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RESEARCH ARTICLE

REVISED Characterization of sacha inchi (*Plukenetia volubilis*) and taro (*Colocasia esculenta*) flours with potential application in the preparation of both gluten-free and high protein foods.

[version 4; peer review: 1 approved, 2 approved with reservations, 2 not approved]

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Abstract

Background

Interest in alternative sources of tubers and legumes has increased due to the search for raw materials with bioactive antioxidant compounds. The objective of this study was to characterize taro (TF) and sachá inchi (*Plukenetia volubilis*) flours obtained by the wet (SIF-WM) and defatted (SIF-DM) methods, as alternatives for the formulation of functional foods.

Methods

Proximal tests were conducted to determine basic chemical composition, quantification of free polyphenols, antioxidant activity using the ABTS* radical method (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) with Trolox as a standard, and rheological analyses, including pasting curves, flow profiles, and viscoelastic properties. Microbiological characterization of the flours was also performed.

Open Peer Review

Approval Status

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Results

The highest protein content was found in sachu inchi flour obtained by the defatted method (72.62%), while carbohydrates were the main component in taro flour (85.4%). In terms of antioxidant activity, taro flour showed higher values of 2.71 $\mu\text{mol ET/g}$ and 7.47 mg EAG/g. Rheological analysis showed that adding taro flour increased the viscosity peak and reduced breakdown, improving gel stability. *Staphylococcus aureus* and *Salmonella spp.* were detected in taro flour.

Conclusions

Taro and sachu inchi flours have great potential for the development of functional products like protein snacks, with good expansion due to taro starch and are naturally gluten-free, making them suitable for people with celiac disease.

Keywords

Rheology properties, Proximal composition, Microbiological quality, Gluten-free foods

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REVISED Amendments from Version 3

The manuscript has been revised to improve clarity and precision. The abstract has been reworded to enhance its structure and readability. The section on rheological results has been refined for better articulation of findings. Additionally, the methodology has been expanded to include detailed procedures for quantifying ash content, phenolic compounds, and antioxidant capacity. The conclusions have also been rewritten to provide a more concise and clear summary of the study's key insights. Furthermore, Figure 5 has been updated according to the reviewer's recommendations for better visual clarity and accuracy.

Any further responses from the reviewers can be found at the end of the article

Introduction

According to Wang et al.,¹ by 2050, if global governance fosters debate and ensures equity in the food system, and if food demand increases significantly due to rapid population growth, intensive land use will be required to meet the demand, particularly for meat and dairy products, which are vital sources of protein, vitamins, and minerals in the human diet.² However, intensive livestock production is environmentally unsustainable due to its high demand for resources, such as land for growing animal feed and fresh water. Furthermore, this activity significantly contributes to greenhouse gas emissions, exacerbating global warming.³ Consequently, a viable strategy to ensure adequate protein intake is to adopt an optimal combination of plant-based proteins.

In this scenario, we must design food systems to contribute to global environmental sustainability and meet nutritional needs, proposing diets based on plants that allow including adjustments according to each region coupled with cultural customs.⁴ Underutilized or orphan crops are used to design original foods, not aimed at international trade, however, because of their high adaptation to the local environment, these crops play an important role in regional nutritional security.⁵ The mixture of plants such as cereals, pseudocereals, legumes, tubers, among others, are used to create different products with adequate balance of protein, carbohydrates, fiber and micronutrients such as vitamins and minerals.

For example, sacha inchi (*Plukenetia volubilis*) known as wild peanut, Inca peanut, sacha peanut or mountain peanut, is an oleaginous plant that belongs to the Euphorbiaceae family.⁶ Today, sacha inchi is still cultivated in the lowlands of the Peruvian Amazon and has been planted for centuries by the indigenous population.⁷ In Colombia, production exceeds 2,400 tons of sacha inchi seed; the department of Putumayo is the largest producer of sacha inchi with 282 hectares, followed by Valle del Cauca, Caquetá and Antioquia.⁸

Sacha inchi is a promising and industrializable food native to the Amazon that has essential unsaturated fatty acids such as omega-3, as alpha-linoleic acid with 47.7% to 51.9% and omega-9 as oleic acid with 7.9 to 8.9% by weight of oil, 27.4% protein, 4% ash and about 50% oil.⁹ These nutritional properties make sacha a favorable food for health and a suitable crop for developing high-protein and gluten-free foods. However, sacha inchi can be unstable due to its high concentration of unsaturated fatty acids, which makes it sensitive to oxidation.¹⁰ In addition, sacha inchi has limiting amino acids such as lysine and tryptophan that can cause low digestibility.¹¹ Likewise, this seed has anti-nutritional factors¹² that limit its use as raw material in the development of new products.

Taro is a tuber abundant in starch of which 17-28% is amylose and the remaining is amylopectin.¹³ Taro has a high content of resistant starch that allows slow digestion with valuable effects on cholesterol and blood glucose regulation.¹⁴ Taro has a high carbohydrate (59.36%) and protein (24.99%) content, with respect to the mineral content of taro, the highest amount present is magnesium 242.373 mg/kg, followed by calcium 94.455 mg/kg, iron 8.351 mg/kg and zinc 6.210 mg/kg, and, in relation to vitamins, is reported content of vitamin C 0.188 mg/100 mg, vitamin B1 0.047 mg/100 mg, and vitamin B3 0.078 mg/100 mg.¹⁵ Compared to other tubers such as sweet potato, potato, cassava and yam; taro has a higher protein and fat content.¹⁵ Likewise, taro has flavonoids and phenolic acids that have antioxidant properties; flavonoids, the largest group of phenolic compounds identified in the whole plant, are associated with reducing many degenerative diseases.¹⁴ However, taro contains antinutrients such as oxalates, which can limit its consumption in fresh form due to the irritation they cause in the throat and mouth.¹⁶ Nevertheless, the extrusion process significantly reduces these drawbacks and simultaneously improves nutritional properties, such as increased protein digestibility. Based on this, combining Sacha inchi and taro is an excellent option to complement each other's nutritional properties, enhancing nutritional value and improving the stability and texture of the final product.

Foodstuffs are complex systems of great nutritional richness and therefore sensitive to attack by microorganisms (bacteria, fungi and yeasts). The main mode of contamination of raw materials is animal defecation, manure fertilization and recontamination persisting in facilities and transport; in addition, insects and rodents are a source of contamination.¹⁷

The low water activity ($a_w < 0.60$) of meal does not favor microbial growth; however, contaminating spores along with inactive microorganisms will remain viable for prolonged periods and constitute a potential health hazard. As a persistence mechanism, *Salmonella spp.* and other pathogens form biofilms that protect against disinfection and increase their tolerance to drying processes.¹⁷

In food matrices there is always a microbial load that must not exceed certain limits, according to Colombian regulations, which must be controlled to avoid the deterioration of the product and the consequent loss of its quality and suitability for consumption.

The design and production of new foods that respond to food and nutritional security in the midst of climate change in low-income countries is a challenge, so this study aimed to characterize the chemical, microbiological, rheological and bioactive properties of individual flours and mixtures of taro and sachá inchi, as potential foods applied in the formulation of different foods.

Methods

The taro tubers were bought in the Municipality of Orito Putumayo, located at 0° 38' North Latitude and 76° 37' West Latitude of Greenwich. Average temperature of 25 °C and relative humidity of 88%. The fresh sachá inchi almonds were supplied by the company Fruty Amazónicos S.A.S. located in the village of La Concordia in the municipality of Valle del Guamuez, located at 00° 25" north latitude and 76° 54" west longitude. Temperatures range between 27 °C and 40 °C.

Obtaining taro flour

Taro flour (TF) was obtained following the methodology described by Quezada Correa et al.¹³ The raw material was received, the peel was removed; it was washed to eliminate impurities and then the edible part was cut into slices to facilitate dehydration; then it was weighed on a FENIX Electronic Weight Only Table Scale to verify yield, subsequently it was dried in a rotary oven (ORVES, Colombia) at a temperature of 60 °C for five hours to eliminate excess moisture. The dried slices were subsequently ground in an electric mill (Quaker City Mill, model 4-E, Philadelphia) and then sieved through a 30-mesh system to achieve homogeneity in the flour particle size. They were then stored in airtight bags until further use.

Obtaining sachá inchi flour

The sachá inchi seeds were received as white kernels, meaning without shell or husk. For the compositional analysis of the kernel, they were manually ground with a mortar to reduce their size, thus facilitating the relevant tests.

Wet method

The sachá inchi flour obtained by the wet method (SIF-WM) was prepared as follows: initially, wet grinding was carried out using a blender (Oster, BLST 4655, Colombia) with a capacity of 1.25 L. For this process, sachá inchi seeds were mixed with water in a 1:3 ratio to ensure effective grinding. This resulted in a milky and homogeneous suspension. Subsequently, a cloth filter was used to separate the insoluble extract (cake) from the water-soluble extract (milk). The cake was then dried in an oven at 65 °C for 3 hours. Finally, once dehydrated, it was immediately packaged in a polyethylene bag with a hermetic seal for preservation.¹⁸

Defatted method

The defatted sachá inchi flour (SIF-DM) was obtained as follows: the sachá inchi kernels were placed in the hopper of an automatic touchscreen oil extraction press (CGLDENWALL, model K28, Shanghai, China), as shown in Figure 1. The process was carried out at a temperature of 124 °C. During the extraction, a screw conveyor pushed the material into the main pressing cylinder. As it moved forward, the design of the screw, with decreasing pitch and spiral depth, reduced the available volume in the chamber, subjecting the material to high pressure and friction against the closed bottom wall of the cylinder. This facilitated efficient oil extraction, with the oil flowing through the cylinder's orifices, while the residual material, known as cake, was expelled through the discharge nozzle. Subsequently, the cake was ground using an electric mill (Quaker City Mill, model 4-E, Philadelphia) and sieved through a system with a No. 30 mesh to achieve uniform particle size. Finally, the resulting flour was packaged in polyethylene bags with airtight seals for preservation.

Proximate analysis

The proximate composition (protein, lipids, dietary fiber, ash and moisture) of taro and sachá inchi flours was determined according to the methods proposed by the AOAC (Association of Official Analytical Chemists, 1990)¹⁹

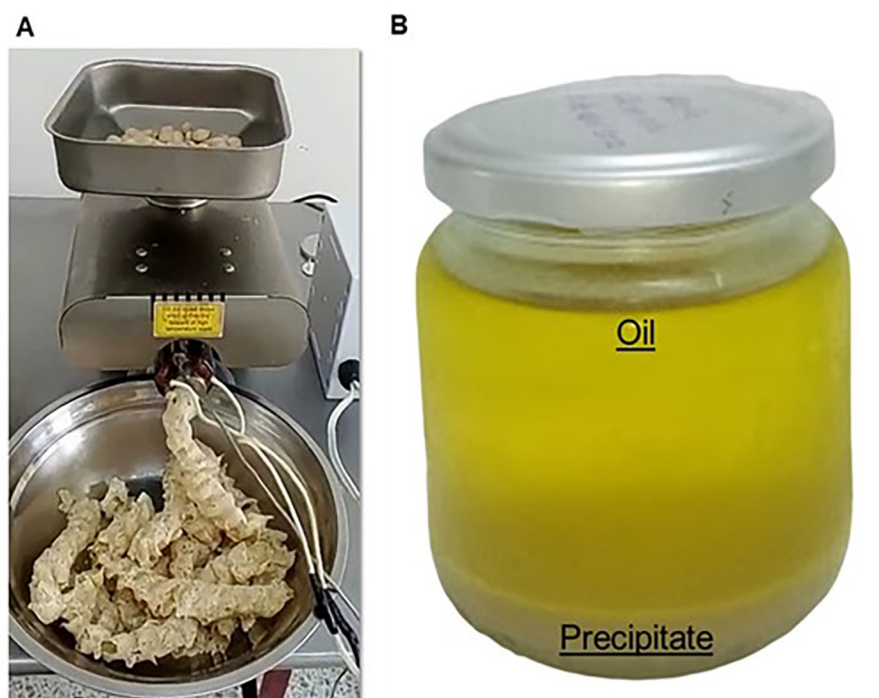


Figure 1. (A) Defatting process of sachu inchi, (B) Oil obtained after mechanical extraction.

and the carbohydrate content was estimated by difference. The details of the method are explained in more detail in the protocols uploaded in the repository.

Water activity measurement

To determine the a_w of the raw materials, the powdered sample was placed in a Decagon Pawkit portable water activity meter until the cuvette was covered. Before reporting the measurement, the equipment was calibrated with standard salts at 0.25 (13.41 mol/Kg LiCl 0.25 a_w) and 0.76 (6.00 mol/Kg NaCl 0.76 a_w).

Protein

For the determination of protein content, the protocol established by AOAC 968.06 was followed. On nitrogen-free paper, 0.2 to 0.8 g of sample plus 1 g of kjeldahl catalyst were weighed and placed in digestion tubes. 10 mL of sulfuric acid were added. Then, gentle heating was initiated until no foaming or splashing was observed, using a temperature ramp as follows: 125 °C for 30 min, 270 °C for 30 min and 400 °C for 140 min.

The samples were digested until they were completely clear and translucent, free of organic matter, in the laboratory Kjeldahl digester (Raypa MBC-6/N, Spain).

Then they were cooled to room temperature in the Raypa distillation unit, after which each sample was titrated with 0.1 N HCl.

