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Chemical composition and physical properties of the fiber fraction of different production batches of *Plukenetia volubilis* L. Cake

Composición química y propiedades físicas de la fracción fibrosa de diferentes lotes de producción de torta de *Plukenetia volubilis* L.

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To determine the chemical composition (dry matter, crude protein, ash, neutral detergent fiber, acid detergent fiber, cellulose, lignin and hemicellulose), and physical properties (solubility, water adsorption capacity and volume) of three batches of *Plukenetia volubilis* L. (Sacha inchi) cake, as well as its mineral profile (Ca, P, Mg, K, Na) and secondary metabolites, a simple classification design was applied and differences were analyzed using ANOVA and Duncan test ($p<0.05$) with the use of INFOSTAT. Results showed significant differences ($p<0.05$) in NDF (batch three: 25.82 % vs. batches one and two: 28.11-29.90 %), DM, ADF, and cellulose, which did not vary among all batches. There were no differences ($p>0.05$) in CP, lignin, hemicellulose, nor in the physical properties. Batch three showed a higher content of macrominerals (except phosphorus) compared to batch two, while batch one showed intermediate values: Ca (0.19 %), P (0.79 %), Mg (0.12 %), K (0.24 %) and Na (0.44 %). Saponins and α-amino groups were identified qualitatively, being abundant in all batches. It is concluded that the chemical composition of Sacha inchi cake varies among batches, except for protein, lignin, and physical properties. Batch three stood out for its lower content of NDF and higher mineral concentration, suggesting its potential preference for nutritional applications.

Keywords: *byproduct, fiber, macrominerals, Sacha inchi, secondary metabolites*

Para determinar la composición química (materia seca, proteína bruta, cenizas, fibra detergente neutro, fibra detergente ácido, celulosa, lignina y hemicelulosa) y propiedades físicas (solubilidad, capacidad de adsorción de agua, volumen) de tres lotes de torta de *Plukenetia volubilis* L. (Sacha inchi), así como su perfil mineral (Ca, P, Mg, K, Na) y metabolitos secundarios se aplicó un diseño de clasificación simple y se analizaron las diferencias mediante ANOVA y prueba de Duncan ($p<0.05$) con el uso del INFOSTAT. Los resultados mostraron diferencias significativas ($p<0.05$) en FDN (lote tres: 25.82 % vs. lotes uno y dos: 28.11-29.90 %), MS, FDA y celulosa, que no variaron entre todos los lotes. No hubo diferencias ($p>0.05$) en PB, lignina, hemicelulosa ni en las propiedades físicas. El lote tres presentó mayor contenido de macrominerales (excepto fósforo) con respecto al lote dos, mientras que el lote uno mostró valores intermedios: Ca (0.19 %), P (0.79 %), Mg (0.12 %), K (0.24 %) y Na (0.44 %). Se identificaron cualitativamente saponinas y grupos α aminos, abundantes en todos los lotes. Se concluye que la composición química de la torta de Sacha inchi varía entre lotes, excepto en proteína, lignina y propiedades físicas. El lote tres se destacó por su menor contenido de FDN y mayor concentración de minerales, lo que sugiere su potencial preferencia para aplicaciones nutricionales.

Palabras clave: *fibra, macrominerales, metabolitos secundarios, Sacha inchi, subproducto*

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Introduction

Oilseeds are one of the most produced, researched, and marketed crops in the world. The harvested seeds are used in the oil industry to obtain oil through various extraction methods (Dellepiane *et al.* 2023). Among them, the most well-known are cylinder press extraction and solvent extraction. As a result, the residual cake is produced, which is generally characterized by having a high content of proteins and ashes (Vichare and Morya 2024).

In Cuba, the Center for Research in Protein Plants and Bio-natural Products (CIPB, initials in Spanish) works on various projects to obtain new products from oil and residual cake from the processing, to make the best use of seeds intended for human consumption. *Plukenetia volubilis* L. (Sacha inchi) is one of the raw materials used.

