

# Physicochemical Properties, Nutritional Composition, Probiotic Viability, and Consumer Acceptance of Yogurt Fortified with Sacha Inchi Flour

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## Abstract

This study investigated the fortification of yogurt with Sacha inchi flour, focusing on its physicochemical properties, nutritional composition, probiotic viability, and consumer acceptance. Yogurt was prepared with varying concentrations of Sacha inchi flour (control, 1, 2.5, and 5% w/v) and stored under refrigeration for 21 d. Fortification significantly increased protein, fat, ash, fiber, and caloric content, while reducing moisture ( $p < 0.05$ ). Viscosity and water-holding capacity were enhanced, whereas syneresis and titratable acidity decreased. Color values ( $L^*$ ,  $a^*$ ,  $b^*$ ) showed a gradual reduction during storage. The viability of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* remained above the therapeutic threshold of  $10^6$  cfu/mL throughout storage, with higher counts observed at increased flour concentrations. Sensory evaluation using a 7-point hedonic scale indicated that yogurt fortified with 1% Sacha inchi flour achieved the highest acceptability, comparable to the control. In contrast, higher concentrations reduced preference due to flavor modifications. Overall, Sacha inchi flour demonstrated potential as a functional ingredient that enhances the nutritional profile of yogurt, supports probiotic viability, and maintains consumer acceptability at lower inclusion levels. These findings highlight its application in the development of functional dairy products with added health benefits.

**Keywords:** Sacha inchi flour, Functional yogurt, Probiotic viability, Nutritional composition

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## Introduction

Probiotic yogurt is a dairy product produced through the acidification and fermentation of milk by viable bacteria, typically *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. This process yields a thicker product with an extended shelf life (Bankole et al., 2023). Probiotic yogurt is considered nutrient-dense, providing essential fatty acids and proteins of major biological significance. It is also rich in protein, vitamins, fatty acids, and minerals, particularly calcium and phosphorus (Azemi et al., 2021).

Yogurt quality is a multifaceted concept influenced by several factors, including the quality of raw materials, fermentation conditions, temperature, and storage. Its assessment requires a combination of methods, focusing on physical, chemical, and sensory attributes. Key physical parameters such as rheology, firmness, and syneresis are essential for evaluating the gel network and are critical to consumer acceptance (Lucey, 2004). Chemical analyses, including pH and titratable acidity, are used to monitor fermentation and product stability. Furthermore, the viability of the lactic acid bacteria (LAB) is a crucial metric, particularly for functional yogurts, as it ensures probiotic benefits are maintained throughout shelf life (Hekmat & McMahon, 1992). The incorporation of novel ingredients such as Sacha inchi directly influences these quality parameters by affecting texture and chemical stability (Villamil et al., 2023), necessitating comprehensive evaluation to validate its use. The utilization of plant extracts containing nutraceutical components has recently emerged as a growing approach for dairy product fortification (Ghasempour et al., 2020). Sacha inchi (*Plukenetia*

*volubilis* L.), also known as Sacha peanut, mountain peanut or Inca peanut is an oleaginous plant of the Euphorbiaceae family (Zakaria et al., 2022). It has gained recognition as a food source due to its high nutritional value and potential health benefits. In Malaysia, the Rubber Industry Smallholders Development Authority (RISDA) has promoted sacha inchi cultivation to increase farmers' income, given its wide range of application (Zakaria et al., 2023). These seeds are high in polyunsaturated fatty acids, particularly omega-3, as well as protein, fiber, antioxidants, and compounds with anti-tumor potential (Cárdenas et al., 2021). When processed into flour, sacha inchi provides a sustainable, plant-based source of nutrients that can enhance food formulations. Its fiber content may exert prebiotic effects by supporting probiotic growth and viability, while its protein fraction contributes to improved texture and nutritional quality (Abdel-Hamid et al., 2024). Incorporating Sacha inchi flour into yogurt therefore holds promise for improving physicochemical properties and offering added health benefits, including gut health promotion and enhanced nutrient intake. These attributes make Sacha inchi flour an attractive fortification material for functional dairy products aligned with consumer demand for health-oriented foods. Sacha inchi is increasingly recognized as a superfood suitable for diverse food products and supplements, particularly as a potential prebiotic supporting probiotic growth due to its high fiber and protein content. Its seeds contain abundant protein content (25–30%), including essential amino acids like cysteine, tyrosine, threonine, and tryptophan (Zakaria et al., 2023).

Several studies have investigated the incorporation of Sacha inchi into yogurt in different forms to enhance its nutritional and functional properties. For instance, yogurt fortified with sacha inchi seeds combined with  $\beta$ -glucans demonstrated higher protein, fat, essential fatty acids, and total solids, while maintaining acceptable sensory attributes (Vanegas-Azuero & Gutiérrez, 2018). Similarly, partial replacement of milk with Sacha inchi oil (1.5–3.5%) produced yogurts with improved fatty acid profiles, higher viscosity, and favorable consumer acceptance (Villamil et al., 2023). More recently, clinical research reported that daily consumption of yogurts enriched with sacha inchi oil positively influenced lipid profiles in healthy adults, confirming its functional potential (Villamil et al., 2024). Sacha inchi seeds have also been incorporated in candied form, with studies demonstrating good acceptability and nutritional contributions, including dietary fiber and phenolic compounds. These findings suggest that Sacha inchi can be used in yogurt production in multiple forms—seeds, oil, and processed derivatives—each contributing distinct nutritional and functional benefits.

Current research on Sacha inchi seeds has primarily focused on the antibacterial, anti-inflammatory, skin-tightening, and anti-aging properties of the seed oil. This emphasis reflects the oil's abundance in lipids, characterized by an exceptionally high proportion of unsaturated fatty acids (85.9–97.5%) (Rashid et al., 2024). However, limited studies have examined the protein and fiber composition of Sacha inchi seeds particularly on its potential as a prebiotic for probiotic bacteria. Maintaining the minimum therapeutic level of probiotics in yogurt during processing and storage remains a challenge due declining survival rate. Contributing factors include the matrix acidity, oxygen levels, the presence of other lactic acid bacteria, and sensitivity to metabolites produced by competing microorganisms (Terpou et al., 2019). Incorporating Sacha inchi flour into yogurt formulations may present challenges due to its high fiber content, which can affect sensory properties. Although Sacha inchi flour enhances nutritional value, its impact on texture and flavor may not align with conventional yogurt. Addressing these concerns is essential to ensure effective incorporation, improve palatability, and enhance market appeal.

The objective of this study was to evaluate the effect of incorporating Sacha inchi flour on the physicochemical properties, probiotic viability, and consumer acceptance of yogurt. The findings demonstrated that Sacha inchi flour effectively enhances the nutritional profile, supports probiotic viability, and maintains high consumer acceptability at a 1% concentration.

## **Materials and methods**

### **1. Preparation of Sacha inchi flour**

Sacha inchi seeds were obtained from MP Bakti Resources plantation in Bachok, Kelantan. The Sacha inchi flour was prepared according to the method described by Vanegas-Azuero and Gutiérrez (2018). Mature seeds were selected, subjected to shell removal and washed with tap water to eliminate foreign materials. The seeds were then dried at 25°C for 1 h to remove residual moisture. Subsequently, the seeds were roasted at 140°C for 20 min using a laboratory oven (QD-09 D, Luxury Electric Deck Oven, China). This roasting condition has been reported to optimize amino acid content, enhance antioxidant activity, and preserve oxidative stability

in Sacha inchi seeds (Morales & Garcia, 2023). The roasted Sacha inchi seeds were milled and sieved through a 60-mesh sieve (approximately 250  $\mu\text{m}$ ) to obtain fine flour suitable for incorporation into yogurt (Dhawi et al. 2020).

## 2. Enumeration of initial probiotic population in commercial yogurt

The screening and enumeration of the initial probiotic population in commercial yogurt involved 3 different product brands from the local market: Brand A, Brand B, and Brand C. Enumeration was performed using the spread plate technique, following the procedure described by Glušac et al. (2015) with modifications. Each yogurt sample (1 mL) was homogenized with 9.0 mL of sterile physiological saline (0.85% NaCl w/v) to create a stock solution. From this, serial dilutions were prepared up to  $10^{-6}$ . A 1.0 mL aliquot of each dilution was spread-plated onto MRS agar adjusted to pH 5.4 for presumptive enumeration of *Lactobacillus delbrueckii* subsp. *bulgaricus* and pH 6.3 for presumptive enumeration of *Streptococcus thermophilus*. The plates were incubated anaerobically at 37°C for 48 h for *L. bulgaricus* and aerobically at 37°C for 48 h for *S. thermophilus*. Although this method does not allow for absolute species identification, it served as an initial screening approach to estimate the viable counts of yogurt starter cultures. The experiments were conducted in triplicate, and colony counts were expressed as log colony-forming units per milliliter (log cfu/mL) of the yogurt sample. Plates containing 15–300 colonies were enumerated and results are presented as mean  $\pm$  standard deviation. A minimum of  $10^6$  viable probiotic bacteria per mL was considered necessary to confer health benefits (Tripathi & Giri, 2014). The results were calculated using the following equation:

$$\text{Log} \left( \frac{\text{cfu}}{\text{mL}} \right) = \log \left( \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume of culture plate}} \right)$$

## 3. Preparation of yogurt fortified with Sacha inchi flour

Yogurt fortified with Sacha inchi flour was prepared using a modified method described by Jovanović et al. (2020). The back-slopping approach was applied, whereby a portion of commercial yogurt was used as inoculum to transfer live probiotic cultures into fresh milk and initiate fermentation. The milk was heated at 85°C for 10 min and subsequently cooled to the inoculation temperature of 43°C. A 5% (v/v) portion of yogurt containing starter cultures was added to the milk, which was then transferred into containers. Sacha inchi flour was incorporated at 3 concentrations: 1.0% (F1), 2.5% (F2), and 5.0% (F3). The inoculated mixtures were homogenized, thoroughly mixed, and incubated at 43°C until the pH reached 4.6 (approximately 2.5 h). Following fermentation, the yogurt samples were stirred and stabilized by cooling at 4°C for 24 h. Two control samples were prepared: yogurt without added sugar (CWTS) and yogurt with added sugar (CWS). These controls were included to assess the effect of sugar on proximate composition and to serve as references for the fortified samples (F1–F3).