Lipids

For lipid testing, 1 g of sample was weighed in the extraction cartridges, then 80 mL of petroleum ether was added, immediately transferred in the rack to the laboratory Soxhlet and Randall Extractor (SX-6MP, RAYPA, Spain). After the extraction time and solvent recovery, the samples were placed in an oven at 60 °C for 1 hour to eliminate the remaining ether.

Fiber

The fiber content was analyzed according to the AOAC 962.09 protocol. For fiber testing, 1-2 g of the degreased sample were transferred to the laboratory fiber extractor (F-6P Fibertest, Spain) and fixed to the angle of the front part of the unit. Then 150 mL of 0.255 N H₂SO₄ was heated in an Erlenmeyer flask, when it was boiling, it was placed on top of the coolant, the heating knob was adjusted to boiling point 3 or 4 and left boiling for 30 minutes. At the end of this time, it was filtered and washed with distilled water and the operation was repeated 3 times using 30 mL of water each time. Then, 150 mL of sodium hydroxide solution preheated to 90 °C was placed in the upper part of the cooler; it was brought to boiling and kept for another 30 minutes. Then it was filtered and washed three times with boiling water. The sample was then placed in an oven at a temperature of 100-110 °C until a constant weight was obtained.

Ash

The ash content was determined following the protocol established by AOAC 942.05. Empty porcelain crucibles were dried in an oven at 100 °C for 3 hours and then transferred to a desiccator to cool. Each crucible was weighed, and the value was recorded. Subsequently, 1 gram of dry sample was weighed into each porcelain crucible and ashed at 500-550 °C for 3 hours in a muffle furnace. After this time, the crucibles were transferred to the desiccator, allowed to cool, and then weighed.

Determination of free polyphenols, FPP fraction

The determination of polyphenols was performed according to Muñoz Pabón et al.,²⁰ with slight modifications. Two grams of the sample were weighed in a 50 mL falcon tube. The first fraction was added to the sample 8 mL of solution with 80% ethanol and 20% water and 1% formic acid (80 mL ethanol + 20 mL water + 1 mL formic acid). The samples were shaken for 25 minutes in a shaker at 200 RPM at room temperature. The samples were then centrifuged at 3500 rpm for 5 minutes at room temperature. Subsequently, the supernatant was taken in a new falcon tube (50 mL) and then 40 microliters of EDTA 2% was added to the supernatant.

For the second extraction, 8 mL of 70% acetone with 1% formic acid (70 mL acetone + 30 mL water + 1 mL formic acid) was added to the pellet from the first extraction. The pellet was shaken in the solution for 25 minutes in the shaker at 200 RPM at room temperature. Subsequently, the samples were centrifuged at 3500 RPM for 5 minutes at room temperature, and the second supernatant was combined with the first supernatant and shaken. The resulting mixture was made up to 20 mL using distilled water. Next, the measurement was carried out using the Folin-Ciocalteu method. 20 µL of the extract was taken and combined with 900 µL of Folin reagent, vortexed, and then 600 µL of NaHCO₃ was added, shaking again to ensure proper dissolution. The reaction was allowed to rest, and finally, the absorbance was measured at 765 nm in the spectrophotometer (Thermo Scientific, Genesys 10S UV VIS).

Determination of antioxidant activity by the ABTS method

In a test tube, 4 mL of ABTS.* solution (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) was added, followed by 135 µL of the standard solution, and it was vortexed for 5 seconds. For the reagent blank, a mixture of 4 mL of 4.5 acetate buffer and 135 µL of ethanol was used. The zero point was prepared with 4 mL of ABTS solution and 135 µL of ethanol. The tube was then capped and allowed to rest for exactly 30 minutes before measuring the absorbance in the spectrophotometer (Thermo Scientific, Genesys 10S UV VIS) at a wavelength of 729 nm.

Rheological analysis

Pasting properties

The pasting properties of each dispersion were determined using a rheometer (TA INSTRUMENTS, AR 1500, New Castel, USA), equipped with a starch pasting cell. Then, a suspension of flour in water with a concentration of 12% (w/w) in 25 g was prepared and exposed to heating and cooling. The following samples were analyzed: 100%TF; 100%SIF-DM; 100%SIF-WM; 25%SIF-DM:75%TF; 50%SIF-DM:50%TF; 75%SIF-DM:25%TF; 25%SIF-WM:75%TF; 50%SIF-WM:50%TF; 75%SIF-WM:25%TF. The shear rate was kept constant at 16.75 s⁻¹, throughout the heating and cooling range (25 °C–90 °C–25 °C) while the heating rate was 10 °C/min. The following parameters were obtained from the rheological analysis maximum viscosity [Pa.s]; minimum viscosity [Pa.s]; breakthrough viscosity [°C] and setback viscosity [Pa.s]. Finally, using the Savitsky-Golay function, the data were smoothed in the GraphPad Prism 8.0.1 program (RRID:SCR_002798).^{21,22}

Flow profile

The flow properties were determined following the methodology outlined by Polo and Roa,²¹ with some modifications. An AR1500 rheometer (TA Instruments, New Castle, USA) was used for this study, and measurements were taken using cylindrical geometry. The average viscosity was determined at 25 °C for 12 minutes, with the shear rate increasing in 4 steps as follows:

Step 1: $1 \times 10^{-3} \text{ s}^{-1}$ to $1 \times 10^{-2} \text{ s}^{-1}$

Step 2: 0.01 s^{-1} to 0.1 s^{-1}

Step 3: 0.1 s^{-1} to 1 s^{-1}

Step 4: 1 s^{-1} to 100 s^{-1}

Subsequently, the sample was heated to 90 °C. Once this process was completed, the average viscosity was determined again at 25 °C, subjecting the sample to the same shear rate.

Flow curves (shear stress versus shear rate) were obtained and fitted to the power law model shown in Equation 1.

$$T = K * y^n \quad (1)$$

Where:

T is the shear stress (Pa).

y is the shear velocity (s^{-1})

K is the coefficient of consistency (Pa. s^{-1})

n is the flow behavior index

The flow behavior index indicates Newtonian flow behavior when $n = 1$, shear thinning behavior when $n < 1$ and shear thickening when $n > 1$). Consistency and creep were determined before and after heating in order to determine the effect of thermal processing on these parameters.

Determination of viscoelastic properties (temperature sweep)

For the determination of viscoelastic properties (temperature sweep), the method described by Roa et al.²³ was used. The viscoelastic properties were determined in a rheometer (TA INSTRUMENTS, AR 1500, New Castel, USA) using a system of parallel flat plates, of 40 mm diameter and 1500 μm distance between plates. The edges of the plates were sealed with vaseline, with the purpose of controlling evaporation and avoiding variations in concentrations of the aqueous suspensions used.

The samples were subjected to a cycle of dynamic heating (25-85 °C), stabilization (85 °C for 2 minutes) and cooling (85-25 °C) at 10°C/min, with a frequency of 0.5 Hz and 0.5% deformation. The profiles of the viscoelastic moduli as a function of temperature were recorded using the equipment's software.

Microbiological characterization

The microbiological characterization of the flours was carried out, following the methodology reported by Muñoz Pabon et al.,²⁴ which was based on parameters established by the Colombian technical standard NTC that applies for each microorganism.

To perform the microbiological characterization, 10 g of sample were taken in triplicate, then diluted in 90 mL of peptonized water and mixed at 150 rpm for 10 min in a shaker (MaxQ 4450 orbital Thermo Fischer Scientific USA), following this, for each microorganism the seeding of the appropriate dilution and selective medium is described. The details of the method are explained in more detail in the protocols uploaded in the repository <https://doi.org/10.5281/zenodo.7582202>.

Coliforms

1 mL of each replicate was taken and seeded in a previously sterile Petri dish by seeding in depth on chromogenic colinstant agar for 24-48 h of incubation at 35 °C.

Mesophiles

1 mL of sample was taken from each replicate, and each dilution was seeded by immersion in previously sterile Petri dishes in Plate Count Agar (PCA) agar for 24-72 h at 30 °C.

Fungi and yeasts

100 µL of each replicate was taken and seeded per surface in previously sterile Petri dish by surface plate seeding on Potato Dextrose Agar (PDA) agar for 24-72 h incubation at 30 °C.

Bacillus cereus

100 µL of each replicate was taken and surface seeded in previously sterile Petri dish by surface plate seeding on Mannitol egg Yolk Polymyxin agar (MYP) agar with egg yolk specific for *Bacillus cereus* for 24 h incubation at 37 °C.

Staphylococcus aureus

100 µL were taken from each replicate and seeded per surface in a previously sterile Petri dish, by surface plate counting on Baird Parker agar at 24 h incubation at 35 °C, and then incubated for 24 h at 35 °C.

Salmonella spp.

25 g of sample were taken in duplicate, then diluted in 225 mL of buffered pepton water and mixed at 150 rpm for 10 min in shaker (MaxQ 4450 orbital Thermo Fischer Scientific USA), once this time was over, they were left in incubation at 37 °C for 18 h, after which 100 µL were inoculated in 10 mL of Rappaport Vassiliadis malachite green broth (RVS medium) at 41. Furthermore, 1000 µL were inoculated in 10 mL of Tetrastate Mueller Kauffmann broth (MKTn medium) at 37 °C for 24 h. After this time, the surface of a Petri dish containing XLD agar selective medium was seeded by means of a loop in such a way that asylated colonies were obtained, Salmonella Shiguelia agar was incubated at 34 °C for 24 h, at the end of which typical colonies of *Salmonella spp.* were observed.

Statistical analysis

A completely randomized design (nine treatments) was used to evaluate the rheological properties of native and formulated flours. The design was based on the type of flour and its inclusion levels in the mix, which were: 100%TF; 100%SIF-DM; 100%SIF-WM; 25%SIF-DM:75% TF; 50%SIF-DM:50%TF; 75%SIF-DM:25%TF; 25% SIF-WM:75%TF, 50%SIF-WM:50%TF, 75%SIF-WM:25%TF. The response variables were viscosity as a function of temperature-shear rate and viscoelastic modulus (G' and G'').

The results were presented as the mean $\pm$ standard deviation of triplicate experiments. One-way analysis of variance (ANOVA) was used to compare means. Differences between means were considered significant at $P < 0.05$ using Tukey's new multiple range test. Data were subjected to analysis with Minitab version 20 (RRID:SCR_014483), <https://www.minitab.com/es-mx/support/downloads/>. Graphs were generated in Graphpad Prism 5.0.

Results and discussion

Proximate analysis

Table 1 shows the results of the proximate analysis for the samples. Plukenetia volubilis seed on dry basis has a high lipid content (52.84%) like oilseeds such as soybean (16.61-24.71%), almond (*Prunus dulcis*) and peanut (*Arachis hypogaea*) whose values vary around 50%.^{25,26}

The lipid results are within the range of values reported by Ruiz et al.,²⁷ Benítez et al.,²⁸ Bueno-Borges et al.,²⁹ and Arévalo et al.³⁰ The protein content of sacha inchi kernel (33.73%) is above this range but close to the value reported by Benítez et al.²⁸ Regarding ash, the value obtained was 3.05%, crude fiber was 3.71%, and carbohydrates accounted for 6.67%. In terms of moisture, sacha inchi seeds presented a content of 6.62%, which is close to the value reported by Arévalo et al.,³⁰ who reported 6.28%.

Table 1. Proximate composition of flours.

Component	Sacha inchi almond	SIF-WM	SIF-DM	TF
% Protein	33.73 ^b ± 0.01	31.54 ^c ± 0.04	72.62 ^a ± 0.10	6.05 ^d ± 0.13
% Ashes	3.05 ^c ± 0.001	2.56 ^d ± 0.06	6.79 ^a ± 0.04	5.18 ^b ± 0.01
% Lipids	52.84 ^a ± 0.62	8.87 ^c ± 0.27	9.84 ^b ± 0.24	0.70 ^d ± 0.05
% Crude fiber	3.71 ^c ± 0.18	19.42 ^a ± 0.32	7.71 ^b ± 0.01	2.65 ^d ± 0.16
% Carbohydrates	6.67	37.61	3.04	85.42

The acronym (TF) refers to taro flour; (SIF-WM) sacha inchi flour by wet method and (SIF-DM) sacha inchi flour by defatted method. Values are presented as mean ± SD. For each parameter, different letters indicate significant differences at $p < 0.05$.

Two methods were used to obtain sacha inchi flour: the wet method and mechanical defatting.

Wet method: The flour obtained by the wet method had an oil content of 8.87% and a protein content of 31.54%, with a protein/fat ratio of 3.56. The high protein values obtained from sacha inchi cake using this mechanical pressing method are the basis for the formulation of protein-rich foods using vegetable sources.

Mechanical defatting method: The sacha inchi flour obtained by the mechanical defatting method had a protein content of 72.62%, exceeding the range of values reported by Ruiz et al.,²⁷ Benítez et al.,²⁸ Bueno-Borges et al.,²⁹ Arévalo et al.,³⁰ and González-Linares et al.,³¹ which vary between 41.49% and 65.6%.

Crude fiber content was 7.71%, while carbohydrate content was 3.04%, which is lower than the 11.25% reported by González-Linares et al.³¹ This difference may result from suspended solids carried in the oil stream.

The comparison of the two methods for obtaining sacha inchi flour (SIF-DM and SIF-WM) made it possible to see the method that presented the highest protein content was SIF-DM with 72.62% compared to a value of 31.55% for SIF-WM. The lipids results were close, 9.84% for the SIF-DM and 8.87% for SIF-WM. As for fiber, SIF-WM presented higher values (19.42%) compared to SIF-DM (7.71%).

