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Dietary Fiber and Fermentability Characteristics of Different *Pili* (*Canarium ovatum*, Engl.) Varieties in the Philippines

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***Pili* nut (*Canarium ovatum* Engl.) is an indigenous fruit-bearing tropical tree nut in the Philippines. This study determined the dietary fiber and *in vitro* fiber fermentability characteristics of the pulp and kernel of seven approved *Pili* nut varieties that are cultivated in single soil conditions. The *Pili* nut pulp is an excellent source of dietary fiber (4.18–14.5 g/ 100 g soluble fiber; 34.0–43.2 g/ 100 g insoluble fiber), while kernel contributes a considerable amount. Short-chain fatty acid (SCFA) acetic acid (0.46–0.93 mmol/g) was the organic acid detected in the *Pili* nut kernel, while the pulp's major organic acids were acetic (0.59–2.49 mmol/g), butyric (0.31–1.68 mmol/g), and propionic (0.22–1.04 mmol/g) after simulating human colonic fermentation. Significant difference in SCFA concentration was observed among varieties ($p < 0.05$). *Pili* pulp total anthocyanidin ranged from 197–305 mg catechin/ 100 g. Aside from the commonly consumed *Pili* nut kernel, the nut pulp is a readily available source of low-cost dietary fiber and health-promoting SCFA.**

Keywords: *Canarium ovatum*, *Pili* nut, dietary fiber fermentability, short-chain fatty acids (SCFA)

INTRODUCTION

Since 1953, when the term dietary fiber was used by Hipsley, numerous studies were reported to establish its chemical nature and physiological benefits. It is an important constituent of a healthy diet and has many health benefits that include management of obesity, type 2 diabetes, cardiovascular and gastrointestinal diseases (Otles and Ozgoz 2014), provision of the intestinal barrier, promotion of immune system development (Topping and Lockette 2016), and enhancement of metabolic health (Lattimen and Haub 2010). It is widely known that dietary fiber is not hydrolyzed by human endogenous enzymes in the small intestine but can be fermented depending on the type of dietary fiber in the colon by gut microbiota (Yang *et al.* 2013). Dietary fiber can be naturally found in food or produced synthetically with or without enzymatic, physical, or chemical modification (Philips 2013) and are non-digestible carbohydrates that escape digestion and absorption that reach the large intestine for partial or complete fermentation by colonic microbiota (Wang *et al.* 2019). They are also known to reduce the rate of macronutrient absorption, reduce postprandial glucose response, provide a positive influence on several blood lipids, and prevent reabsorption of circulating cholesterol, thereby providing balance in physiological health (Perry and Ying 2016; Tan *et al.* 2016). Colonic fermentation of dietary fibers produced energy-yielding metabolites with low molecular weight acids. Acetate, propionate, and butyrate are known major SCFA produced through the fermentation of dietary fiber by gut microflora (Yang *et al.* 2013; Jonathan *et al.* 2012). Several soluble fibers such as β-glucan, oligosaccharides (FOS, GOS), and inulin are considered as "prebiotics" that serve as a

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substrate of health-promoting microorganisms such as *Bifidobacteria* and *Lactobacillus* species in the host large intestine (Kaczmarczyk *et al.* 2012; Mudgil and Barak 2013). During fermentation, colonic microorganisms utilize dietary fiber by producing different enzymes. Colonic fiber fermentation results in the reduction of pH that is associated with the production of low molecular organic acid, thereby enhancing mineral bioavailability (Gibson *et al.* 2004). The physiological function of dietary fiber may be attributed to its physicochemical characteristics such as being undigested in the small intestine, solubility, viscosity, gelling capacity, water holding capacity, binding ability, bulking ability, and ability to be fermented to produce SCFA (Mudgil and Barak 2013). Flavonoids comprise a large group of phenolic compounds; among its subcategories are the anthocyanidins that act as antioxidants to prevent inflammation and several chronic diseases. There are 30 different anthocyanidins; the most commonly studied are cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin, which are naturally found in nature (He and Giusti 2010). Anthocyanidins vary in hydroxyl and methoxyl in 3' and 5' position in the B ring of flavonoid. Anthocyanin produces different metabolites when consumed, *e.g.* Cy-3-glc produces protocatechuic acid and pelargonidin 3-glucoside produces 4-hydroxybenoic acid (Fang 2014). Monomeric anthocyanin from *Pili* pomace was released in the gastric portion that is almost twice its concentration compared to undigested while noting a significant decrease in the duodenal stage (Arenas and Trinidad 2017). Anthocyanins are stable at low pH of gastric condition that is converted into cation form of stable flavylium (Celep *et al.* 2015).

