Philippine Journal of Science
152 (6B): 2343-2357, December 2023
ISSN 0031 - 7683
Date Received: 27 Jul 2023

Effects of Pre-processing Methods of Pili Nuts (*Canarium ovatum* Engl.) on Its Nutritional and Mineral Bioavailability

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Pili* nut is an emerging tree nut among other well-known nuts in the world. It is endemic in the Bicol Region, Philippines. Pre-processing of nuts such as soaking in brine or activation and germination enhances their nutritional quality; however, no scientific substantiation is available to support this claim. Thus, the present study aimed to determine the effects of pre-processing techniques and assess their effects on the nutritional, mineral bioavailability, and antinutritional components. The techniques were: [1] activation through soaking in salt solution (with and without testa) and [2] germination (pre-sprouted and sprouted). The proximate composition reveals that pre-processing *pili* nuts reduces the fat; the same applies to protein, except for soaking with testa. For total macrominerals, soaking increases the level of Na, with the highest levels in those without testa (2984 mg kg^{-1}) and with testa (553 mg kg^{-1}). The level of total Mg was reduced. For total trace elements, pre-processing does not affect the amount of Zn. Necessary control is recommended when selecting salt as a raw material during processing due to the increase in the levels of Cd and Pb after soaking in brine. For mineral bioavailability, treatments do not affect the available K ($p = 0.074$) and Mn ($p = 0.15$). Pre-processing has a negative impact on the bioavailability of Fe. Soaking without testa reduces the phytic acid, whereas germination reduces both tannic and phytic acid. The present research reveals that *pili* nuts pre-processed through soaking and germination have varying effects on the levels of antinutrients, total minerals, and bioavailability.

Keywords: activation, germination, mineral bioavailability, *pili* nut, pre-processing, soaking

INTRODUCTION

The Philippines is the center of diversity for several fruit trees that produce edible nuts. One of the most important of these species is the *pili* (*Canarium ovatum* Engl.), whose geographic spread is still limited in the country. For this reason, the plant's current production is limited to a small area of the Philippines. It typically grows in low and medium-altitude forest areas in southern Luzon, parts of Visayas, and Mindanao. The Bicol Region, where the center of genetic diversity is located, is where the *pili* nut is renowned for growing abundantly – specifically in the provinces of Sorsogon, Albay, and Camarines Sur (PSA 2020). *Pili* nut is a promising indigenous nut that has high commercial potential and is regarded as the Bicol Region's flagship commodity. Aside from the well-known tree nut kernel, export commodities derived from the *pili* nut kernel such as pastries, confectionary products, and

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whole dehydrated kernels are now gaining attraction in domestic and international markets. The *pili* nut fruit is well-known among *Canarium* species due to its large kernel (Coronel 1996). The fruit is composed of pulp, which is covered with an exocarp that changes from green to purplish black during maturity. It is harvested at 3rd maturity (M3) to maximize its nutritional content such as fat (Millena *et al.* 2023). The kernel is enclosed in a stony shell (endocarp) and covered with a papery covering (testa), which is removed during processing.

Several researchers have investigated the *pili* nut's nutritional composition, essential minerals, and trace elements. The *pili* nut has abundant nutritive and bioactive components such as fat content, which is high in monounsaturated fatty acids, tocopherols, and phytosterols; it is also rich in protein, dietary fiber, and phenolic compounds (Millena and Sagum 2018a; Arenas and Trinidad 2017; Pham and Dumandan 2015). Additionally, the *pili* nut kernel is a rich source of vitamins such as vitamin A, niacin, and riboflavin, as well as phytochemicals such as alkaloid and flavonoid (Ogbuagu and Chukuka 2014), and minerals that include Fe, Zn, Cu, Mn, Na, K, Ca, Mg, and P (Millena and Sagum 2018b). Some of the *pili* minerals, specifically Ca and Mg, are highly bioavailable in the small intestine for absorption *in vitro* and can be utilized by the body after exposure to simulated digestion (Millena and Sagum 2018b). However, *pili* nuts also contain antinutrient components such as phytic acid, which is concentrated in the kernel, and tannic acid in the pulp, which are associated with reduced mineral bioavailability (Millena and Sagum 2018b). High levels of phytic acid in *pili* nut kernels may form insoluble complexes that prevent micronutrients from being solubilized in the small intestine, which contributes to the low bioavailability of Fe and Zn in *pili* nut kernels (Millena and Sagum 2018b). Nuts contain appreciable amounts of phytate (inositol phosphate) that cause the chelation of several minerals in the food, which causes low mineral bioavailability in plant sources (Gibson *et al.* 2010). Antinutrients exhibit vital roles in plants such as defense to prevent diseases and pathogens and deter herbivores but may not be beneficial to humans (Faizal *et al.* 2023; Popova and Mihaylova 2019).

With the increasing demand for nutritive foods, several technologies are being adopted to enhance nutrient absorption and aid human health. Many studies have been reported and utilized in different types of food to enhance nutrient absorption by reducing antinutrient content using thermal and non-thermal processing methods. These methods include fermentation, soaking, dehulling, cooking, germination, enzymatic treatment, irradiation, and advanced methods such as ozonation, high-pressure processing, and cold plasma processing (CPP) that show effective antinutrient content reduction (Faizal *et al.* 2023; Popova and Mihaylova 2019; Vanga *et al.* 2017). The activation of seeds, grains, legumes, and beans is widespread among indigenous communities worldwide. A consensus method for activating nuts involves soaking nuts and seeds in salt water for around 12 h, followed by drying at a lower temperature for 24 h (Tunde-Akintunde and Oke 2012). It is claimed that this method of activating the nut may inactivate enzymes and reduce the level of antinutrients in nuts (Mack 2013). At present, limited studies on the processing of nuts support the reduction of antinutrient content and assess its effect on nutritional and mineral bioavailability. Nut activation involves neutralizing enzyme inhibitors found in nuts, which increases nutrient bioavailability, promotes proper digestion, and changes the flavor and texture of the nut (Kumari *et al.* 2020). The information promoting the need for nuts to be "activated" before they may deliver the greatest health advantages has been made available to the general public through lay literature (Kumari *et al.* 2020).

Several claims regarding the benefits of nut activation include the reduction in tannins, oxalic acid, saponin, and phytic acid to varying degrees, as well as inhibitory enzymes like trypsin and amylase inhibitors that result in enhancing macro- and micromineral bioavailability (Faizal *et al.* 2023; Samtiya *et al.* 2020; Popova and Mihaylova 2019; Oghbaei and Prakash 2013; Gibson *et al*. 2010). Several studies on soaking and germination used in amaranth, quinoa, buckwheat, and sorghum reveal techniques that are excellent in minimizing antinutrients and enhancing nutritional, bioactive, and antioxidant potential (Thakur *et al*. 2021; Afify *et al.* 2011), but there are limited studies for nuts. It was contrary to the result found by Kumari *et al.* (2020) that soaking almonds, hazelnuts, walnuts, and peanuts was ineffective in lowering phytate content and nutrient bioavailability. Furthermore, based on the study of Omenna *et al.* (2016), germination increased the composition of cowpea seeds' nutrients and antinutrients.

