

Article

Property Changes in Production of Hybrid Fresh Cheese Rich in Polyunsaturated Fatty Acids from Sacha Inchi (*Plukenetia volubilis*) Oil

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Abstract

Milk fat plays an important role in the flavor and texture of cheese. However, it contains high amounts of saturated fat and cholesterol, which have recently been reported to be unsuitable for maintaining good health. The aim of this study was to evaluate the effects of milk fat's replacement with sachu inchi (*Plukenetia volubilis*) oil in fresh cheese processing on the coagulation properties, nutritional value, and sensory properties, aiming to obtain a hybrid fresh cheese rich in polyunsaturated fatty acids (PUFAs). Milk fat (3.8% in standardized milk) was partly replaced with Vietnamese sachu inchi oil at 20, 40, 60, and 80%. The coagulation time, curd formation, and cheese yield showed no significant differences among the treatments ($p > 0.05$), suggesting that sachu inchi oil did not adversely affect curd formation. The cholesterol levels decreased substantially, from 8.27 ± 0.53 mg/g in control samples to 2.63 ± 0.63 mg/g at 80% fat replacement. An increase in essential fatty acids in the fresh cheese was also found with an increase in the use of sachu inchi oil to replace milk fat, with the oleic acid concentration (OA, C18:1, cis ω -9) rising from $7.88 \pm 0.36\%$ to $23.44 \pm 0.13\%$ and the linoleic acid concentration (LA, C18:2, cis ω -6) from $6.44 \pm 0.68\%$ to $41.75 \pm 2.50\%$ at the highest substitution level. From a nutritional perspective, the replacement of milk fat with sachu inchi oil did not affect the macronutritional values (fat, protein), but it reduced the cholesterol levels and enhanced the overall nutritional value of the fresh cheese with increasing essential fatty acids. The milk fat alternative of sachu inchi oil for cheese processing contributed to a richer and creamier sensory experience of the final products, but no significant differences in the overall liking were found regarding the acceptance of customers. Thus, replacement with sachu inchi oil in PUFA cheese processing is a promising method to improve the nutritional value and sensory quality of fresh cheese.

Keywords: milk; cheese; sachu inchi oil; hybrid cheese; fat replacement; polyunsaturated fatty acid (PUFA)



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1. Introduction

Cheese has a long history as a traditional dairy product worldwide thanks to its nutritional value in the human diet. However, Vietnam lacks a longstanding tradition of dairy farming [1]. Dairy farming appeared in the early 20th century following French colonization, and it has been a commodity production industry since the 1990s. Due to the limited domestic production of raw milk, cheese has not been prioritized in industrial dairy processing. Nevertheless, in recent years, the consumption of cheese in Vietnam has grown; this can be attributed to the popularity of Western fast food items, which have become increasingly favored by Vietnamese consumers, especially the younger generation. The cheese market in Vietnam is projected to grow by 5.45% (2024–2029), resulting in a market volume of USD 284.00 m in 2029 [2].

Recently, the demand for dairy alternatives has increased considerably for various reasons, such as milk allergies, lactose intolerance, cholesterol issues, and other health concerns, as well as the increased demand for sustainable products in order to reduce the environmental footprint and promote human health and animal welfare [3,4]. Milk fat contains a complex mixture of various lipid substances, primarily triglycerides (triacylglycerides) (98% of the total milk fat), diacylglycerides (0.25–0.48%), monoacylglycerides (0.02–0.04%), phospholipids (0.6–1.0%), cholesterol (0.2–0.4%), glycolipids (0.006%), and free fatty acids (0.1–0.4%) [5]. Cow milk fat contains, on average, 60–70% saturated fatty acids. Nowadays, consumers are becoming increasingly concerned about health aspects, and the replacement of dairy fat with plant-based fat can offer advantages. Cheese analogs are cheese-like products in which milk fat, milk protein, or both are partially or wholly replaced with non-dairy-based components [6]. The global cheese analog market size was valued at USD 2.34 billion in 2023 and is expected to expand from USD 2.49 billion in 2024 to USD 4.13 billion by 2032, growing at a compound annual growth rate (CAGR) of 6.5% during the forecast period (2025–2032). Among these products, hybrid cheese is produced from milk and plant-based ingredients, where both components are retained in the final product at varying concentrations. Hybrid cheese is an innovative product that has the benefits of both real cheese and plant-based ingredients, offering numerous advantages over traditional cheese.

Vegetable oils are a significant source of fatty acids, including polyunsaturated fatty acids (PUFAs), which have been revealed to possess many advantages and considerable biological activity [7]. Sacha inchi (*Plukenetia volubilis*) oil is a novel source of oil rich in unsaturated fatty acids, particularly omega-3, omega-6, and omega-9, which contribute to regulating the antioxidant signaling pathways related to cardiovascular health and preventing chronic non-communicable diseases. In 2012, the first sachu inchi seeds were imported to and planted in Vietnam, and the plant's production has now been expanded to many different ecological regions of Vietnam (the northern mountainous region, the north-central region, and the central highlands area) [8]. In recent years, there has been increasing interest in the development of the sachu inchi plant as a novel source of oil, valued for its high content of unsaturated fatty acids, the associated health benefits, and its distinctive sensory characteristics, including its taste and aroma [9].

Vanegas-Azuero and Gutiérrez reported on the physicochemical properties and sensory acceptance of yogurts enriched with natural ingredients such as sachu inchi, aimed at improving the nutritional value of yogurt and fermented milks. Sachu inchi oil, rich in PUFAs, was fortified with two commercial antioxidants (tocopherol and Ecoprol 2020 (ascopyl palmitate and propyl gallate)) and used to increase the shelf-life of fresh cheese from 7 to 14 days [10]. To date, no studies have been reported on the use of sachu inchi oil as a milk fat alternative in the production of fresh cheese. Given that milk is relatively lacking in essential fatty acids, the development of dairy products that are rich in essential

fatty acids from sachu inchi could contribute to enhancing the intake of health-beneficial ingredients among the population. With the robust growth of the Vietnamese cheese market, it is very important to develop new functional hybrid cheese products by improving the quality of fat in cheese using sachu inchi oil as a PUFA-rich substitute. This approach aims to retain the desirable sensory attributes and texture associated with traditional cow's milk cheese while leveraging the sustainability benefits of plant-based ingredients. In attempts to develop a hybrid fresh cheese product combining the nutrition and functional properties of milk and plant-based components, this study investigated the effect of milk fat's replacement by sachu inchi oil on the changes in coagulation properties, nutritional values, fatty acid components, and sensory properties in fresh cheese processing.

2. Materials and Methods

2.1. Materials

The whole cow milk used in the cheese production was collected from Bavimilk, Hanoi, Vietnam, at the morning milking from March to September 2022. Raw milk was kept at 4 °C immediately after milking and transported to the laboratory for the analysis of the dry matter, protein and fat content, acidity, and freshness. The raw milk could be stored for not more than 24 h at 4 °C for cheese production.

Marzyme[®], the liquid microbial rennet (Danisco, International Flavors & Fragrances (IFF), New York, NY, USA) produced by the fermentation of a pure broth of the fungus *Rhizomucor miehei*, was used for milk coagulation. Its optimal pH is 5.5–6.0, its optimal temperature is 36–40 °C, and its enzyme activity is 703–738 IMCU/mL. Its recommended dosage is 24–48 IMCU/L milk.