With the increasing demand for fruit nuts nowadays due to their health benefits, indigenous nut-bearing trees are one of the treasure sources of essential nutrients and health-promoting components. *Pili* nut or *Canarium ovatum* Engl. (Figure 1) is an endemic fruit nut in the Philippines and known for its large kernel among the *Canarium* species. The kernel is considered a high-value commodity in the country and can be eaten raw or can be processed into different delicacies and confectionaries. The pulp is also an edible portion of the fruit nut, and the practice of eating the pulp is widely known in the Bicol region where the center of *Pili* nut genetic diversity is concentrated (Millena and Sagum 2018a). The pulp is soaked and softened in warm water and is eaten either on its own or with different condiments. In the manufacturing of pili nut kernel, the pulp is normally discarded during processing and used as feedstock and compost. Several studies have reported on *Pili* nuts' genetic, nutritional, and chemical components: proximate (Millena and Sagum 2018a), oil and fatty acid profile (Millena and Sagum 2018a; Pham and Dumandan 2015; Zarinah *et al.* 2014), functional properties (Arenas and Trinidad 2017), phytonutrients (Pham and Dumandan 2015), antioxidant and anticancer and immunomodulatory properties (Salvador-Membreve *et al.* 2018), and potential micro/nanocellulose of the pulp for anti-aging ingredient (Bongao *et al.* 2020). However, scientific investigation is still necessary to elucidate the nutritional and functional potential of the said indigenous fruit nut that is vastly available in the Philippines. The study aims to be an essential contribution to the increasing demand for fruit nuts in the world. The research endeavored to determine the amount of both

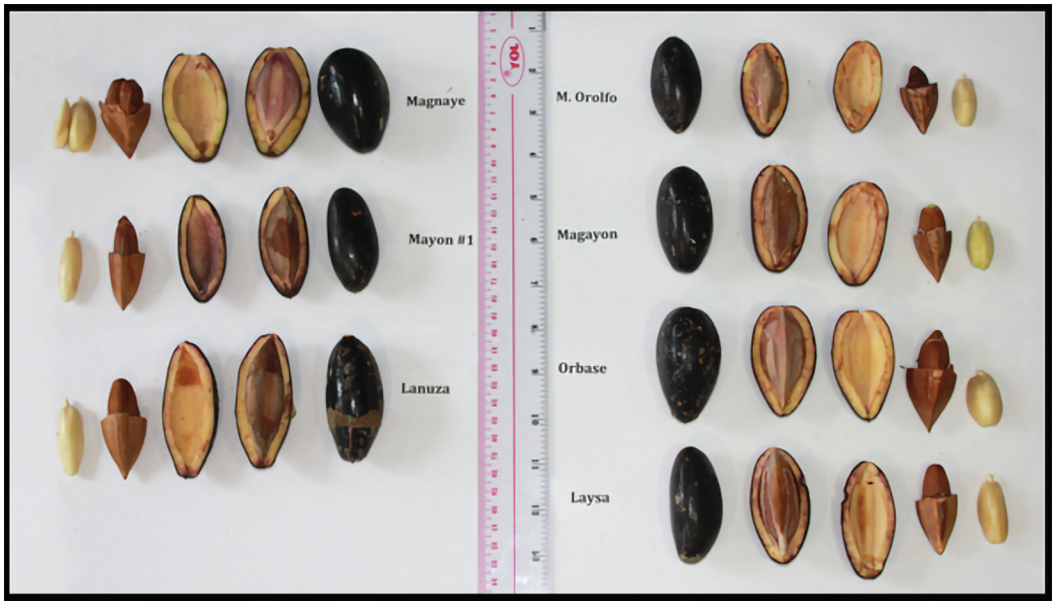

Figure 1. *Pili* nut varieties and their split components (nut fruit, pulp, shell, and kernel with and without testa).

soluble and insoluble fiber, and quantify the major SCFA metabolites after *in vitro* colonic fermentation of kernel and pulp of different *Pili* nut varieties.

MATERIALS AND METHODS

Plant Material

The study made use of the pulp and kernel of seven *Pili* nut varieties that were cultivated in a single soil condition at Albay Research and Development Center, Department of Agriculture Field Office V (latitude 13º18'40" N, longitude 123º38'34" E, East slope of Mayon Volcano). One hundred (100) mature fruits of *Mayon* #1, *Orbase*, *Magayon*, *Magnaye*, *Lanuza*, *Laysa*, *and M. Orolfo* were collected from single mother trees of the different varieties. The maturity of the fruit was determined based on the color of the exocarp. The station agriculturist assisted in the identification and collection of mature fruits that were free from any physical damage. The fruits were washed in distilled water and then dried. Properly labeled per variety, the pulps were manually separated from the shell using stainless steel knife, and the kernels were recovered using a *Pili* nutcracker; the testa or the papery brown coating was also removed from the kernel manually. Edible portions (pulp and kernel) were lyophilized and homogenized. Nylon polyethylene-aluminum plastic was used as the primary container, vacuum-packed, and stored at –40 °C. Based on the related study conducted by the same authors on DNA authentication, the studied varieties belong to the same genus *Canarium ovatum* (Millena and Sagum 2018a).

Reagents and Standards

Deionized water was used for the entire experiment. Merck (Darmstadt, Germany) and Sigma Aldrich (Buchs, Switzerland) brands of chemicals were used and traceable to the National Institute of Standards and Technology (NIST) with 99.99% purity. Enzymes used for total dietary fiber are heat-stable alpha-amylase (No. A3306), protease (No. P3910), and amyloglucosidase (No. A9913) from Sigma-Aldrich. Standard reference material Fortified Breakfast Cereal 3233 by NIST (U.S. Department of Commerce) was used as control during dietary fiber analysis. Supelco certified reference material (CRM46975) of Volatile Free Acid Mix was used as a standard for calibration of high-performance liquid chromatography (HPLC).