## 4. Determination of moisture content

Moisture content was determined using the oven-drying method described in AOAC International (2005), Method 925.23. A crucible was dried in an oven at 105°C for 4 h and subsequently cooled in a desiccator until it reached room temperature. The weight of the empty crucible was recorded as  $W_1$ . Approximately 5 g of yogurt sample was added to the crucible and weighed as  $W_2$ . The crucible containing the sample was dried overnight at 105°C. After drying, it was cooled in a desiccator and weighed promptly upon reaching room temperature ( $W_3$ ). Moisture content (%) was calculated using the following equation:

$$\text{Moisture (\%)} = \left( \frac{W_2 - W_3}{W_2 - W_1} \right) \times 100$$

Where:  $W_1$  = Weight of crucible (g);  $W_2$  = Weight of crucible + wet sample (g);  $W_3$  = Weight of crucible + dried sample (g)

## 5. Determination of ash content

Ash content was determined according to AOAC International (2005), Method 923.03. A crucible was dried in an oven at 105°C for 4 h, cooled in a desiccator to room temperature, and weighed ( $W_1$ ). Approximately 5 g of homogenized sample was placed into the crucible and weighed ( $W_2$ ). The crucible containing the sample was

then incinerated in a muffle furnace at 550°C overnight. After ashing, the crucible was cooled in a desiccator to room temperature and weighed ( $W_3$ ). The percentage of total ash was calculated using the following equation:

$$\text{Ash (\%)} = \left( \frac{W_3 - W_1}{W_2} \right) \times 100$$

Where:  $W_1$  = Weight of crucible (g);  $W_2$  = Weight of sample (g);  $W_3$  = Weight of crucible + ash (g)

## 6. Determination of protein content

Crude protein content was determined by the Kjeldahl method according to AOAC International (2005), Method 991.20. Approximately 1 g of yogurt sample was digested with concentrated sulfuric acid in the presence of a copper catalyst tablet at 400°C until a clear solution was obtained. The digest was then distilled with 32% NaOH, and the released ammonia was collected in a boric acid solution. The distillate was titrated with standardized 0.1 N hydrochloric acid until the endpoint was reached. The percentage of nitrogen (%N) was calculated, and crude protein was obtained by multiplying %N with a conversion factor ( $F = 6.38$  for dairy products). The calculations were performed using the following equations:

$$\%N = \frac{A \times (T - B) \times 14.00 \times 100}{\text{Weight of sample (g)} \times 1000}$$

$$\% \text{Crude Protein} = \%N \times F$$

Where:  $T$  = Volume of acid for sample (mL);  $B$  = Volume of acid for blank (mL);  $A$  = Normality of HCL;  $F$  = Protein conversion factor (6.38 for dairy products)

## 7. Determination of fat content

Fat content was determined by Soxhlet extraction according to AOAC International (2005), Method 991.36. Approximately 3 g of yogurt sample ( $W_1$ ) was weighed, wrapped in filter paper, and placed into the extraction thimble. Extraction was performed using petroleum ether as the solvent. After extraction, the solvent was evaporated, and the extraction cup was dried in an oven at 105°C, cooled in a desiccator, and weighed ( $W_3$ ). The percentage of fat was calculated using the following equation:

$$\% \text{Fat} = \left( \frac{W_3 - W_2}{W_1} \right) \times 100$$

Where:  $W_1$  = Weight of sample (g);  $W_2$  = Weight of extraction cup (g);  $W_3$  = Weight of extraction cup + fat (g)

## 8. Determination of crude fiber content

Crude fiber content was determined according to AOAC International (2005), Method 962.09 using a Fibertherm fiber analyzer. Approximately 1 g of yogurt sample ( $W_2$ ) was weighed into a fiber bag, previously dried and weighed ( $W_1$ ). The sample underwent sequential digestion with 0.15 M sulfuric acid and 0.15 M sodium hydroxide, followed by rinsing with distilled water. The residue was dried at 105°C, cooled in a desiccator, and weighed. The fiber bag containing the sample was then incinerated in a muffle furnace at 550°C for 4 h, cooled, and reweighed. Crude fiber was expressed as the loss in weight of the residue on ashing and calculated using the following equation:

$$\% \text{ Crude Fiber} = \left[ \frac{(W_3 - W_1) - (W_4 - W_5)}{W_2} \right] \times 100$$

Blank value,  $W_5 = W_7 - W_6$

Where:  $W_1$  = Weight of fiber bag (g);  $W_2$  = Weight of sample (g);  $W_3$  = Weight of crucible and fiber bag after digestion (g);  $W_4$  = Weight of crucible and ash (g);  $W_5$  = Weight of blank value of the empty fiber bag (g);  $W_6$  = Weight of crucible (g);  $W_7$  = Weight of crucible and ash of the empty fiber bag (g)

### 9. Analysis of pH value

The pH of the yogurt sample was determined using a benchtop pH meter (Accumet AE150, Fisher Scientific, USA). This instrument incorporates a specialized bulb sensitive to hydrogen ion concentration in the test solution. For each formulation, 2 g of yogurt was transferred into a beaker and mixed with 4 mL of distilled water. The electrode probe was then immersed into the sample and stirred briefly. To ensure accuracy, the pH meter was calibrated prior to use with buffer solutions at pH 4.0, 7.0, and 10.0. Triplicate readings were taken for each sample to enhance measurement precision (Hashim et al., 2009).

### 10. Analysis of titratable acidity

Titratable acidity of yogurt (expressed as lactic acid) was determined using the titration method with 0.1 M sodium hydroxide (NaOH) and phenolphthalein as an indicator, following the procedure described by Mabrouk and Effat (2020). Approximately 10 g of yogurt sample was weighed into a conical flask. Subsequently, 30 mL of distilled water was added, and the mixture was thoroughly shaken. Five drops of phenolphthalein indicator were introduced, and titration was performed using 0.1 M NaOH from a burette until a faint pink tinge appeared, indicating the endpoint. The volume of NaOH used was recorded, and the titration process was repeated 3 times for each sample. Results were expressed as a percentage of lactic acid and calculated using the following equation:

$$\text{Acidity (\%)} = \frac{(\text{Volume of NaOH used}) \times (0.1 \text{ M NaOH}) \times (\text{Milliequivalent factor})}{\text{Weight of sample (g)}} \times 100$$

### 11. Analysis of viscosity

Viscosity of yogurt sample was analyzed using the rotational viscometer (Visco QC100, Aton Parr, Austria) at 20°C, with a shear rate of 34 rpm and spindle No. 4. Triplicate measurements were performed for each sample (Swelam, 2018). Prior to measurement, the instrument was calibrated, and a homogenized sample free of air bubbles was prepared. The yogurt was poured into the sample container, ensuring that the spindle was fully submerged. The spindle was then inserted, the measurement initiated, and viscosity values were recorded once stabilization was achieved. The obtained results were displayed on the monitor in graphical form.

### 12. Analysis of water holding capacity (WHC)

The water holding capacity (WHC) of yogurt samples was determined according to the method described by Ranadheera et al. (2012). WHC was measured using the centrifugation method with a benchtop centrifuge (Centrifuge 5810 R, Eppendorf, Germany). For each sample, 5 g of yogurt was weighed into test tubes. The tubes were centrifuged at 4500 rpm for 30 min at 10°C. Centrifugation separated the liquid whey from the solid components of yogurt: the denser solids migrated to the bottom of the tube, while the liquid whey was displaced to the top. WHC was calculated using the following equation:

$$\text{WHC (\%)} = \left(1 - \frac{W_1}{W_2}\right) \times 100$$

Where:  $W_1$  = Weight of whey after centrifugation (g);  $W_2$  = Weight of yogurt sample (g)

### 13. Analysis of susceptibility to syneresis (STS)

The susceptibility to syneresis (STS) of yogurt samples was determined according to the method described by Isanga and Zhang (2009). Approximately 50 mL of yogurt was weighed and poured onto a filter paper positioned atop a funnel to separate the whey from the solid fraction. Following a 6-h drainage period, the volume of whey collected in a beaker was measured and used as an indicator of syneresis.