The starter culture FD-DVS YF-L812 Yo-Flex, obtained from CHR HANSEN, Denmark, included *L. bulgaricus* and *S. thermophilus* at a ratio of 1:1. Its optimal temperature is 35–45 °C, with a recommended dosage of 100 U/1000 L milk. CaCl₂ salt was obtained from Solvay, Italy. A lactic acid solution (PURAC[®] FCC) was provided by Corbion, the Netherlands.

2.2. Methods

2.2.1. Raw Milk Analysis and Chemical Analysis

The total solids, density, protein content, fat content, and titratable acidity of the milk samples were determined using the gravimetric method, a lactodensimeter, the Kjeldahl method (ISO 8968-1:2014) [11], the Gerber method (ISO 19662:2018) [12], and the Thorner method, respectively, as previously described in the Vietnamese standard TCVN 7405:2018 [13]. The pH of the samples was measured using a Mettler Toledo 1120 pH meter (Mettler Toledo, Greifensee, Switzerland). The properties of the raw milk used in this study were analyzed for all the raw milk samples, and the results are described in Table 1.

Table 1. Physicochemical parameters of raw milk.

No.	Analytical Parameters	Raw Milk	Vietnamese Standard (TCVN 7405:2018)
1	Dry matter (%)	12.9 ± 0.5	≥11.5
2	Fat (%)	4.0 ± 0.4	≥3.2
3	Protein (%)	3.1 ± 0.2	≥2.8
4	Density (g/mL)	1.027	≥1.026
5	pH	6.6	-
6	Acidity (°T)	15–17	-

Sacha inchi oil was obtained from Mekong Megumi (Vietnam) and extracted using the cold pressing method from fresh fruit. The saturated fat, ω 3-6-9, and vitamin E concentrations in the sachu inchi oil were 10.5 g, 8 g, 46 g, 46 g, and 207 mg per 100 g oil, respectively.

2.2.2. Fatty Acid Analysis

The fatty acids in the fresh cheese were methanolized using commercial anhydrous HCl/methanol. The fatty methyl ester content was extracted from the sample matrix with hexane solvent and then analyzed on a GC-MS/MS machine (Trace 1300-TSQ 8000 Evo, Thermo Scientific, Waltham, MA, USA). A total of 0.1 g of the fresh cheese was put into a 10 mL screw-capped glass tube, then 0.2 mL of toluene and 1.5 mL of a HCl/MeOH mixture (85:15, *v:v*) were added and the cap was tightly closed. The mixture was heated in a water bath at 100 °C for about 2 h and then cooled to room temperature. The fatty methyl esters were separated from the cheese and water mixture by adding 1 or 2 mL of hexane to the glass tube, then vortexed and centrifuged at 4500 rpm. The hexane fraction was filtered through a 0.45 μ m filter into a 1.5 mL vial, and then 1 μ L was analyzed on a GC/MS system with a standard of 37 methyl ester compounds (Supelco 37 Component FAME Mix 37, lot no.: LRAD3869; Sigma-Aldrich, St. Louis, MO, USA). The validation of the analytical method using spiked matrices demonstrated a limit of quantification (LOQ) of ~4 mg/kg (0.0004%), with mean recoveries between 97.6% and 102.5% and relative standard deviations (RSDs) ranging from 1.05% to 2.56%, confirming the method's accuracy and precision [14].

2.2.3. Sensory Analysis

Sensory evaluation was conducted by a trained panel comprising 20 assessors with prior experience in cheese tasting. The panel included factory personnel, graduate students, and undergraduate students majoring in food technology, all of whom had completed formal training in sensory analysis.

The samples were equilibrated to room temperature (21 ± 1 °C), cut into uniform $1 \times 1 \times 1$ cm cubes, and served in duplicate on white dishes labeled with randomized three-digit codes. A balanced serving order was applied to minimize potential bias due to the sample sequence. The sensory evaluations were conducted in a laboratory designed in accordance with general guidelines for test room design (ISO 8589:2007) [15], ensuring standardized testing conditions across all the sessions.

****Quantitative Descriptive Analysis****

Quantitative Descriptive Analysis (QDA) was applied to evaluate a total of 21 sensory attributes. The attribute selection and definition followed the ISO 11035:1994 [16] protocol, which guided the generation, screening, and final selection of descriptive terms. A continuous scale anchored with “weak” and “strong” descriptors was used to assess each sensory attribute. During data processing, these values were converted to a 0–5 scale, where 0 represented no detection and 5 the highest level of detection. Prior to the formal evaluation, panelists underwent three calibration sessions using representative samples from preliminary trials to establish a consensus on the attribute interpretation and rating scale usage.

In addition to descriptive analysis, a 5-point hedonic test was conducted to assess the overall liking, comparing the sachu inchi PUFA-enriched fresh cheese samples with the control (non-substituted) sample. The panelists rated each sample as follows: 1 = dislike very much, 2 = dislike slightly, 3 = neither like nor dislike, 4 = like slightly, and 5 = like very much. This dual approach enabled both objective sensory profiling and subjective preference assessment.

Final Lexicon

The final lexicon for the descriptive tests included the following categories:

- ****Appearance****: The color intensity (yellowness), reflection (oiliness), and smoothness (surface ruptures).
- ****Odor and Aroma****: A fresh milk aroma, buttery odor, creamy odor, yogurt odor, or refreshing aroma.
- ****Texture****: The stickiness, meltability, smoothness (mouthfeel), softness, and saltiness.
- ****Flavor****: The tanginess, sweetness, vanilla flavor, and creaminess.
- ****Aftertaste****: The saltiness of the aftertaste, tanginess of the aftertaste, sweetness of the aftertaste, and creaminess of the aftertaste.

2.2.4. Cheese Processing and Experiment Designs

The hybrid fresh cheese was prepared as described previously [17] with several modifications to replace 20–80% of the milk fat with sachai inchi oil. The process is illustrated in Figure 1.

