Obtention, yields, chemical-microbiological properties and amino acids profile in a flour from Sacha Inchi (Plukenetia volubilis L.)

Priscilla. Mora-Aguirre¹, Luis. Romero-Hidalgo¹, Edgar. Landines-Vera¹, Roberto. Ordoñez-Araque*²³, Mario. Valdez-Díaz¹

¹Department of Chemical Engineering, Chemical Engineering Faculty, Universidad de Guayaquil (UG), Av. Delta y Av. Kennedy, Guayaquil, Ecuador
²Department of Nutrition and Dietetics, Faculty of Health and Welfare, Universidad Iberoamericana del Ecuador (UNIBE), Av. 9 de Octubre N25-12 y Colón, Quito, Ecuador
³Gastronomy School, Universidad de las Américas (UDLA), Av. de los Granados y Colimes, Quito-Ecuador

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Abstract
Sacha inchi seed (Plukenetia volubilis) has long been used for its high protein content and for its essential fatty acids (omega 3, 6 and 9). After obtaining the oil, a by-product called dry cake is generated, recent studies have shown that this cake, which is generally wasted, has a high protein content. In this investigation, the residue from the oil extraction from the Sacha Inchi seed was used, this dried cake was subjected to several treatments (dehydration, grinding and sieving) to obtain fine flour. To this product, chemical physical analyzes were performed (moisture, proteins, lipids and ashes), microbiological (microorganisms and mycotoxins) and amino acid profile. The flour had 47% of proteins, 10% of lipid fraction and 20% of fiber, the microbiological result showed that there was no presence of microorganisms due to bad manipulation or pathogens (E. coli), no presence of mycotoxins (furosin, ochratoxin and zearalenone), the most interesting result was found in the amino acid profile, the total amount of non-essential amino acids present in the flour was 601.32 g.kg⁻¹, while the essential amino acids the presence of threonine, valine, leucine and phenylalanine (77, 35, 40 and 50 g.kg⁻¹ respectively). These results allow us to determine that sacha inchi seed can not only provide its benefits with its oil, dry cake represents an interesting unconventional raw material because of its high protein content and can be used in the food industry to enrich different food products low in this macromolecule.

INTRODUCTION
Sacha Inchi (SI) (Plukenetia volubilis L.), also known as “Maní del Inca” is an oleaginous plant of the Euphorbiaceae family, native to the rain forest of the Andean region of South America (Maurer, 2012). This plant is also cultivated in other countries, like Colombia, Japan and Indonesia (Rawdkuen et al., 2016), as well as in Ecuador, a small country that is giving the first steps in the production of SI and the study of the chemical composition is very essential.

This seed is a good source of protein (~27%) and oil (~42%), in fact the quality of this oil is one of the
Table 1: Sacha Inchi moisture reduction by drying

| Weight (g) | Initial moisture in Sacha Inchi cake | Final moisture in Sacha Inchi cake |
|------------|-------------------------------------|----------------------------------|
| 466,525±7,97 | 36,665±0,96 | 7,83±0,09 | 4,775±0,005 | 1,02±0,005

best in this kind of seeds (Hamaker, 1992), has been reported that polyunsaturated fatty acids (PUFAs) has positive benefits regarding the cardiovascular inflammatory diseases and cancer, this has led people to think that these PUFAs are more beneficial than other dietary supplements (Gogus and Smith, 2010), for this reason many small oil seed factories have started the production of this product.

A by-product in the oiling extract process of SI is the pressed cake, the yield of this cake can reach amounts of more than 50% according to the extraction method used (Rawdkuen et al., 2016), for example, in a cold press extraction, the yield of the cake is around 68% (Valdiviezo et al., 2019). In the seed, the percentage of protein is approximately 33%, compared to others oilseeds, is relatively high (Hanssen and Markus, 2011), and this percentage is better in the pressed cake with an approximate value of 59% (Ruiz, 2013), the aminoacidic composition in the SI protein could reach or exceed the recommended amino acid scoring patterns by FAO (FAO, 2013), the SI press cake could give us a good contribution, especially in quality protein (Vásquez, 2017).

Since the SI cultivation is being developed in Ecuador, the residue from the seed after the oil extraction could be used for flour production, and allow the development of foods with composite flours, which are characterized by the quality of the amino acids (Duodu and Minnaar, 2011).