Determination of Soluble and Insoluble Dietary Fiber

The enzymatic-gravimetric method adopted from AOAC 985.29 Official Method of Analysis (2019) was used in the analysis of dietary fiber. Both the pulp and kernel of the seven varieties of the *Pili* nut were used for the analysis. Triplicate samples were subjected to defatting due to their high-fat content (> 10%). Approximately 1.0 g of samples were defatted thrice using 25 mL of diethyl ether with constant agitation. The ether portion was separated and filtered using Whatman No. 541 filter paper, while the residue was evaporated at 60 °C in an air oven overnight. The defatted sample was placed in a 500-mL beaker. MES/TRIS buffer with 40-mL volume was added and stirred until completely dispersed followed by the addition of 100-µL heat-stable α-amylase enzyme. The beaker was covered using aluminum foil and incubated at 95 °C in a shaking water bath for 30 min. After incubation, the digest was cooled down, added with 100-µL protease solution, and covered with aluminum foil. The mixture was again incubated for another 30 min at 60 ± 1 °C. After digestion, the pH was adjusted to 4.35–4.45 and added with 100-µL amyloglucosidase. The mixture was subjected to final incubation for 30 min at 60 ± 1 °C. Analysis was done in parallel with standard reference material 3233 Fortified Cereal by NIST.

Quantification of insoluble dietary fiber (IDF). The pre-weighed crucible with celite was washed with deionized water and dried in an oven at 105 °C for 8 h and cooled for 1 h and weighed. The enzyme digest was filtered in the crucible by suction. The residue was washed twice with 10-mL water, 10-mL ethanol, and 10-mL acetone portion. The crucible containing the residue was dried in an oven at 105 °C for 8 h, cooled for 1 h, and weighed. The duplicate was analyzed for protein (AOAC 950.48) and the other for ash (AOAC 950.49) at 525 °C for 5 h. Equation 1 was used to calculate the amount of IDF:

$$\%\,IDF = \left[\left(\frac{a - b - c}{d}\right)\right] \times 100 - \%_{blank} \qquad (1)$$

where *a* is the mean weight of the residue, *b* is the weight of the protein, *c* is the weight of ash, and *d* is the weight of the sample.

Quantification of soluble dietary fiber (SDF). The filtrate and water washing from the previous experiment were combined and adjusted to 100 g with deionized water in a volumetric flask. The solution was placed in a 500-mL beaker, in which 400 mL of heated 95% ethanol was added. The solution was precipitated at room temperature for another 60 min. A pre-weighed crucible with celite that was washed with 78% ethanol was used for filtration of the precipitate. The precipitate was filtered and washed with the following solutions: three portions of 20 mL of water, two portions of 10-mL ethanol 95%, and two portions of 1-mL acetone. The crucible containing the residue was dried in an oven at 105 °C for 8 h, cooled for 1 h then weighed. The duplicate sample was analyzed for protein and the other for ash at 525 °C. Two sample

blanks (reagent without sample) were included per batch of analysis along with the sample. Certified reference material was analyzed parallel with the sample. The soluble fiber was calculated using Equation 2, and total dietary fiber (TDF) is calculated (see Equation 3) by combining the amount of IDF and total SDF:

$$\%\,SDF = \left[\left(\frac{a - b - c}{d}\right)\right] \times 100 - \%_{blank} \quad (2)$$

where a is the weight of the residue, b is the weight of the protein, c is the weight of ash, and d is the weight of the sample

$$\%\,TDF = \%\,IDF + \%\,SDF \quad (3)$$

In Vitro Fermentability

The seven varieties of *Pili* nut pulp and kernel were subjected to *in vitro* colonic fermentation by adopting the method developed by McBurney and Thompson (1987) and used in the study of Mallillin *et al.* (2008). A portion of the 0.5-g lyophilized original sample was weighed and subjected to fermentation in triplicate. Mixture of 40-mL media [20-mL $NaHCO_3$ buffer solution and 20-mL macromineral solution (0.04 M $NaH_2PO_4.H_2O$ + 0.5 M KH_2PO_4)], 0.1-mL resazurin solution (0.1%), and 2-mL reducing solution (1.25-g cysteine-HCl, 50-pc. KOH pellets, and 1.25-g Na_2S in 100-mL distilled water) were added in each flask and clarified by flushing with high-purity CO_2 gas. The bottles were sealed with butyl rubber and crimped with an aluminum seal and stored at < 0 °C. The next day the samples were thawed for 1 h at 37 °C. Fecal inoculum with 10-mL volume (1:15 dilution) was introduced, covered with a butyl rubber stopper, crimped seal with aluminum, and incubated for 24 h at 37 °C in a water bath. Inocula were prepared from fresh feces collected from three healthy male donors (age ranged from 32–42 yr old) eating an unspecified Filipino diet and having not taken antibiotics for a year. Human fecal inocula as a practical source of microorganisms are used to estimate the fermentation property of the samples. Fresh feces were homogenized in the blender with a 400-mL collection solution (distilled water: fermentation medium: reducing solution ratio at 15:5:2 v/v) and maintained anaerobically with CO_2. Reagent blank was included for correction that includes fecal inoculum. After 24 h, the incubation bottle was opened and 1 mL Merthiolate solution (0.6 g/100 mL) was added to deactivate microorganisms and stop the fermentation. In this study, 24-h *in vitro* fermentation was used to ensure that fermentation of dietary fiber is completed. Based on the study of McBurney and Thompson (1987), there is no significant increase in digestibility occurring beyond 12 h, and the SCFA value was not significantly different at 24 h. Further, no significant change in the amount of organic acid is observed from *in vitro* fermentation at 8 and 24 h for the fermentation of *jucara* pulp (Guergoletto *et al.* 2016). The fermented solution was syringe-filtered using a 0.2-µm Nylon membrane, and SCFAs were quantified *via* HPLC (LC10 Shimadzu). The HPLC conditions were as follows: oven set at 40 °C, detector UV at 210 nm, flow rate at 0.7 mL/min using 0.005N H_2SO_4 mobile phase, and column using BioRAs HPX-87H (300 x 7.8 mm). Negative control (include all the reagent and inoculum except for the sample) was also included. A working standard of 0–10 mM was prepared by serial dilution of the SCFA mixture (Supelco, Philadelphia, PA, USA). Results were blank corrected and expressed as mg/g ± SEM (standard error of the mean).