Scull *et al.* (2022) researched Sacha inchi cake, using seeds harvested in the west of Cuba (Pinar del Río) and later processed at the CIPB. The residual cake showed a high crude protein content (54.41 %), moderate neutral detergent fiber (NDF) (30.97 %) and acid detergent fiber (ADF) (11.84 %), and an adequate ash content (5.05 %). However, a high presence of some secondary metabolites, such as terpenoids and α-amino groups, was identified.

As alternative feed for animals, cakes have two main drawbacks: the presence of dietary fiber and secondary metabolites (Vujetić *et al.* 2025). These two components could cause different physiological effects, complicating their use in feed when present in high concentrations (Srichamnong *et al.* 2018). Moreover, they limit the potential of the byproduct, especially when high doses are used (Zajac *et al.* 2020). Therefore, the objective of this study was to determine the chemical composition and physical properties of the fibrous fraction from different production batches of *Plukenetia volubilis* L. (Sacha inchi) cake.

Materials and Methods

The study was carried out at the Institute of Animal Science (ICA), located in San José de las Lajas municipality, Mayabeque province, Cuba, between 22º 53' N and 82º 02' W at an altitude of 80 m a. s. l. (Herrera 1976).

Obtainig Sacha inchi cake: The residual cake from *Plukenetia volubilis* L. was obtained from seeds harvested in Mayabeque, Cuba, with a planting-harvest interval of seven months. The processing consisted of oil extraction through cold pressing (cylinder extractor press), following the ONO: PO. III.49 protocol from the Center for Research in Protein Plants and Bionatural Products. The resulting cake was stored in polypropylene bags, placed on pallets to avoid contact with the ground, in a refrigerated chamber until its analysis.

Sampling: Three batches of Sacha inchi cake were evaluated, corresponding to different production months: batch one (December 2022), batch two (February 2023), and batch three (May 2023). The number of batches available at the oil extraction plant at the time of sampling was sufficient to evaluate compositional variability and identify differences due to harvest and processing conditions.

Each batch represented an independent experimental unit (n=3). From each, six bags (replicates of primary sampling) were randomly selected. The content of each bag was spread over a sterile, flat surface, and subsamples were collected from the four corners and the center. Subsamples were mixed to obtain 1 kg of homogeneous material per batch, which was then sealed in airtight bags and sent to the Central Unit of Laboratories of the Animal Science Institute (UCELAB) for physical and chemical analysis.

Determination of chemical composition: Contents of dry matter (DM), crude protein (CP), ashes, and macrominerals were determined according to the methodology described by the AOAC (2019). For the extraction of NDF, ADF, lignin, cellulose, and hemicellulose, the fractionation method of Goering and van Soest (1970) was used. Each analysis was performed in duplicate.

Determination of physical properties: Solubility was determined by the method of Seoane *et al.* (1981). For this, samples were screened through a sieve of 0.8 mm. Then, they were placed in an oven at 105 °C overnight.

Solubility: An amount of 60 mL of distilled water was added to 2 g of dry sample, let to rest for 1 hour, filtered, and placed in an oven at 60 ºC for 12 hours. Later, the dry residue was weighed.

This property was determined by the calculation of:

$$Solubility,\ (\%) = \left[\frac{2\ g\ of\ sample - dry\ residue\ weight}{2\ g\ of\ sample}\right] * 100$$

Water adsorption capacity (WAC): It was determined according to Savón *et al.* (1999). The calculation was performed using the formula:

$$WAC,\ \left(\frac{g}{g}\right) = \frac{wet\ sample\ weight\ - dry\ sampe\ weight}{dry\ sampe\ weight}$$

Volume. An amount of 1g of dry sample was taken, centrifuged at 3,000 rpm for 20 minutes, and the volume occupied by the sample after centrifugation was measured. It was expressed in mL/g.

Qualitative composition of secondary metabolites: The content of secondary compounds was qualitatively analyzed according to the methodology of Miranda and Cuellar (2000). To carry out the phytochemical screening, six samples were taken in duplicate of 5 g each and subjected to a maceration extraction process with 90 % v/v ethanol for 48 hours. Then, they were filtered by gravity, and the filtrate was stored in amber glass bottles. Subsequently, the soluble substances in each extract were determined, and the crossing system was used to indicate the presence or absence of metabolites.