The purpose of this research was to obtain flour from the press cake obtained after the oil extraction in SI seeds, watching each parameter to obtain the yields in the process. In addition, a chemical and microbiological analysis were also determined, and a characterization of the protein in the final product. These results will be useful for SI oil seed producers and manufacturers.

**MATERIALS AND METHODS**

**Obtaining Sacha Inchi flour**

In this first step, dry cake samples are received after having extracted the most oil from the seed; as a result, a dry cake with minimal oil remnants is obtained. It must be on sealed plastic containers and avoid the presence of air inside the container.

The cake must not have impurities or contamination. The seeds were harvested in the San Vicente canton - Ecuador

Once the raw material is obtained, it is passed to a dryer; in this phase the excess moisture that could have been left in the raw material of the previous extraction of the oil will be removed. This step will help reduce the possibilities of proliferation of pathogens and mycotoxins that could develop in flour. This dryer is tunnel type, and the temperature was controlled at 65 °C for a period of two hours.

After having removed the excess moisture from the raw material, it is taken to a grinding equipment, for this an endless screw mill is used to achieve a good degree of fineness (100 mesh). The time the dried cake degreased spends in the mill is short, about 2 minutes.

Table 2: Mesh retention percentage

| Mesh size (mm) | Percentage withheld (%) |
|----------------|-------------------------|
| 0.6            | 0.00±0.01               |
| 0.5            | 0.00±0.01               |
| 0.4            | 0.19±0.03               |
| 0.3            | 0.26±0.02               |
| 0.2            | 0.89±0.04               |
| 0.1            | 98.67±0.03              |

Finally, to achieve the uniformity of the flour, it was sifted, separated in different flour fractions by difference in size through meshes with controlled porosities from 100 mesh to 600 mesh. For this step an AS 200 analytical sieve vibrator was used for 10 minutes, the desired degree of fineness is between 100 to 150 mesh, in case of obtaining a degree of fineness greater than this the material will be recirculated to the grinding process.

The following equation will be used to calculate the average particle size,

\[ APS = \sum (M_s \times P_w) \]  

Where APS is the average particle size, Ms is the Mesh size and Aw is the percentage withheld of flour.

**Physicochemical analysis**

**Moisture**
This parameter was determined by the method: AOAC 930.15.

**Protein**
The method used was AOAC 17th 984.13, to determine the percentage of protein present in the cake and flour of Sacha Inchi.

**Lipids**
The content of lipid material was determined using the Modified Folch method.

**Ash**
In this parameter the AOAC 942.05 method was used, to know the presence of inorganic material.

**Fiber**
Using the AOAC 978.10 method the fiber content was determined.

**Microbiological and toxicological analysis in Sacha Inchi flour**
For these analyzes the following parameters were determined:

- **Total aerobes**
The BAM-FDA CAP # 3 2001 method was used.

- **Total coliforms**
The BAM-FDA CAP # 4 2002 method was used.

- **Escherichia coli**
The BAM-FDA method CAP # 4 2002 was used.

- **Yeast and fungi**
The INEN 1529-10 1998 method was used.

- **Mycotoxin furosine**
The BAM-FDA method CAP # 3 2001 was used.

- **Mycotoxin ochratoxin**
The BAM-FDA method CAP # 4 2002 was used.

- **Mycotoxin zearalenone**
The BAM-FDA method CAP # 4 2002 was used.

- **Amino Acid Profile in Sacha Inchi Flour**
For this analysis the AOAC 994.12 method was used. The ACQUITY UPLC system (Waters, Milford, MA, USA) consisting of thermostat, autosampler, high-pressure binary pump and photodiode array detector PDA (an optical detector in the range ultraviolet-visible light that operates between 190 nm and 700 nm) was used for the analysis of 17 AAs. Chromatographic separation was obtained with the AccQ-Tag Ultra C-18 column (2.1 mm 9100 mm; 1.7 lm).

**Statistics analysis**
For the analysis three repetitions of each one were made, and they obtained the means with their respective deviations standard. In addition, a statistical analysis was made using Tukey’s ANOVA test using the Real Statistics program to determine the possible significant differences at $p < 0.05$ level.

**RESULTS AND DISCUSSION**

**Yields in the obtention of Sacha Inchi flour**
For the drying of Sacha Inchi cake, the results were successful, as shown in Table 1, a sample of 466,525 g were dried, which reduced its moisture by 6.81%, according with (Fortin, 2009) this parameter should not exceed from 13%.