It is important to note that the protein content obtained through the wet milling method is lower compared to the mechanical defatting method. This is because, during the wet milling process, a milky suspension is generated, which is then filtered to separate the insoluble extract (cake) from the water-soluble extract (slurry). During filtration, part of the protein may remain in the soluble extract, which explains the lower protein concentration in the cake. In this study, the proximate analysis was performed only on the insoluble extract.^{32,33}

Regarding carbohydrate content, SIF-DM showed a lower percentage compared to other extraction methods. This can be explained by the fact that during the mechanical oil extraction process, not only fat is removed but also some solids, such as proteins, minerals, and certain carbohydrates, which could become trapped in the oil or be eliminated along with the byproducts generated during extraction (see Figure 1B). On the other hand, it is likely that carbohydrates are solubilized in the water used in the wet method, contributing to their higher content in SIF-WM. Additionally, the higher carbohydrate content observed in SIF-WM could be due to a proportional effect: the reduction in proteins during the wet process increases the relative proportion of carbohydrates, which are calculated by difference. Moreover, the wet method may promote the retention of insoluble carbohydrates within the solid matrix, minimizing the loss of hydrophilic compounds and explaining the higher carbohydrate content in SIF-WM.

Taro flour: as shown in Table 1, carbohydrates are the major component of taro with a value of 85.42%. The values of protein (6.05%), ash (5.18%), and lipids (0.70%) from proximate analysis agree with research.^{13,37–41} Crude fiber is the only parameter below the consulted range, with a value of 2.65%, the closest being the 4.38% reported by Calle et al.⁴¹

The study design was based on the type of flour and its inclusion levels in the mixture, considering protein content and carbohydrate percentage as the most relevant factors in each treatment. The proximal composition of the blends was calculated using the individual results obtained from each flour. The analyses showed that the 25%SIF-DM:75%TF blend contained 22.69% protein and 64.83% carbohydrates; the 50%SIF-DM:50%TF blend showed 39.34% protein and 44.23% carbohydrates; and the 75%SIF-DM:25%TF blend reached 55.98% protein and 23.64% carbohydrates. On the other hand, the blends with SIF-WM presented the following values: 25%SIF-WM:75%TF with 12.42% protein and 73.47% carbohydrates; 50%SIF-WM:50%TF with 18.80% protein and 61.52% carbohydrates; and finally, the 75%SIF-WM:25%TF blend with 25.17% protein and 49.56% carbohydrates.

Table 2. Bioactive properties of papachina and sachainchi flour.

Raw material	FPP (mg EAG/g sample)	ABTS ($\mu\text{mol ET/g sample}$)	a_w
TF	$7.47^a \pm 0.27$	$2.71^a \pm 0.02$	0.61
SIF-WM	$2.68^c \pm 0.26$	$0.49^c \pm 0.02$	0.64
SIF-DM	$3.37^b \pm 0.16$	$0.71^b \pm 0.04$	0.54

The acronym (TF) refers to taro flour; (SIF-WM) sachainchi flour by wet method and (SIF-DM) sachainchi flour by defatted method; (FPP) refers free polyphenols.

Values are presented as mean $\pm$ SD. For each parameter, different letters indicate significant differences at $p < 0.05$.

Effect of processing on FPP and the antioxidant activities

Table 2 highlights statistically significant differences in the total polyphenol content among the flours analyzed. Taro flour exhibited the highest polyphenol content, aligning with findings reported by Eleazu et al.⁴² and surpassing other food sources such as plantain flour, as noted by.⁴³ Meanwhile, defatted sachainchi flour demonstrated a higher polyphenol content compared to the flour obtained through the wet method. This result is likely due to the mechanical extraction process, which involves pressure and temperature, facilitating the release of phenolic compounds, as previously observed.⁴⁴

Antioxidants present in foods play an important role in their preservation because they prevent oxidation processes; in addition, these compounds contribute to health promotion through the prevention of pathological processes mediated by oxidative stress. Therefore, the formulation of new foods that present bioactive compounds is attractive for the new trends in the design of new foods.

Regarding the antioxidant activity measured using the ABTS method, taro flour demonstrated significantly higher activity compared to SIF-DM and SIF-WM. This difference can be attributed to taro being a prominent source of antioxidant compounds and containing various biologically active phytoconstituents, such as flavonoids, sterols, and glycosides.⁴⁵

Rheological analysis

Pasting curves

Figure 2 shows the viscosity profile of the different flour blends, where the rheological behavior during the heating and cooling phases can be observed. Samples with a higher taro (TF) content exhibited the highest viscosity peaks due to the presence of starch. Taro contains 85.42% carbohydrates, primarily represented by starch.⁴⁸ Starch granules are not soluble in cold water due to the strong hydrogen bonds that hold the starch chains together. Therefore, when starch is heated in excess water above the gelatinization temperature, it undergoes an order-disorder phase transition known as gelatinization.²¹ This process is associated with water diffusion into the granule, its absorption by the amorphous region, hydration, and radial swelling of the starch granules, leading to an increase in viscosity.¹³ As shown in Figure 2 (C, D, E, F), the higher the presence of a starch source, the higher the viscosity peak. This positive correlation between starch content and viscosity peak is consistent with research on various commercial flours from cereals, pseudocereals, roots, and legumes.⁴⁹

When comparing sachainchi flours obtained using the dry method (SIF-DM) and the wet method (SIF-WM), it is observed that the flour obtained by the dry method (SIF-DM) exhibits higher viscosity, as seen in Figure 2 (A, B, H, I). This is because, in this process, the defatting of the flour allows for better interaction between water, proteins, and starch²²; specifically, SIF-DM flour contains 72.6% protein (see Table 1), which promotes the formation of a more viscous structure by interacting with water.

On the other hand, the particle size of the flour obtained through the wet method (sieved with a standard mesh of 425 to 600 microns) is larger, preventing further size reduction due to the high lipid content. This difference in particle size may also influence the rheological properties of the resulting flour. In this context, the presence of endogenous lipids in taro flour forms a protective layer around carbohydrates and proteins, preventing efficient water absorption and limiting starch swelling during the pasting process.⁵⁰

Next, Figure 3 presents the statistical analysis of the pasting curve properties. In graph 3A, significant differences in viscosity peaks among the different blends are shown. Samples with higher TF content exhibit the highest viscosity peaks due to the presence of starch. This behavior is attributed to the greater amount of water absorbed and the subsequent swelling of starch granules during heating.

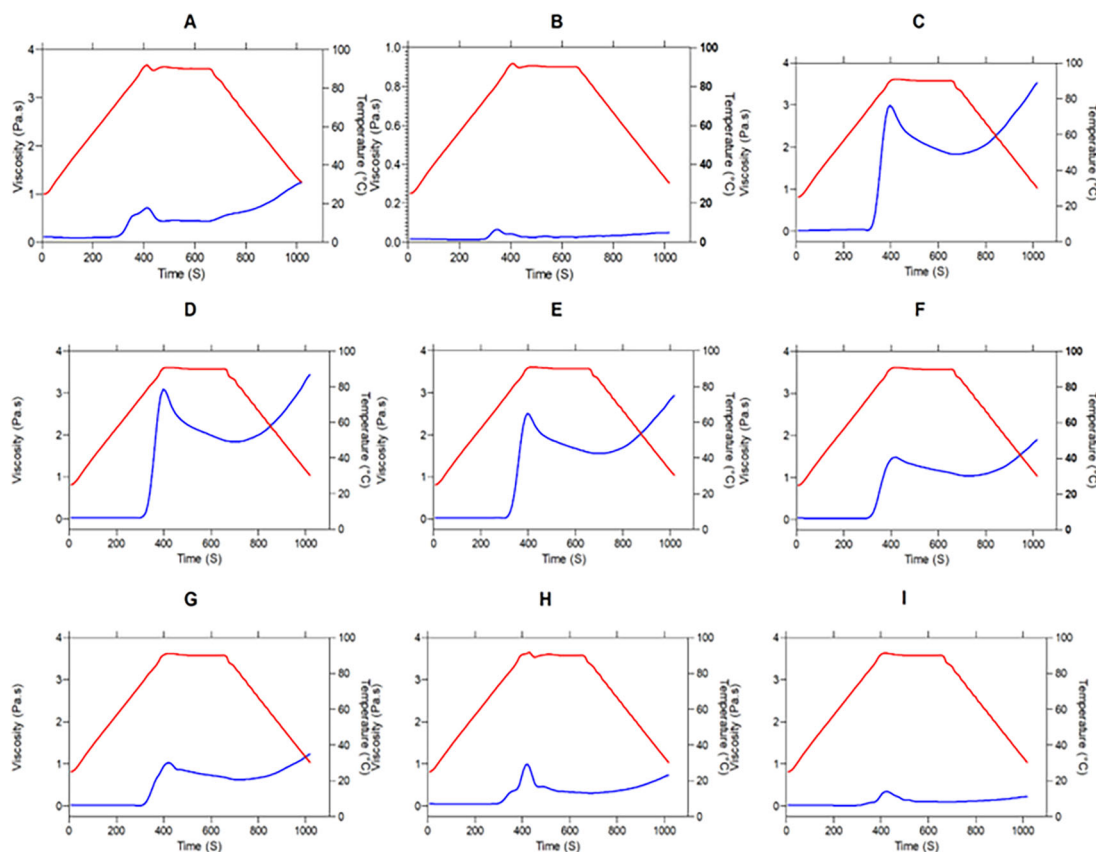


Figure 2. Pasting properties of flour blends. The graphs show the flow profile behavior of: (A) SIF-DM; (B) SIF-WM; (C) TF; (D) 25%SIF-DM:75%TF; (E) 25%SIF-WM:75%TF; (F) 50%SIF-DM:50%TF; (G) 50%SIF-WM:50%TF; (H) 75%SIF-DM:25%TF; (I) 75%SIF-WM:25%TF. The acronym (TF) refers to taro flour; (SIF-WM) sachu inchi flour by wet method and (SIF-DM) sachu inchi flour by defatted method.

Figure 3B shows the behavior of the samples before the onset of retrogradation, that is, the viscosity drop occurring at the end of the constant temperature phase before cooling begins. Samples with higher starch content and lower protein content exhibited a more pronounced viscosity drop. This behavior occurs because once starch reaches its maximum expansion capacity, it releases retained water and solubilizes amylose and amylopectin fractions. Subsequently, during cooling, these macromolecules hydrate individually in excess water and reorganize into a structure similar to the original one.⁵¹

Figure 3C presents the significant differences in the breakdown parameter. Breakdown, or stability, is defined as the difference between the peak viscosity and the trough in the constant temperature section, reflecting the disintegration of starch granules at a maintenance temperature of 95 °C under continuous shear. Lower breakdown values indicate greater shear resistance.⁵³ In this study, samples with higher starch content showed lower stability, while those with lower starch content exhibited greater stability. This behavior can be explained by the properties of starch, as during heating, low-molecular-weight amylose separates from the starch granule, causing it to collapse at a constant temperature as the amorphous portion is dispersed, resulting in a decrease in viscosity.⁵⁴

Finally, Figure 3D presents the analysis of the setback parameter. The setback variable indicates the degree of retrogradation and reorganization of starch molecules during the cooling process, defining the reabsorption of soluble starch polymers and insoluble granular fragments during the cooling phase.⁵⁵ According to the results, blends with higher TF content exhibited higher setback values, which is due to the higher amylopectin content in taro starch (66% amylopectin versus 34% amylose).^{56,57}

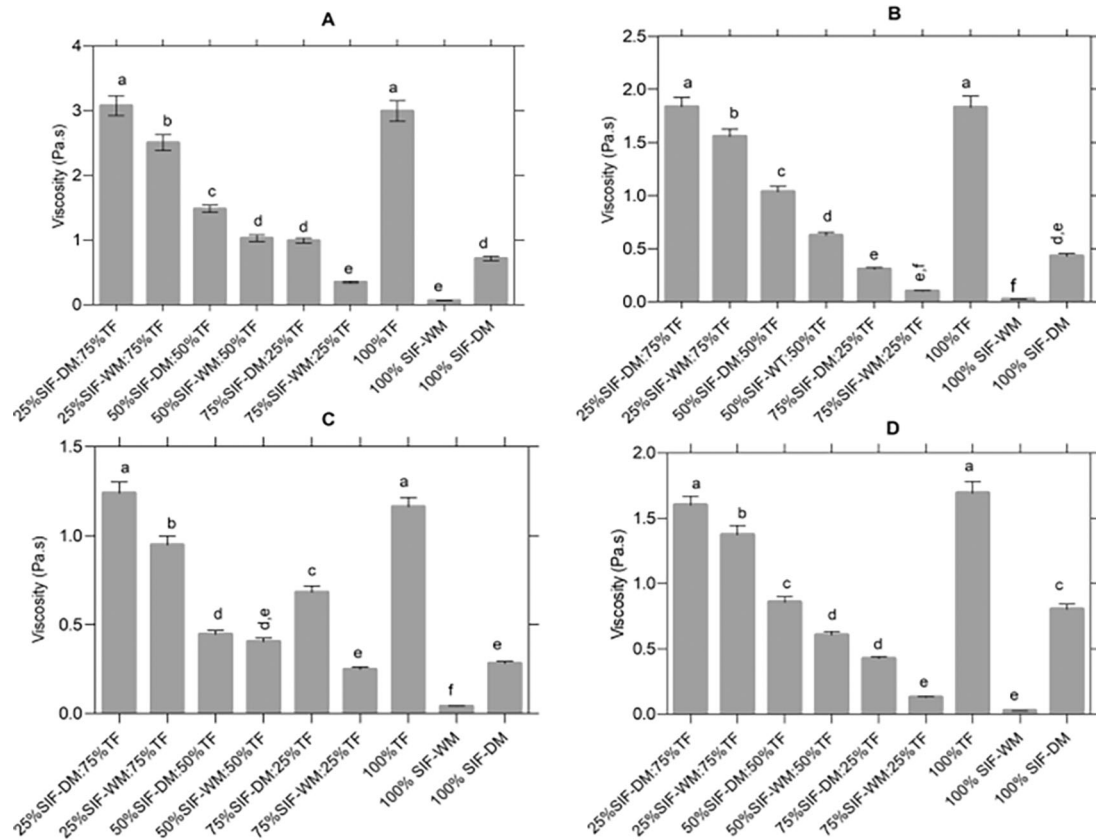


Figure 3. Effect of the different blends on the curves of the pasting curves. The data represent the significant differences in the values of: (A) viscosity peak [Pa.s]; (B) Trough [Pa.s]; (C) Breakdown [°C] and (D) setback [Pa.s]. The acronym (TF) refers to taro flour; (SIF-WM) sachu inchi flour by wet method and (SIF-DM) sachu inchi flour by defatted method.