Table 1. Physical and chemical composition of Sacha inchi cake, produced in Cuba

Indicators, %	**Batches**			**SE±**	**p-value**
	One	**Two**	**Three**		
DM	95.96[b]	96.71[a]	94.48[c]	0.15	<0.0001
CP	53.34	53.40	53.62	0.40	0.8696
Ashes	4.91[ab]	4.55[b]	5.18[a]	0.13	0.0154
NDF	28.11[a]	29.98[a]	25.82[b]	0.68	0.0022
ADF	13.31[b]	16.24[a]	10.18[c]	0.81	0.0004
Cellulose	9.19[b]	12.37[a]	7.00[c]	0.66	0.0002
Lignin	3.41	3.21	2.68	0.28	0.1970
Hemicellulose	14.81	13.74	15.64	0.80	0.2754
Solubility	21.37	20.34	20.48	0.63	0.4740
Water adsorption capacity, g/g	5.66	5.75	5.63	0.11	0.7449
Volume, mL/g	2.38	2.29	2.50	0.07	0.1697

[a,b,c]Values with different letters in the same row differ significantly at P<0.05 (Duncan 1955)

Experimental design and statistical analysis: A simple classification design was used for the variables of chemical composition (DM, CP, ashes, NDF, ADF, cellulose, lignin and hemicellulose), physical properties (solubility, water adsorption capacity and volume), and mineral content (Ca, P, Mg, K and Na) in three batches of raw Sacha inchi cake. Differences among batches were analyzed using ANOVA, and when means were significant (p<0.05), they were compared using Duncan (1955) test. INFOSTAT software (di Rienzo *et al*. 2012) was used for data processing.

Results and Discussion

Table 1 shows the chemical composition and physical properties of the fiber fraction of Sacha inchi cake. These last did not show differences among batches, nor did protein, lignin, and hemicellulose.

DM was elevated, indicating low moisture content in the Sacha inchi cake. This result is important when storing products intended for food, because, according to Sánchez *et al*. (2020), the nutritional quality depends on it. However, batch three showed the lowest value compared to batch two and batch one. The result could be related to the storage time of the cake.

High levels of crude protein were obtained, compared to soy bean (42 %) according to data of Terrien (2017). Results are lower than the 54.41 % of CP reported by Scull *et al.* (2022). This can be related to the harvest period and the region where they were obtained. Unlike these authors, who acquired their cake batches in the second half of 2020 from Pinar del Río province, the batches evaluated in this experiment corresponded to the months of December 2022 and February and May 2023 in Mayabeque province.

CP content obtained was higher than that reported by Alcívar *et al.* (2020) in the Sacha inchi cake obtained in Ecuador (41.49 %), who used the high-temperature cylindrical extractor press method. This contradicts what Mannucci *et al.* (2019) stated about the decrease in protein content of cakes extracted in low tempratures, compared to processing that uses high temperatures, in which concentrations tend to be higher. It is possible that the edaphoclimatic conditions of the plant cultivation influenced the protein levels of each cake.

NDF tenors were lower in batch three compared to the rest. ADF and cellulose were different for all batches, with the highest values in batch two. Differences among batches are related to DM content of the cake, which, in turn, depends on its storage time.

NDF and ADF values were higher than those reported for Sacha inchi cake by Alcívar *et al*. (2020) in Ecuador (16.64 and 12.70 %, respectively). They are within the range of different oilseed cakes that come from plant species, whether from tropical or temperate climates (between 19 and 52, and between 13 and 42 %, respectively). This is also the case for amarula (*Sclerocarya birrea*), macadamia (Integrifolia), and baobab (*Adansonia digitate* L.) in Africa (Solomon 2018), and two canola species (*Brassica napus* and *Brassica juncea*) in Canada (Oryschak *et al*. 2019).

