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Research Article

Nutritional and sensory properties of plant-based milk produced from Sacha inchi seeds (*Plukenetia volubilis* L.)

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Abstract

This work evaluated the nutritional properties as well as sensory properties of the milk produced from roasted seeds and defatted rods, a by-product of the process of cold press oil extraction of Sacha inchi. The protein content of the raw seed decreased to 55.34%. Fat and calorie contents of the defatted nut milk were less than for roasted nut milk. However, the protein and sodium quantity in both fat and non-fat nut milk were found to be at similar levels of approximately 4% per serving size. Nevertheless, Thai FDA has restricted the quantity of defatted rods in food supplements to only 1.5 g per day. Hence, the final product and the sensory aspect of this study focused on only the plant-based roasted Sacha inchi product. The preference test for the roasted Sacha inchi milk, including original, matcha green tea, and cocoa flavor was assessed. The result showed that the cocoa flavor exhibited the highest score. Additionally, the microbial studies, such as the aerobic plate count, and the number of toxic bacteria, and aflatoxins were designated as less than the regulation limit in milk. As a result, the plant-based dairy produced from Sacha inchi was safe and good for the customer's health.

Keywords

Sacha inchi, plant-based milk, sensory, milk allergy, defatted-seed, proximate analysis

Abbreviations

AOAC – Association of Official Analytical Chemists; FDA – Food and Drug Administration; ISO – International Organization for Standardization; JAR – Just-About-Right; SFAA – Soy Foods Association of America; SIM – Sacha inchi milk; USDA – U.S. Department of Agriculture;

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Introduction

Currently, customers are looking for new branded sensory experiences with non-dairy products. More alternatives are available than ever, and the plant-based milk substitute market has grown significantly in the last ten years (Aydar et al. 2020; Pedersen et al. 2013). Because of lactose intolerance and milk allergies from the consumption of cow's milk, there has been significant growth in the plant-based beverages market and a growing preference for vegan milk substitutes. Furthermore, not only because of allergy concerns, customers with hypercholesterolemia or concerns about saturated fat levels likewise prefer vegan/plant-based diets to cow's milk (Sethi et al. 2016). Still, others worry about hormone content and antibiotic usage in dairy livestock. As a result, there has been a steady increase in research and development undertaken for plant-based milk as an alternative to cow's milk. Plant-based milk can be made from numerous plants or nuts such as almonds, cashew, soy, rice, oat, rapeseed, flaxseed, buckwheat, hemp and others (Mattila et al. 2018). The various grains exhibit diverse flavors and distinct health benefits (Thorning et al. 2016). However, most plant proteins have a lower protein quantity than animal-derived proteins. Therefore, if these plant-based products are consumed, this may cause a protein deficiency (Jeske et al. 2017). The high protein content from plant sources can provide the opportunity to formulate plant-based milk alternatives with higher nutritional value (Rukshan et al. 2020).

Plukenetia Volubilis L. is commonly called the Sacha inchi or Inca nut (Fig.1). This healthy fruit is presented in several parts, like star-shaped up to seven ovals. The dark seeds can be shelled, roasted, and consumed as nuts. A significant amount of research emphasizes that Sacha inchi seeds contain up to 60% protein (Sathe et al. 2012; Rawdkuen et al. 2016). The oil extracted from Sacha inchi seed also has a unique fatty acid composition, similar in omega (ω-3) levels and twice as much ω-6 levels compared to flax (Maurer et al. 2012; Rodríguez et al. 2022). The seeds are also rich in hydrophilic and lipophilic antioxidants (Souza et al. 2013; Chirinos et al. 2015). After roasting, the Inca peanut seeds are safe for consumption (Srichamnong et al. 2018; Cárdenas et al. 2021).

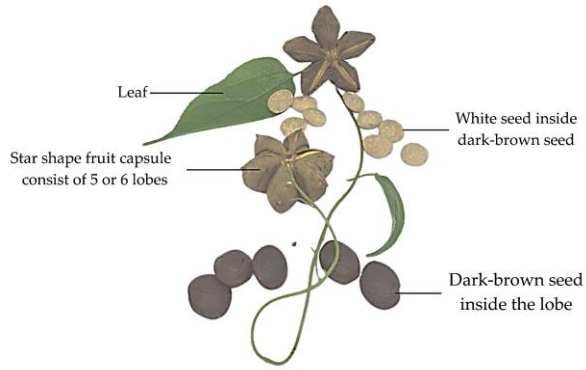


Figure 1. Physical appearance of *Plukenetia Volubilis* L.