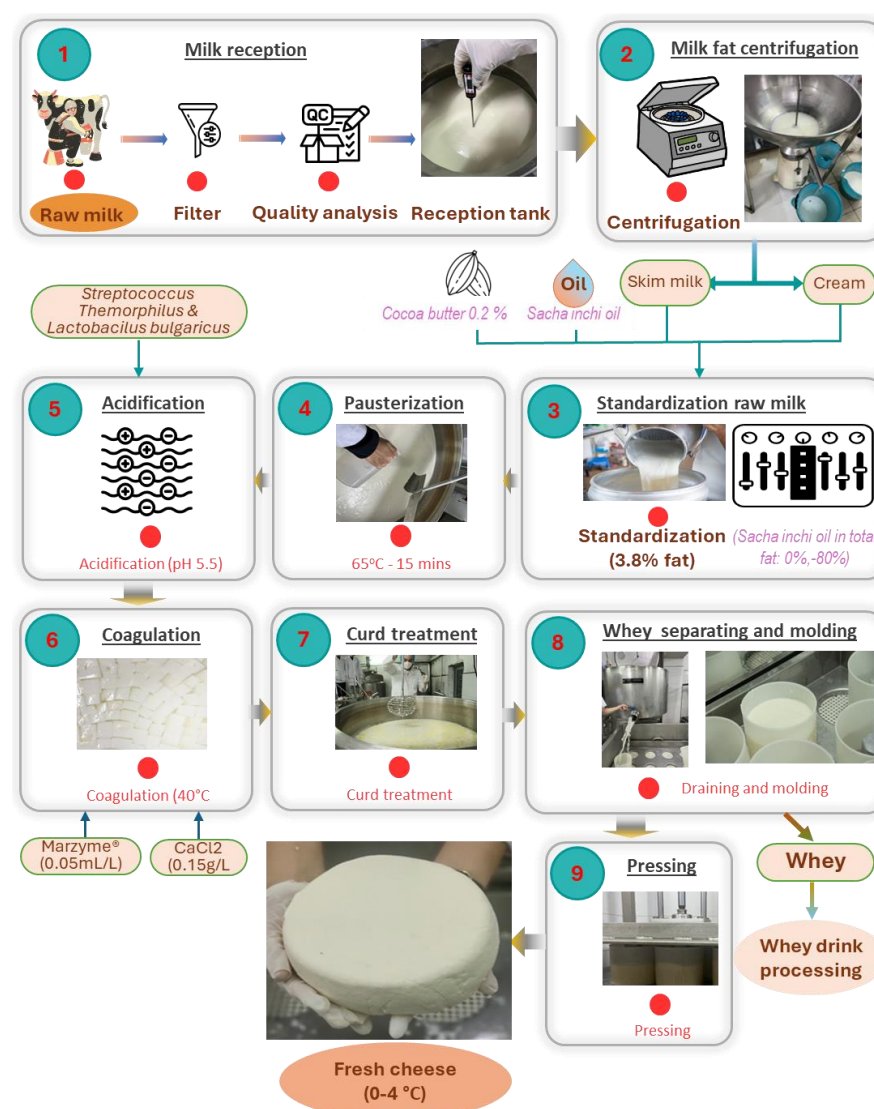


Figure 1. Process flowchart for the preparation of fresh hybrid cheese at 500 kg/batch.

The whole milk was standardized using an Electric Milk Cream Centrifugal Separator (FJ 350 EAR, Drumrahan Retail Ltd., Cavan, Ireland) to obtain cream (60% fat content) and

skim milk (0.4% fat content). A total of 500 kg of standardized milk for cheese processing was prepared for each sample by calculating the extracted amounts of cream, sachu inchi oil, and skim milk, as in the example in Table 2. Next, the sachu inchi oil and cream were homogenized with 100 kg of skim milk at 50 bar using a homogenizer (TA18D, Didacta Italia, Torino, Italy), then mixed with the remaining amount of skim milk for 15 min at 60 rpm in a 1000 L cheesemaking machine (TA503D, Didacta Italia, Torino, Italy). All the samples were standardized to a total fat content of 3.8% (*w/w*), with the milk fat progressively replaced by sachu inchi oil at substitution levels ranging from 20% to 80%, and subsequently used in fresh cheese production.

Table 2. Standardization of raw milk for fresh cheese processing.

	Milk Fat Replacement (%)				
	Control	20	40	60	80
Skim milk (kg)	475.47	476.06	478.41	488.15	482.93
Cream (kg)	26.33	20.19	14.09	8.10	1.94
Sachu inchi oil (kg)	0.00	3.80	7.62	11.69	15.21

The control samples were obtained by mixing cream and skim milk, following the same procedure applied to the experiment samples. Cocoa butter was added at 0.2% of the total fat to stabilize the sachu inchi oil in the hybrid products during storage. After that, the standardized milk was pasteurized at 65 °C for 15 min. The milk was cooled down to 40 °C and inoculated with a starter, FD-DVS YF-L812 Yo-Fle, at pH 5.5. The coagulation enzyme Marzyme® (0.05 mL/kg) was added, along with CaCl₂ (0.15 g/kg), and mixed thoroughly. After coagulation, the curd was cut into 1 cm³ cubes with a cheese knife, and it was gently stirred at 40 °C before draining. The coagulum was heated to 55–60 °C for 10 min without vigorous stirring for the syneresis of the curd. After that the cheese curd was cooled down to room temperature and was transferred to molds with a pressure of 0.03 kg/cm² and kept at room temperature for 1 h. After the whey drainage, the fresh cheese products were withdrawn from the molds, wrapped in plastic bags, and stored at 4 °C.

In attempts to obtain a hybrid fresh cheese product combining the nutrition and functional properties of milk fat and sachu inchi oil, different fresh cheese production procedures with the replacement of milk fat by sachu inchi oil at 20, 40, 60, and 80%, namely 20% R, 40% R, 60% R, and 80% R, were carried out. Each experiment sample was prepared in two replicates on a scale of 500 kg of materials used per batch. The effect of the sachu inchi oil replacement on the coagulation properties, nutritional values, cholesterol, fatty acid components, and sensory properties in fresh cheese processing in comparison with those of the control sample were investigated.

The curd yield was calculated as follows:

$$\text{Curdyield} = \frac{\text{mass of cheese} \times \text{dry matter of cheese}}{\text{mass of milk} \times \text{dry matter of milk}} \times 100 (\%)$$

The cheese yield was calculated as the percentage of cheese obtained per amount of raw milk $\times 100 (\%)$.

2.2.5. Statistical Analysis

In this study, each sachu inchi oil replacement sample was prepared in two replicates on the scale of 500 kg of materials/batch for hybrid cheese processing. The coagulation properties and nutritional values such as the protein and total fat were analyzed twice for each sample of each batch. The total fat content, protein content, fat/protein ratio,

and cheese yield (%) were compared using an analysis of variance (one-way ANOVA) carried out using SPSS version 20 (2011) (IBM Corporation, Armonk, NY, USA). The intergroup differences between the treatments were obtained using a Tukey test at a 5% level of significance.

The fatty acid components and cholesterol were measured in triplicate for each sample in each batch. The mean values and standard deviations were calculated and are described in the results.

The sensory properties were determined by 20 assessors. Multivariate statistical techniques were applied to examine the potential correlations between the sensory characteristics and the sachai inchi oil content added to the product using the R software package version 4.3.1, package FactoMineR version 2.11 (to carry out a PCA).

3. Results

3.1. Effect of Replacement of Milk Fat with Sachai Inchi Oil on Coagulation Properties and Yield of Fresh Hybrid Cheese Products

The sachai inchi oil hybrid cheese production process was conducted with varying levels of milk fat replacement (20%, 40%, 60%, and 80%). The curd coagulation time, curd yield, fat/protein ratio, and cheese yield were determined and are presented in Table 3. There was no difference in the coagulation time after renneting using the same enzymatic process in all the samples. Variations in the sachai inchi oil-to-milk fat ratio did not lead to significant changes in either the curd yield or overall cheese yield. However, a notable decrease in the fat-to-protein ratio was observed in the hybrid cheese formulation containing an 80% replacement of the milk fat.

Table 3. Effect of replacement of milk fat with sachai inchi oil on coagulation properties and yield of fresh hybrid cheese products.