STS was calculated using the following equation:

$$\text{STS (\%)} = \left(\frac{V_1}{V_2}\right) \times 100$$

Where:  $V_1$  = Volume of whey collected after drainage (mL);  $V_2$  = Volume of yogurt sample (mL)

#### 14. Analysis of color

The color of the yogurt samples was determined using the CIE Lab\* system with a Konica Minolta Chromameter (CR-300, Konica Minolta, Japan), following the method of Szajnar et al. (2017) with minor modifications. Prior to analysis, the instrument was calibrated against a standard white ceramic tile. Measurements were conducted under a D65 standard illuminant and a 2° standard observer angle, as recommended for food color evaluation. Colour values were expressed as:  $L^*$  (lightness; 0 = black, 100 = white),  $a^*$  (positive values = redness, negative values = greenness), and  $b^*$  (positive values = yellowness, negative values = blueness). Measurements were performed in triplicate for each sample, and mean values were reported.

#### 15. Determination of probiotic bacteria viability in three weeks refrigerated storage

Probiotic viability was assessed using a modified spread plate technique adapted from Mabrouk and Effat (2020). Yogurt samples (1 mL) were homogenized in 9 mL of sterile physiological saline (0.85% NaCl, w/v) and serially diluted up to  $10^{-6}$ . Aliquots (0.1 mL) of appropriate dilutions were spread in duplicate on MRS agar plates adjusted to pH 5.4 (for presumptive enumeration of *Lactobacillus*-like colonies) and on MRS agar plates adjusted to pH 6.3 (for presumptive enumeration of *Streptococcus*-like colonies). Plates were incubated anaerobically at 37°C for 48 h (pH 5.4) and aerobically at 37°C for 48 h (pH 6.3). Colony counts between 15 and 300 were recorded and expressed as log cfu/mL of yogurt. These represent presumptive counts, as MRS with pH modification does not provide definitive species-level identification. More robust identification of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* typically requires selective media (e.g., M17 agar for *S. thermophilus*) or molecular techniques (Dave & Shah, 1996; Tharmaraj & Shah, 2003). The results were expressed as log colony-forming units (cfu) per milliliter (log cfu/mL) and calculated using the following equation:

$$\text{Log} \left( \frac{\text{cfu}}{\text{mL}} \right) = \log \left( \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume of culture plate (mL)}} \right)$$

#### 16. Sensory evaluation

The sensory evaluation was conducted following approval by the Human Research Ethics Committee of the Universiti Sultan Zainal Abidin (UHREC Code: UniSZA/UHREC/2024/651). A hedonic test was employed to assess sensory acceptance and the sensory characteristics of the yogurt samples. The tasting panel consisted of 50 untrained participants, including students, staff, and lecturers aged 19–50 years from Universiti Sultan Zainal Abidin, Kampus Besut, Terengganu. Every panelist received 5 yogurt samples representing the different formulations and was asked to taste, assess, and comment on sensory attributes during each serving. Samples were coded with three-digit random numbers and presented in randomized order to minimize bias. Plain water was provided between evaluations of samples for mouth rinsing. The attributes evaluated were color, aroma, texture, taste, and overall acceptability, using a 7-point hedonic scale (Jenie et al., 2013). The scale and descriptive attributes for 7-point scale hedonic scale are presented in Table 1.

**Table 1** Scale and description of attribute for 7-point hedonic test

Score	Description
1	Dislike extremely
2	Dislike moderately
3	Dislike slightly
4	Neither like nor dislike
5	Like slightly
6	Like moderately
7	Like extremely

## 17. Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 29.0 (IBM Corp., USA). The effects of different Sacha inchi flour concentration (0%, 1.0%, 2.5%, and 5.0%) and storage time (0, 7, 14, and 21 d) were analyzed using the General Linear Model (GLM) and multivariate analysis. Proximate analysis and sensory evaluation data were assessed using one-way analysis of variance (ANOVA). Duncan's multiple range test was applied to compare sample means, with statistical significance set at  $p < 0.05$ .

## Results and discussion

### 1. Screening from commercial yogurt

According to FAO/WHO standards, probiotic products must contain at least  $10^6$ – $10^7$  cfu/mL of viable microorganisms throughout their shelf life to be classified as probiotic. Similarly, in Malaysia, the Food Safety and Quality Division (FSQD) requires that added probiotic cultures remain viable, with counts of no less than  $10^6$  cfu/mL or cfu/g during the product's shelf life (Siong et al., 2021). The initial screening of probiotic populations in three commercial yogurt brands is presented in Table 2. All three brands exhibited initial population counts exceeding the minimum requirement for probiotic bacteria as established by the FAO/WHO and FSQD. Among the commercial brands, yogurt from Brand B had the highest counts of both *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, whereas Brand C showed the lowest counts. Based on these results, yogurt from Brand B was selected as the starter culture for the fortified yogurt preparation.

**Table 2** Enumeration and viability of probiotic bacteria in commercial yogurt (N = 2)

Yogurt Brand	<i>S. thermophilus</i> (log cfu/mL)	<i>L. delbrueckii</i> subsp. <i>bulgaricus</i> (log cfu/mL)
Brand A	8.32±0.03	7.70±0.03
Brand B	8.39±0.03	7.96±0.09
Brand C	7.21±0.04	7.49±0.06

**Remark:** Values are expressed as mean ± standard deviation (N = 2).

### 2. Physicochemical analysis of yogurt fortified with Sacha inchi flour

Table 3 presents the proximate composition of yogurts fortified with Sacha inchi flour (F1, F2, F3) compared to the control samples (CWTS, CWS). The moisture content of the yogurt samples was 89.39%, 81.93%, 80.12%, 77.62%, and 76.92% for CWTS, CWS, F1, F2, and F3, respectively. Moisture content was highest in CWTS, while F3 exhibited the lowest value. A progressive decrease in moisture content was observed with increasing amounts of Sacha inchi flour. This reduction in moisture is likely attributable to the water absorption properties of the Sacha inchi flour. The higher moisture content observed in CWTS compared to CWS and fortified samples may be explained by the absence of sugar. Sugars possess hygroscopic properties that bind free water, thereby reducing the measurable moisture in the yogurt matrix. In contrast, CWTS (yogurt without sugar) retained a greater proportion of free water, resulting in a higher measured moisture percentage. The progressive reduction in moisture content in fortified samples (F1–F3) is consistent with the addition of Sacha inchi flour, which contributes dry matter and enhances water-binding interactions, thereby lowering the free moisture available in the yogurt. Moisture content plays a critical role in determining yogurt texture and mouthfeel. According to Matela et al. (2019), moisture content in yogurt should be less than 84%, as higher levels can negatively affect texture, palatability, and mouthfeel.

**Table 3** Proximate composition of set yogurt fortified with Sacha inchi flour

Composition (%)	CWTS	CWS	F1	F2	F3
Moisture	89.39±0.09 <sup>a</sup>	81.93±0.81 <sup>b</sup>	80.12±0.40 <sup>c</sup>	77.62±0.23 <sup>d</sup>	76.92±0.11 <sup>d</sup>
Ash	0.58±0.38 <sup>c</sup>	0.75±0.11 <sup>bc</sup>	1.38±0.33 <sup>ab</sup>	1.54±0.23 <sup>a</sup>	1.97±0.01 <sup>a</sup>
Protein	4.17±0.98 <sup>c</sup>	4.33±0.13 <sup>c</sup>	8.08±0.07 <sup>b</sup>	10.06±0.20 <sup>a</sup>	10.53±0.25 <sup>a</sup>
Fat	2.13±0.13 <sup>c</sup>	2.94±0.37 <sup>d</sup>	5.43±0.25 <sup>c</sup>	6.15±0.41 <sup>b</sup>	6.76±0.11 <sup>a</sup>
Fiber	0.09±0.06 <sup>d</sup>	0.05±0.01 <sup>d</sup>	0.70±0.15 <sup>c</sup>	1.73±0.13 <sup>b</sup>	3.00±0.08 <sup>a</sup>
Carbohydrate	3.73±0.56 <sup>b</sup>	10.05±1.19 <sup>a</sup>	5.00±0.04 <sup>b</sup>	4.63±0.31 <sup>b</sup>	3.82±0.25 <sup>b</sup>
Calorie (kcal/100)	50.43±0.75 <sup>d</sup>	83.78±0.99 <sup>c</sup>	98.55±2.40 <sup>b</sup>	107.23±0.06 <sup>a</sup>	106.26±0.71 <sup>a</sup>

**Remark:** Values are expressed as mean ± standard deviation of duplicate determinations. Superscripts (<sup>a-c</sup>) within the same row indicate significant difference ( $p < 0.05$ ). CWTS = control without sugar; CWS = control with sugar; F1 = yogurt containing 1% Sacha inchi flour, F2 = yogurt containing 2.5% Sacha inchi flour, F3: yogurt containing 5% Sacha inchi flour.

The ash content of the samples ranged from 0.58% in CWTS to 1.97% in F3. A significant difference ( $p < 0.05$ ) was observed between the control sample (CWTS) and the fortified samples (F1, F2, F3). Ash content increased proportionally with the concentration of sacha inchi flour, likely due to its relatively high mineral content. Sacha inchi contains 2.70–6.46 g/100 g of ash, composed of minerals such as calcium, phosphorus, sodium, potassium, magnesium, copper, iron, manganese, and zinc (Yanti et al., 2022).