The Sacha inchi seed is rich in protein, combined with good fat, and, as such, is suitable for producing plant-based milk. Therefore, not only the roasted seed but a defatted seed from Sacha inchi oil extraction also has the potential to yield novel health benefits as a drink because of its high abundance in protein (Sathe et al. 2012). The process of extracting Sacha inchi seed oil yielded a defatted granule with a protein of approximately 50-60%, depending on the extracting protocols and the seed quality (Cárdenas et al. 2021; Cordero-Clavijo et al. 2021). However, the Ministry of Public Health, Thai Food and Drug Administration (FDA) announcement about novel food stated that the amount of defatted press-cake from Sacha inchi oil extraction is restricted to only 1.5 g per person per day. This amount could be the major limitation of developing the defatted seed as an alternative plant-based milk. Although even the milk produced from the Inca nut is safe and healthy to drink, this vegan beverage is usually disliked for its beany taste. Therefore, the improvement of the flavor for acceptable consumption is crucial. If a solid, nutty taste is refined, Sacha inchi will be one of the healthiest superfoods for delivering plant-based milk or other products instead of cow's milk.

There are various benefits to making homegrown milk over shelf-stable milk from a carton. First, producing homemade-vegan milk is incredibly simple and inexpensive. Fresh, raw vegan milk, without additives, is better than the store-bought milk in containers, being more refreshing, more nutritional, gluten-free, and has adjustable flavors depending on professional culinary preferences. Most of the commercial plant-based drinks in the market are low in saturated fat and cholesterol (Chirinos et al. 2016). These drinks have high levels of nutrients. Nevertheless, the amount of sugar in vegan-based milk is high. As a result, the total energy content of vegan-based milk can be higher than whole milk, especially since the sugar-sweetened beverage market has been experiencing growth (Kerley 2018). A high amount of added sugar to consumer products has been linked to obesity (Lim et al. 2014). Also, chemicals such as carrageenan, anti-rancid, and food preservatives have been added by manufacturers to enhance taste, appearance, and shelf life (Maersk et al. 2011). Thus, this work aimed to investigate the benefits of producing a simple and healthy plant-based milk from Sacha inchi (*Plukenetia volubilis* L.) and Sacha inchi defatted granules in terms of the nutritional factors, microbiological safety, and consumer perception of the novel alternative milk product.

Materials and Methods

Raw materials of Sacha inchi seeds. The Sacha inchi fruits in this work were harvested from Chiang Mai province, Thailand. First, the star-shaped pods were shelled by the home-built peanut deshelling machine (Friend Energy Limited, Thailand). The process was repeated to de-shell the brown kernels and leave only the white seeds. Next, the seeds were roasted using an oven (model EOT4805K, Electrolux). After roasting, the seeds were cooled to room temperature. Finally, the defatted rods were produced by extracting the nut oil using a screw press oil extractor (model FEA-200 Series, Friend Energy Limited, Thailand). The defatted rods were collected and stored in a desiccator until processing time, within one month.

Proximate and nutritional analysis. The proximate and nutritional composition of the milk samples was determined by the Association of Official Analytical Chemists (AOAC) (Martino et al. 2017), including energy from fat (method 948.15), cholesterol (method 994.10), fiber (method 985.29), sodium (method 984.27), vitamin B1 (method 942.23), minerals, which were sodium, calcium, and iron (method 984.27), moisture (method 925.45), and ash (method 938.08). The protein content (method 981.10) was evaluated by multiplying the total nitrogen content by a factor of 6.25. Other parameters were accessed by the compendium of methods for food analysis, including total energy and total from fat, saturated fat, sugar, and vitamin A. All proximate and nutritional analyses were performed by a certified laboratory (Central Laboratory Co., Ltd., Bangkok, Thailand).