![Figure 1: Drying speed](image)

Figure 1 shows the moisture loss of the wet cake per unit of time and surface area (Ocon and Tojo, 1998).

Using the Equation (1) the average particle size of the Sacha Inchi flour is calculated. As show in Table 2 a greater retention in the 1mm size mesh is evidenced, the average particle size is 1837 $\mu$m.

The yield of flour obtained at the end of the process is 91.6% $\pm 1.45$ the remaining percentage is due to the loss of moisture during the drying process and the particles retained in each equipment (Giraldo-Gómez et al., 2019).

**Physicochemical analysis in Sacha Inchi cake and flour**
As show in Table 3 the moisture decreases by 3.7% by the effect of drying. An increase of 3% is observed for the protein resulting in a flour with a high protein content, (FAO, 1980), indicating that the lower limit for a flour to be considered proteinic is 35%. Comparing these results with the obtained by (Rawdkuen et al., 2016), which hydrolyzed the cake protein giving a result of 45.9% indicates that this parameter is of high value.

The difference of lipids between both compounds does not represent a significant value. The remaining oil (10.5%) has a high content of unsaturated fatty acids (Maurer, 2012), which
Table 3: Sacha Inchi Cake and flour physical-chemical properties

| Parameter      | Sacha Inchi Cake | Sacha Inchi Flour | Units     | Method               |
|----------------|------------------|-------------------|-----------|----------------------|
| Moisture       | 8,18±2,1         | 4,48±2,2          | g.100g-1  | AOAC 930.15          |
| Protein        | 43,64±2,9        | 46,74±3,2         |           | AOAC 17th 984.13    |
| Lipids         | 10,7±1,3         | 10,5±1,2          |           | Modified Folch method|
| Ash            | 3,7±0,3          | 3,1±0,5           |           | AOAC 942.05          |
| Fiber          | 18,13±1,3        | 19,89±1,2         |           | AOAC 978.10          |

Table 4: Results and regulatory limits of microbiological and toxicologic analysis

| Parameter              | Results     | Regulatory limit | Units     | Method used               |
|------------------------|-------------|------------------|-----------|---------------------------|
| Total aerobes          | 20*10-2     | 5                | UFC.g-1   | BAM-FDA CAP # 3 2001     |
| Total coliforms        | <3.0        | 100              | UFC.g-1   | BAM-FDA CAP # 4 2002     |
| Escherichia coli       | <3.0        | 5                | UFC.g-1   | BAM-FDA method CAP # 4 2002|
| Yeasts                 | <100        | 5                | UFC.g-1   | INEN 1529-10             |
| Fungus                 | <100        | 5                | UFC.g-1   | 1998                     |
| Mycotoxin Furosin      | 0           | 1000             | μg.kg-1   | BAM-FDA method CAP # 3 2001|
| Mycotoxin Ochratoxin   | 0           | 5                | μg.kg-1   | BAM-FDA method CAP # 4 2002|
| Mycotoxin Zearalenone  | 0           | 1000             | μg.kg-1   |                           |

Table 5: Amino acid composition

| Essential amino acid   | Sacha Inchi (g.kg-1) | Sacha Inchi flour (g.kg-1) | Sacha Inchi protein* (g.kg-1) | Sacha Inchi Seeds** (g.kg-1) | Sacha Inchi Seeds*** (g.kg-1) |
|------------------------|-----------------------|-----------------------------|--------------------------------|-----------------------------|--------------------------------|
| Threonine              | 77,18±3,3             | 5±0,4                       | 17,335±2,3                     |                             |                                |
| Valine                 | 35,51±4,2             | 27±2,3                      | 33,61±1,3                      |                             |                                |
| Methionine             | 12,52±1,2             | 4±1,3                       | 6,2421±0,3                     |                             |                                |
| Isoleucine             | 40,07±3,4             | 32±2,3                      | 38,73±3,4                      |                             |                                |
| Leucine                | 60,79±3,3             | 76±3,4                      | 72,235±4,3                     |                             |                                |
| Lysine                 | 40,52±2,3             | 110±6,6                     | 178,49±7,8                     |                             |                                |
| Histidine              | 18,67±4,3             | 52±6,7                      | 93,345±2,3                     |                             |                                |
| Phenylalanine          | 50,09±5,4             | 73±2,3                      | 31,835±4,3                     |                             |                                |
| Tryptophan             | -                     | 13±1,5                      | 32,795±2,3                     |                             |                                |
| Total                  | 335,38±12,2           | 392±9,4                     | 504,61±10,5                    |                             |                                |