At present, *pili* nut is sold in different confectioneries, and an immerging product sold as "activated *pili* nut" in the market is available. The information regarding the effects of varying nut pre-processing techniques on the nutritional and mineral bioavailability of *pili* nuts has not yet been validated. Moreover, based on the reviewed literature, there is still conflicting data regarding the effect of activations on mineral bioavailability in nuts that needs to be explored to provide scientific substantiation for the claim. Therefore, the main objective of this study is to elucidate the effects of different nut pre-processing techniques on the nutritional and mineral bioavailability of *pili* nuts by activation through soaking them in salt solution (with and without testa) and germination (pre-sprouted and

sprouted). This study will fill the gap in the absence of scientific evidence to support the claims on nutritional attributes regarding *pili* nut pre-processing techniques. The results of this study conveyed how the techniques influence the nutritional and mineral bioavailability of *pili* nuts, which are also involved in determining the concentration of anti-nutrients such as phytic and tannic acids. The findings of this research will be beneficial in determining which nut pre-processing technique to employ to maximize the nutritional attributes and to provide the necessary information to be communicated to the general public on nut activation.

MATERIALS AND METHODS

Reagents and Consumables

Ultrapure water was utilized during the preparation of solutions and instruments produced by the Milli-Q system (Millipore, Molsheim, France) with a required resistance of 18.2 MΩcm. Nitric acid, hydrochloric acid, sulfuric acid, petroleum ether, and primary standards for minerals and trace elements of analytical grade were used (Merck; Darmstadt, Germany). Phytic acid, tannic acid, enzymes such as α-amylase (G0660), pepsin (P700), pancreatic bile (B8631), and dialysis membrane (23 mm, 14 kDa MWCO) were produced by Sigma-Aldrich (St. Louis, MO, USA). To ensure the accuracy of the results, certified reference materials were used in parallel with the analysis of the samples: LGC CRM 7103 (Queens Road, Teddington, Middlesex, UK), SRM 3233, and SRM 1567b (NIST, Gaithersburg, MD, USA).

Plant Material and Preparation

Two thousand (2000) pieces of *pili* nut fruit cultivars from Buenavista, Bacon District, Sorsogon City were collected from 20 trees. The samples were generously donated by the Philippine *Pili* Industry League Inc. (Sorsogon City). Only *pili* fruits that were fully ripe were harvested and selected to be free from any physical defects, insect infestations, or microbial infestations. The maturity of the *pili* nut fruit was based on the skin color, which is dark purple to black or classified as M3 (Millena *et al.* 2023). The collected *pili* nuts were washed with tap water and soaked overnight for depulping. The soaked nuts are manually separated from the pulp and then rinsed with tap water. The nuts were then divided across the treatments. For control and soaking, the washed nuts were air dried for 2 d. The dried *pili* nuts were cracked using a nutcracker to separate the shell and kernel. The remaining half of the air-dried samples were used for germination. The *pili* nut samples were pre-processed in two ways: activated (soaking) and germination. *Pili* nut kernels with and without testa are subjected to a soaking method in triplicate. For germination, the present study utilizes the pre-sprouted (PS) and sprouted (S) *pili* nut. The dried raw *pili* nut kernel served as the control.

Nut Activation Protocol

The soaking protocol contains two soaking treatments and the experiment was conducted in three independent setups. Two hundred grams (200 g) of *pili* nuts with and without testa were weighed (Ohous, Pine Brook, NJ, USA) and soaked for 12 h in 1 L of solution with a concentration of 20.4 g of commercially available sea salt (purchased at the local grocery store in Legazpi City) at room temperature. The amount of salt to water ratio is based on the lay media described in the study of Afify *et al.* (2011) and Kumari *et al.* (2020). The nuts were fully immersed in the solution at room temperature. The raw or untreated nut serves as control (R), as do raw and dried *pili* nuts, and soaking methods are divided into soaking with testa (SWT) and soaking without testa (SWOT). After soaking, the solutions were drained. For *pili* nuts with testa, the papery covering was manually removed prior to drying.

Nut Germination Protocol

To germinate the *pili* nuts, the nuts were placed in a single layer on the three different wooded germination boxes – which have a size of 1 m in width, 1.5 m in length, and 0.5 m in height – with fine mesh nets covering the whole box. The bottom layer (16 cm) was rocks, the middle layer (16 cm) was filled with mixed sea sand and soil (1:1), and the top layer (16 cm) was decontaminated coco peat, which served as the germination bed. The seeds were laid flat where the heart-shaped, grooved opening or the broadest side of the triangular-shaped nut is facing down. The germination box is located in a partly shaded area. The top layer was sprinkled with water every other day to provide the necessary hydration and maintain the 100% RH for germination activities. The PS and S seeds took an average of 20 and 31 d, respectively, before harvesting. Nuts with 3.8 cm and above require 20 d for the shell cracks and radicle to emerge, whereas the elongated radicle emerged after 31 d (see Appendix I Figure I for reference). The experiment was conducted in three independent setups. Two hundred grams (200 g) of germinated nuts were collected in each germination box. During germination, the hard shell naturally opened, thereby exposing the kernel, and the testa was removed manually. For the germinated nuts, they underwent sanitizing with acidified hypochlorite (500 mg L^{-1}) and washing with distilled water three times (Sun *et al.* 2012). The control and pre-processed *pili* nuts were evenly spread in a single layer on different trays. It was placed in the convection oven (Memmert UN110, Schwalbach, FRG, Germany) and dried for 24 h at 60 °C. The nuts were

then cooled and ground using a laboratory mill (Kniftec KN 295; FOSS Analytical Co. Ltd., P.R. China). The samples were labeled accordingly, placed in airtight glass containers, and stored at –20 °C (Biobase, Shandong, R.P. China) prior to further analysis.

