasa y proteína. Una vez extraído el aceite se calcularon los rendimientos y se realizaron análisis de: índice de acidez, densidad relativa, índice de yodo, índice de peróxido y perfil de ácidos grasos. Estos resultados se compararon con los análisis realizados al aceite de la misma especie, pero de diferentes zonas de cultivo del Perú, aceite de pescado y oliva, conocidos por su alto contenido de ácidos grasos, dando como resultado que el aceite de Sacha Inchi presenta un alto contenido de ácidos grasos polinsaturados y que el método de extracción influye en la calidad del producto. **Palabras clave:** Sacha Inchi, extrusión, índice de peróxido, ácido graso insaturado, índice de yodo.

Abstract

Sacha Inchi (*Plukenetia Volubilis*) (SI) is a plant native from Peru. The fruit of this plant is an oilseed than contains a high content of oil which is rich in unsaturated fatty acids (91.6%), being one of the seeds that contain this type of fat in higher percentage. For the extraction of this oil, the extrusion method was used, using an endless screw that allows the pressure increase in the dough, separating the oil contained in the seed. For this, an experimental extractor was adapted and put into operation to obtain this oil, extracting it at room temperature. To analyze the physicochemical characteristics of the seed, moisture, fiber, ash, fat and protein analyzes were conducted. Once the oil was obtained, the yields were calculated, and the following analyzes were performed: acid index, relative density, iodine value, peroxide index and fatty acid profile. Those results were compared with the analysis made to olive and fish oil, known for their high content of fatty acids, resulting that Sacha Inchi oil is better in both quality indexes, as in percentage of unsaturated fatty acids.

Keywords: Sacha Inchi, extrusion, peroxide index, unsaturated fatty acid, iodine index.

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1 Introduction

Sacha inchi (SI) (*Plukenetia Volubilis Linnaeus*), also known as wild peanuts, sacha peanuts, mountain peanut is an oily plant that belongs to the Euphorceae family. It has been cultivated in the lowlands of the Peruvian Amazon, and has been planted for centuries by the indigenous population, becoming a diet component of several native groups of the region (Gutiérrez et al., 2011; Chirinos et al., 2013). According to Muangrat et al. (2018), the obtaining percentage of this pressed oil at 60 °C is 37.97%, with an approximate percentage of 92% of polyunsaturated fatty acids (GPA), such as alpha linolenic acid (18:3n-3, linolenic acid) and linoleic acid (18:2n-6, α linoleic acid) (Fanali et al., 2011; Cisneros et al., 2014). This type of fatty acids has one or more links between their carbons, and depending on their location they are called α -3, 6 or 9. According to Araujo-Dairiki et al. (2018) these fatty acids have beneficial effects that include the ability to decrease glyceride levels, prevent cardiovascular disorders and have an antithrombotic action, in addition to certain experiments that have shown that this oil has a high antioxidant capacity that helps to reduce DNA damage due to oxidation (Takeyama and Fukushima, 2013). The interest in these nutrients has been due to a set of publications demonstrating that the intake of fat depends on its quality, i.e., the type of predominant fatty acid (Carrillo Fernández et al., 2011). Thus, the study of SI is a challenge both as oily material and also for the biological functionalities that could derive from its oil and/or its extracts (Castaño T. et al., 2012).

On the other hand, the consumption of olive oil (OO) has increased due to the benefits of vegetable oils, since the composition of OO has a large amount of monounsaturated fatty acids, particularly oleic acid. In addition, there are acid α -linolenic (α -3) and linoleic acid (α -6) required by the human body and which cannot synthesize (Piscopo et al., 2016).

Currently, fish oil in capsules is marketed globally because it is rich in polyunsaturated fatty acids α -3 [40.91%, according to Paucar-Menacho et al. (2015)], eicosapentaenoic acid (EPA) (20: 5, n-3) and docosahexaenoic acid

(DHA) (22: 6, n-3) (Van der Tempel et al., 1990). However, environmental pollution has caused the accumulation of heavy metals and dioxins in fish, hence, the benefits of obtaining unsaturated fatty acids from fish (Maurer et al., 2012).