Flow profile analysis

Figure 4 shows the results of the flow analysis with the viscosity behavior before heating (blue line) and after heating (red line) under stress conditions (shear stress, Pa) and shear rate (s^{-1}). We observed the coefficients obtained by regression of the power model, where “n” is the flow index and “K” is the consistency index. According to the consistency index, we see that “K” increases in all samples except for SIF-WM, because of the viscosity gain, being “n” and “K” inversely proportional parameters.

Regarding the flow index, we observed the behavior of the samples with the highest starch content TF were those that presented the greatest change in “n”, decreasing their flow index represented by the gain in viscosity attributed to the behavior of the starch in taro.

In addition, all the mixtures, except for SIF-DM, showed a value of “n” > 1 before heating, suggesting that they exhibit dilatant fluid behavior. In water and food starch suspensions, dilatant or shear-thickening behavior is related to the initial stiffness of starch granules, which resist shear, and to the high concentration of solids, causing particle swelling.⁵⁸ SIF-DM, however, had an “n” value of 0.7709, indicating a pseudoplastic fluid behavior.

For the mixture’s behavior after heating, all the blends, except for SIF-WM, presented an $n < 1$, demonstrating a behavior of a pseudo-plastic fluid, *i.e.*, that changes in temperature affect these mixtures behavior. This behavior occurs because, in the gelatinization process, the starch granules break, releasing amylose to the aqueous medium and, on cooling, these amylose chains align, forming networks that form gels or viscous suspensions.²¹ The n values for the SIF-WM were reduced to 1.064, exhibiting Newtonian behavior. Newtonian flow indicates that viscosity is independent of shear rate. García-Parra et al.⁵⁸ suggest that these flours could be suitable for formulating products such as beverages, where improving the nutritional content is required without affecting viscosity during shearing.

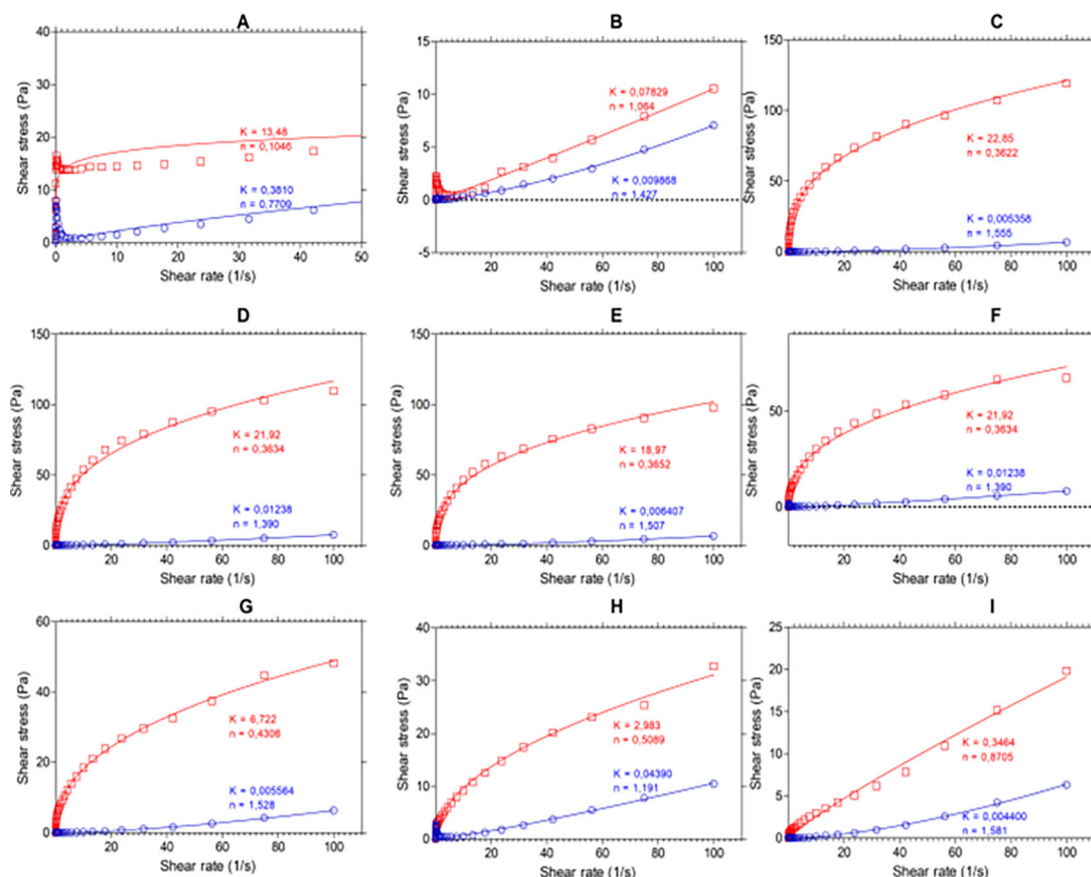


Figure 4. Flow profile of flour blends. The graphs show the flow profile behavior of: (A) SIF-DM; (B) SIF-WM; (C) TF; (D) 25%SIF-DM:75%TF; (E) 25%SIF-WM:75%TF; (F) 50%SIF-DM:50%TF; (G) 50%SIF-WM:50%TF; (H) 75%SIF-DM:25%TF; (I) 75%SIF-WM:25%TF. The acronym (TF) refers to taro flour; (SIF-WM) sacha inchi flour by wet method and (SIF-DM) sacha inchi flour by defatted method.

Viscoelastic properties - Temperature sweeping

Figure 5 presents the moduli G' (red color) and G'' (blue color) as a temperature function ($^{\circ}\text{C}$). We observe the behavior of the elastic and viscous moduli for each mixture, evidencing an increase in the moduli as the temperature sweeps are performed. Except for graphs "5A" and "5H", the mixtures behavior during heating is similar, since the two moduli increase their value, decreasing the difference between them and achieving a crosslinking, where the material ceases to have a viscous character, *i.e.*, it stops being liquid and becomes elastic properties characteristic of a solid. This behavior is characteristic of materials undergoing liquid-solid phase transformations. When the molecules gain weight, in this case, when the starch granules interlock, the loss modulus G'' decreases and the storage modulus G' increases, reaching a point of equilibrium that is its material change of nature, its solidification.

There is a progressive lixiviation of amylose in the swollen granules, where gel-like linkages are formed. Researchers have reported the effect of amylose to increase the firmness of gels during cooling as one of the initial causes of gel firmness. Thus, during the cooling stage, the moduli stay constant with the predominance of the elastic modulus.⁵⁹

The behavior of the flour blends is shown in Figure 5A and 5H, which correspond to 100% SIF-DM and 75% SIF-DM: 25% TF, respectively, shows that the G' and G'' moduli remain constant both during the temperature increase and during subsequent cooling, thus presenting greater stability to temperature variations. This behavior responds to the low starch content of these samples, since starch is key for the viscoelastic behavior of the flours, due to the interaction with water, generating absorption and swelling of the granule and, consequently, greater viscoelasticity. SIF-DM and 75% SIF-DM:25% TF blends did not present a gel point, however, the other blends presented a gel-like behavior, highlighting that the 50% SIF-DM:50% TF and 25% SIF-DM:75% TF blends presented the most elastic gel at a gel temperature of 60°C in both cases, showing similar viscoelastic characteristics.

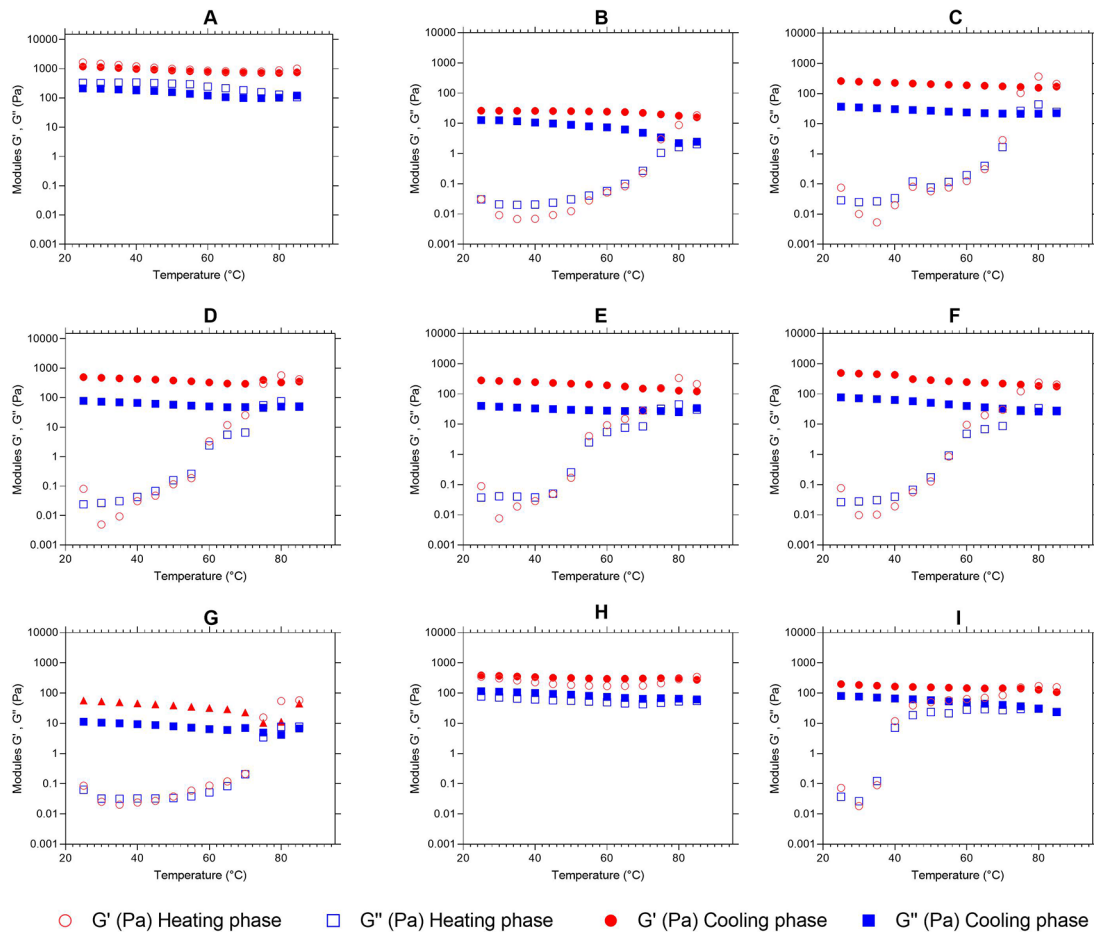


Figure 5. Temperature sweep, G' and G'' modules of the flour blends. The G' (red color) and G'' (blue color) modules are presented for: (A) SIF-DM; (B) SIF-WM; (C) TF; (D) 25%SIF-DM:75%TF; (E) 25%SIF-WM:75%TF; (F) 50%SIF-DM:50%TF; (G) 50%SIF-WM:50%TF; (H) 75%SIF-DM:25%TF; (I) 75%SIF-WM:25%TF. The acronym (TF) refers to taro flour; (SIF-WM) sachu inchi flour by the wet method and (SIF-DM) sachu inchi flour by the defatted method.

We suggest an optimum level of protein inclusion represented by sachu inchi between 25 and 50%, where the consistency of the gel increases and mixtures prepared above this range do not present a gel point, while lower values will have a more liquid behavior.

Microbiological analysis

Table 3 shows the results of the microbiological analysis performed on the three types of flour. For the microbiological analysis, we considered the NTC 6069⁶⁰ standard for quinoa flours and NTC 267⁶¹ for wheat flour.

Table 3. Microbiological analysis

Microorganism	Specification (log UFC/g)	Counts (log UFC/g)		
		TF	SIF-DM	SIF-WM
Total mesophilic aerobic count	5.47	4.21 ± 0.09	4.32 ± 0.09	3.94 ± 0.05
<i>Staphylococcus</i> count	<2	2.38 ± 0.09	1.52 ± 0.10	2.44 ± 0.08
Molds and yeasts count	3.7	1.51 ± 0.10	1.60 ± 0.08	2.02 ± 0.06
Total coliform count	<1	Absence	Absence	Absence
<i>Salmonella</i> in 25g	Absence	Presence	Absence	Absence

The acronym (TF) refers to taro flour; (SIF-WM) sachu inchi flour by wet method and (SIF-DM) sachu inchi flour by defatted method.