Determination of Proximate Composition

The method utilized for proximate analyses was based on the Association of Official Analytical Chemists (AOAC) 21st edition (2019) method for nuts and nut products. Certified reference materials were analyzed in parallel with the sample as part of quality control and assurance during data gathering. Moisture content was analyzed based on the method prescribed by AOAC 925.40. A pre-weighed aluminum dish was prepared, and a 2 g ± 1.0 mg sample was weighed and dried at a temperature of 100 °C for 1 h in a convection oven (Memmert UN75, Schwalbach, FRG, Germany) until constant weight. The moisture content was calculated by weight loss. The ash content was determined gravimetrically, aligned with AOAC 950.49. A 2 g ± 1.0 mg sample was weighed in a pre-weighed beaker. The sample was charred on a hotplate. After charring, the sample was incinerated at a gradual increase in temperature up to 525 °C using a muffle furnace (Ehret 51SP, CA, USA) until it became white or nearly white ash. The ash was cooled in a desiccator for 30 min and weighed until a constant mass was obtained. Protein content was determined using indirect determination (Kjeldahl method) and was performed by adopting AOAC 950.48. A 0.7 g ± 1.0 mg homogenized sample was digested using 15 mL of concentrated sulfuric acid (H_2SO_4) with two Kjeltabs in a digestion block Foss DT 208 (Hilleroed, Denmark) at 420 °C for 1 h. The digested sample was diluted with water and 60 mL of 40% NaOH prior to distillation using Foss Kjeltec 8100 (Hilleroed, Denmark). The distillate was back-titrated against 0.1M HCl using a digital titrator (Titrette, Wertheim, Germany). The protein content was computed by multiplying the percent nitrogen by a factor of 5.30. The fat content was measured based on AOAC 948.22. A 50-mL beaker with a cellulose thimble was used to hold the 2 g ± 1.0 mg sample, which was dried for 5 h at 103 °C. Fat content was extracted using the pre-weighed aluminum cups, with 30 mL of petroleum ether as a solvent, using Foss Soxtec 2045 (Hilleroed, Denmark). After extraction, the aluminum cups with extract were dried in an oven at 103 °C for 1 h. The aluminum cups were cooled in the desiccator for 30 min and weighed until they reached a constant weight. The percent carbohydrate was computed by subtracting 100 from the sum of moisture, ash, fat, and protein. All the analyses were conducted in a minimum of triplicate, unless otherwise specified, and expressed as g 100 $g^{-1.}$ The energy value was computed in accordance with AOAC 991.43 and expressed as Cal 100 g^{-1} by multiplying the carbohydrates, protein, and fat by factors of 4, 4, and 9 Cal, respectively.

Determination of Total Minerals and Trace Elements

For the analysis of minerals and trace metals, the AOAC 2011.14 was utilized with modifications to the instrumentation using microwave plasma–atomic emission spectroscopy (MP-AES), a new generation of nitrogen plasma-based technique, and microwave plasma–atomic emission spectrometry, which can examine multiple elements similar to inductively coupled plasma optical emission spectrometry. A single laboratory method validation was conducted prior to the analysis. Prior to analysis, glassware was thoroughly washed and soaked with 10% nitric acid overnight and then rinsed with ultrapure water three times. The standard solution was prepared by serial dilution using a 1000 mg L^{-1} primary standard traceable to NIST (Merck; Darmstadt, Germany). The calibration ranged from 0–10 mg L^{-1} and 0–2.5 mg L^{-1} for minerals and trace elements, respectively. A minimum of five calibration points were prepared during instrumentation. To ensure accuracy during determination, appropriate CRM was analyzed in parallel with the samples. From the pre-treated, dried, and homogenized samples, 1.00 g ± 1.0 mg was weighed using an analytical balance (Mettler Toledo XPR-204, Greifensee, Switzerland) in a 50-mL beaker. The sample was charred on a hot plate and incinerated in a muffle furnace at 450 °C until the ash was grayish-white. After incineration, the sample was dissolved in 5 mL of 1N HNO_3 and placed on a hot plate to aid dissolution. The digested sample was transferred to a volumetric flask and diluted to 50 mL using 1 N HNO_3. The digestate was filtered with Whatman filter paper no. 42 prior to readings. The samples were quantified using the plasma technique using a microwave plasma–atomic emission spectrometer (MP-AES 4200) (Agilent Technologies, Santa Clara, CA, USA). The acceptance criterion for the calibration curve correlation coefficient (r) was set at > 0.995. The wavelengths (nm) used per analyte are as follows based on the method validation conducted: Na (589.592), Fe (259.94), Ca (393.366), K (766.49), Mg (279.553), Cu (324.75), Ni (352.454), Zn (213.857), Mn (257.610), Pb (368.346), and Cd (228.802). The result was reported after correction from the blank as mg $kg^{-1.}$ A triplicate analysis was conducted per sample.

Determination of Mineral Bioavailability

This study used the *in vitro* method by Millena and Sagum (2018b) involving seven minerals (Ca, K, Mg, Fe, Zn, Mn, and Cu). The *in vitro* approach gives a good approximation of the minerals that are available for absorption. The analysis was carried out using three

independent experiments. The pre-processed *pili* nuts were dried for 24 h at 60 °C. and ground using a laboratory mill (Kniftec KN 295; FOSS Analytical Co. Ltd., P.R. China). To simulate human digestion up to the small intestine, a 10 ± 1.0 mg sample was weighed in a 250-mL Erlemeyer flask, followed by the addition of 90 mL of saliva solution [1% (w/v) α-amylase added with 150 mmol L^{-1} NaCl and dissolved in a 1000 mmol L^{-1} $NaHCO_3$ buffer at pH 6.8] and incubated in a shaking water bath using Foss WB 1024 (Hilleroed, Denmark) for 5 min at 37 °C set to 150 rpm. To simulate the gastric phase, the sample was acidified by 6 N HCl to pH 2.0, gradually added with 3.2 mL of pepsin-HCl solution (8 g pepsin in 50 mL of 0.1 N HCl), and incubated in a shaking water bath for 3 h at 37 °C set to 100 rpm. To determine the amount of sodium bicarbonate to be used in initial dialysis, 20 g of pepsin digest was weighed, and then 5 mL of pancreatin-bile solution (1 g porcine pancreatin and 6.25 g diluted with 0.1 M $NaHCO_3$ to a 250 mL volume) was added. This solution was then titrated potentiometrically against 0.5 M KOH to pH 7.5. The volume of KOH used was multiplied by four, which was equal to the volume of sodium bicarbonate diluted to 100 mL, which served as the initial dialyzing solution. To simulate the small intestine, 20 g of each pepsin digest was weighed in a 30-cm dialysis membrane tubing (Spectra/Por 1, 23 mm, 14 kDa MWCO dialysis membrane tubing) (Spectrum Laboratories Inc., CA, USA), followed by the addition of 5-mL pancreatin bile solution and tied at both ends. The tube was washed three times with distilled water and submerged in the previously prepared sodium bicarbonate solution, which was incubated and dialyzed for 12 h at 37 °C. The dialysate was collected and filtered using Whatman filter paper No. 42 (t_1). The solution was replaced with 100 mL of ultrapure water for the next three cycles and collected every 3 h (t_2, t3, and t_4). After dialyzing and filtration, the dialysate was read using MP-AES (MP-AES 4200; Agilent Technologies, Santa Clara, CA, USA). The percent mineral composition in dialysate was computed using Equation 1, and the percent bioavailability was computed using Equation 2.

$$\begin{matrix} Total\ \mu g \\ mineral\ in \\ dialysate \end{matrix} = \left[\frac{\mu g}{mL} t_1 + \frac{\mu g}{mL} t_2 + \frac{\mu g}{mL} t_n \right] \times 100 \quad (1)$$

$$\begin{matrix} \%\ mineral \\ bioavailabiity \\ (small\ intestine) \end{matrix} = \frac{total\ \mu g\ minerals\ in\ dialysate}{total\ \frac{\mu g}{g}\ in\ test\ sample \times g\ \text{test sample}} \times 100 \quad (2)$$

Determination of Antinutrient Content

The determination of phytic acid was based on AOAC 986.11 with some modifications. One gram (1 g) of sample was digested using a muffle furnace (Ehret 51SP, CA, USA). The sample was dissolved in 50 mL of deionized water and filtered using Whatman no. 42. Aliquot of 1 mL was placed in a 50-mL volumetric flask, added with 25-mL deionized water, 5-mL acetic buffer, 1-mL ascorbic acid (1%), and 1-mL molybdovanadate reagent. The solution was allowed to stand for 10–20 min prior to reading. The solution's absorbance was determined at 660 nm using a UV-VIS spectrophotometer (Thermo Scientific GENESYS™ 150, Wisconsin, USA). A working standard solution with known concentrations of the primary standards for phytic acid was used to quantify the amount of the analyte.

