

## Synbiotic and protein-enriched low-fat Sao Hai rice ice cream

Putthapong Phumsombat<sup>1,2</sup>, Kulanid Trisakwattana<sup>2</sup>, Natcha Ittithanaput<sup>2</sup>, Natchanan Viwatanawanakarn<sup>2</sup>, Chaleeda Borompichaichartkul<sup>2,3\*</sup>

<sup>1</sup>School of Food Industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok, 10520, Thailand; <sup>2</sup>Department of Food Technology, Faculty of Science, Chulalongkorn University, Phayathai Road, Patumwan, Bangkok 10330, Thailand;

<sup>3</sup>Multi-Omics for Functional Products in Food, Cosmetics and Animals, Research Unit, Bangkok, Thailand

**\*Corresponding Author:** Chaleeda Borompichaichartkul, Department of Food Technology, Faculty of Science, Chulalongkorn University, Phayathai Road, Patumwan, Bangkok 10330, Thailand . Email: [chaleeda.b@chula.ac.th](mailto:chaleeda.b@chula.ac.th)

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## RESEARCH ARTICLE

### Abstract

This research aimed to investigate the production of low-fat Khao Sao Hai ice cream fortified with synbiotics, soy protein (SP), and chicken breast protein. The study incorporated Sao Hai rice milk into the ice cream formulations and conducted experiments to analyze the physical, chemical, and sensory characteristics. The application of synbiotic powder, SP, and chicken breast protein was explored, with the results indicating significant variations in the nutritional profile. Specifically, ice cream fortified with chicken breast protein exhibited the highest protein content at 4.20 g, followed by SP at 3.92 g, and the control formulation at 1.38 g. In addition, the survival rate of probiotics, represented by *Lactobacillus acidophilus* LA5, exceeded 98% during storage at  $-20^{\circ}\text{C}$  for 21 days, showcasing the successful encapsulation of probiotics with Konjac glucomannan and soy protein isolate. Furthermore, sensory testing revealed that the control formula received the highest consumer acceptance, followed by SP-fortified ice cream. In contrast, chicken breast protein-fortified ice cream received the least acceptance. These findings highlight the potential of Sao Hai rice in creating nutritionally diverse ice cream formulations, offering insights into consumer preferences and the efficacy of different protein sources in enhancing ice cream quality.

**Keywords:** low-fat ice cream, Sao Hai rice, synbiotics, probiotics, protein enrichment

### Introduction

Thailand takes great pride in its Sao Hai rice, which flourishes in the country's fertile rice-growing regions (Rerkasem, 2017). Alongside the renowned Jasmine rice, varieties like Jek Choey Sao Hai Rice, which descended from the Jek Choey rice lineage, further cement Thailand's position as a leading global rice producer (Suebongsang *et al.*, 2020). Primarily cultivated in the Saraburi Province, Sao Hai rice is distinguished by its slender, firm grains that yield a delightfully crumbly texture upon cooking. Despite its lesser popularity compared to Jasmine rice, Sao Hai rice offers a wealth

of health benefits, including a low glycemic index (GI), making it an excellent choice for individuals with diabetes (Purnomo *et al.*, 2018). Proximate analysis reveals the rich composition of Sao Hai rice, encompassing protein, fat, fiber, ash, and carbohydrates, complemented by antioxidant properties derived from phenolic acids and flavonoids (Jadwong & Therdthai, 2018; Kukusamude *et al.*, 2021).

In the current era, a heightened focus on health-conscious eating prompts exploring innovative, nutritious food options. Traditional dietary supplements often come in capsules or tablets, presenting challenges for continuous

consumption (Thakkar *et al.*, 2020). The idea of producing healthy food, specifically ice cream fortified with protein and synbiotics, is aimed at addressing the above challenges. Ice cream, a universally beloved dessert, typically high in fat and sugar (Beegum *et al.*, 2022), becomes a versatile vehicle for delivering health benefits. When plant-based ingredients are chosen for this endeavor, Sao Hai rice milk takes center stage due to its low GI and nutrient-rich profile. This choice contributes to the overall nutritional value of the ice cream. The incorporation of synbiotics, a dynamic combination of probiotics and prebiotics, further elevates the health-conscious appeal. Such incorporation offers benefits such as improved gut health, weight control, and diabetes management—reflecting the current trend in functional foods (Fuloria *et al.*, 2022). Encapsulation of probiotics with prebiotics was achieved in a synbiotic powder, resulting in high tolerance to various conditions and ensuring the efficacy of the health-enhancing components in the ice cream formulation. This milestone was accomplished through our innovative research and aligns seamlessly with our previous endeavors, further strengthening our dedication to breakthrough nutritional advancement.