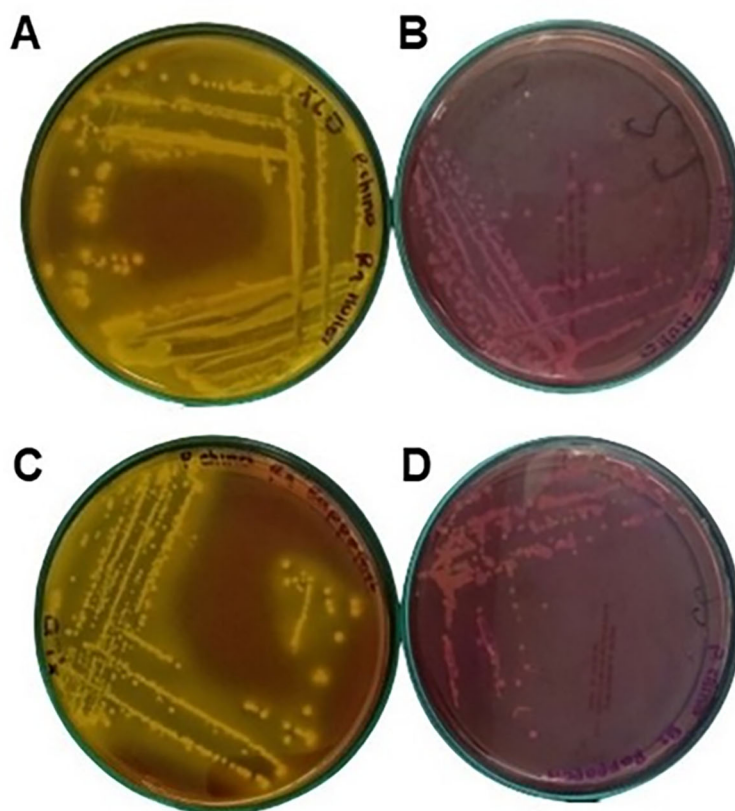


Figure 6. Colonies seeding taro flour sample on XLD agar after 24 h. A: MKTTn broth, C: RVA broth, seeding of taro flour sample on Salmonella-Shigella agar B: MKTTn broth, D: RVA broth.

As can be seen in Table 3, the flours comply with the quality specifications required by Colombian regulations; however, as shown in Figure 6, *Salmonella spp.* is present in the taro flour., possibly due to the persistence of this type of microorganism. Drying this flour at 60 °C preserves certain bioactive compounds but is not sufficient to eliminate it. Larsen et al.¹⁷ indicate that these types of pathogens develop resistance to disinfectants and temperature by forming biofilms, which protect them and allow them to persist on equipment, utensils, and surfaces, leading to food contamination. Additionally, flours such as taro may become contaminated during harvesting or transportation.

Table 2 shows that in foods such as sachu inchi flour and taro flour, where water activity is below 0.85, *Salmonella spp.* and other pathogenic bacteria, such as *Staphylococcus*, can survive in a viable but non-culturable state for extended periods due to their increased resistance to thermal processes. However, although microorganism growth is not sustained at low water activities, foodborne bacteria and fungi can easily contaminate the flour and survive for extended periods with a reduced reproduction rate.²⁴

Conclusions

Mechanical defatting stood out as the most efficient method for obtaining high-protein flours from sachu inchi. However, this process was observed to reduce the preservation of bioactive compounds, decreasing antioxidant activity and total polyphenol content compared to the wet milling method.

The viscosity profile evaluation determined that the starch present in taro provides good viscosity characteristics, making it an important ingredient for the development of foods such as creams, pastes, sauces, among others. These properties are particularly useful in foods that require a heating process to allow starch gelatinization and, therefore, viscosity development. However, mixtures with an excess of taro have a higher rate of retrogradation, which represents a disadvantage for food stability during storage.

Through the viscoelasticity analysis (temperature sweep), it was determined that an inclusion level of defatted sachu inchi flour between 25% and 50% results in a stable and consistent gel, which could be utilized in various food applications.

The drying temperature of taro at 60 °C preserves polyphenols and antioxidant activity at significant levels; however, it does not allow the inactivation of bacteria such as *Salmonella spp.*, which has shown resistance to thermal treatment in recent years.

The development of foods using gluten-free and underutilized raw materials, such as taro and sacha inchi, which easily adapt to regions facing food security challenges, represents a promising alternative for offering the market highly nutritious and gluten-free products.

Future research can formulate foods from sacha inchi flour, which will allow obtaining high protein foods, responding to the trend of obtaining high protein foods from vegetable sources.

Salmonella spp. contamination was present in the taro flour. In response, the facilities and equipment were washed and disinfected with a different product than usual, and again *Salmonella spp.* analyses were performed to guarantee its elimination.

Data availability

Underlying data

Zenodo: Article_sacha. <https://doi.org/10.5281/zenodo.7582202>.⁶³

This project contains the following underlying data:

- Flow profile analysis: folder containing data on the flow analysis of flours in different mixes.
- Pasting folder: containing curves and statistical data of pasting properties
- Viscoelasticity folder: containing curve data and statistical analysis of analyzed viscoelastic properties.

Extended data

Zenodo: Article_sacha. <https://doi.org/10.5281/zenodo.7582202>.⁶³

This project contains the following extended data:

- Protocols on proximate and microbiological analysis

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

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Lorena Sciarini

Universidad Nacional de Cordoba, Córdoba, Cordoba, Argentina

The authors have made most of the requested revisions. However, some issues remain unresolved, and new ones have arisen, especially regarding SIF composition and rheological properties.

Pp 2. Abstract. 7.47 mg GAE/g corresponds to FPP content, not antioxidant activity.

Pp 5. Please, do not start sentences with a number. Check throughout the manuscript.

Pp 5. Protein determination. Which factor was used for protein calculation?

Pp 6. Please change "Fiber" to "Crude fiber."

Table 1. "The lipids results were close, 9.84% for the SIF-DM and 8.87% for SIF-WM." This result is not intuitive. While DM mainly extracts lipids, WM extracts hydrophilic compounds. Why do they have the same lipid content?

In general, the composition of SIF is striking, especially in terms of protein and lipid content (since carbohydrates are calculated by difference, their values are relative). The difference in protein concentration may be due to partial solubilization during the WM process. The difference from the almond is also noteworthy. Another remarkable aspect is the (small) difference in lipid content. I suggest the authors to review these values.

Pp 9. Please unify terminology throughout the text: dry method, defatted method, mechanical pressing.

Pp 9. "On the other hand, it is likely that carbohydrates are solubilized in the water used in the wet method, contributing to their higher content in SIF-WM." This sentence is contradictory. Please revise.

Pp 10. Change "by 43" to "by Sánchez-Rivera 43."

Figure 3B seems to present Trough viscosity values. Please check.

Pp 11. Change "and solubilizes amylose and amylopectin fractions" to "and amylose partially leaches out from granules" (the term "solubilizes" is not correct in this context).

Pp 11. "Reorganize into a structure similar to the original one" is not accurate. Starch gelatinization is an irreversible phenomenon. During cooling and storage, amylose and amylopectin do reorganize into partially crystalline structures, but these are completely different from the original starch granules.

Pp 11. "This behavior can be explained by the properties of starch, as during heating, low-

molecular-weight amylose separates from the starch granule, causing it to collapse at a constant temperature as the amorphous portion is dispersed, resulting in a decrease in viscosity." In general, breakdown positively correlates with peak viscosity: the more water the starch granule absorbs, the more fragile it becomes.

Pp 11. "According to the results, blends with higher TF content exhibited higher setback values, which is due to the higher amylopectin content in taro starch (66% amylopectin versus 34% amylose)." It is unclear what is being compared to what. The starch source is TF, which always has the same AM-AP ratio. Additionally, setback is related to amylose retrogradation, not amylopectin. Finally, in this study, the higher setback is likely related to the higher starch content.

Figure 4. Please clarify the image references (symbols) in the figure caption.

Figures 5A and 5H. The relatively high modulus values from the beginning of the test at room temperature are remarkable. Please comment.

Conclusion. "Mechanical defatting stood out as the most efficient method for obtaining high-protein flours from sacha inchi. However, this process was observed to reduce the preservation of bioactive compounds, decreasing antioxidant activity and total polyphenol content compared to the wet milling method." This seems to be the opposite, as the FPP and ABTS values are higher in SIF-DM than in SIF-WM.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Gluten free bakery, starch properties, cereal properties

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Version 3

Reviewer Report 27 December 2024

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Lorena Sciarini

Universidad Nacional de Cordoba, Córdoba, Cordoba, Argentina

Pp1. Abstract. "The ABTS radical method, which reacts with the phenolic compounds of the food matrix, using Trolox as a standard." Please review the wording (the sentence is incomplete).

Pp 2. Does "Salmonella" correspond to a specific species? Please revise (it could also be Salmonella sp. or Salmonella spp.).

Pp2. It is suggested to change "According to Ref. 1" to "According to Wang et al.¹." Consider

applying this modification throughout the text.

Pp 2. "According to Ref. 1, by 2050, if governance allows for debate and equity in global food and if food demand is very high, driven by rapid population growth, a high intensity of land use will be required to meet the demand for food". The wording is unclear. Please revise.

Pp 4. Please, replace "mNicroorganisms" with "microorganisms"

Pp4. "this, in order to grind the grain. Obtaining a milky and homogeneous" Please revise the punctuation (I understand the period is not appropriate).

Pp4. Deffating method. Was the extraction cake dried? How was it milled afterward? Was it sieved? Please complete the methodology.

Pp5. For the proximate composition, please specify which AOAC method was used in each case.

Pp6. Ref. 20 is incomplete in the References section. Please review all citations.

Pp 6. Polyphenols. "Finally, the supernatant mixture was made up to 20 mL using distilled water and the absorbance was measured in the spectrophotometer". Which colorimetric technique was used? Folin-Ciocalteu? Please clarify.

Pp6. ABTS. "In a test tube 4mL of ABTS.* (2,20-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), solution was added and to start the reaction 135 µL standard solution and vortexed for 5 seconds." Please revise the wording.

Pp6. Flow profile. Which geometry was used?

Pp7. Fungi and yeast. "30°" with "30 °C".

Pp8. Results and discussion. Table 1. The wet method of obtaining Sacha inchi flours results in the solubilization of hydrophilic compounds, such as proteins. This causes the concentration of other components to increase (particularly carbohydrates, according to the authors' findings). However, it is striking that the lipid concentration is so similar, given that the mechanical method specifically extracts the oil. What is the reason for this similarity?

Pp8. "As for carbohydrate content, SIF-DM showed a lower percentage of carbohydrates compared to other extraction methods, due to the fact that in the oil extraction process, not only fat is removed but also some solids such as proteins, minerals, and certain carbohydrates (see Figure 1B). These components may become trapped in the oil or be eliminated with the byproduct generated during extraction. Additionally, it is likely that some soluble carbohydrates dissolve or are removed along with the oil during mechanical extraction". It is more likely that carbohydrates are solubilized in the water used in the wet method rather than in the oil, although some carryover may occur. It is worth exploring the fact that the wet method actually has a higher carbohydrate content because, proportionally, it has fewer proteins, leading to a concentration of carbohydrates (which are calculated by difference).

Pp9. Was the proximate composition of the flour blends measured or calculated?

Pp9. Effect of processing on FPP and the antioxidant activities. Please do not repeat in the text the values already presented in Tables/Figures. Verify this throughout the manuscript. Also, check, as "taro flour presents the highest polyphenol content of 7.50+/-1.73" does not match the value presented in Table 2.

Pp 10. "This difference can be explained because taro is a prime source of antioxidant" What is meant by "prime source"?

Pp10. "... bioactive compounds, which in sacha inchi are present particularly in polyunsaturated essential fatty acids..." ABTS is used to evaluate the radical scavenging capacity of hydrophilic compounds. In fact, the extraction was performed with polar solvents. I find the explanation contradictory.

Pp11. "to the post hoc Tukey analysis five different groups identified with letters from "a" to "e" are classified. In group "a", the samples with the highest viscosity (25% SIF-DM:75% TF and 100% TF) with values of 3.08 Pa.s and 2.99 Pa.s respectively, and in group "e", the samples with the lowest viscosity value (75% SIF-WM:25% TF and 100% SIF-WM) with values of 0.35 Pa.s and 0.64 Pa.s respectively." This type of expression used throughout the manuscript makes the reading tedious and is repeated with what is presented in graphs/tables. Please simplify by focusing on the statistical differences presented between samples.

Pp12. In this page, the results are presented in a disorganized manner. It is suggested to first address Figure 2, and then Figure 3.

Pp12. "According to the above, as shown in Figure 3, the samples that presented a higher viscosity drop are the samples with higher TF content, this is because the crystalline structure (amylopectin) that makes up the granules is altered, when amylose and amylopectin are hydrated individually in excess water, both macromolecules tend to reorganize in a similar structure to the one they had initially." The authors discuss the minimum viscosity (or trough), but I believe there is some confusion here, as this parameter is not associated with retrogradation but with gelatinization. The granules absorb water up to a maximum, and with shear at high temperature, they break, decreasing viscosity. Retrogradation occurs when the temperature decreases.