The processing of homemade SIM. The Sacha inchi seeds were cleaned with tap water and left until dry at room temperature. The dried and washed Sacha inchi seeds were baked at the optimum temperature for the roasting process, 200℃ for 10 min, then left to cool down to room temperature. After the roasting process, the chilled grain was weighed to 125 g and then soaked in 10% w/v Na_2CO_3 solution for at least 12 h, after which the seeds were washed with tap water until the pH of the filtrate water was neutral. The cleaned seeds were soaked in 1.125 mL of lukewarm water at 70℃ (1:10) for one hour before blending in the soymilk maker (model HD2079, Phillips). The soya milk program was selected to produce the milk in which the seeds were blended and boiled for 20 min. The resulting creamy milk was filtered via muslin cloth following a 100-mesh sieve. The schematic of homemade SIM processes are illustrated in Fig. 2. The attained milk was poured into sterilized glass bottles, then the fresh, warm SIM was ready for seasoning (original or sweet, matcha green tea and cocoa flavor) by adding 5% of the seasoning powder into the warm milk and stirring vigorously. Afterward, the ready-to-drink SIM was stored in a refrigerator at 4℃ for further analysis. The milk can be stored for at least one week without adding food preservatives.

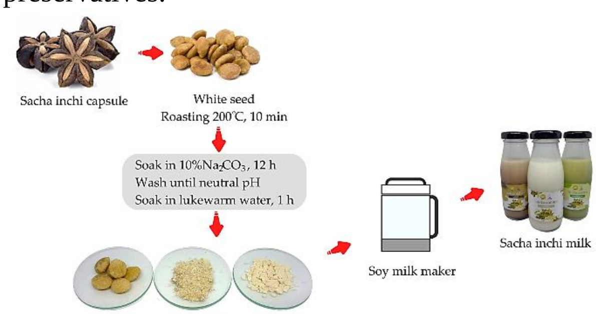


Figure 2. Schematic of homemade SIM processes

Sensory evaluation. Using the preference testing using a 9-pointed hedonic score, consumers were asked to complete the questionnaire regarding the plant-based milk's demographic and consumption behaviors. A panel of 35 judges was recruited based on the following screening criteria; drinks soy milk or other plant-based milk; not allergic to nuts. Sample tastings were divided into two phases. The first sensory evaluation phase was to compare the preference between unsweetened commercial brand almond milk with the unsweetened SIM using a 9-point hedonic scale, ranging from 1 = "dislike extremely" to 9 = "like extremely" with a middle value 5 = "neither like nor dislike". The second phase, the consumers were given information about SIM and were asked to evaluate the three flavors of Inca milk (original but sweetened flavor, matcha green tea, and cocoa-flavored milk) on the same 9-points hedonic scale. The judges performed their examinations in a standardized tasting room equipped with individual booths. Approximately 25 mL samples were served at 5±2℃ (Kanphet 2003).

After the second phase, sensory evaluation using just-about-right (JAR) protocol, the three flavors of SIM, which were original, matcha green tea, and cocoa were evaluated for the JAR scale of five points ranging from 1 = "much less" to 5 = "much more" with median value 3 = "just about right". The intensity percentage of the panel selection is used to ascertain whether a particular attribute is perceived in the developed milk at an extreme, inadequate, or acceptable level (the JAR group). The net scores were calculated based on the JAR score by subtracting the lower-level percentage from the higher-level percentage.

Microbiological and toxicity analysis. Microbiological analysis of the samples was determined by International Organization for Standardization (ISO) protocol (Sriwattana et al. 2016), including *Bacillus cereus* (method 7932: 2004E), *Clostridium perfringens* (method 7932: 2004E), and *Salmonella* spp. (method 6579: 2002/Cor.1 :2004E). Coliform bacteria, *Escherichia coli*, and *Staphylococcus aureus* were determined by the AOAC procedure, method 991.14 (ISO 2004). The aflatoxin B1, B2, G1, G2, and total aflatoxin were quantified using HPLC based on the AOAC official method 2000.08 (AOAC 2012a) accredited ISO/IEC17025:2017 (Do et al. 2007). The aerobic plate count was evaluated according to the Food and Drug Administration (FDA) method (AOAC 2002). All microbial analyses were examined by a certified laboratory (Central Laboratory Co., Ltd., Bangkok, Thailand).

Statistical analysis. All the experiments were performed in triplicate. Data were analyzed by one-way ANOVA and t-test.

Results and Discussion

Nutritional analysis of the SIM. The nutritional composition comparing the roasted seed, and defatted seed produced from roasted-seed milk is shown in Table 1. There were significant differences ($p<0.05$) in protein, fat, ash, and carbohydrate content. In addition, the highest moisture was observed in the non-fat inchi nut milk, whereas the most elevated protein, fat, and carbohydrate contents were found in the roasted-seed milk.