In this study, the selection of Sao Hai rice as the primary raw material for ice cream production was based on its alignment with global preferences for a sweet and cool treat. Ice cream traditionally relies on ingredients like milk, milk fat, sweeteners, stabilizers, and emulsifiers to achieve its unique texture and flavor (Akdeniz *et al.*, 2019). However, lactose intolerance in some consumers necessitates lactose-free alternatives. In response, the formulation of lactose-free ice cream substitutes milk with nutrient-rich plant proteins, maintaining the desirable characteristics of traditional ice cream and accommodates a broader consumer base.

This research aims to develop a process for creating low-fat Sao Hai rice ice cream fortified with synbiotics and protein, utilizing both plant (soy protein, SP) and animal (chicken breast protein) sources. The primary objective is to demonstrate the feasibility of producing ice cream using Sao Hai rice milk as a key ingredient. In addition, we aim to explore the utilization of different protein sources to enhance the physical and chemical properties of the ice cream, while incorporating synbiotic powder for potential health benefits. The study encompasses two ice cream production methods—adding synbiotics before and after curing—to investigate their impact on the physical and chemical characteristics of the ice cream and the survival of probiotics during storage. The objectives include understanding the production process, exploring the effects of protein types on ice cream properties, assessing the survival of probiotics, and examining the sensory acceptance influenced by protein types. The anticipated benefits involve acquiring insights into

Khao Sao Hai ice cream production, understanding the properties when incorporating protein and synbiotics, and discerning the influence of animal and plant proteins on various ice cream attributes. In addition, the study aims to reveal the impact of different probiotic addition steps and frozen storage on ice cream characteristics. The ultimate goal is to extend the research findings to an industrial scale, fostering practical applications and innovations in the production of healthier ice cream alternatives.

## Materials and Methods

### Chemicals and microorganisms

In this study, various chemicals and microorganisms were employed for the production and analysis of low-fat Sao Hai rice ice cream fortified with synbiotics and proteins. Sao Hai rice branded Khum Kha was obtained from TongHua Bua Yai Co., Ltd. (Nakhon Ratchasima, Thailand). Lactose-free soymilk (Vitamilk Champ) was procured from Green Spot Co., Ltd. (Bangkok, Thailand), while coconut cooking oil (Gaysorn Pure) was purchased from Patum Vegetable Oil Co., Ltd. (Pathum Tani, Thailand). Additional chemicals, including sorbitol, maltitol, inulin, gua gum, soy protein isolate (SPI), and salt, were sourced from Bangkok Chemical (Bangkok, Thailand). To determine probiotic viability, bacteriological agar and MRS media were purchased from Himedia (Mumbai, India).

### Experimental design

All abbreviations used in this research have been listed in Table 1. The experimental design consisted of several steps: initially, ice cream formulations with varying percentages of Sao Hai rice milk (100, 75, 50, and 25%) were prepared (Table 2). Subsequently, sensory evaluations were conducted to identify the formulations with the highest acceptance among the panelists. Following the selection of the optimal formulation, ice cream was prepared once more, replacing soymilk with Sao Hai rice milk and fortifying it with either chicken breast protein powder (CK) or SP, while a control formulation remained non-protein fortified (CT). Furthermore, synbiotics were added using two methods, denoted as Method A and Method B. This resulted in six distinct ice cream samples (CT-A, SP-A, CK-A, CT-B, SP-B, and CK-B) for subsequent analysis, which included examining physiochemical properties and probiotic survival rates. It is noteworthy that, after a comprehensive evaluation, only one preparation method was considered suitable based on proximate analysis and the sensory preferences of the panelists.