The method for determining tannic acid was done in parallel to the method of Millena and Sagum (2018b, with some modifications. From the dried sample, 1 g was weighed and extracted using 10 mL of ethanol (23.8%) and 10 mL of metaphosphoric acid (1.5%), which were homogenized using Heidolph Diax 900 (Heidolph, Germany) and brought to 50-mL final volume using ethanol (70%). The solution was filtered using an Agilent Captiva 0.45-µm nylon syringe filter. Two milliliters (2 mL) of the filtrate were transferred into a 25-mL volumetric flask, followed by the addition of 5 mL of Folin-Denis reagent and 5 mL of 1N sodium bicarbonate (Na_2CO_3), which was shaken and diluted to volume using distilled water, and stood for 1.5 h and read at 760 nm using a UV-VIS spectrophotometer (Thermo Scientific GENESYS™ 150, Wisconsin, USA). A calibration curve was established using a working standard solution with known concentrations of the primary standards for tannic acid to quantify the analyte.

Statistical Analysis

Values were calculated using descriptive statistics and expressed as mean $\pm$ standard deviation (SD). A minimum of three replicates through independent experiments were used unless otherwise specified. One-way analysis of variance (ANOVA) was used to establish the significant difference among the treatments and Tukey's test to assess the differences in means. The significance level was set at $p < 0.05$. All statistical calculations were done using IBM SPSS version 17 software (IL, USA).

RESULTS AND DISCUSSION

The effects of different pre-processing techniques on the *pili* nut kernel, as well as the raw kernel after further drying, were subjected to chemical analyses. The results of the analytical studies on the nut kernel carried out on the treated and raw nuts are discussed under the following headings.

Analytical Quality Control

The results of the control samples (LGC CRM 7103 and

SRM 3233/156b) are reflected in Table 1, which are within the certified values both for proximate and elemental composition, as stated in the certificate. The margin of error for the proximate and elemental compositions ranged from –1.56 to +18.64%; all values obtained were within the ranges in the certificate, hence demonstrating good accuracy. Precisions in terms of repeatability expressed as percent relative standard deviations (% RSD) were within the set requirements of the laboratory, ranging from 0.12–4.99%. Standard calibration curves for the elemental analyses were set at a correlation coefficient of not less than 0.995. The calibration curve's standard linearity during the determination ranged from 0.99992–0.99999. The fulfillment of the set criteria for quality assurance and control during measurement was met, hence demonstrating the accuracy of established data.

Proximate Composition

The proximate compositions of pre-processed *pili* kernel using different techniques and the control are presented in Table 2, which are expressed as g 100 g^{-1} and Cal 100 g^{-1} for the energy value on a dry weight basis (DW). The initial moisture content of raw *pili* nut was 2.87 g 100 $g^{-1.}$ The pre-processed samples had moisture contents of 2.38, 5.82, 2.13, and 2.78 g 100 g^{-1} for PS, S, SWT, and SWOT, respectively. In general, raw *pili* nut kernels exhibit superior values in terms of proximate composition, and different pre-processing methods may affect the amount at varying levels. The ash content values ranged from 3.13 to 2.60 g 100 g^{-1}, with the highest value noted in S (3.13 g 100 g^{-1}), followed by SWOT (3.00 g 100 g^{-1}) and raw *pili* nut kernel (2.95 g 100 g^{-1}) that are not significantly different from each other. The ash contents of kernels that undergo SWOT (2.78 g 100 g^{-1}) and PS (2.60 g 100 g^{-1}) had the lowest values. A reduced level of ash during SWOT could lead to leaching out of some water-soluble minerals during soaking (Thakur *et al.* 2021).

For the protein content, no significant difference was observed between raw (11.4 g 100 g^{-1}) and SWT (11.4 g 100 g^{-1}) compared to other treatments. The protein content of the S *pili* kernel (10.8 g 100 g^{-1}) had the lowest value. The obtained results were in good agreement with the data established by Millena and Sagum (2018a), which had a range of 11.5 ± 0.06 to 13.2 ± 0.14 g 100 g^{-1} DW; however, it was slightly lower compared to the S data. One of the reasons for reduced protein is due to its solubility in solutions with high ionic strength due to the addition of salt (Beauchamp and Khajehpour 2012). On the other hand, the testa may provide protection to lessen the effect of solubilizing the protein, as shown in the established data. During sprouting, stored proteins are utilized during seed germination to provide nutrients for seedling development (Wang *et al*. 2007). *Pili* nut kernels provide a good source of essential and non-essential amino acids. It contains 17 amino acids except for tryptophan; the predominant amino acids found in the nut are glutamic acid and aspartic acid, whereas cystine is the most deficient (Ogbuagu and Chukuka 2014).

Table 1. Accuracy performance test of proximate analysis and quantification of metals through the analysis of certified reference materials.

CRM	Analyte	Certified value[a]	Obtained value[b]	% error
LGC CRM 7103	Ash (g 100 g^{-1})	1.599 ± 0.077	1.624 ± 0.193	–1.56
	Total Fat (g 100 g^{-1})	21.17 ± 0.45	21.22 ± 1.03	–0.24
	Protein (g 100 g^{-1})	1.073 ± 0.032	1.078 ± 0.096	–0.47
	Moisture (g 100 g^{-1})	2.88 ± 0.76	2.90 ± 0.28	–0.69
SRM 3233	Ca (mg kg^{-1})	36910 ± 920	36107 ± 327	+ 2.18
	Na (mg kg^{-1})	6830 ± 120	6946 ± 488	–1.70
	K (mg kg^{-1})	3060 ± 140	3014 ± 171	–1.01
	Mg (mg kg^{-1})	1039 ± 37	1140 ± 16.2	+ 1.50
	Fe (mg kg^{-1})	766 ± 36	715 ± 63.3	+ 6.66
	Zn (mg kg^{-1})	628 ± 16	610 ± 50.3	+ 2.87
	Cu (mg kg^{-1})	3.97 ± 0.28	3.23 ± 0.58	+18.64
	Cd (mg kg^{-1})	0.0819 ± 0.0020	0.0751 ± 0.0153	+8.30
SRM 1567b	Pb (mg kg^{-1})	0.0104 ± 0.0024	0.0101 ± 0.0078	+2.88