Fat and carbohydrates. The milk made of full-fatted seeds had more energy than defatted-seed milk, which is expected since the fatted seeds were more abundant in oil. The energy from consuming SIM can be reduced to only 14.56%, while the saturated fat can be cut from 0.38 to 0.15 g by using the defatted seed instead of the roasted seed, as illustrated in Table 1. The results also indicate that most of the energy in both nut milk varieties originates from fat. However, several studies have reported that oil from sacha inchi could be used for nutritional applications. There is no cholesterol or trans-fat for either whole or defatted SIM. The carbohydrate content of Sacha inchi seeds was reported to range from 13.4% to 30.9% (Hanssen et al. 2011). Turning the grain into milk reduced the carbohydrate content to 1.46 g and 0.68 g for the roasted and defatted milk, respectively. Besides, both liquids have a high amount of fiber and calcium.

Protein. The protein content of the Sacha inchi seeds was reported at 24.2-27.0%, while the protein content of defatted seeds ranged from 27.0% to 59.1%, depending on the extraction technique and protein assay used (Rawdkuen et al. 2022; Hanssen et al. 2011). In this work, the protein content of raw seeds, roasted seeds, and the defatted rod was found to be at 58.94%, 26.32%, and 26.92%, respectively. Baking the raw nut can reduce the protein content to the same level as the protein content in the defatted rod, although the raw seeds and leaves contain toxins that are unsuitable for consumption. However, roasting can efficiently diminish these phytotoxins. As a result, thermal processing must be applied before consumption (Srichamnong et al. 2018). In this study, roasting conditions were studied by cooking at different temperatures for lightly roasted (100℃×10 min),

Table 1. Nutritional composition per 100 mL comparing roasted-seed milk and defatted milk

Proximate and nutritional facts	Roasted-seed milk	Defatted milk
Total energy, kcal	58.91	14.56
Energy from fat, kcal	44.55	5.76
Total fat, g	4.95	0.64
Saturated fat, g	0.38	0.15
Cholesterol, mg	Not detected	Not detected
Total carbohydrates, g	1.46	0.68
Protein, g	2.13	1.52
Fiber, g	0.74	0.39
Total sugar g	0.00	<0.50
Fructose	Not detected	Not detected
Glucose	Not detected	Not detected
Sucrose	<0.50	Not detected
Maltose	Not detected	Not detected
Lactose	Not detected	Not detected
Sodium, mg	9.33	8.20
Vitamin A, mg	Not detected	Not detected
Vitamin B1, mg	0.023	0.064
Vitamin B2, mg	Not detected	Less than 0.020
Calcium, mg	20.56	23.00
Iron, mg	0.29	0.19
Ash, g	0.25	0.27

medium roasted (150℃ ×10 min), highly roasted (200℃ ×10 min), and super highly roasted (250℃ ×10 min). No significant differences existed in the protein content of any attributes ($p>0.05$) from the range of studied roasting conditions. Even though the roasting temperature did not affect the protein content, it could stimulate the milk's color and bitter taste. In this work, the optimum condition was 200℃.

After all the nut sorts (roasted seed and defatted rod) were processed into milk, the protein content decreased from 55.59% to 2.92% and 27.03% to 1.26% for roasted grain and defatted seed milk, respectively. We also attempted to produce milk from raw Sacha inchi seed in this work. However, an undesirable beany flavor and unpleasant taste were evident and difficult to suppress.

Sugar. According to the result as illustrated in Table 1, the types and contents of sugars in the unsweetened roasted SIM (original flavor) were estimated. According to the results, fructose, glucose, maltose, and lactose could not be detected, while sucrose content was less than 0.5 g.100 mL^{-1}.

Microbiology for food safety test. The organisms responsible for contaminated milk can come from several sources including disease conditions like mastitis, external udder surfaces, and the dairy industry. Several kinds of bacteria can cause mastitis infection, such as *Staphylococcus*. Therefore, the presence of harmful organisms in many dairy products points to them being potentially unsafe for consumers. A count of less than 100 cfu.mL^{-1} is considered acceptable for milk before consumption (Wang et al. 2018). Table 2 displays the microbiological test results for food safety in the homemade milk produced in this work.