Table 1. List of abbreviations and acronyms used in the article.

| Abbreviations | Definition                                                                                                        |
|---------------|-------------------------------------------------------------------------------------------------------------------|
| CT            | Control (no protein fortified)                                                                                    |
| SP            | Soy protein                                                                                                       |
| CK            | Chicken breast protein                                                                                            |
| CT-A          | Low-fat Sao Hai ice cream fortified with synbiotics, controlled formula prepared with method A                    |
| SP-A          | Low-fat Sao Hai ice cream fortified with synbiotics and soy protein prepared with method A                        |
| CK-A          | Low-fat Sao Hai ice cream fortified with synbiotics and soy protein chicken breast protein prepared with method A |
| CT-B          | Low-fat Sao Hai ice cream fortified with synbiotics, controlled formula prepared with method B                    |
| SP-B          | Low-fat Sao Hai ice cream fortified with synbiotics and soy protein prepared with method B                        |
| CK-B          | Low-fat Sao Hai ice cream fortified with synbiotics and soy protein chicken breast protein prepared with method B |
| KGM           | Konjac glucomannan                                                                                                |
| KGMH          | Konjac glucomannan hydrolysate                                                                                    |
| SPI           | Soy protein isolate                                                                                               |
| GI            | Glycemic index                                                                                                    |
| CFU           | Colony-forming unit                                                                                               |

Table 2. Composition variation in ice cream recipes based on Sao Hai rice milk content.

| Ingredient          | Amount (%w/w) |      |      |      |
|---------------------|---------------|------|------|------|
|                     | 100%          | 75%  | 50%  | 25%  |
| Sao Hai rice milk   | 71.6          | 53.7 | 35.8 | 17.9 |
| Soymilk             | 0             | 17.9 | 35.8 | 53.7 |
| Inulin              | 6.5           | 6.5  | 6.5  | 6.5  |
| Maltitol            | 6             | 6    | 6    | 6    |
| Sorbitol            | 6             | 6    | 6    | 6    |
| Erythritol          | 5             | 5    | 5    | 5    |
| Coconut cooking oil | 3.5           | 3.5  | 3.5  | 3.5  |
| Gua gum             | 0.3           | 0.3  | 0.3  | 0.3  |
| Salt                | 0.1           | 0.1  | 0.1  | 0.1  |

Sample preparation

To ensure the appropriate preparation of samples for experimentation, several ingredients required specific procedures.

Synbiotic preparations

The synbiotic powder used in this study had been previously optimized. It was prepared through the encapsulation of *L. acidophilus* LA5 using the FreeZone 6 Freeze Dryer System (Labconco, Missouri, USA) at -50°C and a pressure below 130 mbar for 3 days. The coating material for encapsulation was a combination of SPI and Konjac Glucomannan Hydrolysate (KGMH) at a 1:1 ratio. KGMH was prepared by hydrolyzing Konjac glucomannan with mannanase (Amano Enzyme, Nagoya, Japan) at a concentration of 200 IU/g KGM for 30 minutes at 70°C. The total solid content for freeze drying was 20%.

Chicken breast protein powder

Deboned and frozen lean chicken breast was processed by grinding with a meat mincer, followed by a 15-minute boiling process in a pressure cooker. The minced and pressure-cooked chicken meat underwent vacuum drying at 65°C, 0.09 MPa for 4 hours (Hotpack Model 273600, CT, USA). The resulting dried chicken breast meat was ground into powder and sieved to achieve a uniform particle size of 50 meshes.

Sao Hai rice milk

Sao Hai rice milk was prepared by cooking rice with water at a ratio of 1:2 in a rice cooker set to auto mode. After cooking, the rice was blended with water at a ratio of 1:6 (w/w) for 1 minute. The resulting mixture was strained through a sieve to remove larger grains, producing the rice milk used in ice cream preparation.