Quantification of Anthocyanidin

Due to the pulps' pigmentation in some varieties (see Figure 1), the authors quantified the amount of anthocyanidin of the seven varieties to differentiate the anthocyanin contents. Only the anthocyanidin is quantified since based on the study of Aril-dela Cruz *et al.* (2018), the major flavonoid from the crude extract of *Pili* nut fruit exocarp is anthocyanin. The method by Sun and Kirvanta (1998) was adopted for the quantification of total anthocyanidin. A 0.5-g lyophilized pulp was weighed in a 125-mL Erlenmeyer flask and diluted with 25-mL (70%) acidified ethanol and shaken for 1 h in a shaking water bath at room temperature. After 1 h, the mixture was added with 25-mL acetone (70 %) and again agitated for 1 h. The mixture was centrifuged at 2000 g for 15 min. An aliquot of 1-mL supernatant was placed in a test tube followed by the addition of 2.5 mL (1% vanillin in ethanol) and 2.5 mL (9N HCl in ethanol). The solution was incubated at 30 °C for 20 min and quantified using UV-Vis (ultraviolet-visible) spectrophotometer (Shimadzu UV-1601) at 500 nm. Standard catechin (+) hydrate (Sigma Aldrich) was prepared by serial dilution and used as the standard. The concentration of the standards prepared ranged from 10–200 ppm, and the set acceptable linearity was < 0.995.

Statistical Analysis

Statistical calculation was performed through the SPSS version 17 software. A minimum of three trials was used during the experimentation except for *in vitro* digestion, which used quadruplicate trials. Descriptive statistics were used to calculate the mean and SEM. One-way analysis of variance was used for multiple comparisons and Tukey's honestly significant difference test was used to determine the difference between means. Significance was accepted at $p < 0.05$ probability.

Table 1. Accuracy performance using standard reference materials and blanks.

Parameters	Soluble fiber	Insoluble fiber	Total dietary fiber
	g/ 100 g	g/ 100 g	g/ 100 g
[a]NIST-SRM 2322 certified value	2.71 ± 0.84	6.60 ± 0.45	9.19 ± 0.94
[b]Obtained value	2.54 ± 0.26	6.82 ± 0.21	8.67 ± 0.29
% Error	6.27	3.33	5.66
[b]Blank	0.02 ± 0.01	0.03 ± 0.03	0.04 ± 0.01

[a]Certified value ± uncertainty
[b]Mean ± SD from three parallel determination

Table 2. *Pili* nut kernel dietary fiber and major *in vitro* fermentation metabolites of human colonic microbiota.

***Pili* nut kernel variety**	**Soluble DF**	**Insoluble DF**	**Total DF**	**pH**	**Acetate**	**Propionate**	**Butyrate**
	g/ 100 g	g/ 100 g	g/ 100 g		mmol/g	mmol/g	mmol/g
Mayon#1	$5.56 + 1.12^{b}$	$1.95 + 0.24^{ab}$	$7.51 + 1.12^{b}$	$5.27 + 0.03^{bc}$	$0.67 + 0.10^{bcd}$	ND	ND
Orbase	$2.99 + 0.33^{c}$	$2.31 + 0.22^{a}$	$5.30 + 0.33^{c}$	$5.26 + 0.04^{bc}$	$0.74 + 0.14^{abc}$	ND	ND
Magayon	$7.82 + 0.85^{a}$	$2.16 + 0.40^{ab}$	$9.97 + 0.85^{a}$	$5.30 + 0.10^{abc}$	$0.87 + 0.01^{ab}$	ND	ND
Magnaye	$7.48 + 0.51^{a}$	$1.86 + 0.07^{ab}$	$9.34 + 0.51^{ab}$	$5.33 + 0.10^{abc}$	$0.64 + 0.02^{cd}$	ND	ND
Lanuza	$1.98 + 0.31^{c}$	$1.46 + 0.36^{b}$	$3.43 + 0.31^{c}$	$5.21 + 0.05^{ac}$	$0.93 + 0.09^{a}$	ND	ND
Laysa	$2.79 + 0.04^{c}$	$1.97 + 0.00^{ab}$	$4.76 + 0.04^{c}$	$5.35 + 0.05^{abc}$	$0.49 + 0.08^{d}$	ND	ND
M. Orolfo	$2.44 + 0.19^{c}$	$1.64 + 0.17^{b}$	$4.08 + 0.19^{c}$	$5.33 + 0.07^{ab}$	$0.46 + 0.05^{d}$	ND	ND
p	**	**	**	**	**		
n	3	3	3	4	4	4	4