[a]Certified value ± uncertainty (U)
[b]Mean ± U, n = 3, U-expanded uncertainty at 95% confidence

Table 2. Proximate composition of pili nut kernels from different pre-processing techniques

Pre-processing method	Ash content (g 100 g^{-1})	Protein N = 5.30 (g 100 g^{-1})	Fat (g 100 g^{-1})	Carbohydrate (by difference, g 100 g^{-1})	Total energy (Cal 100 g^{-1})
SWT	3.00 ± 0.09^{a}	11.4 ± 0.80^{a}	63.3 ± 1.3^{c}	27.5 ± 1.2^{a}	658 ± 6.9^{e}
SWOT	2.78 ± 0.06^{bc}	11.2 ± 0.13^{b}	63.2 ± 1.4^{c}	20.1 ± 1.2^{b}	689 ± 3.7^{c}
PS	2.60 ± 0.05^{c}	11.1 ± 0.06^{b}	68.3 ± 0.47^{b}	15.6 ± 0.48^{c}	722 ± 2.2^{b}
S	3.13 ± 0.16^{a}	10.8 ± 0.02^{c}	61.7 ± 1.2^{c}	18.6 ± 1.4^{b}	672 ± 5.1^{d}
R	2.95 ± 0.08^{ab}	11.4 ± 0.05^{a}	72.6 ± 0.40^{c}	10.2 ± 0.36^{d}	740 ± 2.3^{a}
p	**	**	**	**	**

Reference: [SWT] soaking with testa, [SWOT] soaking without testa, [PS] pre sprouted, [S] sprouted, and [R] raw. Data are based on pre-processed samples and presented as mean ± SD g 100 g^{-1} dry weight (DW). Values with different superscript letters in each column indicate significant differences (one-way ANOVA test and *post hoc* Tukey's test, $p < 0.05$) in nutritional components measured among varieties. **Significant at $p < 0.05$.

Previous studies revealed that fat content is a major component of *pili* nuts, which agrees with the present study (Millena and Sagum 2018a; Kakuda *et al.* 2000). The fat content of the pre-processed *pili* nut kernels ranged from 61.7–72.6 g 100 $g^{-1.}$ Both soaking in brine solution and germination methods reduced the level of fat compared to raw *pili* kernels. The same result was observed on the reduction of fat during activation for almonds, hazelnuts, peanuts, and walnuts (Chua 2019). The value for pre-processed *pili* nut kernel is lower compared to the result found by Millena and Sagum (2018a), which ranged from 67.2–74.1 g 100 g^{-1} DW. However, the value for the raw kernel agrees with the previous study. A reduction in the fat content during germination may be attributed to lipolytic activity that hydrolyzed the fat and provided the essential energy for protein synthesis and growth activities of the plant (Thakur *et al.* 2021; Chauhan *et al.* 2015). Pre-processing techniques such as soaking and germination help to reduce the level of fat compared to control that meets the requirement (PNS/FDA 28:2011), which is set at maximum 70% fat for processed *pili* nuts. The *pili* nut is a good source of health promoting fat; the predominant fatty acid in the *pili* nut kernel are monounsaturated fatty acids oleic (C18:1 *cis*-9) (Millena and Sagum 2018a; Pham and Dumandan 2015; Kakuda *et al.* 2000).

The amount of carbohydrates in pre-processed *pili* kernels ranged from 15.6–27.5 1 g 100 $g^{-1.}$ The highest values were from SWT (to 27.5 1 g 100 g^{-1}) and SWOT (20.1 g 100 g^{-1}) activation methods. The values of carbohydrates and energy for raw *pili* kernels are in good agreement with that established by Millena and Sagum (2018a). The caloric value of *pili* nut kernels subjected to different pre-processing methods ranged from 658–722 Cal 100 g^{-1}, which was negatively reduced compared to the control of 740 Cal 100 $g^{-1.}$ The pre-processing methods utilized in the present study had a positive effect on the level of carbohydrates, which increased significantly but had a negative impact on the energy value. The reduction in total energy and increase in total carbohydrates may be associated with a reduction in the level of fat, which is a dense source of energy compared to other macronutrients. Further, during germination, a reduction in energy may be attributed to growth activities and other biochemical activities in the seed (Zhang *et al.* 2015).

Mineral and Trace Metal Composition

The amount of macrominerals in *pili* nut kernels subjected to different pre-processing techniques and controls is depicted in Table 3, expressed as mg $kg^{-1.}$ Results show that K is the most predominant macromineral in the *pili* nut kernel, with values ranging from 13467–25793 mg $kg^{-1.}$ Pre-processing had a negative effect on the level of K except for SWT, which increases the amount significantly. The reduction of minerals such as K can be attributed to the passive diffusion process or leaching out into a soaking solution (Gibson *et al.* 2010). For SWT, the level of K enhanced the level by 117%, which may be attributed to the reabsorption of K from the testa. In terms of Mg level, which is the second dominant macromineral, the value ranged from 2582–2765 mg $kg^{-1.}$ The level of Ca obtained in the present study ranged from 847–1051 mg $kg^{-1.}$ Pre-processing of the kernel had no significant effect on the amount of Ca, except for the PS technique, which increased by 120%. Both soaking and germination techniques reduced the level of Mg significantly compared to the control (3482 mg kg^{-1}), so the utilized pre-processing techniques had a negative impact on the amount of Mg. It is notable that soaking methods increased the level of Na content significantly, with the highest significant value in SWOT by a factor of 30 (2984 mg kg^{-1}) and SWT by a factor of 5.6 (554 mg kg^{-1}) compared to the control. According to the Philippine Daily Reference Intake (PDRI) 2015 for adults aged 19–20 yr old, the recommended intake of Na is 500 mg.

Table 3. Macromineral composition of *pili* nut kernels from different pre-processing methods.

Pre-processing method	Na	Ca	K	Mg
	(mg kg^{-1})			
SWT	554 ± 22^{b}	909 ± 38^{b}	25793 ± 1077^{a}	2765 ± 10^{b}
SWOT	2984 ± 229^{a}	926 ± 46^{b}	13467 ± 1104^{d}	2585 ± 18^{c}
PS	93 ± 6^{a}	1051 ± 13^{a}	16779 ± 1278^{c}	2581 ± 18^{c}
S	123.4 ± 6^{c}	487 ± 22^{b}	15366 ± 1483^{cd}	2724 ± 30^{b}
R	99 ± 18^{c}	853 ± 20^{b}	21950 ± 885^{b}	3482 ± 17^{a}
p	**	**	**	**

Reference: [SWT] soaking with testa, [SWOT] soaking without testa, [PS] pre sprouted, [S] sprouted, and [R] raw. Data are based on pre-processed samples and presented as mean ± SD. Values with the same letter in each column are not significantly different by Tukey's test. **Significant at $p < 0.05$.