Sachai Inchi Oil Replacement in Raw Milk						
No.	Parameters	Fresh Cheese	20%	40%	60%	80%
1	Curd coagulation time	2'47 s	2'47 s	2'47 s	2'47 s	2'47 s
2	Curd obtained (%)	16	18	16	17	17
3	Fat/protein ratio	1.64 ± 0.11 ^a	1.57 ± 0.05 ^{ab}	1.58 ± 0.07 ^{ab}	1.46 ± 0.02 ^{ab}	1.33 ± 0.02 ^b
4	Cheese yield (%)	11.42 ± 0.54 ^a	12.67 ± 0.86 ^a	11.85 ± 0.58 ^a	11.50 ± 0.16 ^a	12.07 ± 0.00 ^a

Means with the same letter in the same row are not significantly different ($p > 0.05$).

3.2. Effect of Replacement of Milk Fat with Sachai Inchi Oil on Nutritional Values and Fat Recovery in Hybrid Cheese Products

Raw milk containing varying levels, 20–80%, of sachai inchi oil as a milk fat replacer was used for the production of fresh cheese. Figure 2 demonstrates that a high concentration of the sachai inchi oil replacement increased the water retention in the coagulum, leading to a higher amount of water in the final products. However, a slight decrease in the water content was observed at the 80% replacement level. There was no significant effect on changes in the nutritional values in all the samples.

The fat recovery was calculated based on the total amount of fat in the cheese products in comparison with the total fat content in the raw milk before processing. A reduced fat content caused by substituting sachai inchi oil at 20 to 80% showed no significant effect on the total fat recovery in the cheese products. The total fat recovery in the fresh cheese products was approximately 89% in the control samples and 84% in the hybrid cheese sample produced with 80% milk fat replacement.

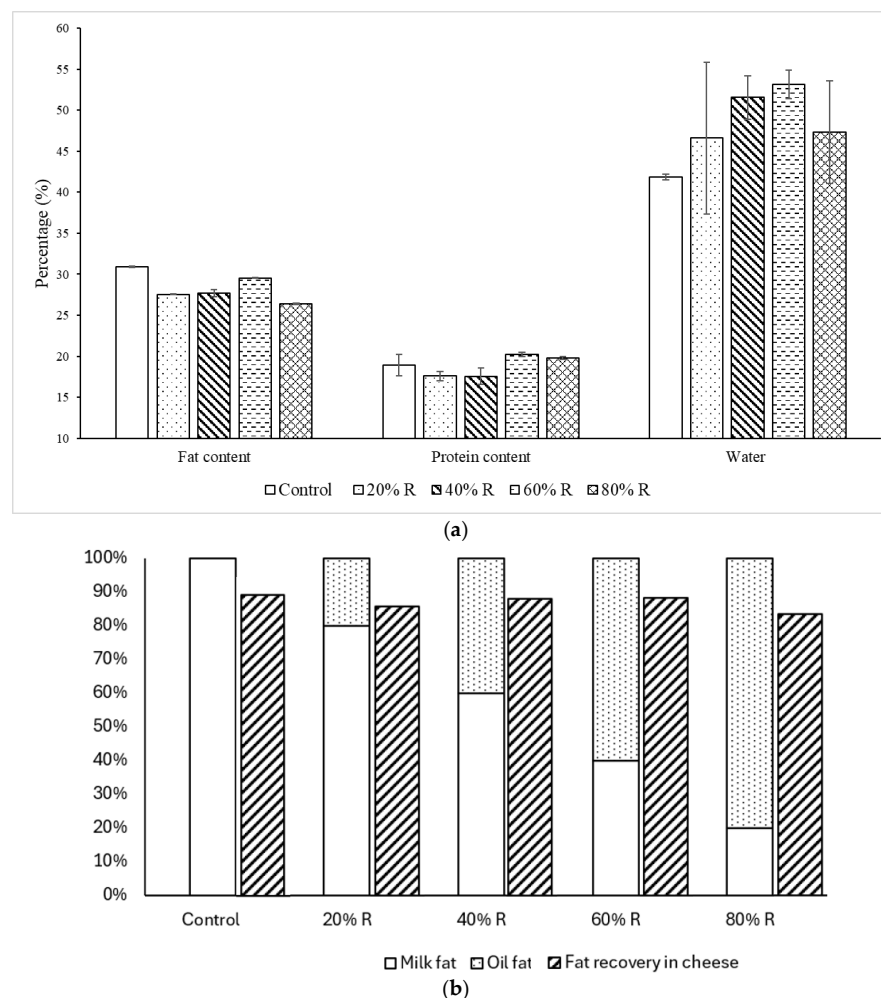


Figure 2. Effect of replacement of milk fat with sachu inchi oil on changes in nutritional values of fresh cheese products (a) and fat recovery in hybrid cheese products (b).

3.3. The Effect of the Replacement of Milk Fat with Sachu Inchi Oil on the Nutrition Properties of Fresh Cheese Products

3.3.1. Fatty Acid Profile of Sachu Inchi Oil

The fatty acid constituents of sachu inchi oil were analyzed by GC/MS. Sachu inchi oil is a rich source of PUFAs, primarily linoleic acid (LA, C18:2, cis ω -6) ($43.81 \pm 2.50\%$) and oleic acid (OA, C18:1, cis ω -9) ($37.9 \pm 0.79\%$). Sachu inchi oil has small amounts of linolenic acid (C18:3 ω -3) ($6.26 \pm 0.42\%$) and saturated fatty acids such as palmitic acid (C16:0) ($6.18 \pm 0.67\%$) and stearic acid (C18:0) ($1.41 \pm 0.25\%$) and trace amounts ($<1\%$) of elaidic acid (C18:1, trans n9), arachidic acid (C20:0), cis-11-eicosanoic acid (C20:1 ω -9), cis11,14-eicosadienoic acid (C21:2, cis-11,14), behenic acid (C22:0), erucic acid (C22:1 ω -9), lignoceric acid (C24:0), and nervonic acid (24:1 ω -9). With high amounts of unsaturated fatty acids, sachu inchi oil represents a valuable ingredient for the promising development of PUFA-rich hybrid fresh cheese, offering potential health benefits due to its functional lipid profile.

3.3.2. Effect of Replacement of Milk Fat with Sachu Inchi Oil on Fatty Acid Profiles and Cholesterol Level in Hybrid Fresh Cheese Products

The fatty acid profiles of the hybrid fresh cheese products were analyzed to evaluate the possibility of producing hybrid fresh cheese rich in PUFAs from sachu inchi oil. The results in Table 4 indicate that replacing milk fat with sachu inchi oil increased the levels of linoleic acid (LA, C18:2, cis ω -6) and oleic acid (OA, C18:1, cis ω -9) in all the hybrid fresh

cheese samples. The LA concentration was $6.44 \pm 0.68\%$ of the total fatty acids in the control sample, but a higher level of this PUFA was obtained in the sachu inchi hybrid cheese, with concentrations of $13.02 \pm 0.00\%$, $21.02 \pm 0.69\%$, $35.55 \pm 0.73\%$, and $41.75 \pm 2.50\%$ of the total cheese fat with milk fat replacements of 20, 40, 60, and 80%, respectively. The oleic acid concentration (OA, C18:1, cis ω -9) in the total cheese fat increased from $7.88 \pm 0.36\%$ in the control sample to $23.44 \pm 0.13\%$ in the hybrid fresh cheese with 80% replacement. On the other hand, decreases in palmitic and myristic acid, which are associated with milk fat, were found in proportion to the increase in the sachu inchi oil replacement.

Table 4. Changes in fatty acid profiles in hybrid fresh cheese products made with sachu inchi oil.