Protein content ranged from 4.17% in CWTS to 10.53% in F3. A progressive increase was observed with higher proportions of Sacha inchi flour, reflecting its high protein content. Raw Sacha inchi seeds contain approximately 22–30% protein, with major soluble fractions including albumin, glutelin, globulin, and prolamin proteins. The seeds also provide essential amino acids such as leucine, tyrosine, isoleucine, lysine, and tryptophan (approximately 64, 55, 50, 43, and 43 mg/g of protein, respectively), and are notable for higher levels of sulfur-containing amino acids compared to other oilseed crops (Rodzi & Lee, 2022). According to Codex Alimentarius standards, yogurt should contain no less than 2.70% protein (Matela et al., 2019). In this study, all samples exceeded this requirement, particularly the fortified yogurts.

Fat content ranged from 2.13% in CWTS to 6.76% in F3. Significant differences were observed between the control samples (CWTS, CWS) and the fortified samples (F1, F2, F3), indicating that incorporation of Sacha inchi flour increased fat levels. A slight difference was noted between CWTS (yogurt without sugar) and CWS (yogurt with sugar), which may be attributed to sugar's effect on solids composition, altering proportional fat calculations on a wet-weight basis. Nevertheless, both controls remained within the typical range reported for set yogurt. Sacha inchi flour was the primary contributor to elevated lipid content in fortified formulations. Sacha inchi seeds contain 33–54% lipids, consisting of approximately 77.5–84.4% polyunsaturated fatty acids (PUFAs), 8.4–13.2% monounsaturated fatty acids (MUFAs), and 6.8–9.1% saturated fatty acids (SFAs) (Wang et al., 2018; Rodzi & Lee, 2022). Fat plays a vital role in yogurt quality, improving texture, appearance, flavor, and taste (Matela et al., 2019). According to the Codex Alimentarius, yogurt should contain less than 15% fat. All 5 yogurt formulations complied with this standard.

There were no significant differences in the fiber content between the control samples (CWTS and CWS). However, the fiber content increased markedly with the incorporation of sacha inchi flour, confirming that sacha inchi flour is a significant source of dietary fiber. Fiber content ranged from 0.05% in CWS to 3.00% in F3. The total dietary fiber in Sacha inchi seeds is estimated at 72.4% insoluble dietary fiber, while roasted sacha inchi contains approximately 23.88% fiber, which is higher than that in fresh sacha inchi (13.03%) and raw seeds (18%) (Sethuraman et al., 2020). Fiber contributes positively to yogurt quality by improving textural properties, enhancing water-holding capacity, reducing lipid retention, and lowering caloric content through its bulking effect (Hashim et al, 2009).

Carbohydrate content was significantly higher in CWS compared to CWTS and the fortified samples. The carbohydrate contents of the yogurt samples were 3.73%, 10.05%, 5.00%, 4.63%, and 3.82% for CWTS, CWS, F1, F2, and F3, respectively. The elevated carbohydrate content in CWS (yogurt with sugar) was expected due to the direct contribution of added sucrose. CWTS (yogurt without sugar) contained only the natural lactose present in milk. Sucrose, a disaccharide composed of glucose and fructose, contributed directly to the carbohydrate content in CWS.

The addition of sacha inchi flour did not significantly increase the carbohydrate content. Instead, the carbohydrate levels decreased with increasing flour fortification (F1–F3). This trend reflects the nutrient composition of sacha inchi flour, which is rich in protein, fat, and dietary fiber but relatively low in digestible carbohydrates (D'Andrea et al., 2023). As more flour was incorporated, the relative proportion of carbohydrate decreased on a wet-weight basis. Furthermore, the carbohydrate fraction naturally present in milk, primarily lactose, was reduced due to partial substitution with Sacha inchi flour. Similar reductions in carbohydrate content have been reported in yogurts fortified with plant-based flours or protein-rich ingredients (Güler-Akın et al., 2007; D'Andrea et al., 2023). Previous studies reported carbohydrate content in roasted Sacha inchi was 21.36% (Sethuraman et al., 2020). Carbohydrates, specifically lactose, are the major constituents of milk and are converted to lactic acid during fermentation, accounting for the observed decrease in carbohydrate content (Amove et al., 2019).

Table 3 also shows that yogurts fortified with sacha inchi flour had significantly higher calorific values compared to the control samples ( $p < 0.05$ ), primarily due to their higher fat content. Fat is well established as the most energy-dense macronutrient, storing more energy than carbohydrates and proteins (Vanegas-Azuero & Gutiérrez, 2018).

### 3. pH value

Table 4 presents the pH values of control samples and fortified yogurt samples (CWTS, CWS, F1, F2, F3) measured at different storage times (0, 7, 14, 21 d). A general trend of decreasing pH was observed across all samples during, indicating increasing acidity. For each yogurt sample, pH values changed significantly over time. For example, in CWTS, the pH decreased from 4.58 at day 0, to 4.28 at day 7, 3.93 at day 14, and 3.83 at day 21. A similar decreasing trend was observed in F3. F1 and F2 exhibited significant reductions in pH until day 14, but no significant differences were detected between days 14 and 21, suggesting stabilization of acidity at later stages of storage. Significant differences in pH were also observed between the samples at the same storage time. At day 0, CWTS and F3 had the highest initial pH values (4.58), which were significantly higher than those of other samples, while CWS had the lowest initial pH value (4.49). By day 21, CWS exhibited the lowest pH value (3.71), whereas F3 maintained the highest pH (4.08). The primary factor contributing to acidity development in yogurt is the fermentation of milk by lactic acid bacteria, specifically *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*. During fermentation, these probiotic bacteria convert lactose into lactic acid, thereby lowering pH and increasing acidity in the yogurt mix (Sidhu et al., 2020).

In the present study, the pH of all yogurt samples decreased significantly during 21 d of storage, reflecting the continued metabolic activity of lactic acid bacteria (LAB). However, yogurts fortified with Sacha inchi flour (F1–F3) exhibited slightly higher final pH values (3.91–4.08) compared to the sugar control (CWS: 3.71). This suggests that Sacha inchi flour may have provided buffering compounds, such as proteins and minerals, which slowed acidification during storage. Similar trends have been reported in fiber-enriched yogurts. For instance, Fernández-García et al. (1998) observed that the addition of date fiber increased yogurt pH compared to the control. Conversely, Shori (2020) reported a decrease in pH with increasing concentrations of apple pomace flour. In some cases, dietary fibers such as inulin have shown little or no effect on pH (Sendra et al., 2010). These findings indicate the impact of prebiotic fortification on yogurt pH depends largely on the chemical composition of the added ingredient. Thus, the effect of Sacha inchi flour appears comparable to other fiber-based fortifiers, contributing to a more stable pH during storage. The higher pH values observed in yogurts fortified with Sacha inchi flour may also be attributed to the rise in protein content. Increased protein enhancing buffering capacity, enabling the yogurt matrix to resist changes in pH more effectively. Consequently, the starter cultures must produce more acid to reach the same pH level (Marand et al., 2020). Previous studies have similarly reported that protein fortification raises yogurt pH, such as whey proteins (Akalin et al., 2012) and *Spirulina platensis* (Barkallah et al., 2017) which aligns with the findings of the present study.

### 4. Titratable acidity

The titratable acidity of each yogurt sample, as shown in Table 4, was inversely correlated with pH values. All samples exhibited a significant increase in titratable acidity over the storage period, indicating progressive acidification. Similar to the pH trend, the continuous increase in titratable acidity across all yogurt treatments extended storage time can be attributed to the high metabolic activity of the starter cultures and the conversion of residual lactose into lactic acid (Mabrouk & Effat, 2020).

**Table 4** pH and titratable acidity values of yogurt samples over storage time

Analysis	Sample	Storage (Day)			
		0	7	14	21
pH value	CWTS	4.58±0.00 <sup>aA</sup>	4.28±0.01 <sup>bB</sup>	3.93±0.02 <sup>cC</sup>	3.83±0.00 <sup>cD</sup>
	CWS	4.49±0.00 <sup>dA</sup>	4.25±0.05 <sup>bB</sup>	3.82±0.01 <sup>dC</sup>	3.71±0.04 <sup>dD</sup>
	F1	4.51±0.01 <sup>cA</sup>	4.33±0.03 <sup>bB</sup>	3.93±0.01 <sup>bC</sup>	3.91±0.01 <sup>bC</sup>
	F2	4.54±0.00 <sup>bA</sup>	4.32±0.04 <sup>bB</sup>	3.96±0.00 <sup>bC</sup>	3.95±0.00 <sup>bC</sup>
	F3	4.58±0.00 <sup>aA</sup>	4.44±0.04 <sup>aB</sup>	4.23±0.01 <sup>aC</sup>	4.08±0.01 <sup>aD</sup>
Titratable acidity	CWTS	1.78±0.03 <sup>cC</sup>	2.19±0.04 <sup>cB</sup>	2.29±0.05 <sup>bAB</sup>	2.39±0.04 <sup>dA</sup>
	CWS	1.83±0.04 <sup>cC</sup>	2.01±0.04 <sup>dB</sup>	2.31±0.08 <sup>bA</sup>	2.25±0.00 <sup>cA</sup>
	F1	2.19±0.06 <sup>bB</sup>	2.25±0.00 <sup>bcB</sup>	2.39±0.00 <sup>bA</sup>	2.45±0.00 <sup>cA</sup>
	F2	2.26±0.06 <sup>abB</sup>	2.32±0.00 <sup>abB</sup>	2.57±0.04 <sup>aA</sup>	2.61±0.03 <sup>bA</sup>
	F3	2.31±0.01 <sup>aC</sup>	2.39±0.04 <sup>aB</sup>	2.67±0.01 <sup>aA</sup>	2.71±0.01 <sup>aA</sup>

**Remark:** Values are expressed as mean ± standard deviation of triplicate determinations. Superscripts (<sup>a-c</sup>) within the same column (sample) indicate significant differences ( $p < 0.05$ ). Superscripts (<sup>A-D</sup>) within the same row (storage time) indicate significant differences ( $p < 0.05$ ). CWTS = control without sugar; CWS = control with sugar; F1 = yogurt containing 1% Sacha inchi flour; F2 = yogurt containing 2.5% Sacha inchi flour; F3 = yogurt containing 5% Sacha inchi flour.