According to Alcívar (2022), the composition of carbohydrates in Sacha inchi cake is influenced by factors such as plant genetic, which is directly affected by the climate of the area in which it is grown. Furthermore, it can vary depending on the form in which the oilseed is presented, whether in meal, by grinding the seed directly, or in the byproduct of oil extraction. The latter also depends on the type of processing the seed undergoes to obtain the oil.

However, generally speaking, indicators of fibrous fractionation are considered low when compared to other fibrous oilseeds such as royal palm nuts, with values of NDF and ADF of 72.55 % and 55.84 %, respectively (Martínez-Pérez *et al.* 2021). Hemicellulose:cellulose ratio was 1.11, 1.61, and 2.23 for batches two, one, and three, respectively. According to Marrero (1998), the higher this ratio in food, the more the fibrous component could be degraded by the animal. This aspect, combined with low cell wall composition, could indicate better digestibility of Sacha inchi cake in monogastric animals, aspects that should be researched in future studies. Results of the physical properties showed intermediate solubility values. In the *Sacha inchi* cake produced in Ecuador, Alcívar *et al*. (2022) reported lower values (7.96 %). Wang *et al*. (2018) noted that, in Sacha inchi seeds, insoluble dietary fiber represented 72.40 % and soluble fiber, 9.0 %. Differences among studies may be related to the methods for obtaining the cake that were previously discussed and to the relationship of the different chemical compounds.

Lignin values were low and perhaps the insoluble hemicelluloses prevail. These components, as stated by Martínez-Pérez (2010), are part of the insoluble fiber fraction. Furthermore, they are responsible for regulating the gastrointestinal tract function of monogastric animals. According to Hua *et al.* (2019), insoluble dietary fiber promotes peristalsis, increases fecal volume, interferes with nutrient digestibility, and absorbs and eliminates fat, certain metals, and other toxic substances.

Sacha inchi cake showed that it is a bulky byproduct and has low water absorption capacity. According to Wrolstad (2012), insoluble dietary fiber is characterized by a lower capacity for water retention, as observed in the present study. Mudgil (2017) suggested that water absorption capacity is nothing more than the ability of fiber to swell and retain water in its fibrous matrix, which is related to the fibrous content and depends on the relative proportions of the polysaccharides that compose it. Hemicellulose has greater hygroscopic power than cellulose, and it is almost zero in lignin (González *et al.* 2007). This explains the performance of WAC in Sacha inchi cake, which showed low hemicellulose and lignin levels.

The composition of macrominerals was higher in batch three compared to batch two (table 2). Batch one did not differ from these two for calcium and potassium, while it decreased and increased, respectively, for phosphorus. For magnesium, it was similar to batch one. These results correspond with those recorded in the ash concentration (table 1), which is low when compared to soybean, rapeseed, and sunflower cakes (6.8, 5.6-7.0, 5.7-7.5 %, respectively) (Arrutia *et al*. 2020). Ash content and, therefore, minerals present in the cake can be attributed to the edaphoclimatic characteristics of the plant cultivation (Martínez 2022).

As demonstrated in table 2, phosphorus was the most abundant macromineral in the cake. The calcium-phosphorus relation is essential, as when serum calcium concentrations increase, phosphorus concentrations decrease and vice versa. This relation varies depending on animal species and life stage. Calcium and phosphorus compete with each other in intestinal absorption. Both are essential for bone health and maintaining the optimal mineral balance in the body. They are essentially located in bones, and they participate in the formation of the skeleton and teeth (Dolores 2020).

Calcium acts in muscle contraction, nervous excitability, blood coagulation, and in the secretion of exocrine glands. Phosphorus has important metabolic functions, participating in enzyme regulation and energy storage. It is a component of nucleic acids and phospholipids present in tissues (Carbajal 2017).

Magnesium is involved in the maintenance of bones, teeth, and heart. It participates in energy metabolism, glucose release, and protein formation. It also regulates the function of muscles and the nervous system, blood pressure, and electrolyte balance. It contributes to DNA formation and to the reduction of tiredness and fatigue. Magnesium is involved in bone formation and it is essential for a large number of enzymatic reactions (Sarli 2015).