People who cannot eat fish daily or do so infrequently, can supplement their intake of fatty acids α -3 with vegetable oils (Strobel et al., 2012). However, as these fatty acids are easily oxidized at high temperatures, their potential applications are limited. As such, the analysis of new sources of fatty acids α -3, would be extremely beneficial from the point of view of human health (Takeyama and Fukushima, 2013). The aim of this research is to extract the SI oil by a cold pressing and conduct its characterization to be able to compare between oils rich in unsaturated fatty acids, such as fish oil and olive oil.

2 Materials and methods

2.1 Process for obtaining the oil

The seeds of SI (*Plukenetia volubilis Linnaeus*) coming from San Vicente, Manabí, Ecuador, once cultivated were stored hermetically, then it was proceeded to extract manually the shell from the seed, and were ready for the analysis and extraction processes. Finally, to separate the non-lipid dry mass, a Dutch extruder "Piteba" brand was used from the oily part, in which its limitation lies in the use of seeds with total fat content of more than 25% gross weight. According to Fanali et al. (2011), the presence of oil in SI seed was between 37% and 47%. This extraction was carried out at room temperature in order to generate the least possible impact on the unsaturated structures of fatty acids. Once the crude oil is extracted, which has suspended solids because during the extrusion process certain solid material particles were retained, it is left to rest for a few hours in a container that was properly sealed and without light in order to avoid accelerated oxidation. Once the two phases are separated (solid of the dry) it was proceeded to conduct the filtration of the first layer with a standard filter paper, thus leaving the oil ready for its storage as shown in Figure 1.

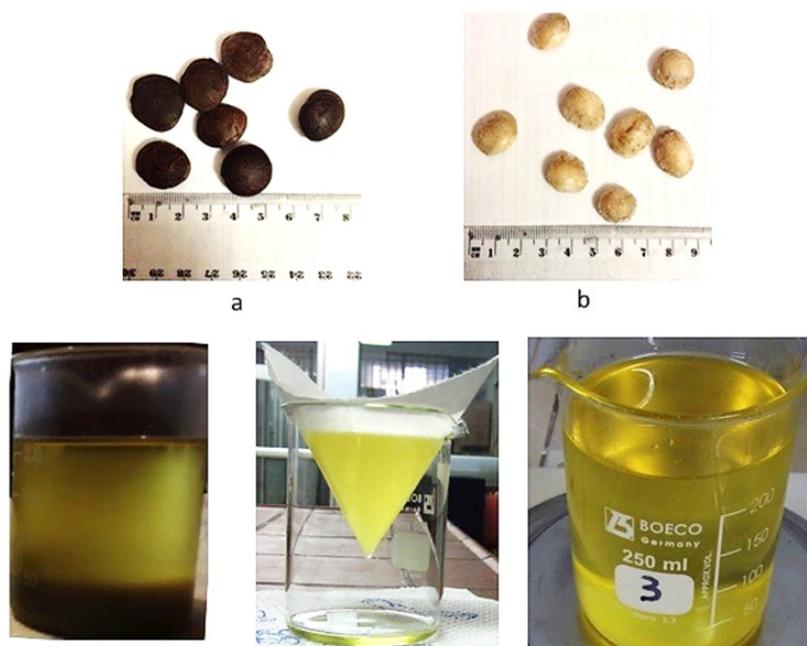


Figure 1. a) SI seed with shell. b) SI seed without shell. c) Product obtained after the extrusion. d) Oil filtration. Filtered oil.

2.2 Chemical-physical analysis of Sacha Inchi

Each test was performed following the parameters of the AOAC Official Methods of Analysis and the American Oil Chemists' Society (AOCS). In case of needing to weigh a sample, an AE Adam balance of 1000 grams was used, which was properly calibrated.

2.2.1 Percentage of humidity

Following the guidelines of the AOCS-94 Cde 13a-63 method, 5 grams approximately were weighed, then these were taken to a Thermo Fisher Scientific stove for approximately 5 hours at 110 °C and then were weighed again. The percentage of humidity in the sample was determined by using Equation (1).

$$\% \text{Humidity} = \left(1 - \frac{\text{Weight of dry sample}(g)}{\text{weight of humidity}(g)} \right) * 100 \quad (1)$$

2.2.2 Determination of fat

For this type of analysis, the AOAC 18th 922.06 method was followed by weighting 2 grams of the sample after the extraction of moisture, and the raw fat is separated from the sample by soxhlet and was weighted. The total fat percentage in the sample was determined by using

Equation (2).

$$\% \text{fat} = \frac{\text{Sample weight of the fat obtained}(g)}{\text{weight of the sample seed}(g)} * 100 \quad (2)$$

2.2.3 Percentage of raw fiber and ash

The fiber determination was followed by the parameters of the AOAC method, 2005, 962.09 using 1.25% of diluted sulphuric acid, 1.25% of sodium hydroxide solution, 95% of ethyl alcohol and petroleum ether. The percentage of ash was determined by the AOCS-94 Ba 5-4 method, in which a Thermo scientific Lindbergh blue m flask was used.