The titratable acidity (TA) of all yogurt samples increased progressively during refrigerated storage, reflecting the continued metabolic activity of lactic acid bacteria. This increase is characteristic of post-acidification, whereby residual lactose is fermented into lactic acid even under refrigeration (Ranadheera et al., 2010). Among the controls, CWS (with sugar) exhibited higher TA values compared to CWTS (without sugar), likely due to the presence of additional fermentable sugars that enhanced bacterial acid production. Interestingly, yogurts fortified with sachu inchi flour (F1–F3) showed slightly higher TA values than the controls throughout the storage period. This may be attributed to the nutritional composition of Sacha inchi flour, particularly its protein and carbohydrate fractions, which can support bacterial metabolism and stimulate acid production. These findings are consistent with previous reports that the incorporation of plant-based ingredients can modulate bacterial activity and influence the acidification profile of yogurt (Jayaweera et al., 2025).

### 5. Viscosity of yogurt

The viscosity of all yogurt samples decreased gradually during storage (Table 5), consistent with the expected structural weakening of the yogurt gel matrix over time. This reduction can be attributed to post-acidification, proteolysis, and rearrangement of casein micelles during storage, which collectively reduce gel firmness (Lucey, 2002). The viscosity of both control samples and the fortified yogurts initially increased, peaking around day 7 (except for F1), before decreasing over time. This initial thickening explained by the continued slow rearrangement of the casein protein network, as residual metabolic activity of the starter cultures strengthens the gel structure during the early stages of cold storage (Guzmán-González et al., 2000). Following this phase, viscosity declined due to post-acidification, whereby continued lactic acid production further lowered pH, leading to gel shrinkage and increased syneresis (Undugoda, & Nilmini, 2019). Additionally, the proteolytic activity of *Lactobacillus delbrueckii* subsp. *bulgaricus* contributed to casein micelle breakdown, directly weakening the gel and reducing firmness.

Among the controls, CWS (yogurt with sugar) consistently exhibited higher viscosity values than CWTS (yogurt without sugar). This difference can be explained by the higher carbohydrate content of CWS (Table 3), as sugars enhance water-binding capacity and contribute to a thicker consistency. Yogurts fortified with Sacha inchi flour (F1–F3) also showed higher viscosities than CWTS throughout storage, with F3 (5% sachu inchi flour) displaying the greatest value at day 0 (22.47 Pa·s). The increase in viscosity with sachu inchi flour addition can be attributed to its fiber and protein components, which enhance water retention and gel strength, thereby reinforcing the 3-dimensional network and increasing resistance to flow (Marand et al., 2020). According to Zannini et al. (2018), fiber and protein compounds can enhance water retention capacity, resulting in viscous gel formation and higher viscosities. Similar findings have been reported in studies

incorporating apple pomace flour (Jovanović et al., 2020), soy flour (Rinaldoni et al., 2012), and oat  $\beta$ -glucan (Amaya-Llano et al., 2008) into yogurt, all of which improved viscosity and texture due to the stabilizing effects of plant-derived polysaccharides and proteins. Although viscosity decreased across all treatments during storage, fortified samples—particularly F2 and F3—retained relatively higher values compared to the controls. This suggests that Sacha inchi flour contributes to stabilizing yogurt structure during refrigerated storage, consistent with other plant-based fortification strategies (Pasephol et al., 2008).

**Table 5** Viscosity of set yogurt fortified with Sacha inchi flour over storage time

Sample	Storage (Day)			
	0	7	14	21
CWTS	13.50±0.37 <sup>cB</sup>	16.77±1.00 <sup>bA</sup>	12.09±0.87 <sup>bB</sup>	11.83±1.75 <sup>bB</sup>
CWS	18.70±0.49 <sup>bA</sup>	19.21±0.29 <sup>abA</sup>	16.14±0.62 <sup>abB</sup>	15.93±1.43 <sup>abB</sup>
F1	15.00±1.75 <sup>cA</sup>	16.68±1.87 <sup>bA</sup>	18.00±3.25 <sup>aA</sup>	12.71±1.75 <sup>abA</sup>
F2	17.47±0.26 <sup>bA</sup>	19.94±4.99 <sup>abA</sup>	17.74±2.87 <sup>aA</sup>	15.62±0.87 <sup>abA</sup>
F3	22.47±0.42 <sup>aA</sup>	23.85±0.13 <sup>aA</sup>	18.18±1.00 <sup>ab</sup>	14.12±1.49 <sup>abC</sup>

**Remark:** Values are expressed as mean  $\pm$  standard deviation of triplicate determinations. Superscripts (<sup>a-c</sup>) within the same column (sample) indicate significant differences ( $p < 0.05$ ). Superscripts (<sup>A-C</sup>) within the same row (storage time) indicate significant difference ( $p < 0.05$ ). CWTS = control without sugar; CWS = control with sugar; F1 = yogurt containing 1% Sacha inchi flour; F2 = yogurt containing 2.5% Sacha inchi flour; F3 = yogurt containing 5% Sacha inchi flour.

## 6. Water holding capacity (WHC)

The relationship between WHC and syneresis is inversely proportional, as yogurts with higher WHC generally exhibit lower syneresis due to the stronger ability of the protein gel matrix to retain water molecules. Conversely, a reduction in WHC during storage leads to increased syneresis, as less water is entrapped within the gel structure, resulting in greater whey separation (Amatayakul et al., 2006; Sodini et al., 2004). WHC is a crucial quality attribute in yogurt, as it influences texture, mouthfeel, and overall consumer acceptance.

As shown in Table 6, all samples exhibited a general decrease in WHC over time, which is expected as syneresis increases during storage. In this study, the control without sugar (CWTS) consistently showed lower WHC and higher syneresis compared to the control with sugar (CWS). The addition of sugar is expected to enhance water-binding interactions within the protein network, thereby improving gel stability and reducing whey expulsion. These findings suggest that sugar plays a functional role in strengthening the yogurt gel structure, leading to improved water retention and decreased syneresis over the storage period. Among the fortified samples, F3 consistently demonstrated the highest WHC, making it the most desirable formulation for maintaining yogurt quality during storage. Conversely, CWTS exhibited the lowest WHC, indicating poor water retention, greater whey separation, and a less desirable texture. F1 and F2 perform moderately, with F2 showing better stability over time compared to F1.

The WHC is closely related to the ability of proteins and dietary fibers to retain water within the yogurt structure. Fat globules in the milk may also contribute to water retention. In this study, yogurts fortified with sacha inchi flour exhibited a significantly higher WHC compared to the control samples (CWTS), likely due to the higher protein and dietary fiber content in the formulation. The increased WHC is attributed to enhanced compactness of the protein network, immobilization of free water, and reduced susceptibility to water release (Marand et al., 2020). Overall, these findings highlight that both sugar and Sacha inchi flour contribute to improving yogurt stability by reducing syneresis through enhanced WHC.

## 7. Susceptibility to syneresis (STS)

Syneresis, an undesirable property in yogurt products, refers to the separation of liquid from the yogurt curds (Wu et al., 2000). It is considered a key indicator of yogurt quality, with higher levels of syneresis generally signifying lower product quality. Consistent with the WHC results, syneresis was higher in the control samples (CWTS, CWS) compared to the fortified yogurts. Moreover, syneresis tended to decrease with increasing concentrations of Sacha inchi flour. This reduction may be attributed to the additional proteins present in the fortified yogurts, which influenced protein–water interactions and intrinsic protein factors such as amino acid composition, protein conformation, and surface polarity or hydrophobicity (Isanga & Zhang, 2009).

Previous studies have also demonstrated that higher fat content in yogurt is associated with lower syneresis values, as fat globules contribute to water retention and gel stability (Keogh & O’Kennedy, 1998). These findings suggest that both protein and fat components play critical roles in reducing syneresis, thereby improving yogurt quality.