Ice cream preparation

The ice cream was prepared by weighing all ingredients according to the specified proportions (Table 2). Sao Hai rice milk was used in varying percentages (100%, 75%, 50%, and 25% w/w) in place of soymilk to determine the effect on the final product. All ingredients were mixed until homogeneous. The preparation involved pasteurization, cooling, homogenization, and subsequent storage at -20°C for further use (Sabet-Sarvestani *et al.*, 2021). Two different methods, Method A and Method B, were used to study the effect of temperature on probiotic survival. Method A involved adding synbiotics into 4°C ice cream after storage in a refrigerator for 18 hours. Method B involved adding synbiotics into 37°C ice cream after cooling it in an ice bowl. In Method A and Method B, the ice cream formulations were fortified with either SP or chicken breast powder (CK), as specified in Table 3.

Table 3. Abbreviations and sources of protein for the six formulas of low-fat Sao Hai rice ice cream fortified with synbiotics and protein.

| Sample                                                                                     | Abbreviations | Source of protein (% w/w)     |                     | Synbiotic powder (%w/w) |
|--------------------------------------------------------------------------------------------|---------------|-------------------------------|---------------------|-------------------------|
|                                                                                            |               | Chicken breast protein powder | Soy protein isolate |                         |
| Method A: add synbiotic after aging the ice cream mix                                      |               |                               |                     |                         |
| Low-fat Sao Hai ice cream fortified with synbiotics, controlled formula                    | CT-A          | 0                             | 0                   | 1                       |
| Low-fat Sao Hai ice cream fortified with synbiotics and soy protein                        | SP-A          | 0                             | 3                   | 1                       |
| Low-fat Sao Hai ice cream fortified with synbiotics and soy protein chicken breast protein | CK-A          | 3                             | 0                   | 1                       |
| Method B: add probiotics before aging the ice cream mix                                    |               |                               |                     |                         |
| Low-fat Sao Hai ice cream fortified with synbiotics, controlled formula                    | CT-B          | 0                             | 0                   | 1                       |
| Low-fat Sao Hai ice cream fortified with synbiotics and soy protein                        | SP-B          | 0                             | 3                   | 1                       |
| Low-fat Sao Hai ice cream fortified with synbiotics and soy protein chicken breast protein | CK-B          | 3                             | 0                   | 1                       |

### Amino acid profile

The amino acid profiles were analyzed to determine the composition of essential and nonessential amino acids in both SP powder and chicken breast powder samples. The analysis was conducted by the Food Research and Testing Laboratory, Faculty of Science, Chulalongkorn University, Bangkok, Thailand. All 20 amino acids, including both essential and nonessential ones, were quantified using standard procedures (European Commission, 1998). The amino acid content of both samples was reported as grams per 100 g of product.

### Preliminary trials for sensory screening

In this comprehensive study, two distinct sensory evaluations were incorporated to understand the sensory characteristics of Sao Hai rice ice cream. The first evaluation focused on discerning the impact of substituting Sao Hai rice milk for soymilk, while the second aimed to gauge the overall preference of the panelists for the final product. Both evaluations followed a uniform and meticulous method, ensuring consistency in the assessment process. Sensory evaluation employing a nine-point hedonic scale was conducted to meticulously assess the sensory preferences associated with Sao Hai rice ice cream (Omar *et al.*, 2020). Prior to the evaluation, each ice cream sample (10 g) was thoughtfully dispensed into individual plastic cups to ensure consistency in serving size. All sensory evaluation sessions were meticulously conducted under controlled ambient conditions to minimize external factors that might have influenced panelist perceptions. The esteemed panel of sensory evaluators, with 30 individuals comprising 15 males and 15 females, and with a age range of 20–45 years, was included to encompass diverse perspectives. This deliberate diversity

in the panel was expected to enhance the robustness and representativeness of the sensory data collected during the evaluation process. Each ice cream sample was systematically labeled with random three-digit codes to eliminate potential bias. Each ice cream sample was systematically labeled with random three-digit codes to eliminate potential bias and then presented to the panelists in a randomized sequence. Panelists were provided with explicit guidelines instructing them to assign liking scores for various sensory attributes, including color, smell, taste, texture, and overall acceptance. Each attribute was systematically rated on a numerical scale, with values ranging from 9 (indicating “liked extremely”) to 1 (indicating “disliked extremely”).