Table 4. Total micromineral and trace metal composition of *pili* nut kernels from different pre-processing.

Pre-processing method	Zn	Mn	Fe	Cu	Ni	Cd	Pb
	(mg kg^{-1})						
SWT	40.8 ± 1.5^{a}	46.9 ± 0.74^{a}	27.2 ± 4.2^{a}	14.2 ± 0.03^{c}	0.36 ± 0.01^{a}	0.595 ± 0.07^{a}	1.59 ± 0.54^{a}
SWOT	42.0 ± 1.3^{a}	42.4 ± 0.39^{b}	26.8 ± 4.1^{a}	14.2 ± 0.12^{c}	0.12 ± 0.01^{d}	0.655 ± 0.4^{a}	1.22 ± 0.39^{a}
PS	43.9 ± 3.4^{a}	46.7 ± 0.74^{a}	29.6 ± 2.9^{a}	15.0 ± 0.04^{a}	0.27 ± 0.02^{b}	0.275 ± 0.03^{b}	0.453 ± 0.18^{b}
S	43.4 ± 3.1^{a}	42.4 ± 0.33^{b}	23.6 ± 2.4^{ab}	14.5 ± 0.06^{b}	0.19 ± 0.00^{c}	0.223 ± 0.03^{b}	0.433 ± 0.12^{b}
R	44.8 ± 2.5^{a}	47.9 ± 0.52^{a}	21.9 ± 1.6^{b}	13.8 ± 0.04^{d}	0.17 ± 0.00^{c}	0.285 ± 0.13^{b}	0.427 ± 0.17^{b}
p	0.089	**	**	**	**	**	**

Reference: [SWT] soaking with testa, [SWOT] soaking without testa, [PS] pre-sprouted, [S] sprouted, and [R] raw. Data are based on pre-processed samples and presented as mean ± SD. Values with the same letter in each column are not significantly different by Tukey's test. **Significant at $p < 0.05$.

Consumption of 30 g *pili* nut (SWOT) as a serving size for nuts can contribute to around 18% of the daily mineral intake of Na, which is considered a high source. This may add up to the daily intake of Na in the diet, which may have a negative impact on human health; thus, consumption in moderation is suggested. A lower amount of absorbed Na can be achieved by soaking the nut with intact testa, which serves as a barrier to absorbing too much Na. Pre-processing by germination has no significant effect on the level of Na. Other methods utilized in nuts such as chopping before soaking in brine significantly increase the level of Na due to the increased surface area exposed to the brine solution (Kumari *et al.* 2020). The same trend in terms of macrominerals K > Mg > Ca > Na is observed from the previous study of Millena and Sagum (2018b) for *pili* nut kernel, except for the treatment under soaking technique that enhances the Na level to a greater extent.

The amounts of microminerals and trace elements in pre-processed and raw *pili* kernels are reflected in Table 4. Among the established micromineral components, Zn and Mn are predominant. The pre-processing techniques employed do not significantly affect the level of Zn, with a range of 40.8–44.8 mg kg^{-1} (p = 0.089). For Mn, SWOT and sprouting significantly reduced the level to the same value of 42.4 mg kg$^{-1.}$ No significant change in Mn was observed in pre-processing utilizing SWT and pre-germination. For the total Fe, the value ranged from 29.6 to 21.9 mg kg^{-1}, and a significant increase was noted for all the treatments except S, which had no significant difference from the control. Almost the same trend in Fe was observed in Cu; the level significantly increased using all treatments compared to the control (13.8 mg kg^{-1}), which had the lowest amount. The same observation of an increase in Cu both for soaking and germination was noted in the study of Thakur *et al.* (2021) for cereals. For the trace metals Ni, Cd, and Pb, the levels significantly increase with soaking techniques, whereas sprouting has no effect compared with the control. The Cd and Pb content significantly increased during soaking both with and without testa *pili* kernels – up to (0.595–0.655 and 1.22–1.59 mg kg^{-1}, respectively – compared to the control. Traces of heavy metals in the nuts may be attributed to volcanic inputs, as the Bicol region is surrounded by five volcanoes. Other possible sources of heavy metals can be correlated with agricultural practices such as the addition of pesticides, insecticides, and inorganic supplements, which can be absorbed by plants and deposited in fruits

and other parts (Alengebawy *et al.* 2021; Barraza *et al.* 2017; Aikpokpodiom *et al.* 2013). Some ingredients in the formulation of glyphosate-based herbicides and other pesticides such as petroleum products contain traces of heavy metals such as Cd, Pb, Ni, and other hazardous heavy metals (Defarge *et al.* 2018). The application of excessive or uncontrolled synthetic inputs may result in inaggregation or bioaccumulation through time in the soil that is available for plant absorption (Alengebawy *et al.* 2021). Purified salt is recommended in food production, as it contains a lower level of heavy metals (Cheraghali *et al.* 2010). In the present study, the addition of sea salt – which is the traditional practice in soaking *pili* kernels – contributes to elevated amounts of heavy metals in terms of Cd and Pb compared to the control. Pre-processing using germination techniques does not increase the heavy metal significantly compared with control. Several studies show that unrefined salt contains higher levels of heavy metals (Soylak *et al.* 2008; Dim *et al.* 1991), which do not comply with Codex legislation requirements of 2 mg Pb kg^{-1} and 0.5 mg Cd kg^{-1}. Salt monitoring utilized in food processing should be strengthened. The Philippine law [Republic Act (RA) No. 8172] requires that salt for human consumption contain no more than 0.1 mg Hg kg^{-1}, 0.5 Cd kg^{-1}, and 2.0 mg Pb kg^{-1}. Since most of the salt used around the globe comes from mines, heavy metal contamination might concern table salt (Cheraghali *et al.* 2010). It is quite alarming that activation through SWT and SWOT gives a higher amount of Pd and Cd – which are known dangerous metals – without any known helpful influences in the biological systems of living organisms, animals, and plants. Aside from essential minerals, food may also contribute to exposure to several toxic chemicals, and exposure beyond its threshold level may pose a threat and lead to several illnesses. Thus, the utilization of salt for food preparation should be considered by selecting a salt provider that complies with the necessary purity requirement set by RA 8187 to reduce the risk of potential heavy metals and ensure food safety.