Data are presented as mean ± SEM
Values with the same letter in each column are not significantly different by Tukey's test.
ND – not detected
**Significant at $p < 0.05$

Table 3. *Pili* nut pulp total anthocyanidin, dietary fiber, and major *in vitro* fermentation metabolites of human colonic microbiota.

***Pili* nut pulp variety**	**Total anthocyanidin**	**Soluble fiber**	**Insoluble fiber**	**Total DF**	**pH**	**Acetate**	**Propionate**	**Butyrate**
	mg catechin/ 100 g	g/ 100 g	g/ 100 g	g/ 100 g		mmol/g	mmol/g	mmol/g
Mayon # 1	$235 + 2.6^{c}$	$11.2 + 0.45^{b}$	$38.8 + 0.35^{abc}$	$50.0 + 0.45^{abc}$	$3.86 + 0.25^{c}$	$1.68 + 0.15^{b}$	$0.72 + 0.02^{b}$	$0.76 + 0.01^{b}$
Orbase	$275 + 1.1^{b}$	$10.9 + 1.0^{b}$	$36.5 + 1.8^{bc}$	$47.4 + 1.0^{cd}$	$5.18 + 0.02^{a}$	$0.67 + 0.05^{d}$	$0.27 + 0.03^{d}$	$0.31 + 0.04^{c}$
Magayon	$233 + 1.9^{c}$	$10.4 + 0.35^{b}$	$34.0 + 0.91^{c}$	$44.4 + 0.35^{de}$	$3.57 + 0.09^{d}$	$2.31 + 0.28^{a}$	$1.04 + 0.01^{a}$	$0.36 + 0.05^{c}$
Magnaye	$305 + 1.6^{a}$	$12.0 + 0.63^{ab}$	$40.4 + 0.30^{ab}$	$52.4 + 0.65^{ab}$	$3.53 + 0.08^{de}$	$1.26 + 0.01^{bc}$	$0.67 + 0.08^{bc}$	$1.68 + 0.02^{a}$
Lanuza	$197 + 1.6^{e}$	$4.18 + 0.32^{c}$	$39.2 + 0.38^{abc}$	$43.4 + 0.33^{e}$	$3.40 + 0.05^{e}$	$2.49 + 0.09^{a}$	$0.86 + 0.12^{ab}$	$0.50 + 0.01^{c}$
Laysa	$228 + 0.16^{c}$	$6.26 + 0.72^{c}$	$43.2 + 0.47^{a}$	$49.4 + 0.70^{bc}$	$4.99 + 0.25^{ab}$	$0.59 + 0.04^{d}$	$0.22 + 0.03^{d}$	$0.51 + 0.06^{c}$
M. Orolfo	$207 + 0.29^{d}$	$14.5 + 0.01^{a}$	$38.5 + 1.6^{bc}$	$53.1 + 0.01^{a}$	$5.03 + 0.09^{b}$	$1.25 + 0.04^{c}$	$0.48 + 0.07^{c}$	$0.39 + 0.05^{c}$
p	**	**	**	**	**	**	**	**
n	3	3	3	4	4	4	4	4

Data are presented as mean ± SEM
Values with the same letter in each column are not significantly different by Tukey's test.
**Significant at $p < 0.05$

RESULTS

The values obtained from the certified reference material (3233 Fortified Cereal by NIST) that were analyzed in parallel with the samples are within the acceptable range provided in the certificate of analysis (see Table 1). Correlation coefficients during HPLC instrumentation and UV-Vis were 0.9990 minimum and 0.9987, respectively. The blanks were utilized for the corrected values obtained. Amounts of SDF, IDF, and TDF – as well as SCFA produced after *in vitro* fermentation of *Pili* nut kernel and pulp – are presented in Tables 2 and 3, respectively. *Pili* nut kernel contained a considerable amount of total dietary fiber that ranged from 3.43–9.97 g/ 100g, where a significant difference was observed among varieties. *Pili* nut pulp appeared to be a high source of total dietary fiber (43.4–53.1 g/ 100g). The varieties of *M. Orolfo*, *Magnaye*, and *Mayon* #1 had similar significant amounts of total dietary fiber, while Lanuza had the least. The varieties *Magayon* and *Magnaye* had the highest amounts of total dietary fiber (9.97 and 9.34 g/ 100 g, respectively) as well as soluble fiber (7.82 and 7.28g/ 100 g, respectively). Higher amounts of soluble fiber had been found in the pulp compared to the kernel. The ratio of soluble and insoluble fiber in the kernel also varied among varieties. *Mayon* #1, *Magayon*, and *Magnaye* varieties had a 1:3 ratio while other varieties had a 1:1 ratio of SDF and IDF. The soluble fiber in the pulp ranged from 4.18–14.5 g/ 100 g. The ratio of soluble and insoluble fiber also varied among varieties as 1:3 was noted for *Mayon* #1, *Orbase*, *Magnaye*, and *M. Orolfo* with 1:5 for the rest of the varieties. The amounts of common plant pigment anthocyanidin in *Pili* nut pulp varieties are presented in Table 3. Significant differences observed in the content may be attributed to the nut's varieties and pulp characteristics. The fiber in the pulp was dominated by insoluble fiber (34.0–43.2 g/ 100g). Among the varieties, the pulps of *M. Orolfo* and *Magnaye (*14.5 and 12.0 g/ 100 g, respectively) had the significantly highest amounts of soluble dietary fibers compared to other varieties. Lesser variability was observed in the amount of IDF. In terms of anthocyanidin content of the pulp, *Magnaye* had the highest amount (305 mg catechin/ 100 g) among varieties, which was ~ 30% higher compared to *Lanuza* (197 mg catechin/ 100 g).