### Analysis of physical properties

In the analysis of the physical properties of Sao Hai rice ice cream, a conscientious examination of various parameters was conducted. The viscosity of the ice cream mixture was determined at 4°C using rotational viscometer (Fungilab Premium series, Barcelona, Spain) equipped with an R5 probe, which was rotated at 100 rpm, with readings being recorded over 30 seconds (Bolliger *et al.*, 2000). This process was repeated three times to ensure accuracy and expressed in centipoise (cP). In addition, sweetness was assessed using a refractometer (0–40° Brix), calibrated with distilled water before each measurement, and readings were taken in triplicate for each sample. The assessment of % overrun involved the weighing of ice cream after an 18-hour incubation at 4°C, and a comparison was made to the weight of the liquid ice cream before it underwent hardening in an ice cream maker. This measurement was used to quantify the volume increase upon freezing, and the formula for its calculation was: % Overrun = (Volume of ice cream – Volume

of mix used)/Volume of mix used  $\times$  100% (Marshall *et al.*, 2003).

Ice cream color was measured using the CIE system ( $L^*$ ,  $a^*$ ,  $b^*$ ) after the samples had hardened at  $-20^\circ\text{C}$  for 24 hours. A Colorimeter CR-400 (Konica Minolta, Tokyo, Japan) was employed for this purpose, and measurements were calibrated with a standard color sheet before each reading.

Furthermore, the study of the ice cream's melting characteristics entailed the observation of its dissolution over 60 minutes at  $25^\circ\text{C}$  (Muse and Hartel, 2004). Before the analysis, the ice cream samples underwent a hardening process at  $-20^\circ\text{C}$  for 24 hours. For each sample, 40–45 g were weighed and placed on a sieve, and the weight of the melting ice cream was recorded every 5 minutes. The dissolution percentage was calculated from the graph of ice cream volume versus time.

Lastly, ice cream hardness was determined using a TA.XTplusC Texture Analyzer (Stable Micro System, Godalming, United Kingdom) equipped with a 6 mm diameter probe (P/6) and a 1 kg load cell. Measurements were conducted before the curing of the liquid ice cream, and the probe was pressed into the ice cream with a maximum penetration of 15 mm. The temperature of the ice cream was monitored at 1 cm from the surface and was maintained at  $-3.0 \pm 1.0^\circ\text{C}$  using a digital thermometer. All measurements were performed in triplicate to ensure precision.

### Determination of pH during storage

This study analyzed the chemical properties to assess various aspects of low-fat Sao Hai rice ice cream samples supplemented with synbiotics, SP, and chicken breast protein. The pH measurement was carried out after the ice cream underwent a hardening process at  $-20$  degrees Celsius, with each sample analyzed three times. The ice cream samples were analyzed during storage over 3 weeks to provide insights into their acidity.

### Probiotic survival rate determination

The survival rate of probiotics within the synbiotic microencapsulation system incorporated into the ice cream was a crucial aspect of this study. To assess probiotic survival, 10-g portions of each ice cream sample were collected using aseptic techniques. These samples were subjected to standard plate count analysis using MRS agar. The probiotic survival rate was then calculated using the formula: Survival rate (%) =  $(\log N / \log N_0) \times 100$ , where  $N$  represents the probiotic cell count

in ice cream after storage (CFU/g), and  $N_0$  represents the probiotic cell count in ice cream before storage (CFU/g). This experiment was performed weekly over 3 weeks, and the results were expressed in CFU/g.

### Proximate analysis

Since there are no significant differences in terms of physicochemical properties and probiotic survival between both preparation methods, only ice cream prepared with method A (adding synbiotic after aging the ice cream mix) was used for proximate analysis. The proximate analysis included the examination of total energy, carbohydrates, moisture, ash, fat, protein, and crude fiber in the ice cream samples. The analysis was conducted in accordance with established methods (AOAC International; 1993, ISO 6731:2010 (E)/IDF21:2010(E), AOAC(2019) 945.46, AOAC(2019) 989.05, AOAC(2019) 991.20, and AOAC(2019) 978.10). These proximate analyses were carried out at the Food Research and Testing Laboratory, Faculty of Science, Chulalongkorn University, Bangkok, Thailand.