Potassium and sodium also showed elevated values, followed by phosphorus (table 2). Sodium-potassium pump is an important physiological process that takes place among cells, during which an exchange of potassium ions for sodium ions occurs. It is known as an active transport process,

Table 2. Macromineral composition of Sacha inchi cake

Indicators (%)	Batches			SE±	p-value
	One	Two	Three		
Ca	0.19[ab]	0.17[b]	0.22[a]	0.010	0.0124
P	0.79[b]	0.72[c]	0.85[a]	0.020	0.0006
Mg	0.12[b]	0.13[b]	0.14[a]	0.003	0.0045
K	0.24[ab]	0.23[b]	0.26[a]	0.010	0.0255
Na	0.44	0.49	0.38	0.090	0.6586

[a,b,c]Values with different letters in the same row differ significantly at P<0.05 (Duncan 1955)

essential for cellular function. The main function of potassium (intracellular element), sodium, and chlorine (extracellular elements) lies in the regulation of cellular osmotic pressure (Montagud 2020).

The qualitative analysis of the secondary metabolites is shown in table 3. The three batches showed similar results, with no differences for each group of studied compounds. The saponin test conducted on the sample from each batch showed positive results, with a sustained foam index of approximately 2 cm for more than 5 minutes. These compounds have surfactant properties, meaning that they reduce the surface tension of a liquid and create foam (Góngora-Chi *et al*. 2023). The abundant presence of saponins in the three batches can be associated with the variety of the plant or the cropping conditions, such as soil, temperature, and irrigation.

Saponins can decrease food palatability, inactivate the dietary metabolism of proteases, lipases, and amylases, which are necessary for digestibility (Lozada-Salcedo 2017). The saponins present in oilseed cakes such as soybean, sunflower, and hemp have been attributed negative effects. Hemolysis, interference with bile acids, liposoluble vitamins, cholesterol, and dietary lipids are described (Ancuta and Sonia 2020).

The presence of α-amino groups in the samples was positive for all three batches and took on a bluish-purple coloration. Among the secondary metabolites that are present in these groups are lectins, protease inhibitors (trypsin and chymotrypsin), and non-protein amino acids. These have different anti-nutritional effects when found in high concentrations.

Trypsin inhibitors, for example, may reduce protein digestibility when their presence is abundant in oilseed cakes such as soybean, sunflower, sesame, peanut, hemp, and flax (Ancuta and Sonia 2020). In soybean cake, the abundance of these compounds can inhibit growth (Loeffler *et al*. 2013) and increase pancreas size in growing chickens (Beukovic *et al*. 2012).

In the consulted scientific literature, there were not enough studies on Sacha inchi cake, where antinutritional effects in animals are associated with high concentrations of secondary metabolites. Hurtado-Ramírez *et al*. (2014) linked hepatotoxic effects and changes in the normal levels of hematocrit, hemoglobin, and alanine aminotransferase in Hy Line Brown laying hens, which consumed Sacha inchi cake, due to the abundant presence of saponins. For these reasons, it is necessary to use different processing methods to reduce the content of secondary metabolites.

The presence of phenols in the samples from the three batches was negative, and there was no variation when adding $FeCl_3$. The absence of a change in coloration indicates that evaluated cakes do not contain phenols in significant amounts, with concentrations too low to be detected in the test. In the analyzed samples corresponding to the three batches, alkaloids and flavonoids were also not identified. The result for tannins was inconclusive, due to their low concentration, or the presence of other compounds that may have interfered with the reaction.

Table 3. Qualitative analysis of the secondary metabolites in *Sacha inchi* cake (batches 1, 2 y 3)

Trial	Results (Batches 1, 2 and 3)
Saponins	+++
α amino groups	+++
Phenols	---
Tannins	+/-
Alkaloids	---
Flavonoids	---

+++: Positive ---: Negative +/-: Unfinished

Conclusions

According to the findings, it is concluded that the chemical composition of *Plukenetia volubilis* L. (Sacha inchi) raw cake varies among production batches, except for the content of protein, lignin, hemicelluloses, and the physical properties of the fibrous fraction. This demonstrates the importance of considering the differences among batches when working with this by-product in future research.

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