The microbiological criteria for dairy products were also set by the Institute of Medicine and National Research Council (U.S.) Committee in the review of the use of scientific criteria and performance standards for safe food, as shown in Table 2 (Ruegg 2003). All of the bacteriological quantities found in both Sacha inchi seed and SIM were lower than the acceptable limit of 2.0×104 cfu.g^{-1} recommended as the general bacterial count limit in soymilk by the Soy Foods Association of America (SFAA) (IMNRC 2003) and also below the permissible limit for pasteurized milk (3×10^4 $cfu.mL^{-1}$). Several crops, such as cereals, spices, and especially nuts, are prone to aflatoxin contamination, which is an indication of their carcinogenic potential in humans. As a result, the U.S. Department of Agriculture (USDA) and the Food and Drug Administration (FDA) set the tolerance limit of 20 ppb of aflatoxins on food products (Oluwole et al. 2020). Among four types of aflatoxins, B1, B2, G1, and G2, B1 is the most concerning due to its high toxicity. The limit of aflatoxin levels in milk is lower than in food, at 0.5 ppb (Sarma et al. 2017). According to this study, the total aflatoxin in Sacha inchi seeds was less than 20 ppb, at 3.29 ppb, though aflatoxins cannot be detected in SIM.

Sensory analysis. The preliminary examination of consumer behavior and attitude towards SIM and comparison of preferences between almond milk and inchi milk was performed. The questionnaires were completed by 35 subjects regarding plant-based milk's demographic and consumption behaviours. The reasons for drinking plant-based milk from 35 subjects were i) allergic to cow milk, ii) plant-based milk is good for one's health and is tasty. The preferences for almond milk and SIM were evaluated for color, aroma, flavor, sweetness, body, and overall liking (Table 3). Almond milk was liked significantly more than SIM in all investigated aspects except color ($p<0.05$). Furthermore, almond milk was in the range of slightly liked to moderately liked (except for sweetness, which was neither liked nor disliked), while SIM was in the range of disliked somewhat to neither liked nor disliked (except for color, which was moderately appreciated). Thus, Inca milk needed to be improved for its bean scent and flavor, sweetness, and body compared to commercial samples.

Table 2. Microbiological and toxicity properties of the Sacha inchi seed and its milk product

Microbiological tests	**Limit in milk**	**Sacha inchi seed**	**SIM**
Aerobic plate count, $cfu.mL^{-1}$	10^5	< $8.8\times x10^2$	< 2.5×10 EAPC
Bacillus cerus, $cfu.mL^{-1}$	100	<1	<1
Clostridium perfringens, $cfu.mL^{-1}$	100	<1	<1
Coliforms bacteria , $cfu.mL^{-1}$	5	<10 est.	<1 est.
Escherichia coli , $cfu.mL^{-1}$	10^5	<10 est.	<1 est.
Salmonella spp., $cfu.25mL^{-1}$	$5x10^4$	Not detected	Not detected
Staphylococcus aureus , $cfu.mL^{-1}$	425	<10 est.	<1 est.
Yeast and molds , $cfu.mL^{-1}$	-	-	<1 est.
Aflatoxin B1 , $\mu g.kg^{-1}$	-	1.43	Not detected
Aflatoxin B2 , $\mu g.kg^{-1}$	-	1.86	Not detected
Aflatoxin G1 , $\mu g.kg^{-1}$	-	Not detected	Not detected
Aflatoxin G2 , $\mu g.kg^{-1}$	-	Not detected	Not detected
Total aflatoxin , $\mu g.kg^{-1}$	0.5	3.29	Not detected

The preferences for original, matcha green tea and cocoa SIM are shown in Table 3. The ANOVA: single factor was performed to evaluate the differences in overall liking between the three groups. The statistical results showed that the means of the three groups (original, matcha green tea, and cocoa) had no significantly different means)p>0.05(. The colors of the three samples were slightly liked to moderately liked. Other attributes were not well-liked. Although not significantly different in liking, the cocoa flavor seemed to have higher scores than the original and green tea flavors. Information about its health benefits may not affect the preference for SIM. However, it was not explicitly shown in this study due to the fact that the Inca milk served before and after giving the information was different in sweetness. Thus, the slight increase in liking for the original flavor of SIM could be due to the amount of sugar and flavor added (Almoselhy 2023).