Fatty Acid	Effect of Replacement of Milk Fat with Sachu Inchi Oil on Fatty Acid Components of Cheese Fat				
	Control	20%	40%	60%	80%
Butyric acid (C4:0)	0.42 ± 0.02	0.58 ± 0.12	0.42 ± 0.11	0.53 ± 0.04	0.47 ± 0.05
Hexanoic (C6:0)	n.d	n.d	n.d	n.d	n.d
Octanoic (C8:0)	n.d	n.d	n.d	n.d	n.d
Decanoic (C10:0)	1.16 ± 0.10	0.68 ± 0.05	1.02 ± 0.05	0.39 ± 0.34	0.20 ± 0.05
Undecanoic (C11:0)	0.3 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Lauric (C12:0)	2.60 ± 0.08	2.67 ± 0.10	1.78 ± 0.08	0.63 ± 0.12	0.24 ± 0.08
Tridecanoic (C13:0)	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.01	n.d	n.d
Myristic (C14:0)	10.24 ± 0.07	7.13 ± 0.20	7.10 ± 0.26	3.85 ± 0.22	1.50 ± 0.12
Miristoleic (C14:0, n5)	1.05 ± 0.00	0.69 ± 0.05	0.66 ± 0.05	0.34 ± 0.00	0.09 ± 0.00
Pentadecanoic (C15:0)	0.74 ± 0.10	0.48 ± 0.08	0.45 ± 0.10	0.19 ± 0.02	0.06 ± 0.00
Cis-10_pentadecenoic (C15:1, cis n5)	0.58 ± 0.00	0.38 ± 0.01	0.36 ± 0.08	0.14 ± 0.04	0.04 ± 0.00
Palmitic (C16:0)	28.35 ± 1.22	19.67 ± 0.82	22.50 ± 1.13	22.50 ± 1.87	9.26 ± 0.11
Palmitoleic (C16:1, n7)	1.71 ± 0.00	1.10 ± 0.00	1.13 ± 0.17	0.57 ± 0.04	0.27 ± 0.05
Heptadecanoic (C17:0)	0.30 ± 0.00	0.18 ± 0.01	0.23 ± 0.01	0.20 ± 0.01	n.d
Cis-10_heptadecenoic (C17:1, cis n7)	0.32 ± 0.02	0.20 ± 0.03	0.25 ± 0.01	0.11 ± 0.02	0.05 ± 0.00
Stearic (C18:0)	8.05 ± 0.25	7.52 ± 0.50	7.37 ± 0.16	7.66 ± 0.25	3.30 ± 0.29
Elaidic (C18:1, trans n9)	2.85 ± 0.23	1.79 ± 0.15	2.58 ± 0.12	2.26 ± 0.15	1.21 ± 0.13
Cis-9_oleic (OA, C18:1, cis ω -9)	7.88 ± 0.36	11.47 ± 0.25	19.79 ± 0.37	16.23 ± 0.22	23.44 ± 0.13
Linolelaidic (C20:0)	n.d	n.d	n.d	n.d	n.d
Linoleic (LA, C18:2, cis ω -6)	6.44 ± 0.68	13.02 ± 0.00	21.02 ± 0.69	35.55 ± 0.73	41.75 ± 2.50
Arachidic (C20:0)	0.10 ± 0.00	0.03 ± 0.00	0.33 ± 0.02	0.24 ± 0.06	0.25 ± 0.02
Gamma-linolenic (GLA, C18:3 ω -6)	n.d	n.d	n.d	n.d	n.d
Cis-11-eicosanoic (C20:1 ω -9)	0.12 ± 0.00	0.18 ± 0.02	0.23 ± 0.00	0.194 ± 0.06	0.13 ± 0.07
Linolenic (C18:3 ω -3)	1.14 ± 0.06	0.85 ± 0.00	0.84 ± 0.06	1.64 ± 0.16	3.95 ± 0.44
Heneicosanoic (C21:0)	n.d	n.d	n.d	n.d	n.d
Cis11,14-eicosadienoic (C21:2, cis-11,14)	0.06 ± 0.00	0.05 ± 0.00	0.09 ± 0.00	0.08 ± 0.00	0.05 ± 0.02
Behenic (C22:0)	n.d	n.d	0.29 ± 0.03	0.35 ± 0.05	0.18 ± 0.02
Cis-8,11,14-eicosatrienoic (DGLA, C20:3 ω -6)	n.d	n.d	n.d	0.013 ± 0.00	0.01 ± 0.00
Cis-13-docosenoic (Erucic, C22:1 ω -9)	0.03 ± 0.00	n.d	0.28 ± 0.02	0.36 ± 0.06	0.20 ± 0.03
Cis-11,14,17-eicosictraenoic (C20:3 ω -3)	n.d	n.d	n.d	n.d	n.d
Tricosanoic (C23:0)	n.d	n.d	0.010	n.d	n.d
Cis-5,8,11,14-eicosictraenoic (C20:4 ω -3)	0.08 ± 0.00	0.04 ± 0.00	0.05 ± 0.02	n.d	n.d
Cis-13,16-docosadienoic (C22:2—cis 13,16)	n.d	n.d	n.d	n.d	n.d
Lignoceric (C24:0)	n.d	n.d	0.14 ± 0.04	0.13 ± 0.02	0.09 ± 0.00
Cis-5,8,11,14,17-eicosapentaenoic (EPA, C20:5 ω -3)	0.03 ± 0.00	0.01 ± 0.00	0.02 ± 0.02	n.d	n.d
Nervonic (24:1 ω -9)	n.d	n.d	0.14 ± 0.03	0.15 ± 0.02	0.08 ± 0.00
Cis-4,7,10,13,16,19-docosahexaenoic (DHA, C22:6 ω -3)	n.d	n.d	n.d	n.d	n.d

n.d. means not detected; all data are expressed as mean $\pm$ standard deviation.

Similar results were observed in the case of cholesterol reduction with increasing replacement with sachu inchi oil. The cholesterol contents in the hybrid fresh cheese samples with 20%, 40%, 60%, and 80% milk fat replacement were 6.95 ± 0.47 mg/g, 4.93 ± 0.42 mg/g, 4.18 ± 0.49 mg/g, and 2.64 ± 0.63 mg/g, respectively. These values were

lower than those of the whole-milk cheese sample (8.27 ± 0.53 mg/g) (Figure 3). Moreover, a linear relationship was observed between the replacement with plant oil and the reduction in the cholesterol content in the fresh cheese products. Specifically, the replacement of 80% of the milk fat in the raw milk led to a reduction of 60% in the cholesterol in the fresh cheese products.