After 24 h of fermentation, a significant reduction in pH was noted specifically for the pulp compared to the blank, which was at 5.84 ± 07. In general, a significant reduction of pH was observed in the *Pili* nut pulp compared to the kernel, which may be due to the higher production of metabolites. At the same fermentation condition, the pH reduction of samples and the blank group was in the following order: blank < *Pili* kernel < *Pili* pulp. The values of SCFA obtained both for the pulp and kernel are higher compared to the blank. Among the SCFAs quantified, only acetic acid was detected in the *Pili* nut kernel after being subjected to simulated colonic conditions. The amount of acetic acid produced from *Pili* nut kernel of different varieties ranged from 0.46–0.93 mmol/g, and a significant difference in the concentration was observed ($p < 0.05$). The *Lanuza* variety had the highest amount of acetate (0.93 mmol/g) produced, which is statistically comparable with those of *Magayon* (0.64 mmol/g) and *Orbase* (0.74 mmol/g). The *Pili* nut kernel is not propionic- and butyric acid-producing. For the *Pili* nut pulp, a significant amount of SCFAs was produced after simulating the colonic condition. The total SCFA ranged from 1.25– 3.85 mmol/g, and the *Lanuza*, *Magayon*, *Magnaye*, and *Mayon* #1 (3.85, 3.71, 3.61, and 3.16 mmol/g, respectively) varieties had the highest total SCFA produced compared to those of *Orbase* and *Laysa* (1.25 and 1.32 mmol/g, respectively), which were comparably inferior. The ratio of acetate, propionate, and butyrate varied among varieties. Acetate > propionate > butyrate was observed for most of the SCFA produced by *Pili* nut pulp varieties; however, distinct ratios were observed in *Magnaye*, where the ratio of butyrate > acetate > propionate was 45:35:20 and the acetate > butyrate > propionate ratio of *M. Orolfo* was 45:40:15. The pulps of *Magayon* and *Lanuza* varieties respectively had the highest and significantly similar amount of acetic (2.41 and 2.49 mmol/g) and propionic acid (1.04 and 0.86 mmol/g). *Magnaye* had the highest butyric acid produced (1.68 mmol/g) among varieties. It was observed that most of the varieties are high acetate-producing compared to other SCFAs. However, *Magnaye* was found to be a superior variety in terms of butyric acid production compared to the pulps of the other six *Pili* nut varieties.

From the previous study conducted by the same authors, it was concluded that the seven *Pili* nut varieties have the same chemical profiles – such as fatty acid, proximate, and mineral composition – although these may vary in concentration (Millena and Sagum 2018a, b). The *Pili* pulp, as shown in Table 3, also revealed that most of the parameters studied vary in concentration. Contrasting amounts of SCFAs may also be attributed to the nuts' distinct varieties. Each variety may have a distinctive amount of soluble fiber, and the authors hypothesized that the amount of various specific dietary fiber may also affect the concentration of metabolites produced during colonic fermentation.

DISCUSSION

Food may contain fiber with different types of carbohydrates and proportions of soluble and insoluble fiber; thus, it is necessary to eat varieties of food that are rich in dietary fiber. It is also necessary to characterize fiber in different