**Table 6** Water holding capacity (WHC) and susceptibility to syneresis (STS) of set yogurt fortified with Sacha inchi flour over storage period

Analysis	Sample	Storage (Day)			
		0	7	14	21
WHC	CWTS	60.00±0.00 <sup>cA</sup>	58.00±2.83 <sup>aA</sup>	58.00±2.00 <sup>cA</sup>	52.00±0.00 <sup>cB</sup>
	CWS	72.00±0.00 <sup>abA</sup>	68.00±0.00 <sup>bA</sup>	64.00±0.00 <sup>bB</sup>	60.00±0.00 <sup>bB</sup>
	F1	70.00±2.83 <sup>bA</sup>	66.00±2.83 <sup>bA</sup>	64.00±0.00 <sup>bAB</sup>	59.00±1.41 <sup>bB</sup>
	F2	70.00±2.83 <sup>bA</sup>	69.00±1.41 <sup>bAB</sup>	66.00±2.83 <sup>bAB</sup>	63.00±1.41 <sup>abC</sup>
	F3	76.00±0.00 <sup>aAB</sup>	79.00±1.41 <sup>aA</sup>	73.00±1.41 <sup>aB</sup>	66.00±2.83 <sup>aC</sup>
STS	CWTS	42.40±0.57 <sup>aD</sup>	52.40±0.57 <sup>aC</sup>	57.50±1.56 <sup>aB</sup>	70.80±2.83 <sup>aA</sup>
	CWS	32.30±3.25 <sup>bC</sup>	51.60±0.57 <sup>aB</sup>	55.00±0.00 <sup>abB</sup>	61.30±1.56 <sup>bA</sup>
	F1	36.40±0.00 <sup>bC</sup>	50.00±2.83 <sup>aB</sup>	52.80±0.00 <sup>abAB</sup>	55.50±0.99 <sup>cA</sup>
	F2	34.80±1.70 <sup>bC</sup>	49.40±0.57 <sup>aB</sup>	50.50±0.71 <sup>bcAB</sup>	52.70±0.42 <sup>cdA</sup>
	F3	27.00±1.41 <sup>cC</sup>	35.20±1.13 <sup>bB</sup>	45.50±4.95 <sup>cA</sup>	50.00±0.00 <sup>dA</sup>

**Remark:** Values are expressed as mean ± standard deviation of triplicate determinations. Superscripts (<sup>a-c</sup>) within the same column (sample) indicate significant differences ( $p<0.05$ ). Superscripts (<sup>A-C</sup>) within the same row (storage time) indicate significant difference ( $p<0.05$ ). CWTS = control without sugar; CWS = control with sugar; F1 = yogurt containing 1% Sacha inchi flour; F2 = yogurt containing 2.5% Sacha inchi flour; F3 = yogurt containing 5% Sacha inchi flour.

## 8. Color analysis

It is essential to specify both the illuminant and the standard observer used in color measurements, as these parameters directly influence the accuracy and reproducibility of CIE *Lab*\* values. Providing this information ensures that results can be reliably compared across different studies and experimental conditions (Pathare et al., 2013).



**Fig. 1** The images of yogurt samples. CWTS = control without sugar; CWS = control with sugar; F1 = yogurt containing 1% sachu inchi flour; F2 = yogurt containing 2.5% sachu inchi flour; F3 = yogurt containing 5% sachu inchi flour.

A comparison of the L\*, a\*, and b\* values for color between different yogurt formulations during storage is presented in Table 7. In terms of lightness (L\*), statistical analysis revealed significant differences among yogurt samples and across the storage period. The L\* values decreased as storage time progressed, indicating gradual darkening. Fortification with Sacha inchi flour also contributed to reduced lightness, with higher concentrations producing more pronounced darkening. The whiteness of milk is attributed to fat globules and casein micelles, which scatter white light in the visible spectrum (Sidhu et al., 2020). Therefore, variations in milk composition and Sacha inchi flour fortification may explain the observed differences in L\* values. Similar findings were reported in yogurt fortified with flaxseed powder, where L\* values decreased as powder concentration increased (Marand et al., 2020).

Regarding the a\* (red–green) values, all yogurt samples shifted towards greener hues over storage, with control samples exhibiting the highest proportion of green. Meanwhile, the b\* (yellow–blue) values generally increased over time, indicating a stronger yellow hue as storage progressed. The visually yellowish-brown color of Sacha inchi flour, contributed to the more yellow appearance of fortified yogurts, particularly at higher flour concentrations, as reflected in elevated b\* values. This phenomenon is attributed to the high carotenoid content in Sacha inchi seeds. Carotenoids have the ability to absorb light in the visible spectrum, resulting in yellow, orange, or red hues perceived by the human eye (Amorim et al., 2022).

**Table 7** Color analysis (L\*, a\*, b\*) of set yogurt fortified with Sacha inchi flour over storage time

Analysis	Sample	Storage (Day)			
		0	7	14	21
L*	CWTS	87.57±0.09 <sup>aA</sup>	80.49±0.02 <sup>bB</sup>	76.28±0.59 <sup>aC</sup>	75.01±0.93 <sup>aC</sup>
	CWS	84.38±0.14 <sup>bcA</sup>	85.50±0.45 <sup>aA</sup>	74.45±0.18 <sup>aB</sup>	71.42±3.33 <sup>abB</sup>
	F1	85.96±1.32 <sup>ba</sup>	85.22±0.13 <sup>aA</sup>	75.89±2.28 <sup>aB</sup>	72.78±0.33 <sup>abB</sup>
	F2	85.75±0.24 <sup>ba</sup>	84.45±0.76 <sup>aB</sup>	73.80±0.15 <sup>aC</sup>	72.13±0.30 <sup>abD</sup>
	F3	83.70±0.33 <sup>ca</sup>	79.89±0.24 <sup>bB</sup>	73.99±0.78 <sup>aC</sup>	68.46±1.35 <sup>bD</sup>
a*	CWTS	-2.95±0.03 <sup>cdC</sup>	-1.91±0.00 <sup>aA</sup>	-1.87±0.03 <sup>dA</sup>	-2.29±0.01 <sup>dB</sup>
	CWS	-3.01±0.02 <sup>dC</sup>	-2.75±0.11 <sup>cC</sup>	-1.65±0.00 <sup>cA</sup>	-2.31±0.16 <sup>dB</sup>
	F1	-2.81±0.11 <sup>cC</sup>	-2.85±0.08 <sup>cC</sup>	-1.66±0.12 <sup>cA</sup>	-1.99±0.12 <sup>cB</sup>
	F2	-2.48±0.00 <sup>bD</sup>	-2.23±0.06 <sup>bC</sup>	-1.33±0.50 <sup>bB</sup>	-1.16±0.07 <sup>ba</sup>
	F3	-1.75±0.08 <sup>aC</sup>	-1.98±0.00 <sup>aD</sup>	-1.03±0.08 <sup>aB</sup>	-0.50±0.08 <sup>aA</sup>
b*	CWTS	6.33±0.35 <sup>ba</sup>	2.40±0.02 <sup>dD</sup>	3.97±0.08 <sup>dC</sup>	5.68±0.04 <sup>bcB</sup>
	CWS	6.24±0.13 <sup>ba</sup>	5.34±0.45 <sup>bB</sup>	3.46±0.01 <sup>dC</sup>	4.99±0.02 <sup>cB</sup>
	F1	6.68±0.95 <sup>baB</sup>	7.64±0.06 <sup>aA</sup>	5.28±0.43 <sup>cB</sup>	6.23±0.37 <sup>abAB</sup>
	F2	6.76±0.64 <sup>ba</sup>	7.40±0.32 <sup>aA</sup>	6.71±0.18 <sup>bB</sup>	6.48±0.21 <sup>aA</sup>
	F3	8.55±0.06 <sup>aA</sup>	5.97±0.25 <sup>bB</sup>	8.34±0.45 <sup>aA</sup>	6.61±0.47 <sup>aB</sup>

**Remark:** Values are expressed as mean ± standard deviation of triplicate determinations. Superscripts (<sup>a-c</sup>) within the same column (sample) indicate significant differences ( $p < 0.05$ ). Superscripts (<sup>A-C</sup>) within the same row (storage time) indicate significant difference ( $p < 0.05$ ). CWTS = control without sugar; CWS = control with sugar; F1 = yogurt containing 1% Sacha inchi flour; F2 = yogurt containing 2.5% Sacha inchi flour; F3 = yogurt containing 5% Sacha inchi flour.