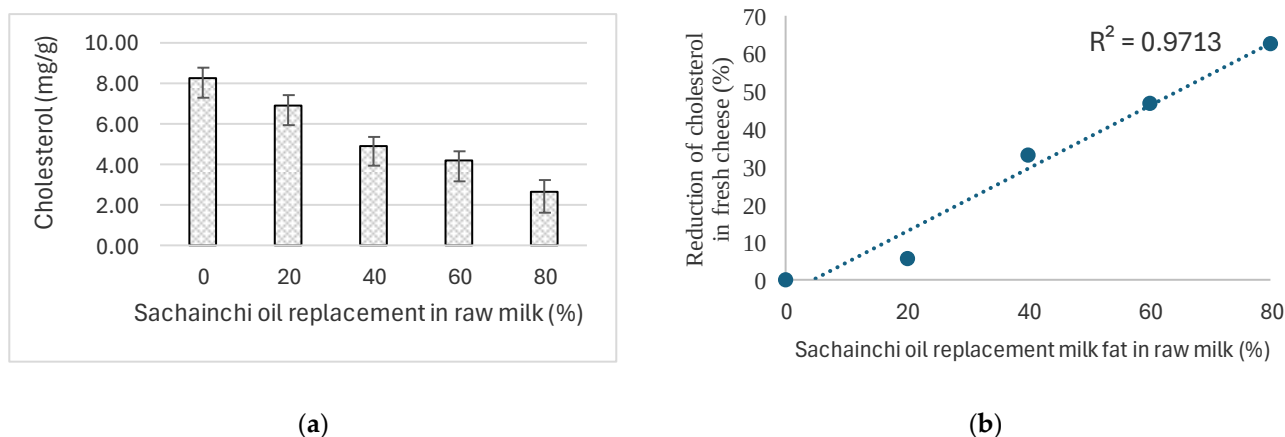


Figure 3. Effect of the replacement of milk fat with sachai inchi oil (a) on the cholesterol content in the fresh cheese products and (b) on the relationship between the replacement with plant oil and the reduction in the cholesterol content in the fresh cheese products.

3.4. Effect of Replacement of Milk Fat with Sacha Inchi Oil on Sensory Properties of Hybrid Fresh Cheese Products

Sensorial and descriptive analysis are frequently used to better comprehend and accommodate consumer demands for the research and development of new food products [18]. To develop new cheese products that align with Vietnamese consumers' expectations, a fresh cheese lexicon for descriptive analysis was established with twenty-one categories. This lexicon was used to investigate the effect of milk fat's replacement by sachai inchi oil on the sensorial properties of the hybrid fresh cheese. A comparison of the individual description scores of all the sensory characteristics is given in Figure 4. The results showed that the color of the sachai inchi cheese and its milk aroma were different from those of the control sample. Similar scores were observed for the flavor, texture, and aftertaste of the sachai inchi cheese to those of the control sample. Although several sensory properties of the hybrid fresh cheese changed compared with those of the original fresh cheese, there was no significant difference in the overall liking scores, reflecting the acceptance of the customers of the hybrid fresh cheese products.

The sensory radar chart demonstrates distinct differences in the sensory attributes among the four cheese samples, particularly between the control (fresh cheese) and those supplemented with sachai inchi oil (hybrid fresh cheese). In terms of their appearance, samples with higher levels of sachai inchi oil substitution exhibited a noticeably greater color intensity (yellowness) compared to the fresh cheese, with a progressive increase observed from the control to the 60% replacement sample. The oiliness peaked in the 40% substitution sample. The surface smoothness showed minimal variation across the samples, although the 60% substitution sample exhibited slightly superior performance.

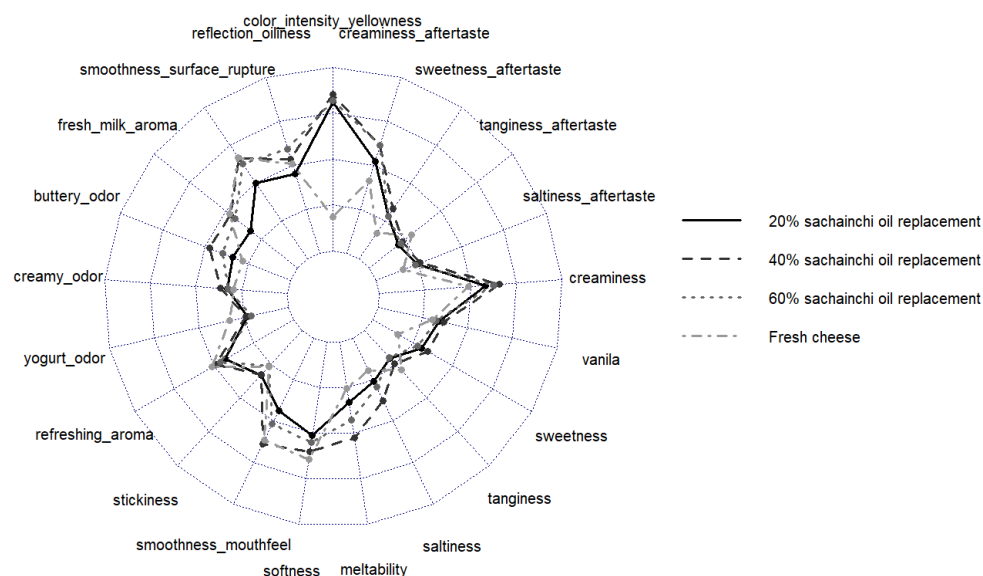


Figure 4. Effect of replacement of milk fat with Sacha Inchi oil on sensory properties of hybrid fresh cheese.

For the odor and aroma attributes, the sensory radar chart reveals a clear trend. The fresh cheese exhibited the most pronounced yogurt odor and refreshing aroma, indicative of a more traditional dairy sensory profile. As the proportion of Sacha Inchi oil increased, there was a consistent rise in the fresh milk aroma and buttery odor, with the highest intensities recorded for the 60% replacement sample. Conversely, the intensity of the creamy odor decreased with an increasing oil content. These changes suggest a shift in the aromatic profile from the tangy, fresh character of the control to the richer, more buttery profile of the oil-enriched formulations.

The texture profile also evolved notably with increasing levels of oil substitution. The sample containing 60% Sacha Inchi oil showed the highest intensities across most texture attributes, particularly the meltability, smoothness, softness, and saltiness, indicating an overall enhancement in the textural quality. In contrast, the fresh cheese displayed the lowest scores in all the texture dimensions except the softness, reflecting a firmer, less creamy consistency.

Flavor attributes such as the sweetness, vanilla flavor, and creaminess exhibited a consistent increase with higher levels of oil replacement. The 60% substitution sample recorded the highest intensities, particularly for the creaminess and vanilla flavor. Conversely, the fresh cheese exhibited the highest tanginess alongside the lowest scores for the sweetness, vanilla flavor, and creaminess. These findings indicate that Sacha Inchi oil contributes to a smoother, sweeter, and more aromatic flavor profile, while diminishing tangy notes.

Regarding the aftertaste, most attributes demonstrated a consistent enhancement with increasing oil substitution. The creaminess of the aftertaste showed the most significant increase, particularly in the 40% and 60% replacement samples. The sweetness of the aftertaste also increased steadily, peaking at 60% substitution. In contrast, the tanginess of the aftertaste was the most intense in the hybrid fresh cheese and declined modestly with an increasing oil content. The saltiness of the aftertaste remained relatively consistent across the samples.

In the principal component analysis (PCA) of the sensory data, the first two principal components accounted for 90% of the total variance among the samples. Principal Component 1 (PC1) alone explained 63.08% of the variation and contributed the most to the differentiation among the cheese samples (Figure 5a). According to PC1, the cheese products containing Sacha Inchi oil (hybrid fresh cheese) were clearly separated from the

non-substituted (fresh cheese) sample. This separation was primarily associated with sensory attributes such as the saltiness of the aftertaste, the color intensity (yellowness), the sweetness, the sweetness of the aftertaste, and the creaminess, all of which were characteristic of the oil-substituted group (Figure 5b). In contrast, the tanginess of the aftertaste and a yogurt odor were typical of the fresh cheese sample.