food matrices and its fermentability in the large intestine to evaluate its contribution to human nutrition and health. The study conducted by Mallillin *et al.* (2008) revealed that root crops (*kamote*, *gabi*, potato, *tugi*, *ube*, and *casava*) and legumes (mungbean, soybean, peanut, pole *sitao*, cowpea, chickpea, green pea, lima bean, kidney bean, and pigeon pea) are good sources of dietary fiber. The present study provides conclusive information that the *Pili* pulps' soluble and insoluble fibers are higher compared to those of the studied root crops and legumes. Soluble fiber constitutes 10% of *Pili* nut pulp, where *Magnaye* and *M. Orolfo* varieties have the superior amount. Soluble fibers are known to be readily and entirely utilized by probiotics in the gut compared to insoluble fibers (Prasad and Bondy 2019). Dietary fiber is widely known as an essential component in the human diet that has several health benefits in reducing the risk of cardiovascular diseases, obesity, diabetes mellitus, and colon cancer (Brownlee *et al.* 2017; Mayengbam *et al.* 2017; Nie *et al.* 2018). The *Pili* pulp is an excellent source of dietary fiber that can provide a reduced energy per volume of food. Most of the physiological benefits of dietary fiber can be attributed to its physicochemical characteristics such as solubility, viscosity, gelling capacity, and ability to produce short-chain volatile acids (Mudgil and Barak 2013). The viscosity of dietary fiber serves as a physical barrier to reduce the absorption of fats and enhances fecal bile acid excretion, thus resulting in reduced circulating cholesterol (Liu *et al.* 2021). The viscous property of dietary fiber is also responsible for regulating obesity and diabetes mellitus by reducing energy intake that affects the absorption of macronutrients such as fats. Viscous fiber forms gels that prevent the action of lipases and bile in micelle formation, thus reducing absorption. Some dietary fiber can bind to bile acids, which prevent cholesterol reabsorption and increases fecal bile excretion that results in reduced circulating cholesterol (Gibson *et al.* 2017). The *Pili* nut pulp contains a higher amount of insoluble fiber than the kernel; this type of fiber helps increase fecal bulk, induce hydration of stool that enhances bowel movement (laxative), and reduce fecal transit time, thus preventing large bowel ailments such as diverticulitis, constipation, and reducing the risk of several bowel cancers (Mudgil and Barak 2013). Aside from commonly known root crops and legumes in the Philippines, the *Pili* nut pulp is an excellent and vastly available source of low-cost dietary fiber. Consumption of 30-g pulp (4–5 pieces) can compensate the Filipinos' daily nutritional requirement of dietary fiber of 20–25 g/d based on the 2015 Philippine Dietary Reference Intake. Thus, regular but moderated consumption of *Pili* nut pulp can provide beneficial effects to human health.

The *Pili* nut pulp, which comprises almost 60% of the fruit and is usually discarded during processing, can be still be used as a potential source of nutrients and non-nutrients. The present study established that *Pili* nut pulp, specifically the *Magnaye* variety, can be a natural source of anthocyanidin that can be used in different food products. The pulp contains a considerable amount of anthocyanidin that is almost seven times lower compared to that of its exocarp (17.5 mg CE/g) and is a high source of phenolics (8.8 mg AAE/g) and flavonoids (2.2 mg CE/g). The extract of *Pili* nut exocarp also exhibited potential antioxidant activities and can be utilized as a functional food colorant in certain food such as yogurt (Aril-dela Cruz *et al.* 2018). A study conducted by Arenas and Trinidad (2018) *in vitro* simulated digestion revealed that the anthocyanidin level of *Pili* pomace was almost doubled in gastric digestion and reduced significantly in the small intestine and in the colon. Several studies have also shown that some of the phenolic compounds such as anthocyanidins and gallic acid stimulate the growth of probiotics such as *Bifidobacterium* and *Lactobacillus* spp. (Guergoletto *et al.* 2016). Even though the *Pili nut* pulp has lower anthocyanidin content, it can be used in food products to enhance the physical, chemical, rheological, and sensory properties of certain products. The water-holding capacity and gelling property of the fiber helps in maintaining freshness and moisture in food.

The present study is the first to assess the *in vitro* fermentation of different varieties of *Pili* nut pulp and kernel using human colonic inoculum. Colonic fermentation should be done *in vivo* ideally. However, due to the difficulty in performing the method, several *in vitro* methods can provide a reliable estimate to mimic colonic conditions.

It is well known that during fermentation, colonic microorganisms produce several enzymes that can degrade polysaccharides, which are indigestible by gastric and small intestinal enzymes. Thus, the fermentation of *Pili* nut kernel and pulp by colonic microbiota was studied. During fermentation, a reduction in pH value was observed largely in the pulp than in the kernel. This may be correlated to the metabolites produced from the carbohydrates in the pulp fermented by gut microflora (Huang *et al.* 2020). Other beneficial effects of the decrease in pH value in the colon are the following: first, it limits the growth of pathogenic microorganisms such as *E. coli* and other genera in the gut; second, it promotes the growth of health-modulating microbial species; and third, it enhances the bioavailability of certain minerals (Fu *et al.* 2018; Gibson *et al.* 2017). Several fruit pulps such as *jeriva* (*Sygrus romanzoffiana*) and *macaúba* palm fruit (*Acrocomia aculeata*) are known as high sources of dietary fiber that can enhance the growth of beneficial microorganisms like *Bifidobacterium lacti*, *Lactobacillus casei*, and *Lactobacillus acidophilus* (Andrade *et al.* 2020). Reduction in pH is also an inhibiting factor for the

growth of pathogenic microorganisms that cause intestinal problems and colorectal cancer (Slavin 2013).

The difference in fermentability of specific soluble fiber was not assessed, which is one of the limitations of the present study. A 24-h *in vitro* colonic fermentation was adopted in this study, and considering that the colonic residence time takes as long as 72 h, further fermentation and production of the metabolite are expected. Different metabolites such as formate, lactate, valerate, hexanoate, caproate, acetate, propionate, and butyrate can be produced during colonic fermentation (Liu *et al.* 2021). Among these, the most abundant are acetate, propionate, and butyrate, which are considered in the present study.