Secondary sensory evaluation was processed by the JAR. The result obtained in the JAR evaluation is shown in Fig. 3. In the original flavor, the color parameter was mainly scored in the JAR group at 80%, and the higher intensity was observed in the matcha flavor at 85%. However, the % intensity of the JAR group in the cocoa flavor decreased to 65.71%. The result related to the net effect score stated that the color of the original and cocoa should be slightly increased. In this study, the beany odor was the most worrying topic. However, adding flavor did not improve the aroma much. According to the JAR score, 40% was in the JAR group for the original flavor but adding matcha green tea, the % intensity of JAR dropped to 34.29%. However, flavoring with matcha and cocoa can improve the flavor scores from 37% in the original flavor to 40% and 50% for matcha green tea and cocoa, respectively. The body parameter demonstrated the lower difference between original and matcha flavor.

Table 3. Mean and standard deviation of liking scores for two phases

Phase I						
Samples	**Color**^{s}	**Aroma**	**Flavor**	**Sweetness**	**Body**	**Overall liking**
Almond	6.8±1.3	7.7±1.1^{a}	6.5±1.5^{a}	5.6±2.0^{a}	6.2±1.3^{a}	6.8±1.3^{a}
Original	7.1±1.0	4.7±1.8^{b}	4.2±2.2^{b}	4.0±2.1^{b}	5.0±2.1^{b}	4.5±1.9^{b}
Phase II						
Samples	**Color**^{s}	**Aroma**^{s}	**Flavor**^{s}	**Sweetnes**^{s}	**Body**^{ns}	**Overall liking**^{ns}
Original	6.9±1.1	5.3±1.9	5.4±1.9	5.2±1.8	5.7±1.7	5.7±1.7
Matcha	6.7±1.7	5.8±2.1	5.8±1.8	5.3±2.0	5.9±1.7	5.9±1.8
Cocoa	6.6±1.6	6.1±1.8	6.2±1.8	5.4±1.9	6.3±1.7	6.4±1.7

Note*:* Different letter denotes a significant difference *(*$p<0.05$*)*

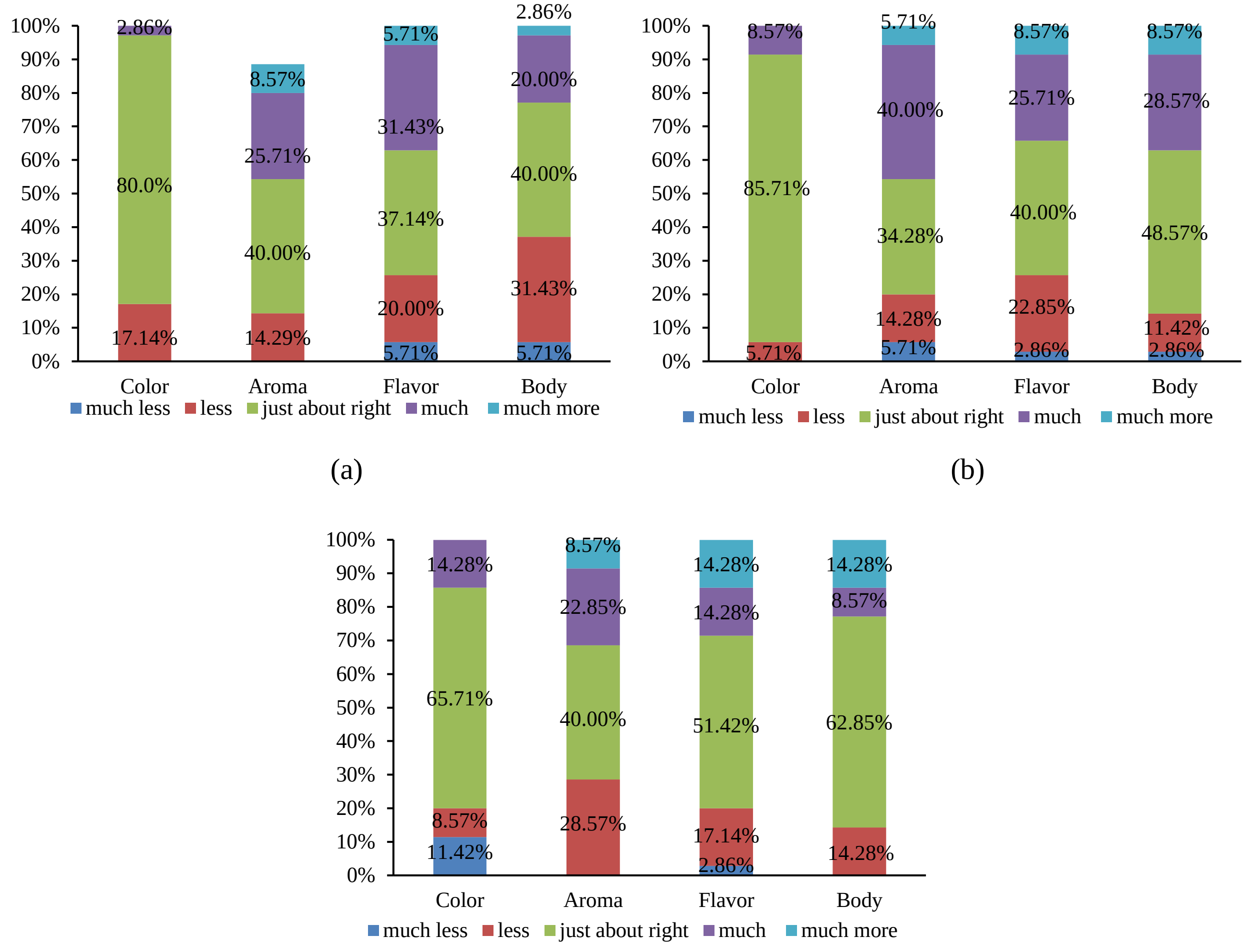
The negative net effect (Table 4) of all three flavors, including original, matcha green tea, and cocoa, indicated that increasing the sweetness in the final product is strongly recommended. If adding sugar positively influenced the preference for SIM then increasing the sweetness to a level that matches Thai consumers' preference could be an opportunity to develop SIM further. The disliking of SIM was mainly due to its lacking sweet taste and having a characteristically strong bean odor. The JAR results suggest that the cocoa flavor may be more suitable for masking and improving the aroma and flavor of SIM. The body of SIM was somewhat just-right, although consumers commented that it was sandy and left particles in the mouth after swallowing.