### Statistical analysis

The statistical analysis of the experimental data was carried out using a completely randomized design (CRD). The experiments were conducted in duplicate to ensure the reliability of the results. Mean values were compared using Duncan's New Multiple Range Test (DNMRT) at a significance level of 95%. IBM SPSS Statistics version 22 (IBM Corporation, NY, USA) was employed for the statistical analysis.

## Results and Discussion

### Effect of using Sao Hai rice milk instead of soymilk

This study comprehensively evaluated the effect of using Sao Hai rice milk instead of soymilk in ice cream formulations through a sensory analysis involving 30 panelists. These evaluations were conducted using a nine-point hedonic scale, considering various sensory attributes, including color, smell, taste, texture, and overall acceptance, as summarized in Table 4. Notably, the sensory assessment revealed significant differences ( $p \leq 0.05$ ) between the ice cream samples made with 100% Sao Hai rice milk and those formulated with other combinations. The 100% Sao Hai rice milk ice cream received lower scores across all sensory attributes. This lower rating was attributed to the ice cream's white color and unique rice milk aroma, both of which were unfamiliar to the panelists. In contrast, ice cream formulations containing both

**Table 4.** Sensory evaluation of ice cream using Sao Hai rice milk instead of soymilk.

| Sao Hai rice milk | Color                   | Smell                   | Taste                   | Texture                 | Overall acceptance      |
|-------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| 100%              | 5.13 <sup>b</sup> ±2.15 | 5.16 <sup>b</sup> ±1.64 | 5.53 <sup>b</sup> ±1.70 | 4.67 <sup>b</sup> ±1.47 | 5.13 <sup>b</sup> ±1.66 |
| 75%               | 7.63 <sup>a</sup> ±1.10 | 6.76 <sup>a</sup> ±1.15 | 6.77 <sup>a</sup> ±1.36 | 7.47 <sup>a</sup> ±1.20 | 7.47 <sup>a</sup> ±0.90 |
| 50%               | 8.00 <sup>a</sup> ±0.87 | 7.08 <sup>a</sup> ±1.30 | 6.60 <sup>a</sup> ±2.01 | 7.63 <sup>a</sup> ±1.13 | 7.37 <sup>a</sup> ±1.10 |
| 25%               | 7.68 <sup>a</sup> ±1.21 | 6.96 <sup>a</sup> ±1.31 | 6.90 <sup>a</sup> ±1.24 | 7.23 <sup>a</sup> ±1.35 | 7.20 <sup>a</sup> ±1.37 |

The values in the table are presented as mean ± SD from three replicate analyses. Different letters (a, b, ...) denote statistically significant differences ( $p \leq 0.05$ ) between vertical averages.

Sao Hai rice milk and soymilk exhibited more favorable sensory characteristics, particularly in terms of color, smell, and taste. Panelists showed a preference for the cream-yellow color, soy-based aroma, and familiar taste, as soy-containing products are well-recognized in Thai cuisine (Akesowan, 2009). Regarding texture, 100% Sao Hai rice milk ice cream exhibited a significantly rougher texture due to the high water content in rice milk, leading to larger ice crystals. In contrast, soymilk, rich in fat and milk solid nonfat proteins, contributed to a smoother texture with reduced ice crystal formation (Basharat *et al.*, 2020; Zanhj and Jideani, 2012). As reported by Abou-Dobara *et al.* (2016), who studied the sensory attributes of rice milk compared to other types of milk, rice milk exhibited an intense white color, garnering higher scores for color and appearance compared to cow milk with its yellow hue. Despite its favorable color and appearance, rice milk scored lower in smell, taste, and mouthfeel compared to cow milk. This decline in scores may be attributed to the vegetarian grainy taste of rice milk and its very low fat content, consistent with previous reports by Basharat *et al.* (2020). Overall, the 75% Sao Hai rice milk ice cream formulation, which garnered the highest overall acceptance scores, was selected for further experimentation. This high score and selection reflect its potential as a preferred alternative to traditional soy-based ice cream products.