Non-essential amino acid

| Cysteine               | -                     | 21±3,2                      | 55,24±2,5                      |                             |                                |
| Tyrosine               | 63,29±2,2             | 61±2,3                      | 100,25±7,8                     |                             |                                |
| Glycine                | 40,52±3,3             | 12±1,4                      | 36,735±4,5                     |                             |                                |
| Glutamic acid          | 155,73±5,5            | 69±4,4                      | 79,825±6,7                     |                             |                                |
| Aspartic acid          | 113,16±6,6            | 33±4,6                      | 55,79±3,4                      |                             |                                |
| Arginine               | 132,05±3,3            | -                           | -                              |                             |                                |
| Alanine                | 48,49±6,6             | 13±1,4                      | 12,88±2,3                      |                             |                                |
| Proline                | -                     | 17±3,2                      | 13,36±1,3                      |                             |                                |
| Serine                 | 111,33±3,3            | 9±0,4                       | 16,62±1,7                      |                             |                                |
| Total                  | 601,32±9,8            | 153±6,5                     | 370,7±11,5                     |                             |                                |
have anti-inflammatory properties and offer protection against heart disease and high blood pressure (Williams, 2011).

The ash have a minor percentage for the Sacha Inchi flour (3.1%) and is within the limit recommended by (FAO, 1980).

The presence of fiber in Sacha Inchi flour (19.89%) makes it a good food supplement, because it generates an increase in peristaltic movements of the intestine, facilitates transit and intestinal distention (Badui, 2006).

Microbiological and toxicological results in Sacha Inchi flour

The results of the microbiological tests as shown in Table 4, determine that according to the limits established by (CODEX-FDA), the samples satisfy the parameters necessary for their use as an ingredient in the production of various food products.

Amino acid composition and comparison with other studies

The amino acid content in the flour from the Sacha Inchi cake is higher in these four essential amino acids Threonine, Valine, Methionine and Isoleucine compared to the samples from the seed and protein extract shown in Table 5, (Rawdkuen et al., 2016, 2018), however the content of other essential amino acids such as Leucine, Lysine, Tryptophan and Histidine was much higher in the samples of protein extract and Sacha Inchi seeds. The comparison of the essential amino acids present in different samples of Sacha Inchi not only indicate their differences, it also allows to demonstrate the high content of essential amino acids they have and determine that they can be used as ingredients of food products such as granola bars, cookies, noodles and drinks as described by (Jagersberger, 2013), in addition to comparing the results with the dietary requirements of essential amino acids in humans, it can be determined that the consumption of 100 grams of Sacha Inchi cake flour per day could accomplish the needs of 10 mg.kg⁻¹.day⁻¹ of histidine, 39 mg.kg⁻¹.day⁻¹ of leucine, 20 mg.kg⁻¹.day⁻¹ of isoleucine, 15 mg.kg⁻¹.day⁻¹ of methionine, 25 mg.kg⁻¹.day⁻¹ of phenylalanine, 30 mg.kg⁻¹.day⁻¹ of lysine, 15 mg.kg⁻¹.day⁻¹ of threonine, mg.kg⁻¹.day⁻¹ of tryptophan and 26 mg.kg⁻¹.day⁻¹ of valine (Joint FAO/WHO/UNU Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition (Geneva, 2002). Finally, regarding the content of non-essential amino acids, Sacha Inchi cake flour has a total of 601.32 g.kg⁻¹, compared to the total content of Sacha Inchi protein isolate 153 g.kg⁻¹ and the seed of Sacha Inchi 370.7 g.kg⁻¹; These results demonstrate that the Sacha Inchi cake flour has a greater amount of non-essential amino acids than the other two types of products of Sacha Inchi.

CONCLUSIONS

The process of obtaining Sacha Inchi flour gives a high yield (91.6%) which provides a high content of proteins, fiber and lipids with a high percentage of unsaturated fatty acids. The proportion of essential amino acids such as Leucine, Lysine, Tryptophan and Histidine is higher compared to other studies. This product meets the daily requirements of most essential amino acids.

Conflicts of interest

The authors declare that they have no conflict of interest for this study.

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