2.2.4 Percentage of protein

A Thermo Fisher Scientific Brand Flash 2000 analyzer was used for this type of test, in which 1 milligram of the sample was weighed and carried to the instrument input, then combustion around 900°C and then it was heated in approximately 900 °C, and then a serpentine-shaped column is done and was finally detected by a Thermal Conductivity Detector (TCD) which automatically provides protein percentage data.

2.3 Chemical Physical Analysis of the Oil

The guidelines described in point 2.2 were also used for these analyses. In case of needing to weigh a sample, an AE Adam balance of 1000 grams was used, which was properly calibrated.

2.4 Free acidity index

A direct titration was conducted according to the AOCS-94 Cd 3a-63 method, 10 grams of the oil sample were weighed in an Erlenmeyer and 50 ml of ethyl alcohol was added, then about 3 drops of phenolphthalein indicator were added. It was then titrated with a 0.1 N sodium hydroxide (NaOH) solution until the color changed to pink. Finally, the calculation was performed with Equation (3). Where AI is the acidity index, V is the volume in ml of the valued NaOH solution used to neutralize the free fatty acids in the sample, N is the normal concentration of NaOH and W the weight in grams of the oil sample.

$$IA = \frac{V * N * 56,1}{W} \quad (3)$$

2.4.1 Relative Density Determination

Method AOAC-90 920-212 was used for this analysis. In a properly titrated specimen, 100 ml of the oil sample were placed and then this amount was weighed to obtain the result with the Equation (4), obtaining the same in the units of gr/ml.

$$\% \text{Relative density} = \frac{\text{weight of the oil}(g)}{100 \text{ ml}} \quad (4)$$

2.4.2 Peroxide index

Following the guidelines of the Method AOAC-90 965-33, first two grams of the oil sample were weighed, then 24 ml of 1:3 chloroform-acetic acid solutions were added, then 0.4 ml of potassium iodide solution and 24 ml of distilled water, the sodium thiosulphate solution with 0.01 N potassium permanganate was assessed and titration continued until the blue color disappeared. The peroxide index was calculated with Equation (5) and the result showed active oxygen mill equivalents per kilograms of fat. Simultaneously, a white sample is performed performing the same procedure with water. Where IP is the peroxide index, V the milliliters of sodium thiosulphate solution used in the test, V' the milliliters of sodium thiosulphate solution consumed in the white, N is the normal sodium thiosulphate solution and P is the normal weight in grams of the sample.

$$IP = \frac{(V - V') * N * 1000}{P} \quad (5)$$

This test was carried out for 13 days with the aim to study the oxidation progression of this oil to the weather, environmental temperature and exposed to light. The analyses were carried out on days 1-4-6-8-11-13. Error bars were used as a method of statistical analysis.

2.4.3 Iodine Index

The measurement of this parameter was performed according to the parameters set out in method AOAC-90 920-159. First 0.1 g of SI oil was weighed in a 250 ml Erlenmeyer, the oil was dissolved in 10 ml of chloroform and 10 ml of the Wijs solution, then it was left to rest for 30 minutes in the darkness stirring occasionally; then 5 ml of potassium iodide solution at 15% were added stirring vigorously, and 100 ml of freshly boiled and flared water were added, washing any residue of the existing solution in the edges. Finally, it was titrated with sodium thiosulphate 0.1 N, using starch as an indicator. At the same time, analysis of a white sample was performed. The calculation of the iodine index was done using Equation (6). In which V_s are the milliliters of sodium thiosulphate used in the white, V_m are the milliliters of sodium thiosulphate used in the sample and N is the normality of sodium thiosulphate.

$$\text{Iodine index} = \frac{(V_s - V_m) * N * 12,67}{\text{weight of the sample}} \quad (6)$$

2.5 Fatty Acid Profile

It was performed in accordance with the AOCS Ce 1B-89 procedure using a TRACE 1310 Mainframe gas chromatograph with a Trace TR-FAME 260M137P capillary column (25m x 0.32mm x 0.25um). The working conditions were performed as described by Wang and Kakuda (2018).