### Amino acid profile

The analysis of the amino acid profile revealed significant differences between SPI powder and chicken breast powder. SPI exhibited higher levels of lysine, methionine, and phenylalanine, essential amino acids crucial for protein synthesis and various metabolic processes (Matthews, 2020), with lysine at 8.68 g/100 g, methionine at 0.37 g/100g, and phenylalanine at 4.75 g/100g. Conversely, chicken breast powder contained elevated levels of leucine, isoleucine, and valine, key branched-chain amino acids essential for muscle protein synthesis and repair (Kaspy *et al.*, 2023), with leucine at 8.27 g/100 g, isoleucine at 4.64 g/100 g, and valine at

5.07 g/100 g. These findings highlight the complementary nature of soy and chicken proteins, suggesting their potential to enrich the nutritional profile of ice cream formulations. In addition, the absence of hydroxylysine and hydroxyproline in both protein sources indicates their minimal collagen content (Hu *et al.*, 2010), potentially influencing the structural properties of the ice cream. Overall, the amino acid profile analysis provides valuable insights into the nutritional composition of protein-enriched ice cream, emphasizing the importance

**Table 5.** Amino acid profile of soy protein isolate powder and chicken breast powder.

| Typical quality | Amino acid composition (g/100 g product) |                       |
|-----------------|------------------------------------------|-----------------------|
|                 | Soy protein isolate powder               | Chicken breast powder |
| Alanine         | 3.90                                     | 5.33                  |
| Arginine        | 6.69                                     | 6.44                  |
| Aspartic acid   | 10.08                                    | 9.06                  |
| Cystine         | 0.98                                     | 1.01                  |
| Glutamic acid   | 17.47                                    | 13.52                 |
| Glycine         | 3.81                                     | 4                     |
| Histidine*      | 2.64                                     | 3.04                  |
| Hydroxylysine   | Not detected                             | Not detected          |
| Hydroxyproline  | Not detected                             | < 0.5                 |
| Isoleucine*     | 4.07                                     | 4.64                  |
| Leucine*        | 7.04                                     | 8.27                  |
| Lysine*         | 8.68                                     | 7.55                  |
| Methionine*     | 0.37                                     | 2.84                  |
| Phenylalanine*  | 4.75                                     | 4.14                  |
| Proline         | 4.32                                     | 3.40                  |
| Serine          | 4.72                                     | 3.99                  |
| Threonine*      | 3.44                                     | 4.51                  |
| Tryptophan*     | 0.92                                     | 1.04                  |
| Tyrosine        | 3.51                                     | 3.54                  |
| Valine*         | 4.71                                     | 5.07                  |
| Total           | 92.1                                     | 91.89                 |

\* Essential amino acids.

of diverse protein sources in optimizing product quality and nutritional value.

### Physical properties of low-fat Sao Hai ice cream fortified with synbiotics

The physical properties of low-fat Sao Hai ice cream fortified with synbiotics were precisely examined. As indicated in Table 6, it was observed that ice cream formulations containing SP (SP-A and SP-B) exhibited higher total solid content compared to those without SP. This difference can be attributed to the higher solubility of SPI in water, enabling it to dissolve more readily and contribute to an increased total solid content in the ice cream (O' Flynn *et al.*, 2021). Furthermore, the viscosity of ice cream containing SP was significantly higher than that of the other samples. This phenomenon can be explained by the protein's capacity to absorb water molecules, resulting in the formation of a protein network that encapsulates water, ultimately elevating the ice cream's viscosity. Taha *et al.* (2019) also noted that SPI possesses a strong affinity for the oil–water interface to enhance the ability to bind with water and create a gel-like structure. It is noteworthy that this increase in viscosity was accompanied by a reduction in overrun, particularly in soy-containing ice cream samples, although overrun is influenced by several factors, including protein content, fat, and emulsifiers (Sharma *et al.*, 2017). In addition, the presence of foam in ice cream can impact its viscosity; however, excessively high viscosity may lead to decreased overrun due to reduced air incorporation during the mixing process. A comparative analysis of animal-based and plant-based proteins in ice cream characteristics revealed that protein structure plays a pivotal role in determining ice cream viscosity. Animal-based proteins, such as myofibrils found in chicken breast protein powder used

in the present study, share similarities with fibrous protein characteristics and connective tissue observed in the study by Ito *et al.* (2004). In contrast, SP comprises globulins, which are globular proteins sensitive to heat and prone to dissolution. This suggests that SP can interact with water molecules, reducing free water content and resulting in higher viscosity, in contrast to chicken breast protein, which remains insoluble in water.