Mineral Bioavailability in the Small Intestine

In reality, the amount of macro and microminerals in food is not 100 % absorbable by the body when consumed. As a result, the current study investigated the influence of various nut pre-processing techniques on mineral bioavailability through *in vitro* digestion. It simulates the gastrointestinal tract by adjusting the pH and ionic strength, adding endogenous enzymes, and using physical action to simulate digestion. Good estimates of absorbable mineral nutrients are necessary to assess the quality of food, address nutrient deficiencies, and support body homeostasis. In the current study, only the availability of Ca, K, Mg, Cu, Fe, Zn, and Mn was assessed. The study simulates the human digestive process up to the small intestine only because most of the nutrients are absorbed in the small intestine, whereas only a portion of them is absorbed in the colon (Millena and Sagum 2018b). After the *in vitro* digestion, the percent mineral bioavailability decreases in order – Cu > Ca > K > Mg > Fe > Zn > Mn – as graphically presented in Appendix II Figure II and numerical values presented in Appendix II Table I. The mineral bioavailability of different pre-processing methods utilized in the present study varied significantly, except for some minerals. Based on the established data of the present undertaking, except for K ($p = 0.069$) and Mn ($p = 0.15$), the level of bioavailability of each mineral varies significantly depending on the pre-processing method utilized. The bioavailable Ca ranged from 13.0–22.2%, wherein pre-processing by sprouting and SWOT had the highest percentage of availability with 19.3 and 22.2%, respectively. The percent bioavailability of Mg ranged from 12.9–19.3%; activation increased significantly except for PS (17.4%) and raw (12.9%), which gained the lowest values. Both soaking methods enhance the bioavailability of Mg. In terms of the level of Cu availability, both soaking techniques had a positive effect, with almost 50% availability compared to germination (37.5–43%) and control (40.7%). All the pre-processing techniques had a significant negative effect on the level of Fe compared to the control (21.3%) and almost no effect on the bioavailable Zn except for sprouting, which was enhanced by around 50%. The result is in good agreement with the data established by Kumari *et al.* (2020) for the reduction of Fe bioavailability during activation. Several studies suggest that phytate and mineral molar ratios affect mineral bioavailability, where a molar ratio greater than 1 significantly affects the bioavailability of minerals (Faizal *et al.* 2023; Ma *et al.* 2005). Reduced bioavailability may be attributed to the antinutrient contents such as phytic acid and oxalic acid, which form insoluble complexes with positively charged di- or trivalent elements – particularly Ca^{2+}, Zn^{2+}, $Fe^{2+, 3+}$, Mg^{2+}, Mn^{2+}, and Cu^{2+} (elements that retard its absorption) (Faizal *et al*. 2023; Millena *et al.* 2018b). The phosphate forms ligands with the minerals as phytate, and the molecule serves as the storage of minerals in the seeds (Silva *et al.* 2021). Based on the study, soaking and germination did not promote Fe absorption. It was suggested that the effect of phytate on Fe is dose-dependent. The molar ratio of phytate to iron above 1 affects the bioavailability of Fe (Hurrell and Egli 2010), which is in good agreement with the present study. Another contributing factor is the competition of minerals for absorption in the small intestine, which may lead to a reduction in the availability of some minerals. With the present established information, it shows that pre-processing may enhance the bioavailability of some minerals, whereas some minerals such as K and Mn may have no effect and have a negative effect on bioavailable Fe and Cu.

Table 5. Antinutrient components of the pre-processed *pili* nut kernel.

Pre-processing method	Antinutrient	
	Phytic acid (mg 100 g^{-1})	Tannic acid (mg 100 g^{-1})
SWT	1074 ± 21^a	0.235 ± 0.027^b
SWOT	852 ± 56^b	0.169 ± 0.027^b
PS	649 ± 64^c	0.742 ± 0.049^a
S	627 ± 62^c	0.773 ± 0.030^a
R	1113 ± 41^a	0.238 ± 0.034^b
p	**	**

Reference: [SWT] soaking with testa, [SWOT] soaking without testa, [PS] pre-sprouted, [S] sprouted, and [R] raw. Data are based on pre-processed samples and presented as mean ± SD. Values with the same letter in each column are not significantly different by Tukey's test. **Significant at $p < 0.05$.

Antinutritional Components

Table 6 presents the amounts of antinutrient components in *pili* nuts when exposed to different pre-processing techniques. The level of phytic acid is higher compared to the tannic acid in *pili* nut kernels, which is in good agreement with the study of Millena and Sagum (2018b). Soaking the *pili* nut kernel with testa in salt solution does not reduce the level of phytic acid significantly, with values of 1113 and 1074 mg 100 g^{-1} for raw and soaked kernels with testa, respectively. SWOT or by removing the coating of the nuts reduces the level of phytic acid by 23%. Exposing the surface areas of nuts allows phytic molecules to leach out in salt solutions. The reduction of phytic acid during SWOT is likely due to the passive leaching or diffusion and hydrolysis of phytate (Elgi *et al.* 2002; Hotz and Gibson 2007). The same result was observed in soaking walnuts in brine solution – reducing the level by 5–12% – but not in almonds, hazelnuts, or peanuts (Kumari *et al.* 2020). Differences in the effect of phytic acid reduction in different nuts may be attributed to the structural and biochemical differences between nuts (Kumari *et al.* 2020). Several antinutrients are effectively reduced by heat treatment methods, but phytic acid is heat stable, so other non-heat treatments may be used such as physical methods, fermentation, irradiation, ozonation, and CPP, to name a few (Faizal *et al.* 2023; Samtiya *et al.* 2020). For the germination techniques, it reduces the level of phytic acid by 40%. According to Ertop and Bektaş (2018), during germination, endogenous enzyme activity increases, which allows the breakdown and reduction of antinutrients such as phytic acid. Endogenous enzymes phytase break down phytic acid into P, myo-inositol, and mineral contents for utilization in different metabolisms during plant growth (Afify *et al.* 2011). The reduction of phytic acid during SWOT can be explained by partial hydrolysis of phytic acid by the endogenous enzyme phytase or through leaching in the solution. Traces of tannic acid were noted in the kernel, which ranged from 0.169–0.773 mg 100 $g^{-1.}$ Previous studies strengthen the observation that tannic acid is higher in *pili* pulp than in the kernel (Millena and Sagum 2018b). Both SWT and SWOT do not significantly increase the level of tannic acid, but germination enhances the level of tannic acid.

There are several approaches to reducing antinutrients in different food products to enhance nutritional bioavailability. The present study reveals that soaking the *pili* kernel with testa does not reduce the level of phytic acid but reduces it by removing the testa to a certain level, which is further reduced by almost half during germination. Further, tannic acid is reduced not only by soaking methods but also significantly through germination. Numerous studies show that soaking in brine for 12 h is most effective, and germination or combination reduces antinutrients, enhances nutrition, and increases bio-active components in foods such as cereals, seeds, and grains (Thakur *et al.* 2021; Singh *et al*. 2017). The present study supports the claim of reducing the antinutrient in *pili* nuts, but the effect on nutritional composition and mineral bioavailability may vary to some extent. The reduction in phytic acid in the present undertaking showed a positive effect to some extent on the bioavailability of Ca, Cu, and Mg. Several mechanisms could be responsible for the reduced antinutrient; soaking may have solubilized it, resulting in passive diffusion or leaching (Elgi *et al.* 2002); another mechanism could be the hydrolysis of phytate by the endogenous enzyme phytase through fermentation but was not assessed in the present study (Faizal *et al*. 2023). Other studies suggested that antinutrients, through their chelating properties, reduce the maximum utilization of nutrients by obstructing some biochemical reaction in the body that affects the optimal bioavailability of the nutrients for absorption within the intestinal lumen (Ertop and Bektaş 2018).