2.6 Statistical analysis

For the physicochemical seed and oil analyses, three replications of each were made, and the means were obtained with their corresponding standard deviations. In addition, a statistical analysis was performed using Tukey's ANOVA test and the Real Statistics program to determine potential significant differences at $p < 0,05$ level. For the elaboration of graphs, the software 'R' was used.

3 Results and Discussion

3.1 Yields on the extraction of Sacha Inchi seed oil

As observed in Table 1, it can be seen that taking 1000 grams of SI seed a lot of this total weight is lost in the shell, so the weight of the seed will be taken without

shell as a calculation basis for the other parameters. The dry mass (extraction residue) obtained after the extraction (68.08%) gives a very high value, so it represents a very important percentage as it can be used later. The percentage of oil yield shown in Table 1 is 26.92%, which com-

pared to the results provided by Muangrat et al. (2018) who obtained oil yields between 37.97% and 40.63%, it is evidenced that the performance in this study was lower, because during oil extraction in this investigation the temperature did not increase.

Table 1. Yields on obtaining SI seed oil.

Oil obtained (%p/p)	Sediments (%p/p)	Seed residue (%p/p)	Seeds without shell (g)	Shelled seed (g)	Semilla con cáscara (g)
26,93 ±1,67	3,8±0,18	68,08 ±7,46	653,17 ±13,83	347,2 ±14,4	1000,3 ±0,6

3.2 Physicochemical analysis of Sacha Inchi seed

As can be seen in Table 2, this seed contains a high oil content: 42.0% which according to Wang and Kakuda (2018) is in the estimated range (33.4% - 54.3%). The amount of protein: 29.78%, suggests that this seed once extracted the oil will have a very high protein remnant. The ash percentage is 2.9% which is slightly lower as reported by Gutié-

rrez et al. (2011) who obtained a percentage of 4%. The amount of moisture is relatively low: 6.72% as it is a dry fruit. According to James (1995) it is within the range 0-10% for processing and storing without degradation of microorganisms to triacylglycerides. The amount of fiber obtained in this study was 18% on a dry sample, which is higher than the one reported by Muangrat et al. (2018), who obtained a percentage of 13.86% on a dry sample.

Table 2. Physicochemical characteristics of SI seed

Characteristics	SI Seeds
Moisture	6,72 ±0,1
Fat	42,03 ±0,2
Ash	2,9 ±0,025
Fiber	18,0 ±0,095
Protein	29,78 ±1,6

3.3 Physical-chemical analysis of Sacha Inchi seed oil

3.3.1 Acidity Index

As observed in Table 3, the acidity index of this oil is 0.38 mg KOH/g. According to FAO/OMS (2015), it should not be higher than 4 mgNaOH/g for cold-pressed vegetable oils. The low acidity of this acid reflects the low refining and good quality of SI oil. The good quality of this oil is corroborated compared to the olive oil that according to Paucar-Menacho et al. (2015) has a value of 1.14 mgNaOH/g, and the fish oil that according to Nascimento et al. (2015) has a value of 11.72 mgNaOH/g,

ding to Gutiérrez et al. (2011) this is due to the amount of unsaturated fatty acids present in it. Compared to the olive oil that according to Paucar-Menacho et al. (2015) gives a value of 0.9252, there is a similarity between these two oils due to the high unsaturation in both.

3.3.2 Relative density

In Table 3 is presented the result of this parameter with a value of 0.91, which reflects that this oil is light. Accord-

3.3.3 Iodine index

According to Muangrat et al. (2018) this characteristic of the oil is related to its unsaturation, which as can be seen in Table 3 it gives a value of 192.5 I₂/100g. Comparing it with the reported by Paucar-Menacho et al. (2015) for olive oil (56.15 g I₂/100 g) and by Nascimento et al. (2015) for fish oil (93.92 g I₂/100 g), the SI oil shows very high value in the iodine index, reflecting that this oil has greater unsaturation compared to the other two oils.