Pp12. "As shown in Figure 3, mixtures with higher inclusion of starch present in TF and less protein contributed by sachá inchi result in more free water for starch molecules to swell and solubilize presenting higher viscosity; on the contrary, at high proportions of protein its interaction with starch granules could stabilize the amylose leaching channel of starch granules, which resulted in lower swelling power and solubility." It would be useful to consider, in the mixtures, the effect of dilution of the starch present in TF. On the other hand, proteins also develop viscosity, which could explain why TF+SIF-DM has higher viscosity than TF+SIF-WM.

Pp12. "until the amorphous part is solubilized, decreasing viscosity". In general, when referring to starch in water, the term "dispersion" is used, not "solubilization."

Pp12. Flow profile analysis. Please, unify: "flowability index" or "flow index".

Pp12. Two consecutive paragraphs begin with "Regarding the Flow index." Please review the

wording.

Pp12. "dilettantish fluid" or "dilatant fluid"?

Pp13. Viscoelastic properties - Temperature sweep. Figure 5 needs more details. First, the color assigned to each module should be clarified in the figure caption. Second, it should be specified which curve corresponds to the heating phase and which to the cooling phase. Without this information, it is not possible to follow the discussion.

Pp13. "progressive exudation" by "progressive lixiviation".

Pp 14. "where a_w is less than 0.85". Lower?

Pp 15. Conclusions. "The method that allows for the highest concentration of protein in sacha inchi is mechanical defatting with a protein result of 73%, compared to that obtained by the wet method, which resulted in 31%." This does not belong in the Conclusion section, but rather in the Results and Discussion section.

Pp 15. "However, during the production of sachá flour with the mechanical method, bioactive compounds are lost, which is why lower values of total polyphenols and antioxidant activity are obtained, compared to taro flour." In this sentence, methods for obtaining Sacha Inchi flour are compared, but it is compared with taro flour. Please review the wording.

Pp 15. "The viscosity profile evaluation determined that the starch contained in taro provides good viscosity characteristics, being this an important ingredient for the development of foods such as creams, pastes, sauces, among others." This is useful for foods that require a heating process to allow starch gelatinization and hence viscosity development.

Pp15. "the optimum level of inclusion". In fact, the optimal level will depend on the food that is being formulated.

Pp16. "Although taro's drying temperature of 60 °C maintains polyphenols and antioxidant activity". We do not know if they "maintain," as it was not compared against other drying temperatures.

Pp16. "using gluten-free orphan raw materials". What do authors mean by "orphan"?

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Gluten free bakery, starch properties

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 05 Feb 2025

Karen Muñoz

Dear Dr. Lorena Sciarini,

We would like to express our sincere gratitude for the valuable contributions you made during the evaluation process of our article. Your suggestions have been highly enriching, and we have taken them into account in order to enhance the quality and clarity of our work. Below we respond to each of the suggestions made.

Pp1. Abstract. "The ABTS radical method, which reacts with the phenolic compounds of the food matrix, using Trolox as a standard." Please review the wording (the sentence is incomplete).

Response: Thank you for your observation. We have reviewed and corrected the wording of the sentence to make it clearer and more complete, while also complementing the main idea. The revised version is as follows: **"Methods:** Proximal tests were conducted to determine basic chemical composition, quantification of free polyphenols, antioxidant activity using the ABTS* radical method (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) with Trolox as a standard, and rheological analyses, including pasting curves, flow profiles, and viscoelastic properties. Microbiological characterization of the flours was also performed."

Pp 2. Does "Salmonella" correspond to a specific species? Please revise (it could also be Salmonella sp. or Salmonella spp.).

Response: We have reviewed the entire document and replaced 'Salmonella' with the correct designation 'Salmonella spp.' to refer to the corresponding species.

Pp2. It is suggested to change "According to Ref. 1" to "According to Wang et al.¹."

Consider applying this modification throughout the text.

Response: Thank you for your observation. We have taken your suggestion into account and have adjusted the referencing format throughout the text accordingly

Pp 4. Please, replace “mNicroorganisms” with “microorganisms”

Response: Thank you very much for the observation. It has been replaced with the appropriate term “microorganisms”.

Pp4. “this, in order to grind the grain. Obtaining a milky and homogeneous” Please revise the punctuation (I understand the period is not appropriate).

Response: The suggestion was taken into account, and the wording was improved to enhance the clarity of the text. The revised version is as follows: “The sachu inchi flour obtained by the wet method (SIF-WM) was prepared as follows: initially, wet grinding was carried out using a blender (Oster, BLST 4655, Colombia) with a capacity of 1.25 L. For this process, sachu inchi seeds were mixed with water in a 1:3 ratio to ensure effective grinding. This resulted in a milky and homogeneous suspension. Subsequently, a cloth filter was used to separate the insoluble extract (cake) from the water-soluble extract (milk). The cake was then dried in an oven at 65 °C for 3 hours. Finally, once dehydrated, it was immediately packaged in a polyethylene bag with a hermetic seal for preservation [18].”

Pp4. Deffating method. Was the extraction cake dried? How was it milled afterward? Was it sieved? Please complete the methodology.

Response: La requested information was completed and the text's wording was improved. The modified version is as follows: “The defatted sachu inchi flour (SIF-DM) was obtained as follows: the sachu inchi kernels were placed in the hopper of an automatic touchscreen oil extraction press (CGLDENWALL, model K28, Shanghai, China), as shown in Figure 1. The process was carried out at a temperature of 124 °C. During the extraction, a screw conveyor pushed the material into the main pressing cylinder. As it moved forward, the design of the screw, with decreasing pitch and spiral depth, reduced the available volume in the chamber, subjecting the material to high pressure and friction against the closed bottom wall of the cylinder. This facilitated efficient oil extraction, with the oil flowing through the cylinder's orifices, while the residual material, known as cake, was expelled through the discharge nozzle. Subsequently, the cake was ground using an electric mill (Quaker City Mill, model 4-E, Philadelphia) and sieved through a system with a No. 30 mesh to achieve uniform particle size. Finally, the resulting flour was packaged in polyethylene bags with airtight seals for preservation.”

Pp5. For the proximate composition, please specify which AOAC method was used in each case.

Response: The provided suggestion was taken into account, and in response, the AOAC method used for each compositional analysis was included in each case.

Protein: "For the determination of protein content, the protocol established by AOAC 968.06 was followed."

Fiber: "The fiber content was analyzed according to the AOAC 962.09 protocol."

Ash: "The ash content was determined following the protocol established by AOAC 942.05."

Pp6. Ref. 20 is incomplete in the References section. Please review all citations.

Response: All references have been reviewed and completed where necessary.

Pp 6. Polyphenols. "Finally, the supernatant mixture was made up to 20 mL using distilled water and the absorbance was measured in the spectrophotometer". Which colorimetric technique was used? Folin-Ciocalteu? Please clarify.

Response: The raised concern was taken into account, and the wording was complemented and improved to ensure greater clarity, resulting in the following enhanced version: "For the second extraction, 8 mL of 70% acetone with 1% formic acid (70 mL acetone + 30 mL water + 1 mL formic acid) was added to the pellet from the first extraction. The pellet was shaken in the solution for 25 minutes in the shaker at 200 RPM at room temperature. Subsequently, the samples were centrifuged at 3500 RPM for 5 minutes at room temperature, and the second supernatant was combined with the first supernatant and shaken. The resulting mixture was made up to 20 mL using distilled water. Next, the measurement was carried out using the Folin-Ciocalteu method. 20 µL of the extract was taken and combined with 900 µL of Folin reagent, vortexed, and then 600 µL of NaHCO₃ was added, shaking again to ensure proper dissolution. The reaction was allowed to rest, and finally, the absorbance was measured at 765 nm in the spectrophotometer (Thermo Scientific, Genesys 10S UV VIS)."

Pp6. Ref. 20 is incomplete in the References section. Please review all citations.

Response: All references have been reviewed and completed where necessary.

Pp 6. Polyphenols. "Finally, the supernatant mixture was made up to 20 mL using distilled water and the absorbance was measured in the spectrophotometer". Which colorimetric technique was used? Folin-Ciocalteu? Please clarify.

Response: The raised concern was taken into account, and the wording was complemented and improved to ensure greater clarity, resulting in the following enhanced version: "For the second extraction, 8 mL of 70% acetone with 1% formic acid (70 mL acetone + 30 mL water + 1 mL formic acid) was added to the pellet from the first extraction. The pellet was shaken in the solution for 25 minutes in the shaker at 200 RPM at room temperature. Subsequently, the samples were centrifuged at 3500 RPM for 5 minutes at room temperature, and the second supernatant was combined with the first supernatant and shaken. The resulting mixture

was made up to 20 mL using distilled water. Next, the measurement was carried out using the Folin-Ciocalteu method. 20 μ L of the extract was taken and combined with 900 μ L of Folin reagent, vortexed, and then 600 μ L of NaHCO₃ was added, shaking again to ensure proper dissolution. The reaction was allowed to rest, and finally, the absorbance was measured at 765 nm in the spectrophotometer (Thermo Scientific, Genesys 10S UV VIS)."

Pp6. ABTS. "In a test tube 4mL of ABTS.* (2,20-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), solution was added and to start the reaction 135 μ L standard solution and vortexed for 5 seconds." Please revise the wording.

Response: The suggestion made was taken into account, and the wording was improved, resulting in the following: "In a test tube, 4 mL of ABTS.* solution (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) was added, followed by 135 μ L of the standard solution, and it was vortexed for 5 seconds. For the reagent blank, a mixture of 4 mL of 4.5 acetate buffer and 135 μ L of ethanol was used. The zero point was prepared with 4 mL of ABTS solution and 135 μ L of ethanol. The tube was then capped and allowed to rest for exactly 30 minutes before measuring the absorbance in the spectrophotometer (Thermo Scientific, Genesys 10S UV VIS) at a wavelength of 729 nm."

Pp6. Flow profile. Which geometry was used?

Response: The question raised was answered, and the wording was improved. The modified version is as follows: "The flow properties were determined following the methodology outlined by Polo and Roa [21], with some modifications. An AR1500 rheometer (TA Instruments, New Castle, USA) was used for this study, and measurements were taken using cylindrical geometry. The average viscosity was determined at 25 °C for 12 minutes, with the shear rate increasing in 4 steps as follows:...)

Pp7. Fungi and yeast. "30°" with "30 °C".

Response: Thank you for the observation, the corresponding correction has already been made.

Pp8. Results and discussion. Table 1. The wet method of obtaining Sacha inchi flours results in the solubilization of hydrophilic compounds, such as proteins. This causes the concentration of other components to increase (particularly carbohydrates, according to the authors' findings). However, it is striking that the lipid concentration is so similar, given that the mechanical method specifically extracts the oil. What is the reason for this similarity?

Response: The similarity in lipid concentration between the flours obtained through the wet and mechanical methods can be explained by the specific characteristics of each process. In the wet method, sachá inchi seeds are mixed with water, and while there is no initial loss of solids, some hydrophilic compounds, such as proteins and carbohydrates, migrate into the solvent during the soaking and homogenization stages. During filtration, the cake retains the lipids, while soluble compounds are removed with the liquid byproduct. Additionally, the

cake obtained through the wet method does not undergo the compression applied by the mechanical press, meaning the necessary pressure to fully extract the oil is not achieved. As a result, a greater amount of lipids is retained in the final cake from the wet method, which explains the similarity in lipid concentration values between the two methods.

Pp8. "As for carbohydrate content, SIF-DM showed a lower percentage of carbohydrates compared to other extraction methods, due to the fact that in the oil extraction process, not only fat is removed but also some solids such as proteins, minerals, and certain carbohydrates (see Figure 1B). These components may become trapped in the oil or be eliminated with the byproduct generated during extraction. Additionally, it is likely that some soluble carbohydrates dissolve or are removed along with the oil during mechanical extraction". It is more likely that carbohydrates are solubilized in the water used in the wet method rather than in the oil, although some carryover may occur. It is worth exploring the fact that the wet method actually has a higher carbohydrate content because, proportionally, it has fewer proteins, leading to a concentration of carbohydrates (which are calculated by difference).

Response: Thank you very much for the observations. We took the suggestions into account, resulting in the following version: "Regarding carbohydrate content, SIF-DM showed a lower percentage compared to other extraction methods. This can be explained by the fact that during the mechanical oil extraction process, not only fat is removed but also some solids, such as proteins, minerals, and certain carbohydrates, which could become trapped in the oil or be eliminated along with the byproducts generated during extraction (see Figure 1B). On the other hand, it is likely that carbohydrates are solubilized in the water used in the wet method, contributing to their higher content in SIF-WM. Additionally, the higher carbohydrate content observed in SIF-WM could be due to a proportional effect: the reduction in proteins during the wet process increases the relative proportion of carbohydrates, which are calculated by difference. Moreover, the wet method may promote the retention of insoluble carbohydrates within the solid matrix, minimizing the loss of hydrophilic compounds and explaining the higher carbohydrate content in SIF-WM."

Pp9. Was the proximate composition of the flour blends measured or calculated?