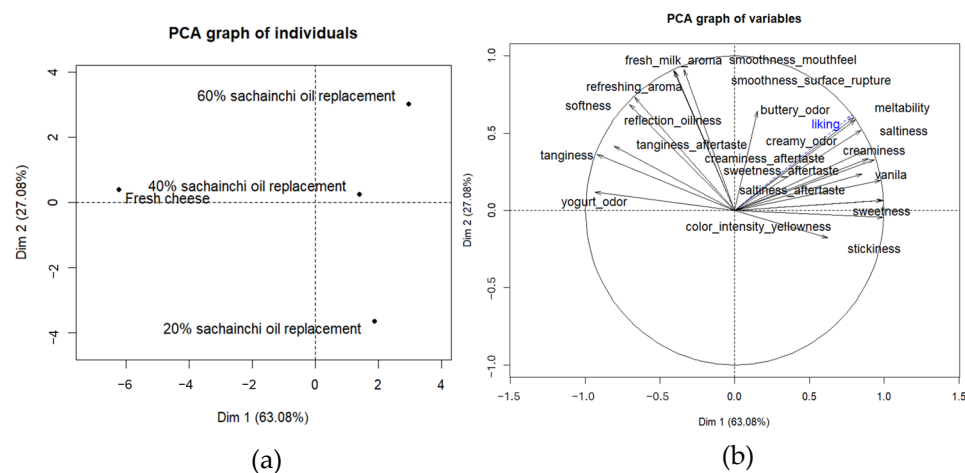


Figure 5. Principal component analysis (PCA) of descriptive sensory data for hybrid fresh cheese. (a) Score plot of cheese samples. (b) Loading plot of sensory attributes (solid black arrows); overall liking was included as supplementary variable (dashed blue arrow).

While PC1 distinguished the samples based on the presence or absence of sachainchi oil, Principal Component 2 (PC2) captured variation associated with increasing levels of oil substitution. As the concentration of sachainchi oil increased, sensory characteristics such as the smoothness (mouthfeel), fresh milk aroma, and smoothness related to the extent of surface ruptures became more pronounced.

Furthermore, when the overall liking scores were included as a supplementary variable in the PCA, a strong positive correlation was observed with sensory attributes such as a creamy odor and meltability. This suggests that enhancing these sensory properties may improve consumers' acceptance. When mapped in the same product space, the overall liking scores tended to increase with higher levels of sachainchi oil substitution. This trend is further supported by the overall liking scores for the different formulations: the 60% sachainchi oil sample received the highest score (4.9), followed by the 40% (4.5) and 20% (4.1) substitution samples and the fresh cheese sample (3.9).

4. Discussion

Cheese is a well-known nutrient product supplying high-quality protein and great sources of fat and calcium for the daily diet. Cheese also contains relatively high levels of saturated fatty acids and cholesterol, which might be associated with increased blood cholesterol levels, coronary heart disease, and several chronic diseases related to obesity such as cardiovascular disease and type-2 diabetes [19,20]. The replacement of milk fat in fresh cheese processing by sachainchi oil, which contains more than 80% polyunsaturated fatty acids, might result in a hybrid fresh cheese product containing health-benefiting components from an alternative fat source [21]. However, cheese is a complex system, and changes in the fat components of raw milk might lead to a change in the cheese networks and interactions in the cheese matrix [22].

The protein matrix in cheese is the continuous phase in the network, with fat globules as particle fillers. Therefore, the effects of modifying the fat in cheese analogs depend on the sort of oil used, the percentage of fat modification, and the fat globule size [23]. The results

obtained in this study demonstrated that replacement with sachu inchi oil did not change the coagulation properties and yield of the hybrid fresh cheese products. The replacement of milk fat (the main fat component in cheese) seemed not to affect the protein network in the hybrid fresh cheese, with no significant difference in the coagulation time and yield of the products in all the samples (Table 3). In this study, the addition of cocoa butter at 0.2% of the total fat in cheese processing remarkably supported the stability of the hybrid fresh cheese products and reduce the migration of oil to the cheese surface for 7 days of storage. However, an increase in the sachu inchi oil replacement to 80% resulted in a reduction in the fat/protein ratio in the hybrid fresh cheese products (Table 3, Figure 2). This result was due to differences in the fat properties between the milk fat globules and sachu inchi oil drops filling the casein network, leading to the migration of the alternative oil from the protein matrix of the fresh cheese (Figure 3). Previous studies reported the use of vegetable oil, such as canola oil [24] and palm oil [25], to produce low-saturated-fatty-acid and low-cholesterol cheese products. Our current study suggested that hybrid fresh cheese can be successfully produced by replacing up to 80% of the milk fat in raw milk with sachu inchi oil, without significant changes in the coagulation properties or final product yield.

Although milk fat generally accounts for 20–30% of the total composition in cheese products, this natural component contains relatively high amounts of saturated fatty acids and cholesterol [5]. Many researchers have made efforts to obtain health-benefiting alternative cheese products, such as plant-based cheese and partial or full analogs of cheese products, adapting to the demand of modern society for eco-friendly products and improving the diversity of cheese products in the global market. Sachu inchi oil is well-known as a functional ingredient with food, medicinal, and cosmetic applications with various beneficial health properties and a unique taste and flavor [9,26]. Lourith et al. indicated that Thai sachu inchi oil was noted to be high in α -linolenic (51.72%) and linoleic (24.3%) acids and palmitic acid (4.16%) oil. In agreement with this previous study, Vietnamese sachu inchi oil was also shown to be a major source of polyunsaturated fatty acids (Table 2) but was different in terms of its fatty acid components in comparison with Thai sachu inchi oil [26]. Our results implied that Vietnamese sachu inchi oil is rich in linoleic acid (LA, C18:2, *cis* ω -6) ($43.81 \pm 2.50\%$) and oleic acid (OA, C18:1, *cis* ω -9) ($37.9 \pm 0.79\%$) and contains small amounts of linolenic acid (C18:3 ω -3) ($6.26 \pm 0.42\%$), palmitic acids (C16:0) ($6.18 \pm 0.67\%$), and stearic acid (C18:0) ($1.41 \pm 0.25\%$). The results obtained from this study suggested that Vietnamese sachu inchi oil is a promising alternative ingredient to milk fat in cheese processing that can be used to obtain a hybrid fresh cheese rich in polyunsaturated fatty acids.

Although sachu inchi oil has been used as a functional antioxidant ingredient to increase the shelf-life of fresh cheese in addition to its nutritional content [21], its application as an alternative ingredient to milk fat in cheese processing has not yet been reported. In this study, the partial substitution of the milk fat components of raw milk in fresh cheese processing resulted in a reduction in the milk saturated fatty acids (myristic acid (C14:0), palmitic acid (C16:0)) and cholesterol content and an increase in bioactive components from sachu inchi oil such as linoleic acid (LA, C18:2, *cis* ω -6) and oleic acid (OA, C18:1, *cis* ω -9) in the products. These PUFAs may regulate the antioxidant signaling pathway and modulate inflammatory processes [27]. Linoleic acid (LA, C18:2, *cis* ω -6) is an essential fatty acid which cannot be synthesized by humans or other higher animals and plays key roles in regulating body homeostasis and protecting humans against inflammatory diseases, cancer, cardiovascular diseases, and other chronic diseases [28]. Oleic acid (OA, C18:1, *cis* ω -9) can enhance the activity of antioxidants, protect against cardiovascular disease, help to prevent cancer and Alzheimer's disease, and lower cholesterol [29].