### 9. Viability of probiotic bacteria over three weeks of refrigerated storage

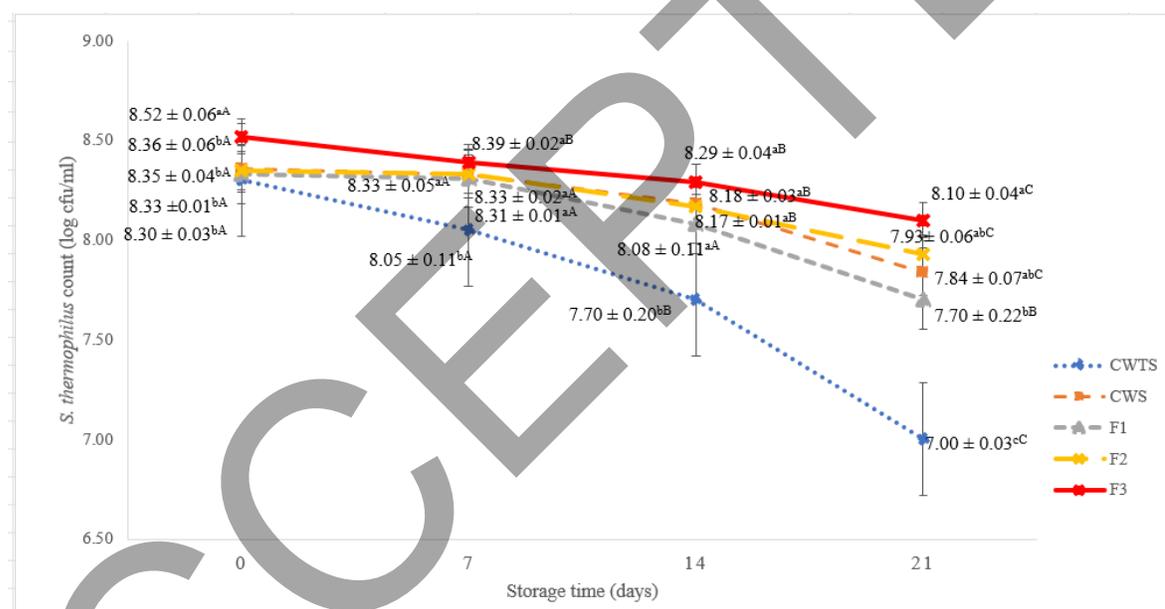
Probiotic viability was evaluated using MRS agar at different pH values, which provides presumptive counts of *Lactobacillus*- and *Streptococcus*-like colonies. This approach allowed monitoring of survival trends of yogurt starter cultures during storage; however, it does not permit definitive species identification. Selective media such as M17 agar for *S. thermophilus* or molecular methods would be required for precise differentiation, as highlighted in previous studies (Dave & Shah, 1996; Tharmaraj & Shah, 2003).

The fermentation patterns observed in the control samples (CWTS and CWS) differed from those in the formulations containing Sacha inchi flour (F1–F3). CWTS exhibited the lowest LAB viability over

storage, probably due to the absence of sugar as an additional fermentable substrate, which limited the metabolic activity of the starter cultures. In contrast, CWS maintained higher LAB counts because sugar supported continued microbial growth and survival during storage (Shori, 2016).

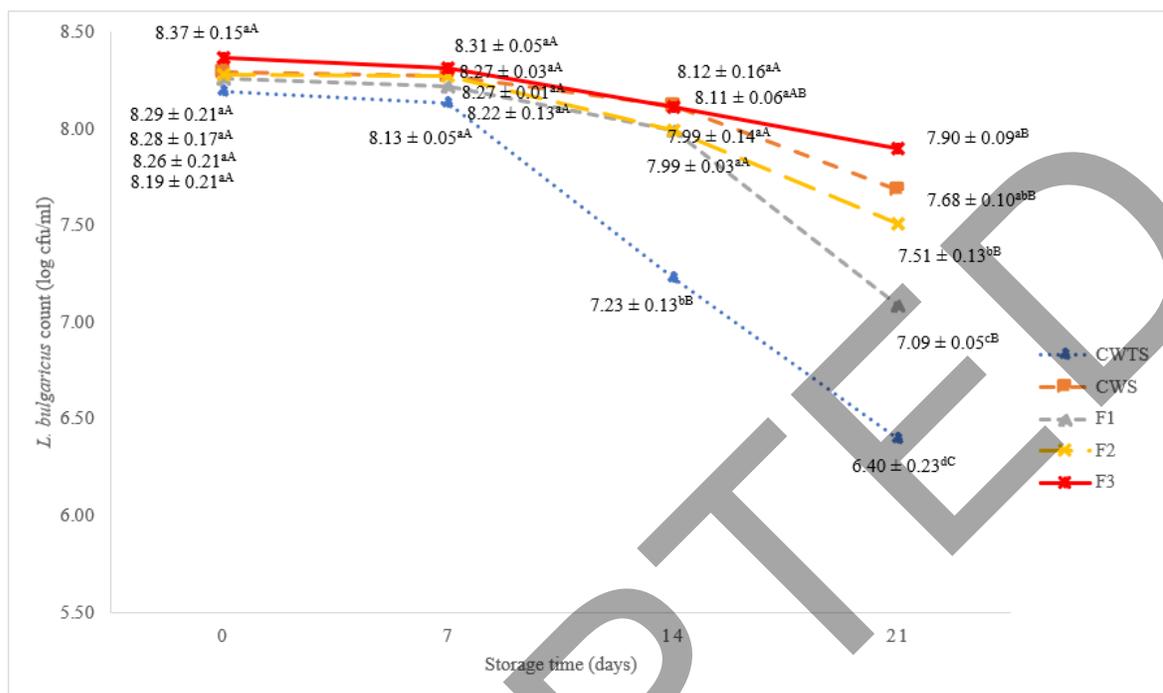
Yogurts fortified with Sacha inchi flour (F1–F3) demonstrated improved LAB viability compared to the controls. This effect can be attributed to the nutritional contribution of sachu inchi flour, including proteins and peptides that serve as nitrogen sources, dietary fibers that may act as prebiotics (Huang et al., 2025), and an enhanced buffering capacity that slowed the decline in pH (Oliveira et al., 2009). These factors collectively created a more favorable environment for LAB survival. Among the fortified formulations, F2 and F3, containing higher levels of Sacha inchi flour, provided greater protection to the LAB cultures, thereby maintaining higher viable counts throughout storage compared to CWTS and CWS.

As shown in Fig. 2 and Fig. 3, the viability of *S. thermophilus* and *L. bulgaricus* in yogurt formulations with 1%, 2.5%, and 5% sachu inchi flour, as well as in control samples (CWTS, CWS), remained relatively stable throughout the three-week storage period. Although there was a significant reduction ( $p < 0.05$ ) in the viability of both probiotic bacteria during storage, the final probiotic count in all yogurt formulations remained above the minimum therapeutic threshold of 6 log cfu/mL.



**Fig. 2** Viability of *S. thermophilus* (log cfu/mL) in set yogurt fortified with sachu inchi flour over storage time. CWTS = control without sugar; CWS = control with sugar; F1 = yogurt containing 1% sachu inchi flour; F2 = yogurt containing 2.5% sachu inchi flour; F3 = yogurt containing 5% sachu inchi flour.

**Remark:** Values are expressed as mean ± standard deviation of triplicate determinations. Superscripts (<sup>a, b, c</sup>) within the same column (sample) indicate significant differences ( $p < 0.05$ ). Superscripts (<sup>A, B, C</sup>) within the same row (storage time) indicate significant difference ( $p < 0.05$ ).



**Fig. 3** Viability of *L. bulgaricus* (log cfu/mL) in set yogurt fortified with sachu inchi flour over storage time. CWTS = control without sugar; CWS = control with sugar; F1 = yogurt containing 1% sachu inchi flour; F2 = yogurt containing 2.5% sachu inchi flour; F3 = yogurt containing 5% sachu inchi flour.

**Remark:** Values are expressed as mean ± standard deviation of triplicate determinations. Superscripts (<sup>a-c</sup>) within the same column (sample) indicate significant differences ( $p < 0.05$ ). Superscripts (<sup>A-C</sup>) within the same row (storage time) indicate significant difference ( $p < 0.05$ ).

The viability trends of *S. thermophilus* and *L. bulgaricus* in the yogurt samples were notably similar. Among all formulations, the CWTS (control without sugar) exhibited the most significant decline in viability over the 21-d storage period, indicating it was the least effective in maintaining probiotic survival. In contrast, CWS, F1, and F2 showed better stability, though all experienced notable reduction by day 21. The F3 formulation demonstrated the highest stability, preserving the greatest viability of both *S. thermophilus* and *L. bulgaricus* throughout storage. Similar findings were reported yogurt fortified with quinoa flour, where high flour concentrations supported greater probiotic viability (Mabrouk & Effat, 2020).

Fig. 2 and Fig. 3 show that CWTS consistently had the lowest viability for both starter cultures. This outcome is primarily attributed to elevated acid stress, which inhibits bacterial growth by acidifying the cytoplasm and disrupting enzymatic reactions (Shabala et al., 2006). CWTS also exhibited significantly higher acidity compared to fortified yogurts (F1, F2, and F3), with the exception of CWS. This increased acidity likely impaired starter culture viability. Low pH, nutrient depletion, and lactic acid accumulation during storage are known to hinder probiotic survival, reducing their effective dosage for consumption. Organic acids such as lactic acid can penetrate microbial cells in their non-dissociated form; once inside, they dissociate, releasing  $H^+$  ions, disrupting cellular functions inhibiting growth (Maia et al., 2023).

In the CWS formulation, adequate sugar levels provided a steady energy supply, supporting bacterial activity throughout the shelf life. While lactose in milk serves as the primary sugar, added sugars offer additional nutrients that enhance probiotic growth. Carvalho et al. (2004) observed that bacterial growth in the presence of various sugar substrates produces cells with distinct morphological and physiological traits, potentially increasing stress resistance. Glucose and other growth promoters are commonly added to dairy products to improve the growth rate of probiotic species such as *Lactobacilli* and *Bifidobacteria*, which typically grow less efficiently in milk. These supplements significantly enhance probiotic survival during storage (Tripathi & Giri, 2014).

Conversely, fortified yogurts (F1–F3) showed higher viability of both starter cultures across the 21-d period, with viability increasing alongside Sacha inchi flour concentration. This enhancement is attributed to the flour's high protein content, which supports probiotic growth by supplying essential amino acids, improving resistance to acidic conditions, and promoting protease activity. A consistent supply of these nutrients creates a more favorable environment for probiotic proliferation.