Table 3. Physical-chemical characteristics of SI oil

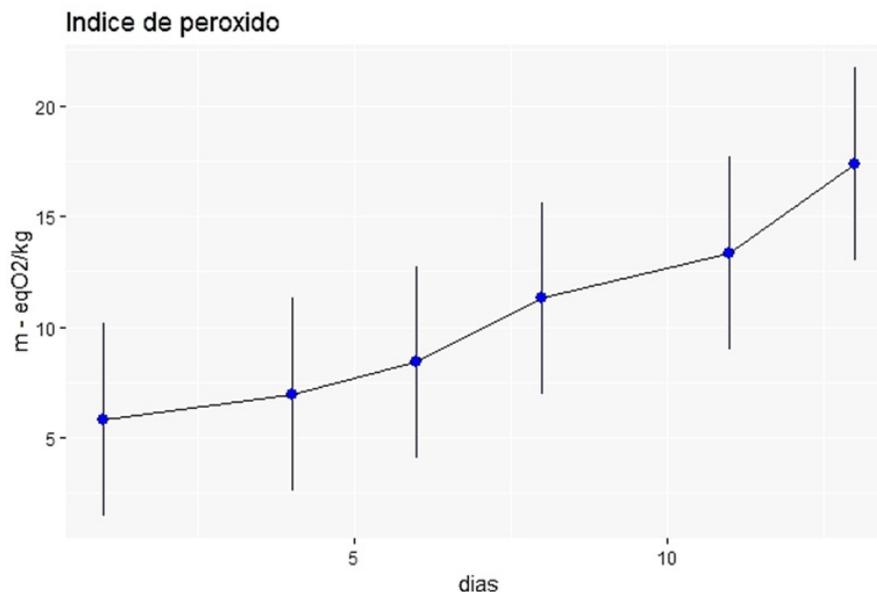
Property	Oil type		
	Sacha Inchi	Olive	Fish ³
Acidity index (mg KOH/g)	0,38 ± 0,02	1,14 ± 0,037 ¹	11,72 ± 0,1
Relative density	0,91	0,9252 ± 0,0014 ¹	N/D
Peroxide index (m-eq O ₂ /Kg)	5,81 ± 0,5	6,91 ± 3,44 ²	7,22 ± 1,1
Iodine index (I ₂ /100g)	192,5 ± 0,7	56,15 ± 0,14 ¹	93,92 ± 0,7

¹ (Paucar-Menacho et al., 2015), ² (Piscopo et al., 2016), ³ (Nascimento et al., 2015).

3.3.4 Peroxide index

As mentioned by Cebi et al. (2017) this test directly measures the concentration of hydroperoxide resulting from the primary oxidation in the oxidation of products, which according to FAO/OMS (2015) it must not exceed 10 mill

equivalents of oxygen peroxide/kg in the case of vegetable oils and animal origin. The result obtained in this study was 5.81 m-eq O₂/Kg, which gives a lower value compared to olive oil with a value of 6.91 m-eq O₂/Kg according to Piscopo et al. (2016), and a value of 7.22 m-eq O₂/Kg in fish oil according to Nascimento et al. (2015).

**Figure 2.** Oxidation index (m-eq O₂/Kg) vs time (days).

To find out how vulnerable SI oil is to oxidation, the procedures described in 2.2.3 at room temperature (25 °C) were performed, proving that this oil is sensitive to develop rancidity if it is exposed for a long time to light and the weather. As reported by Takeyama and Fukushima (2013) which exposed the oil to a certain UV radiation of 300 nm for 15 days and obtained an increase in the oxidation of the same, obtaining values of 250 m-eq O₂/Kg.

As reported by Maurer et al. (2012), who obtained a value of 100 m-eq O₂/Kg at 65 °C for 15 days, which is very different compared to the analysis carried out in this research which was performed at 25 °C (16 m-eq O₂/kg for 15 days). All these results indicate that this oil has a high sensitivity to oxidation due to exposure to ultraviolet light and temperature.

3.4 Fatty acid profile

Table 4. Oil fatty acid profile.