A challenge in hybrid cheese production is forming a protein network to achieve the desired structure in the final product [30]. In cheese, fat or oil droplets are emulsified within a continuous protein matrix. When the milk fat was replaced with sachu inchi oil at levels below 80%, only the ratio of the sachu inchi oil to the milk fat was altered, while the total fat and milk protein content in the raw milk remained constant. This led to modifications in the fatty acid composition of the resulting hybrid cheese products, without causing significant changes in the protein/fat ratio, cheese yield, or fat recovery. The linear reduction in myristic and palmitic acids, the most abundant saturated fatty acids in milk, in tandem with increasing milk fat replacement with sachu inchi oil is important evidence for the feasibility of this cheese analog processing method. It could be used as an effective approach to reduce the saturated fats in foods while maintaining other desirable properties of the products. In recent years, with the public's increased awareness of the dangers of cholesterol found in animal fats increasing the risk of chronic non-communicable diseases, alternative cheese products in which the milk fat is replaced with vegetable fat have gained increased popularity. The hybrid fresh cheese products obtained in this study with a high amount of health-benefiting fatty acids from sachu inchi oil will reduce the amount of saturated fat and cholesterol in foods, enhance the value of local agricultural products, and provide nutritional products for the community. The substitution of milk fat with plant oil, which has a lower carbon footprint, makes hybrid cheese production more sustainable in comparison with the production of traditional fresh cheese products.

Most dairy products have well-defined labeling requirements, but other nutritional ingredients can be added to the analogs to improve their health outlook. Nevertheless, developing dairy substitutes that match dairy products' sensory and nutritional attributes remains challenging, and its success affects consumers' acceptance. Scientists and researchers are extensively exploring innovative approaches that enhance plant-based dairy analogs' physical and sensory properties [3]. The observed sensory changes may indicate complex interactions due to the physicochemical properties of sachu inchi oil during cheese-making. For instance, the heightened sensory perceptions of creaminess, sweetness, and meltability in the higher-substitution samples may be attributed to the emollient properties of the oil, which alter the fat distribution and texture of the product. Furthermore, the changes in the aroma profile suggest that the addition of lipid-derived volatiles from sachu inchi oil contributes to a richer and creamier sensory experience. The findings of this study were further supported by principal component analysis, which revealed distinct differences between the oil-substituted cheese samples and the fresh cheese. The strong correlation between consumer preferences and specific sensory characteristics, such as creaminess and meltability, highlights the potential of sachu inchi oil to enhance consumers' acceptability of hybrid fresh cheese. This relationship suggests that consumer preferences may be more strongly influenced by texture and aroma attributes than by traditional milk characteristics, such as acidity or a yogurt-like odor.

Solanke et al. defined a fat replacer as a material that replaces all or some of the fat properties in food and gives it a taste, texture, or mouthfeel identical to that of full-fat food. Since producing analog cheese requires the substitution or addition of various ingredients, the processing methods often need to be altered to achieve similar properties to those of conventional cheese [31]. It has generally been found that such analogs may exhibit an unsatisfactory textural quality. Interestingly, no significant difference was observed in the overall acceptability during the consumer sensory testing. More systematic investigations are needed, both in terms of the investigation of the protein interactions needed to provide the target textures and sensory analysis to ensure the satisfaction of consumer preferences. Nowadays, consumers are more concerned about the health aspects of food, and the replacement of dairy fat with plant fat can be an advantage [32]. Although the stability of

health-benefiting hybrid fresh cheese products needs to be investigated, the results of this study showed great potential for developing hybrid fresh cheese rich in polyunsaturated fatty acids from sachu inchi oil that is satisfactory in terms of its taste and nutrition. In comparison with traditional fresh cheese processing methods, the production of sachu inchi hybrid fresh cheese requires additional centrifugation and homogenization steps, both of which are commonly employed in standard dairy processing facilities.

The PCA loading plot provides insights into how the sensory attributes interrelated. Attributes located close to each other and oriented in the same direction had a strong positive correlation, meaning that increases in one attribute were associated with increases in the other. Notably, consumer liking was closely associated with a creamy odor, meltability, sweetness, creaminess, and a vanilla flavor, suggesting these are key to consumer preferences. Additionally, saltiness, the sweetness of the aftertaste, and the creaminess of the aftertaste had an important role in the overall sensory acceptance. In contrast, attributes such as a yogurt odor, tanginess, and the tanginess of the aftertaste appeared on the opposite side of the plot, showing negative correlations with liking. This suggests that traditional dairy characteristics related to sourness or fermentation may reduce the consumer appeal when sachu inchi oil is incorporated. The positioning of the smoothness (mouthfeel) and fresh milk aroma suggests a potential sensory linkage, as both intensified with higher oil substitution. Overall, these findings indicate that sachu inchi oil not only enhances specific sensory attributes but also alters their interrelationships, shifting the sensory profile toward characteristics that are more positively perceived by consumers.

Although the hybrid fresh cheese was produced at 500 kg material/batch, further research steps are needed to improve the stabilization of sachu inchi oil in the protein network in hybrid fresh cheese products during storage, in order to extend the shelf-life of the products. In addition, hybrid fresh cheese containing sachu inchi oil is abundant in polyunsaturated fatty acids, which can easily oxidize during storage. Thus, the oxidation and quality of the sachu inchi oil will also affect the quality of the final products. While commercial sachu inchi oil was applied in this research, further research should address the oxidation of sachu inchi oil in hybrid fresh cheese processing and storage. Nevertheless, the results of this study for 500 kg material/batch presents promising potential for application at an industrial scale.

5. Conclusions

The findings of this study demonstrate that sachu inchi oil can be used as a fat alternative for fresh cheese production to obtain hybrid fresh cheese rich in PUFAs, without significant changes in coagulation properties such as the coagulation time, curd yield, and cheese yield. The replacement of milk fat by sachu inchi oil did not affect the macronutritional values (fat, protein) and contributed to a richer and creamier sensory experience of the final products. From a nutritional perspective, the replacement of milk fat by sachu inchi oil offered a more balanced lipid profile, significantly lowered the cholesterol content from 8.27 ± 0.53 mg/g in fresh cheese to 2.63 ± 0.63 mg/g at 80% substitution, and enhanced the overall nutritional quality by markedly increasing the PUFAs, such as oleic acid (OA, C18:1, *cis* ω -9) (from $7.88 \pm 0.36\%$ to $23.44 \pm 0.13\%$) and linoleic acid (LA, C18:2, *cis* ω -6) (from $6.44 \pm 0.68\%$ to $41.74 \pm 2.50\%$). This hybrid approach shows strong potential to support public health nutrition by offering consumers a more health-conscious dairy alternative. In future studies, we will investigate a green processing method for the stabilization of hybrid fresh cheese products (preventing oil oxidation, increasing the shelf-life) without oil separation on the surface of the cheese products during storage.

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Abbreviations

The following abbreviations are used in this manuscript:

PUFA Polyunsaturated fatty acid
PCA Principal component analysis

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