CONCLUSION

Soaking and germination are low-cost traditional non-heat pre-processing methods utilized in reducing antinutrients common in food grains, but there is very limited information for nuts. Several studies have shown that germination and soaking enhance the nutritional quality of cereals and grains. Conflicting literature in different nuts was observed on the effect of pre-processing. As of the time of this present study, no undertaking has been carried out to assess the effect of soaking and germination on the nutritional, total mineral, antinutrient composition, and mineral bioavailability of *pili* nuts through simulated digestion. Subjecting the nuts to

different pre-processing techniques reveals variations in the proximate composition, mineral composition and its bioavailability, and anti-nutritional factors. Removal of testa prior to soaking in brine reduces the level of the antinutrient phytic acid but not tannic acid. Germination is an excellent method for reducing the level of antinutrients, both phytic acid and tannic acid. In germinating nuts, one may consider the required timeliness for the production of germinated nuts and the final sensory characteristics of the final product, which may be affected. Control must be in place for soaking by ensuring the quality of the salt, which may lead to elevated levels of heavy metals such as Pb and Cd in the final product. The present study reveals that activating *pili* nuts through soaking and germination has advantages in lowering the level of antinutrients, reducing the level of fat (< 70 %) to comply with PNS/FDA 28:2010 requirement, and providing advantages and disadvantages on total and mineral bioavailability at varying levels.

DECLARATION OF INTEREST

The authors declare that they have no known conflicting personal or financial relationships or conflicts of interest that could influence the work presented in this paper.

Funding
This research did not receive any funding support.

ACKNOWLEDGMENT

The authors would like to acknowledge Mr. Joeriz Olbes of the Philippine *Pili* Industry League Inc. for generously providing the *pili* nut samples. The technical assistance of the Department of Science and Technology Regional Office No. V–Regional Standards and Testing Laboratories, the Department of Agriculture–Albay Research and Development Center, and Bicol University College of Science are kindly acknowledged. The invaluable support of Bernardo A. Altavano Jr. during the final editing and proofreading of the manuscript is likewise acknowledged.

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APPENDIX I

Figure I. Germinated *pili* nut: [a] pre-sprouted and [b] sprouted.

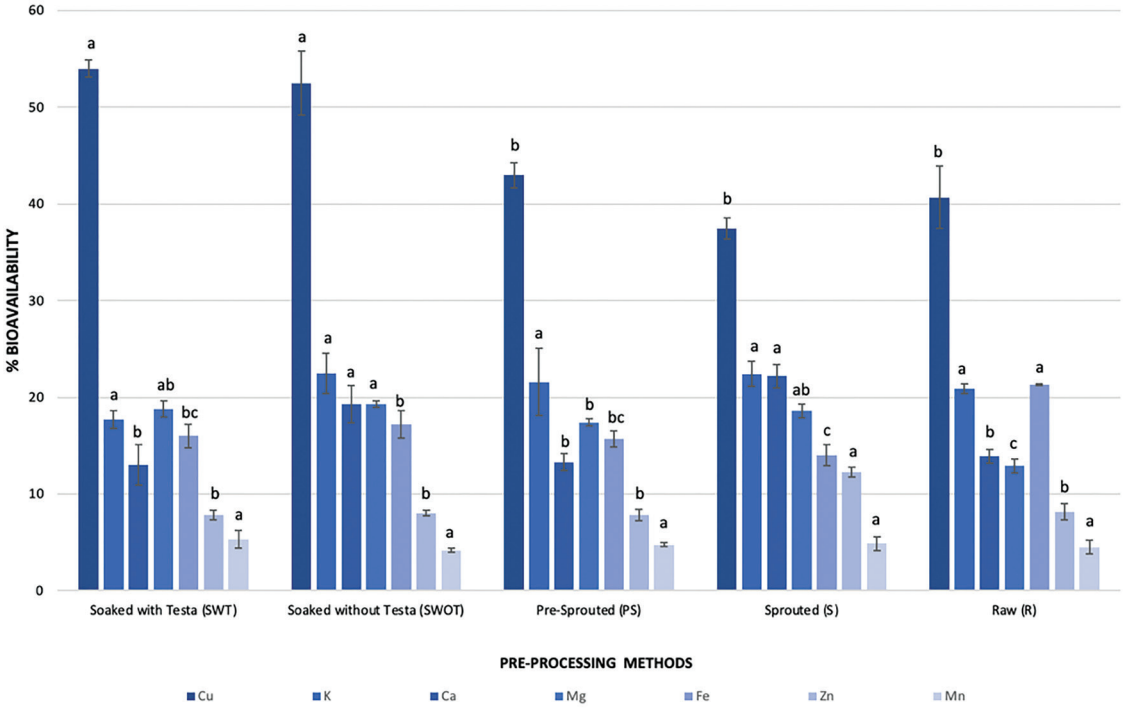

Figure II. Mineral bioavailability in different pre-processing methods after *in vitro* simulated digestion up to the small intestine.

Table I. In vitro mineral availability of pre-processed pili nuts up to the small intestine.

Pre-processing method	**Availability in %**						
	Ca	K	Mg	Cu	Fe	Zn	Mn
SWT	13.0 ± 2.1^{b}	17.7 ± 0.9^{a}	18.8 ± 0.8^{ab}	54.0 ± 0.9^{a}	16.0 ± 0.3^{bc}	7.80 ± 0.5^{b}	5.30 ± 0.9^{a}
SWOT	19.3 ± 1.9^{a}	22.5 ± 2.1^{a}	19.3 ± 0.3^{a}	52.5 ± 3.3^{a}	17.2 ± 1.4^{b}	8.03 ± 0.3^{b}	4.15 ± 0.2^{a}
PS	13.3 ± 0.9^{b}	21.6 ± 3.5^{a}	17.4 ± 0.4^{b}	43.0 ± 1.3^{b}	15.7 ± 0.8^{bc}	7.80 ± 0.6^{b}	4.75 ± 0.2^{a}
S	22.2 ± 1.2^{a}	22.4 ± 1.3^{a}	18.6 ± 0.7^{ab}	35.5 ± 1.1^{b}	14.0 ± 1.1^{c}	12.3 ± 0.5^{a}	4.88 ± 0.7^{a}
R	13.9 ± 0.7^{b}	20.9 ± 0.5^{a}	12.9 ± 0.7^{c}	40.7 ± 3.2^{b}	21.3 ± 0.1^{a}	8.16 ± 0.8^{b}	4.49 ± 0.7^{a}
p	**	0.069	**	**	**	**	0.15

Reference: [SWT] soaking with testa, [SWOT] soaking without testa, [PS] pre-sprouted, [S] sprouted, and [R] raw. Data are based on pre-processed samples and presented as mean ± SD. Values with the same letter in each column are not significantly different by Tukey's test. **Significant at $p < 0.05$.