The present study reveals that consumption of *Pili* nut pulp and kernel can also provide health-promoting metabolites. After mimicking *in vitro* colonic fermentation of the pulp, it was found out that acetate is the major fatty acid produced among other SCFAs. The concentration of acetate also varies depending on the *Pili* variety. However, in the colonic fermentation of the kernel, it was found out that acetate is the only major SCFA produced. Several enteric microorganisms like *Bacteroides* spp., *Bifidobacterium* spp., *Prevotella* spp., *Clostridium* spp., *Streptococcus* spp., and *Ruminococcus* spp. are associated with the production of acetate (Louis *et al.* 2014). Acetate serves as a substrate to some metabolic pathways such as gluconeogenesis and lipogenesis and an important energy source in peripheral tissues and the liver (Zambell *et al.* 2003). Acetate can also inhibit the growth of enteropathogenic bacteria that are related to microbiota-brain-cell that can enter the blood-brain barrier to reduce appetite (Holscher 2017).

Aside from providing energy for colonic cells, propionate and butyrate contribute to major physiological activities in human health. Propionate and butyrate have important roles in stimulating immune cells, inhibiting intestinal inflammation, and stimulating the production of satiety hormone (Wang *et al.* 2019). The concentration of propionate, which is only detected in *Pili* nut pulp, is of considerable value. Propionate is produced by several bacterial species, such as *Akkermansia munciniphila*, *Megasphaera elsdenii*, *Coprococcus catus*, *Bacteroides* spp., *Dialister* spp., and *Veillonella* spp. (Louis *et al.* 2014). Propionate that is absorbed in the intestinal wall is required for several metabolic functions such as gluconeogenesis, reduction of cholesterol synthesis, and activation of G protein receptors that release satiety hormone (Cheng and Lai 2000; Sayago-Ayerdi *et al.* 2019). *In vivo* studies have also shown that propionate can help reduce the glucose-induced secretion of insulin (Ximenes *et al.* 2007). Similar to propionate, butyrate is only detected in the *Pili* pulp where the *Magnaye* variety outstood other varieties significantly. Butyrate is considered a valuable volatile acid that is known to be produced by *Clostridia* clusters of obligate anaerobes, *Faecalibacterium prausnitzii*, *Coprococcus comes*, and *Coprococcus eutactus* (Louis *et al.* 2014). It is used as energy by colonocytes. It inhibits inflammation, is essential in maintaining colonic cell integrity, and inhibits the growth and development of tumor cells through apoptosis of damaged DNA (Ogawa *et al.* 2004; Charoensiddhi *et al.* 2016). Generally, the chemical properties of polysaccharides affect the utilization of colonic microbiota to produce SCFA. These chemical properties include arrangement, glycosidic linkages, sugar composition, branch chain, molecular weight, solubility, and viscosity (Jonathan *et al.* 2012). The differences in the amount of metabolite distribution among the *Pili* nut parts and varieties studied may be due to the differences in the type and amount of specific carbohydrates found in the food sample (Henningsson *et al.* 2001; Yang *et al.* 2013; Guergoletto *et al.* 2016). Further, SCFA produced during anaerobic fermentation is dependent on the fermentable fiber present and the composition of gut microbiota (Huang *et al.* 2020; Louis and Flint 2017). With the comprehensive studies conducted on dietary fiber, some soluble fibers are classified as prebiotics – a substrate that can promote the growth of several beneficial microorganisms that improves host health (Gibson *et al.* 2017). The present study is in agreement with the study of Guergoletto *et al.* (2016) on the *in vitro* fermentation of *jucara* pulp (*Euterpe edulis*) that also produces acetate, propionate, and butyrate after 24 h of fermentation, with only a difference in the quantification of metabolites in different time points. This study yields that the *Pili* pulp has superior metabolites produced compared to the *jucara* pulp.

CONCLUSION

Aside from the scientific evidence on the *Pili* nut's nutritional quality, the present study furthered the functional property of *Pili* nut pulp and kernel in terms of dietary fibers and their fermentability in colonic conditions. The study demonstrated that significant differences in the amount of SDF and IDF, anthocyanin, and SCFA can be attributed to the fruit's distinct varieties. The consumption of *Pili* nut pulp, which has been practiced by people living in the Bicol region, can be an excellent source of dietary fiber aside from other plant-based food. The dietary fiber found in *Pili* nut pulp can serve as a substrate of colonic microorganisms in the gut to produce health-promoting metabolites. Among the SCFAs quantified, acetate is the sole major fatty acid produced after *in vitro* fermentation of *Pili* nut kernel, while *Pili* pulp produces varying ratios of acetate, propionate, and butyrate. The *Magnaye* variety has the most distinct property compared to other varieties due to its high butyrate production. The *Pili* pulp

that is commonly a waste by-product during processing can be utilized as a source of low-cost dietary fiber and can be used to produce fiber-rich products that can contribute to human nutrition. Future researches on the isolation and characterization of different carbohydrates in *Pili* nut pulp is recommended to further elucidate and quantify the potential prebiotics found in the pulp. Further, identifying the specific polysaccharides in the *Pili* nut pulp is necessary to substantiate the differences in concentration of metabolites produced during *in vitro* colonic fermentation.

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STATEMENT ON CONFLICT OF INTEREST

The authors declared no conflict of interest.

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