Across all samples, viable counts for *S. thermophilus* were consistently higher than those for *L. bulgaricus* after storage. This trend aligns with previous studies reporting greater stability of *S. thermophilus* during yogurt storage. The use of plastic containers in this study may have contributed to these results, as oxygen permeability can affect microbial viability (Ranadheera et al., 2012). Although both cultures are facultative anaerobes, *S. thermophilus* is also microaerophilic and can tolerate low oxygen levels. As a result, increased oxygen penetration from plastic packaging does not significantly impact the viable count of *S. thermophilus*. On the other hand, the oxygen permeability of the packaging may negatively affect the growth of *L. bulgaricus*, which is more sensitive to oxygen exposure (Sidhu et al., 2020).

#### 10. Sensory evaluation

The results of the sensory analysis for all yogurt formulations were presented in Table 8. Overall, color scores were high across all samples. However, CWTS received a significantly lower score compared to the other formulations, indicating its color is less preferred. In contrast, CWS, F1, F2, and F3 received statistically similar scores, suggesting a consistent and favorable appearance. Although increasing the concentration of Sacha inchi flour darkened the yogurt's color—due to the flour's natural yellowish to brown hue—panelists appeared to prefer the slightly darker tones of the fortified yogurts. A similar trend was reported in modified banana flour yogurt, where darker color was positively received (Jenic et al., 2013). Consumer acceptance of Sacha inchi-fortified yogurt was closely linked to changes in physicochemical properties. Color analysis (Table 7) showed that increasing Sacha inchi flour concentration led to a gradual decrease in L\* (lightness) and a\* (green–red) values, resulting in a darker, more yellow–brown color. This shift aligns with the sensory findings, where panelists preferred the fortified yogurts' color over that of the control without sugar.

In terms of aroma, fortified yogurt scores ranged from 4.90 in F1 to 3.72 in F3., both significantly lower than the control samples (CWTS and CWS). The evaluation used a 7-point hedonic scale, where 1 indicated “dislike extremely” and 7 indicated “like extremely.” A score of 4 represented a neutral response (“neither like nor dislike”). These results suggest that the aroma of F1 was slightly above neutral acceptability, whereas F3 approached the lower end of the scale, indicating reduced aroma preference at higher levels of Sacha inchi flour fortification. This reduction in aroma acceptance may be attributed to the distinctive aroma of Sacha inchi flour, which can overpower the natural yogurt aroma. Panelists noted that the fortified samples had a masked or unfamiliar odor, which may be off-putting to those unaccustomed to such flavors. A similar decline in aroma preference was reported in bread formulations where wheat flour was partially replaced with Sacha inchi flour (Marínez & Ramos-Escudero, 2024).

**Table 8** Sensory evaluation of yogurt fortified with different concentrations of Sacha inchi flour

Attributes	CWTS	CWS	F1	F2	F3
Colour	6.00±1.01 <sup>b</sup>	6.42±0.64 <sup>a</sup>	6.42±0.64 <sup>a</sup>	6.42±0.64 <sup>a</sup>	6.42±0.64 <sup>a</sup>
Aroma	5.50±1.25 <sup>a</sup>	5.90±0.93 <sup>a</sup>	4.90±0.93 <sup>b</sup>	4.46±1.09 <sup>b</sup>	3.72±1.33 <sup>c</sup>
Texture	5.24±1.19 <sup>b</sup>	6.14±0.86 <sup>a</sup>	4.88±1.22 <sup>b</sup>	4.34±1.22 <sup>c</sup>	4.00±1.64 <sup>c</sup>
Taste	5.08±1.55 <sup>b</sup>	6.38±0.83 <sup>a</sup>	4.22±1.47 <sup>c</sup>	3.26±1.23 <sup>d</sup>	3.06±0.93 <sup>d</sup>
Overall acceptability	4.98±1.42 <sup>b</sup>	6.32±0.84 <sup>a</sup>	4.18±1.26 <sup>c</sup>	3.32±1.19 <sup>d</sup>	3.02±1.15 <sup>d</sup>

**Remark:** Sensory scores were evaluated using a 7-point hedonic scale, where 1 = dislike extremely and 7 = like extremely. Values are expressed as mean ± standard deviation of triplicate determinations. Superscripts (<sup>a–d</sup>) within the same row indicate significant differences among samples ( $p < 0.05$ ). CWTS = control without sugar; CWS = control with sugar; F1 = yogurt containing 1% Sacha inchi flour; F2 = yogurt containing 2.5% Sacha inchi flour; F3 = yogurt containing 5% Sacha inchi flour.

The texture attribute scores indicated that fortification with Sacha inchi flour resulted in lower ratings compared to CWS. However, the difference between F1 and CWTS was not statistically significant ( $p < 0.05$ ). Fortifying yogurt with fiber-containing ingredients typically improves water-holding capacity and viscosity (Dabija et al., 2018). Although this trend was observed in the present study, the significantly low texture score at the highest fortification level (5%) was not associated with syneresis. Instead, it may be attributed to the graininess and poor mouthfeel caused by tiny fragments of sacha inchi flour particles ( $p < 0.05$ ). Similar findings were reported in yogurt fortified with flaxseeds, where higher fiber content negatively affected texture perception (Marand et al., 2020).

The viscosity and syneresis results (Tables 5 and 6) provide important context for consumer perception of texture. While samples with higher flour concentrations, such as F3, exhibited the highest initial viscosity (22.47 cP) and water-holding capacity (76.00%), they also received significantly lower texture acceptability score in sensory analysis. This suggests that viscosity, although generally considered a desirable quality, was not the primary driver of texture preference at higher concentrations. Instead, the negative perception was likely due to the graininess and poor mouthfeel caused by the high fiber content of the flour particles, which overshadowed the benefit of increased thickness.

Taste scores followed a similar trend, with CWS receiving the highest ratings while F3 the lowest. Taste or flavor is one of the most crucial attributes influencing consumer acceptability. In dairy products, sensory properties depend largely on the balance of flavor compounds derived from milk proteins, fats, and carbohydrates. The distinctive flavor of yogurt is primarily due to lactic acid and a complex mixture of volatile compounds produced during lactic fermentation (Krastanov et al., 2023). Fortification with Sacha inchi flour may impart a pronounced bitterness, leading to an unpleasant aftertaste that overshadows the natural sweetness of yogurt.

Overall acceptability scores for Sacha inchi-fortified yogurt were significantly lower ( $p < 0.05$ ) compared to the control samples. CWS ranked highest with a score of 6.32, followed by CWTS, F1, F2, and F3. Among the fortified samples, F1 achieved the highest acceptability score. The reduction in overall acceptability with increasing Sacha inchi flour concentration can be explained by the combined effects of enhanced Sacha inchi aroma, oil-derived flavor notes, and grainy texture. At higher fortification levels, these distinctive flavor compounds become overwhelming, negatively impacting taste and overall acceptability. The lowest scores observed in F3 strongly suggest that excessive fortification led to a less pleasant flavor profile at overshadowing the yogurt's natural sweetness.

## Conclusion

This study evaluated the fortification of yogurt with Sacha inchi flour in terms of physicochemical properties, probiotic viability during refrigerated storage, and consumer acceptance. Incorporation of Sacha inchi flour enhanced physicochemical attributes by increasing viscosity and water-holding capacity (WHC) while reducing susceptibility to syneresis (STS), with improvements most pronounced at higher concentrations (5%). Fortification also significantly increased the ash, protein, fat, and fiber content, thereby improving the nutritional profile of the yogurt. Probiotic viability was positively influenced by Sacha inchi flour, with fortified samples maintaining *S. thermophilus* and *L. bulgaricus*, counts above the minimum therapeutic threshold of  $10^6$  cfu/mL throughout storage. This outcome highlights the potential prebiotic effect of Sacha inchi flour, which supports probiotic proliferation and gut health. Despite these nutritional and functional benefits, fortification altered sensory characteristics, particularly at higher concentrations. Among the fortified samples, F1 (1% Sacha inchi flour) achieved the highest acceptability score, closely resembling the control formula. In contrast, F3 (5% Sacha inchi flour) provided greater nutritional and functional improvements, but was associated with lower sensory scores, largely due to bitterness, distinctive aroma, and grainy texture. Future studies should explore strategies to enhance the sensory appeal at higher-fortification levels, such as incorporating natural sweeteners or flavor enhancers, blending Sacha inchi flour with other plant-based flours, or applying microencapsulation techniques to improve flavor release and texture. This study contributes to sustainable development efforts by aligning with SDG no. 3 (Good Health and Well-being). Further investigations could examine the production of short-chain fatty acids (SCFAs) by probiotics in Sacha inchi-fortified yogurt, providing deeper insights into nutrient bioavailability and health-promoting properties. Additionally, optimizing complementary flavors and natural sweeteners could enhance consumer acceptance.

and market potential. Comparable studies with chia seed, flaxseed, and soy flour fortification have reported similar effects on texture, probiotic survival, and sensory acceptance, suggesting that Sacha inchi flour offers comparable promise as a functional fortification, while uniquely contributing  $\omega$ -3 fatty acids.

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### Conflicts of interest

The authors declares that there is no conflict of interest regarding the publication of this paper.

### Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used the AI tool to enhance grammar given that we are non-native speakers. After using this service, the authors reviewed and edited the content as required and took full responsibility for the content of the publication.

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