Fatty acid profile				
Composition of fatty acid	Sacha Inchi(%)	Sacha Inchi(%) ¹	Olive(%) ²	Fish(%) ³
C14:0	N.D	N.D	N.D	10,22
C16:0	5,44	4,7	13,56	22,8
C16:1	N.D	N.D	1,43	10,54
C17:0	N.D	N.D	242	0,85
C18:0	3,94	3,3	1,86	4,4
C18:1	17,12	8,9	71,22	9,62
C18:2	34,67	34,1	10,13	1,51
C18:3	38,84	48,2	618	0,99
C20:0	N.D	N.D	356	N.D
C20:5n3	N.D	N.D	N.D	20,28
C22:6n3	N.D	N.D	N.D	19,64
C20:1	N.D	N.D	284	2,56
C20:2	N.D	N.D	102	N.D
C22:1	N.D	N.D	N.D	1,62
Total α -3	38,84	48,2	618	40,91
Total α -6	34,67	34,1	10,13	1,51
Total α -9	17,12	8,9	71,22	9,62
Total Saturated	9,38	8	15,88	37,42
Total Unsaturated	90,63	91,2	83,92	62,58
Total mono unsaturated	17,12	8,9	73,17	20,16
Total Poly unsaturated	73,51	82,3	10,75	42,42

¹ (Cisneros et al., 2014), ² (Romero Aroca, 2011), ³ (Paucar-Menacho et al., 2015).

The fatty acid profile can be seen in Table 4. The high content of unsaturated fatty acids can be observed in all the three oils, the highest being SI (90.63%) similar to the one reported by Fanali et al. (2011) who obtained a value of 92%. Within them linolenic acid α -3 (38.84%) is the most present, which helps to reduce the risk of cardiovascular disease in humans (Araujo-Dairiki et al., 2018). The fatty linoleic acid α -6 is the second with the highest percentage within SI oil (34.67%) which contributes to the prevention of inflammatory diseases (Saiki et al., 2017) and decreases body fat in children (Racine et al., 2010). In addition, compared to the values obtained in this trial with those provided by Cisneros et al. (2014), it is observed that there is a small difference in the composition of fatty acids, reaching higher values in linolenic acid α -3 (48.2%) and decreasing in oleic acid α -9 (8.9%). There is no greater difference in the case of linoleic acid α -6.

According to Romero Aroca (2011) olive oil has a total saturated percentage of 15.88%, and a total unsaturated percentage of 83.92%, emphasizing that oleic acid α -9 is the most present in this type of oils, reaching a percen-

tage of 71.22%, which is stable to oxidation because it is a fatty acid with only a double bond (Paucar-Menacho et al., 2015). According to Paucar-Menacho et al. (2015) the fatty acid profile of fish oil has a total saturated percentage of 37.42%, and total unsaturated percentage of 62.58%. It should be emphasized that linolenic acid α -3 is the most present in this type of oil, reaching 40.91%.

Comparing these three oils, it can be shown that SI oil provides a higher amount of unsaturated fatty acids, which differ in the amount between linolenic acid α -3, linoleic α -6 and oleic α -9, the first two being the ones with more presence. It can be said that SI oil has a beneficial health effect, reducing the amount of triglycerides in the blood. These can help in the control of certain diseases such as diabetes mellitus, obesity and also work as possible cytotoxic agents for certain tumor cells (Rodríguez-Cruz et al., 2005).

4 Conclusions

The oil extraction percentage by extrusion (26.92%) compared to that obtained in physicochemical analyses (42.03%) shows that this extraction method has a low yield, taking as an alternative other types of extraction to obtain more oil.

SI seed has a high oil content, and in this research it was determined to contain a high content of unsaturated fatty acids (90.63%); and compared to the results obtained with seeds from Peru, it has a slight variance in the linolenic (9.36% difference) and oleic fatty acids (8.22% difference), concluding that the composition of polyunsaturated and monounsaturated fatty acids varies depending on the origin of the seed.

Compared to olive and fish oils which are commonly known for their high unsaturation level, it has a higher level of polyunsaturated fatty acids: 62.76% compared to olive oil and 31.09% compared to with fish oil. However, olive oil is rich in oleic acid α -9 and fish oil in linolenic acid α -3, which are beneficial depending on the consumer's need. By performing a physicochemical analysis of the three oils, it was possible to determine that the quality of SI oil is higher compared to the other two oils, being a less processed and lighter product.

Due to the high protein content present in SI oil, a subsequent study is recommended to obtain a possible product from the residue after the oil extraction.

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