According to the questionnaire, 14% of the consumers had heard about Inca nut, while 60% had consumed Inca leave tea and roasted Inca nut before. However, 86% of the judges had never heard about Sacha inchi. The consumers who may not buy or be interested in SIM indicated that the product was not tasty, had an intense beany odor, and was too bland (not sweet enough). They were also not familiar with the product. Those who may buy SIM indicated that the product was new, easy to drink, good for health, and high in protein.

They also showed it could potentially be masked with other flavors. Those who were not sure (28%) about their purchase decision indicated that the product had a solid beany aroma and was bland. 34% of the judges may buy the novel product, while 29% may not. However, other flavors such as cocoa could mask the beany smell and improve the flavor. Therefore, reducing the beany odor and flavor is highly recommended by using different elements to mask and process the Sacha inchi to diminish its strong aroma and flavor. In addition, sweetness may result in increased liking and could enhance the potential of the product to be a new high-protein milk option that is a good source of protein for health-conscious consumers and those allergic to cow's milk.

Conclusions

This work examined the nutritional benefits of SIM produced from roasted and defatted seeds. The



(a) (b) (c)

Figure 3. Just-about-right of responses for (a) original; (b) matcha green tea; (c) cocoa-flavored SIM

Table 4. The net effect of JAR evaluation

Samples	Colour	Odor	Flavor	Sweetness	Body
Original	-14.28	8.57	11.43	-71.43	-14.29
Matcha	2.88	25.71	8.57	-57.14	22.88
Cocoa	-5.71	2.86	8.57	-71.43	8.57

results of this research support the idea that Sacha inchi seed is a good source of nutritional benefit and could be developed as a novel plant-based milk that is good for health-conscious consumers and those who are allergic to cow's milk. The current product was not well-liked due to its intense beany odor and flavor and its lack of sweet taste. Furthermore, based on the sensory study results, the cocoa powder is most suitable to apply to SIM production because seasoning with the cocoa flavor reduced the beany taste and enhanced the flavor of SIM, which led to increased acceptability by panellists. There are several possibilities for further development of this alternative product, such as peanut butter or alternative high protein flour for baking.

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