Response: The wording was adjusted based on the observation made. Below, the revised and corrected version is presented: "The study design was based on the type of flour and its inclusion levels in the mixture, considering protein content and carbohydrate percentage as the most relevant factors in each treatment. The proximal composition of the blends was calculated using the individual results obtained from each flour. The analyses showed that the 25% SIF-DM:75% TF blend contained 22.69% protein and 64.83% carbohydrates; the 50% SIF-DM:50% TF blend showed 39.34% protein and 44.23% carbohydrates; and the 75% SIF-DM:25% TF blend reached 55.98% protein and 23.64% carbohydrates. On the other hand, the blends with SIF-WM presented the following values: 25% SIF-WM:75% TF with 12.42% protein and 73.47% carbohydrates; 50% SIF-WM:50% TF with 18.80% protein and 61.52% carbohydrates; and finally, the 75% SIF-WM:25% TF blend with 25.17% protein and 49.56%

carbohydrates.”

Pp9. Effect of processing on FPP and the antioxidant activities. Please do not repeat in the text the values already presented in Tables/Figures. Verify this throughout the manuscript. Also, check, as "taro flour presents the highest polyphenol content of 7.50+/-1.73" does not match the value presented in Table 2.

Response: We have taken the suggestions into account and corrected the corresponding values. The modified version is as follows: “Table 2 highlights statistically significant differences in the total polyphenol content among the flours analyzed. Taro flour exhibited the highest polyphenol content, aligning with findings reported by Eleazu et al. [42] and surpassing other food sources such as plantain flour, as noted by [43]. Meanwhile, defatted sachu inchi flour demonstrated a higher polyphenol content compared to the flour obtained through the wet method. This result is likely due to the mechanical extraction process, which involves pressure and temperature, facilitating the release of phenolic compounds, as previously observed [44].”

Pp 10. “This difference can be explained because taro is a prime source of antioxidant” What is meant by "prime source"?

Response: We aimed to replace the term 'primary source' with a more context-appropriate expression, resulting in the following revision: “Regarding the antioxidant activity measured using the ABTS method, taro flour demonstrated significantly higher activity compared to SIF-DM and SIF-WM. This difference can be attributed to taro being a prominent source of antioxidant compounds and containing various biologically active phytoconstituents, such as flavonoids, sterols, and glycosides [45].”

Pp10. “... bioactive compounds, which in sachu inchi are present particularly in polyunsaturated essential fatty acids...” ABTS is used to evaluate the radical scavenging capacity of hydrophilic compounds. In fact, the extraction was performed with polar solvents. I find the explanation contradictory.

Response: You are absolutely right; the explanation was contradictory. Therefore, we have revised the analysis and discussion of results, resulting in the following version. “In this study, a drying temperature of 60 °C was used for the taro flour (TF), which allows for achieving the maximum antioxidant activity in taro flours [46]. The SIF-DM showed the lowest antioxidant activity, which can be attributed to the loss of polar hydrophilic compounds, such as polyphenols, during the mechanical extraction. These compounds are the main contributors to the radical scavenging capacity measured using the ABTS

Pp11. “to the post hoc Tukey analysis five different groups identified with letters from “a” to “e” are classified. In group “a”, the samples with the highest viscosity (25% SIF-DM:75% TF and 100% TF) with values of 3.08 Pa.s and 2.99 Pa.s respectively, and in group “e”, the samples with the lowest viscosity value (75% SIF-WM:25% TF and 100% SIF-WM) with values of 0.35 Pa.s and 0.64 Pa.s respectively.” This type of expression

used throughout the manuscript makes the reading tedious and is repeated with what is presented in graphs/tables. Please simplify by focusing on the statistical differences presented between samples.

Response: We took the suggestion into account throughout the manuscript and simplified some expressions to make the reading more fluid and improve its quality

Pp12. In this page, the results are presented in a disorganized manner. It is suggested to first address Figure 2, and then Figure 3.

Response: Thank you for your suggestion. We have organized the results as you recommended and rewritten them to improve clarity.

Pp12. "According to the above, as shown in Figure 3, the samples that presented a higher viscosity drop are the samples with higher TF content, this is because the crystalline structure (amylopectin) that makes up the granules is altered, when amylose and amylopectin are hydrated individually in excess water, both macromolecules tend to reorganize in a similar structure to the one they had initially." The authors discuss the minimum viscosity (or trough), but I believe there is some confusion here, as this parameter is not associated with retrogradation but with gelatinization. The granules absorb water up to a maximum, and with shear at high temperature, they break, decreasing viscosity. Retrogradation occurs when the temperature decreases.

Response: The suggestion was taken into account, the information was organized, and the wording was improved, resulting in the following structured idea: "In graph 3.A, significant differences in viscosity peaks among the different blends are shown. Samples with higher TF content exhibit the highest viscosity peaks due to the presence of starch. This behavior is attributed to the greater amount of water absorbed and the subsequent swelling of starch granules during heating.

Figure 3.B shows the behavior of the samples before the onset of retrogradation, that is, the viscosity drop occurring at the end of the constant temperature phase before cooling begins. Samples with higher starch content and lower protein content exhibited a more pronounced viscosity drop. This behavior occurs because once starch reaches its maximum expansion capacity, it releases retained water and solubilizes amylose and amylopectin fractions. Subsequently, during cooling, these macromolecules hydrate individually in excess water and reorganize into a structure similar to the original one [51]."

Pp12. "As shown in Figure 3, mixtures with higher inclusion of starch present in TF and less protein contributed by sachinchi result in more free water for starch molecules

to swell and solubilize presenting higher viscosity; on the contrary, at high proportions of protein its interaction with starch granules could stabilize the amylose leaching channel of starch granules, which resulted in lower swelling power and solubility.” It would be useful to consider, in the mixtures, the effect of dilution of the starch present in TF. On the other hand, proteins also develop viscosity, which could explain why TF+SIF-DM has higher viscosity than TF+SIF-WM.

Response: The observation was taken into account, and the text was adjusted as follows: “...Conversely, in blends with a higher protein content, proteins interact with starch granules, stabilizing the amylose leaching channel and reducing both swelling and solubility of starch. This behavior is influenced by the dilution of starch in taro flour (TF), as a lower starch concentration in the blend limits its ability to absorb water and form viscous structures [52]. Additionally, proteins play a crucial role in viscosity development, which explains why TF+SIF-DM blends exhibit higher viscosity compared to TF+SIF-WM blends. In TF+SIF-DM blends, the preservation of functional proteins promotes more effective interaction with water and starch molecules, resulting in higher viscosity. In contrast, in TF+SIF-WM blends, the loss of proteins during the wet extraction process reduces their contribution to viscosity, thereby lowering the rheological performance of the blend.”

Pp12. “until the amorphous part is solubilized, decreasing viscosity”. In general, when referring to starch in water, the term “dispersion” is used, not “solubilization.”

Response: Thank you for your observation. We have replaced the term 'solubilization' with 'dispersion' to refer to starch in water.

Pp12. Flow profile analysis. Please, unify: “flowability index” or “flow index”.

Response: Within the paragraph, we unified the term "flow index", thank you for the observation.

Pp12. Two consecutive paragraphs begin with "Regarding the Flow index." Please review the wording.

Response: Thank you for the suggestion; we took your recommendation into account and improved the writing throughout the text.

Pp12. “dilettantish fluid” or “dilatant fluid”?

Response: Thank you for the observation; we changed the term "dilent fluid" to the correct term "dilatant fluid".

Pp13. Viscoelastic properties - Temperature sweep. Figure 5 needs more details. First, the color assigned to each module should be clarified in the figure caption. Second, it

should be specified which curve corresponds to the heating phase and which to the cooling phase. Without this information, it is not possible to follow the discussion.

Response: You are absolutely right, Figure 5 needs more details. Therefore, we have specified the color assigned to each modulus and clearly indicated which curve corresponds to both the heating phase and the cooling phase.

Pp13. “progressive exudation” by “progressive lixiviation”.

Response: You are absolutely right, the change was made using the correct term: progressive lixiviation. “There is a progressive lixiviation of amylose in the swollen granules, where gel-like linkages are formed. Researchers have reported the effect of amylose to increase the firmness of gels during cooling as one of the initial causes of gel firmness. Thus, during the cooling stage, the moduli stay constant with the predominance of the elastic modulus [59].”

Pp 14. “where aw is less than 0.85”. Lower?

Response: Thank you for the observation. The requested change has been made by using the term “below” instead of “lower,” which improves the accuracy of the wording. The modified version is as follows:

Table 2 shows that in foods such as sachu inchi flour and taro flour, where water activity is below 0.85, *Salmonella* spp. and other pathogenic bacteria, such as *Staphylococcus*, can survive in a viable but non-culturable state for extended periods due to their increased resistance to thermal processes. However, although microorganism growth is not sustained at low water activities, foodborne bacteria and fungi can easily contaminate the flour and survive for extended periods with a reduced reproduction rate [24].

Pp 15. Conclusions. “The method that allows for the highest concentration of protein in sachu inchi is mechanical defatting with a protein result of 73%, compared to that obtained by the wet method, which resulted in 31%.” This does not belong in the Conclusion section, but rather in the Results and Discussion section.

Response: You are right, this statement “The method that allows for the highest concentration of protein in sachu inchi is mechanical defatting with a protein result of 73%, compared to that obtained by the wet method, which resulted in 31%” does not belong in the conclusions section, so we have taken your suggestion into account and have properly relocated it.

Pp 15. “However, during the production of sachu flour with the mechanical method, bioactive compounds are lost, which is why lower values of total polyphenols and antioxidant activity are obtained, compared to taro flour.” In this sentence, methods for obtaining Sachu Inchi flour are compared, but it is compared with taro flour. Please review the wording.

Response: Thank you for the observation. We have improved the wording based on the provided recommendation. "Mechanical defatting stood out as the most efficient method for obtaining high-protein flours from sacha inchi. However, this process was observed to reduce the preservation of bioactive compounds, decreasing antioxidant activity and total polyphenol content compared to the wet milling method."

Pp 15. "The viscosity profile evaluation determined that the starch contained in taro provides good viscosity characteristics, being this an important ingredient for the development of foods such as creams, pastes, sauces, among others." This is useful for foods that require a heating process to allow starch gelatinization and hence viscosity development.

Response: We took the suggestion into account and have modified it as follows: "The viscosity profile evaluation determined that the starch present in taro provides good viscosity characteristics, making it an important ingredient for the development of foods such as creams, pastes, sauces, among others. These properties are particularly useful in foods that require a heating process to allow starch gelatinization and, therefore, viscosity development. However, mixtures with an excess of taro have a higher rate of retrogradation, which represents a disadvantage for food stability during storage."

Pp15. "the optimum level of inclusion". In fact, the optimal level will depend on the food that is being formulated.

Response: You are absolutely right; the term used was not appropriate. Therefore, we have attached an improved version of the conclusion below: "Through the viscoelasticity analysis (temperature sweep), it was determined that an inclusion level of defatted sacha inchi flour between 25% and 50% results in a stable and consistent gel, which could be utilized in various food applications."

Pp16. "Although taro's drying temperature of 60 °C maintains polyphenols and antioxidant activity". We do not know if they "maintain," as it was not compared against other drying temperatures.

Response: You are absolutely right; the term used was not appropriate. Therefore, we have attached an improved version of the conclusion below: "The drying temperature of taro at 60 °C preserves polyphenols and antioxidant activity at significant levels; however, it does not allow the inactivation of bacteria such as *Salmonella spp.*, which has shown resistance to thermal treatment in recent years."

Pp16. "using gluten-free orphan raw materials". What do authors mean by "orphan"?

Response: We have replaced the term 'orphan' and improved the wording of this conclusion: "The development of foods using gluten-free and underutilized raw materials, such as taro and sacha inchi, which easily adapt to regions facing food security challenges, represents a promising alternative for offering the market highly nutritious and gluten-free products."

Competing Interests: No competing interests were disclosed.

Reviewer Report 23 November 2024

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Elisa Julianti 

Department of Food Science Faculty of Agriculture, Universitas Sumatera Utara, Medan, North Sumatra, Indonesia

Thank you for the opportunity to review the revised article and the author responses. I have no further comments to make at this time.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Flour and Starch from Root and Tuber Crops as Alternatives to Wheat Flour

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 17 September 2024

<https://doi.org/10.5256/f1000research.155702.r317396>

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**Umi Purwandari**

University of Trunojoyo Madura, Madura, Indonesia

The paper was written carefully enough and data were analyzed thoroughly using recent related publication. However, microbiological analysis did not seem relevant to the work, and therefore it would be better deleted.

The term 'flour size' was confusing. Was it starch granule size, and if it was so, did the authors measure it, or is that any supporting argument for judging the size.

Some parts were not correctly written, such as in page 10 where it was refer to Table2 for oil concentration. While Table 2 was about antioxidant activity. Also, statistical notation for Table 2, ABTS did not look right.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.**Reviewer Expertise:** Food science, food microbiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 31 Oct 2024

Karen Muñoz

Dear Dr Purwandari, Thank you very much for your thoughtful and constructive feedback on our manuscript. We appreciate the time and effort you dedicated to reviewing our work, as well as your insightful suggestions, which have greatly helped us improve our paper. Below, we address each of your comments in detail:

1. The paper was written carefully enough and data were analyzed thoroughly using recent related publication. However, microbiological analysis did not seem relevant to the work, and therefore it would be better deleted.

Regarding the microbiological analysis, it is important to note that this was conducted to evaluate the initial state of the obtained flours concerning their microbiological composition. Since these flours are intended for food consumption, it is essential to know the level of microbial growth and to ensure that they fall within the permissible ranges established by current regulations governing such products.

2. Some parts were not correctly written, such as in page 10 where it was referred to Table 2 for oil concentration. While Table 2 was about antioxidant activity. Also, statistical notation for Table 2, ABTS did not look right.

With the purpose of addressing the question raised, the information was supplemented using the correct term ;granulometry.; Additionally, the article mentions the following textually "Comparing SIF-DM flour with SIF-WM, the defatting method produces a flour with higher viscosity due to the interaction of water with proteins and starch, considering that SIF-DM has 72.62% protein. This result can be attributed to the fact that the lower viscosity of sachu inchi flour obtained by the wet method is due to the sample's particle size (sieving with a standard mesh size of 425 to 600 microns), as it was not possible to reduce particle size due to its high fat content, and the resulting granules from the liquefaction and subsequent drying processes were used. Therefore, the endogenous lipids present in the taro flour form a type of protective layer around the aggregate of carbohydrates and proteins, preventing water absorption and the subsequent swelling of starch".

3 Some parts were not correctly written, such as in page 10 where it was referred to Table 2 for oil concentration. While Table 2 was about antioxidant activity. Also, statistical notation for Table 2, ABTS did not look right. Regarding the suggested corrections, these have been properly adjusted, where Table 1 was referenced and the scientific notation was modified.

Competing Interests: No competing interests were disclosed.

Reviewer Report 10 September 2024

<https://doi.org/10.5256/f1000research.155702.r317400>

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Elisa Julianti

Department of Food Science Faculty of Agriculture, Universitas Sumatera Utara, Medan, North Sumatra, Indonesia

The subject of this manuscript ("Characterization of sacha inchi (*Plukenetia volubilis*) and taro (*Colocasia esculenta*) flours with potential application in the preparation of both gluten-free and high protein foods") falls inside the general scope of the F1000 Research. The study presents an interesting exploration of sacha inchi and taro flours as alternatives in gluten-free and high-protein food formulations. The choice of materials is commendable due to their nutritional benefits and potential applications. However, in my point of view, the paper does need some revision before indexing, as follows:

1. In the conclusion of abstract, Based on the characteristics obtained, what type of food is suitable for the application of sacha inchi and taro flour? For example, would it be bread, pasta, biscuits, or other types of products?
2. In the introduction, it should be justified what are the drawbacks of using sacha inchi and taro as single food ingredients? Why do you want to combine sacha inchi and taro flour as food ingredients?
3. In the methods : The method does not describe the analytical procedure for sacha inchi almonds. Was any treatment applied to the seeds before analysis?
Is there a reason for using the ratio of SIF and TF?
4. In the discussion :
 - It should be explained what causes the difference in protein content obtained through the wet method and the mechanical defatting method.
 - Sacha inchi flour obtained through the wet method is flour derived from the dried, water-insoluble components. The water-soluble carbohydrate components are certainly not included in SIF. However, why is the carbohydrate content in sacha inchi almonds lower than in SIF-DM? Additionally, why is the carbohydrate content in SIF-DM lower, even though only the fat is removed during its production? Please explain.
 - In SIF-DM, there is a release of phenolic compound due to involving pressure and temperature, but why is the FPP content in SIF-DM higher than in SIF-WM? Does the released FPP not get lost during the processing?
 - It would be more meaningful if the researcher could show the starch content of each sample with different SIF and TF ratios.
 - Based on Table 3, the aw for all three types of flour is <0.85, which should prevent the growth of microorganisms. How do you explain the presence of Salmonella and mold in your flour products?
5. In conclusion : What is the recommended ratio of SIF (to TF) ?

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

No

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Flour and Starch from Root and Tuber Crops as Alternatives to Wheat Flour

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 31 Oct 2024

Karen Muñoz

Dear Dr Elisa, Thank you very much for your thoughtful and constructive feedback on our manuscript. We appreciate the time and effort you dedicated to reviewing our work, as well as your insightful suggestions, which have greatly helped us improve our paper. Below, we address each of your comments in detail:

1. In conclusion of abstract, Based on the characteristics obtained, what type of food is suitable for the application of sachu inchi and taro flour? For example, would it be bread, pasta, biscuits, or other types of products?

In response to Question 1, the conclusion of the document has been adjusted to mention that, based on the obtained characteristics, "the analyzed flours represent a raw material with great potential for the development of products such as protein snacks due to the protein contribution of sachu inchi, good expansion due to taro starch, and being naturally gluten-free for individuals with celiac disease".

2. In the introduction, it should be justified what are the drawbacks of using sachu inchi and taro as single food ingredients? Why do you want to combine sachu inchi and taro flour as food ingredients?

In response to Question 2, the introduction has been supplemented with information about some drawbacks or limitations of both sachu inchi and taro. "Sachu inchi can be unstable due to its high concentration of unsaturated fatty acids, making it sensitive to oxidation. Additionally, sachu inchi has limiting amino acids, such as lysine and tryptophan, which may cause low digestibility. Likewise, this seed contains antinutritional factors that limit its use as a raw material in the development of new products. On the other hand, taro contains antinutrients such as oxalates, which can restrict its fresh consumption due to the irritation they cause in the throat and mouth. However, the extrusion process significantly reduces these issues while simultaneously enhancing nutritional properties, such as increased protein digestibility. Based on this, combining sachu inchi and taro is an excellent option to complement their nutritional properties, increasing nutritional value and improving the stability and texture of the final product".

3. In the methods : The method does not describe the analytical procedure for sachu inchi almonds. Was any treatment applied to the seeds before analysis? Is there a reason for using the ratio of SIF and TF? In relation to Question 3, in the section on methods used to obtain sachu inchi flour, additional information is provided about the process followed prior to the proximal characterization of the sachu inchi kernel. It is explained verbatim that "the sachu inchi seeds were received as white kernels, meaning without shell or husk. For the compositional analysis of the kernel, they were manually ground with a mortar to reduce their size, thus facilitating the relevant tests". Furthermore, the reason for using the SIF and TF ratio lies in their high nutritional value (carbohydrates and protein) and the fact that both raw materials have had limited scientific and industrial advancement, presenting a significant opportunity to leverage their potential.

4. 4. In the discussion :

- It should be explained what causes the difference in protein content obtained through the wet method and the mechanical defatting method.

To address this question, additional information has been included to explain the cause of differences in protein content obtained through the wet milling method compared to the mechanical defatting method. The document states verbatim: "It is important to note that the protein content obtained through the wet milling method is lower compared to the mechanical defatting method. This is because, during the wet milling process, a milky suspension is generated, which is then filtered to separate the insoluble extract (cake) from the water-soluble extract (slurry). During filtration, part of the protein may remain in the soluble extract, which explains the lower protein concentration in the cake. In this study, the proximate analysis was performed only on the insoluble extract".

- Sachu inchi flour obtained through the wet method is flour derived from the dried, water-insoluble components. The water-soluble carbohydrate components are certainly not included in SIF. However, why is the carbohydrate content in sachu inchi almonds lower than in SIF-DM? Additionally, why is the carbohydrate content in SIF-DM lower, even though only the fat is removed during its production? Please explain.

Regarding the question, why is the carbohydrate content in sachu inchi kernels lower than in SIF-DM? Table 1 shows that the kernel contains 6.67% carbohydrates, while SIF-DM

showed 3.04% carbohydrates. In relation to the question of why the carbohydrate content in SIF-DM is lower, despite the fact that only fat is removed during its production: as shown in the following image, it is important to mention that during the oil extraction process, not only fat is removed but also some solids such as proteins, minerals, and some carbohydrates. These components may become trapped in the oil or be removed with the byproduct generated during extraction. Additionally, it is likely that some soluble carbohydrates dissolve or are removed along with the oil during mechanical extraction.

- In SIF-DM, there is a release of phenolic compounds due to involving pressure and temperature, but why is the FPP content in SIF-DM higher than in SIF-WM? Does the released FPP not get lost during the processing?

Regarding the question of why the free total phenol content (FPP) in SIF-DM is higher than in SIF-WM, it is important to consider the differences in the extraction processes. During the wet milling process (SIF-WM), phenolic compounds, which are water-soluble, can migrate into the liquid solvent during the maceration stage, resulting in a significant loss of these bioactive compounds in the solvent and thus decreasing the concentration of phenols in the final flour. In contrast, the mechanical defatting process (SIF-DM), a more physical method that does not involve liquid solvents, results in much lower phenolic compound loss. Without a significant aqueous phase, the phenols remain largely within the solid matrix of the flour.

- It would be more meaningful if the researcher could show the starch content of each sample with different SIF and TF ratios.

To complement this information in the article, the following text has been added detailing the carbohydrate percentage and protein content in each mixture with different inclusion levels. "The study design was based on the type of flour and its inclusion levels in the mixture, taking protein content and carbohydrate percentage as the most relevant factors in each treatment. The following results were obtained: the 25% SIF-DM:75% TF mixture had 22.69% protein and 64.83% carbohydrates; the 50% SIF-DM:50% TF mixture showed 39.34% protein and 44.23% carbohydrates; and the 75% SIF-DM:25% TF mixture yielded 55.98% protein and 23.64% carbohydrates. Meanwhile, the mixtures with SIF-WM presented the following values: 25% SIF-WM:75% TF with 12.42% protein and 73.47% carbohydrates; 50% SIF-WM:50% TF with 18.80% protein and 61.52% carbohydrates; and finally, the 75% SIF-WM:25% TF mixture with 25.17% protein and 49.56% carbohydrates".

- Based on Table 3, the aw for all three types of flour is <0.85, which should prevent the growth of microorganisms. How do you explain the presence of *Salmonella* and mold in your flour products?

As mentioned in Table 3, the analyzed flours exhibit low water activity, with values below 0.85.

This level of water activity is not suitable for the growth of most microorganisms, as the available water is insufficient to sustain basic metabolic processes. However, while low water activity inhibits the active proliferation of microorganisms, it does not guarantee the total absence of pathogens. Spores of certain microorganisms and bacteria, such as *Salmonella*, can survive under adverse conditions, remaining viable for extended periods. Additionally, many pathogens possess persistence mechanisms that allow them to survive in hostile environments. In this case, *Salmonella* can form protective structures called biofilms, which enable microorganisms to adhere to surfaces and resist disinfection treatments. Moreover, these biofilms not only act as physical barriers but also increase the tolerance of microorganisms to physical processes such as drying, protecting bacterial cells from environmental stress.

5. In conclusion : What is the recommended ratio of SIF to TF ?

To address this question, the conclusions state that through the viscoelasticity analysis (temperature sweep), it was determined that the optimal inclusion level of defatted sachu inchi is between 25% and 50%, which allows for the formation of a stable and consistent gel. Complementing this conclusion, the 25% SIF-DM:75% TF mixture has 22.69% protein and 64.83% carbohydrates, while the 50% SIF-DM:50% TF mixture shows 39.34% protein and 44.23% carbohydrates. These values are relevant for the development of products such as extruded snacks, where the protein contribution from sachu inchi is crucial, and the expansion capability provided by taro starch allows both ingredients to complement each other effectively.

Competing Interests: No competing interests were disclosed.

Version 1

Reviewer Report 04 September 2023

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Diego Salazar 

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² Universidad Complutense de Madrid, Madrid, Community of Madrid, Spain

The work presented is interested in using unconventional sources, their characterization, and their nutritional components. However, the work requires management of scientific writing, the information presented needs to be revised, and the correct use of units of measurement is missed, for example, %, degrees Celsius, among others. On the other hand, the methodology needs to be more precise and allow a straightforward reading of what has been developed.

Finally, the results must be rewritten, and consider that critical analysis and discussion of the results must be carried out, not only using the data from the tables and rewriting them.

Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

No

Are all the source data underlying the results available to ensure full reproducibility?

No

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Food Science and Technology. Research in new sources of foods, andean crops, legumes, tubers.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 05 Sep 2023

Karen Muñoz

The units are adjusted and the methodology is clarified.

We kindly ask you to specify in which paragraph it is necessary to adjust the analysis, since in each test a comparative and statistical analysis of the results is performed.

Competing Interests: No competing interests were disclosed.

Author Response 04 Mar 2024

Karen Muñoz

Thank you for your valuable comments.

I would like to continue the process of evaluation and correction of the article, for this I ask for new corrections, in order to be able to make them.

Best regards

Competing Interests: No competing interests were disclosed.

Reviewer Report 04 September 2023

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Oluwaseun P Bamidele 

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I worked on the PDF format of this manuscript and there is a fundamental error in the design of this study. The work would have been good if the authors work on compositing the two raw materials. There is no scientific contribution with way the study is presented. Although the Sacha inchi is new but not Taro.

The major challenge with the manuscript is the design. Compositing the root (Taro) and the Sacha at different ratios or percentages will make a difference in flour, which is rich in protein and other bioactive compounds.

Also, the manner in which the author reported the analyses is poor. Reporting on Sacha is not enough, using it in food preparation will be the best.

I recommend that the authors should proofread their manuscript. The English is not good enough.

Please find the annotated pdf with further comments [here](#).

Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

No

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Food Chemistry, Food processing and preservation, Encapsulation of bioactive compounds, Functional foods, Gamma irradiation.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 05 Sep 2023

Karen Muñoz

The corrections suggested in the pdf of the manuscript were adjusted.

A characterization of the two raw materials taro and sachu inchi is made.

This research was done in order to pave the way for new projects on the development of food based on vegetable matrices.

Mixtures with inclusion levels of 25, 50 and 75% of sachu inchi flour and taro 75, 50 and 25% were worked in order to evaluate the bioactive, rheological and proximal properties.

We kindly request to specify the paragraphs or sentences that should be adjusted to improve the analysis.

In addition, improvements were made in the English language.

Competing Interests: No competing interests were disclosed.

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