Table 6 presents the findings of the ice cream hardness analysis, revealing that the control ice cream exhibited softer properties compared to the ice creams enriched with SP and chicken breast protein ( $p \leq 0.05$ ). This observation aligns with a study by Yan *et al.* (2021), which highlighted the significance of rise value in determining ice cream hardness. A higher rise in value indicates increased air content in the ice cream, resulting in a softer and fluffier texture. Conversely, higher solids content, particularly protein content, leads to chewier and denser ice cream. This correlation underscores the role of protein content in influencing ice cream texture. Furthermore, the denser texture observed in SP- and chicken breast protein-enriched ice creams suggests enhanced protein–solid interactions, contributing to their firmer consistency. This finding is consistent with recent literature emphasizing the impact of protein characteristics on ice cream properties (Patel *et al.*, 2006; Roy *et al.*, 2022).

The results from the ice cream melting rate analysis, as outlined in Table 6, offer insightful comparisons that deepen our understanding of ice cream properties. Interestingly, the addition of soy protein concentrate (SPC) in ice cream formulations, as indicated by Dervisoglu *et al.* (2005), resulted in a significantly higher pH across all samples, leading to notable alterations in melting behavior. This increase in pH, attributed to the

**Table 6.** Physical properties of low-fat Sao Hai rice ice cream fortified with synbiotics.

| Sample | Total soluble solid (°Brix) | Viscosity (cP)             | Overrun (%)                | Hardness (kg)             | Melting rate (g/min)    | Color                     |                          |                          |
|--------|-----------------------------|----------------------------|----------------------------|---------------------------|-------------------------|---------------------------|--------------------------|--------------------------|
|        |                             |                            |                            |                           |                         | $L^*$                     | $a^*$                    | $b^*$                    |
| CT-A   | 28.55 <sup>cd</sup> ±0.21   | 848.0 <sup>c</sup> ±351.0  | 35.33 <sup>ab</sup> ±15.51 | 1.98 <sup>c</sup> ±0.61   | 4.98 <sup>a</sup> ±0.61 | 78.14 <sup>ab</sup> ±0.45 | 0.32 <sup>ab</sup> ±0.16 | 6.86 <sup>c</sup> ±0.58  |
| SP-A   | 32.00 <sup>a</sup> ±0.28    | 3044.7 <sup>a</sup> ±301.1 | 11.56 <sup>b</sup> ±10.83  | 12.83 <sup>a</sup> ±1.98  | 0.76 <sup>b</sup> ±0.01 | 76.00 <sup>b</sup> ±1.62  | 0.63 <sup>a</sup> ±0.06  | 9.14 <sup>a</sup> ±0.41  |
| CK-A   | 28.75 <sup>cd</sup> ±1.90   | 995.2 <sup>c</sup> ±16.0   | 29.03 <sup>ab</sup> ±5.95  | 11.42 <sup>ab</sup> ±0.62 | 4.36 <sup>a</sup> ±0.26 | 78.99 <sup>ab</sup> ±0.18 | -0.07 <sup>b</sup> ±0.11 | 7.80 <sup>b</sup> ±0.49  |
| CT-B   | 28.20 <sup>d</sup> ±0.14    | 908.4 <sup>c</sup> ±67.4   | 40.06 <sup>a</sup> ±8.36   | 2.69 <sup>c</sup> ±1.69   | 5.37 <sup>a</sup> ±0.77 | 79.69 <sup>a</sup> ±0.42  | -0.05 <sup>b</sup> ±0.12 | 7.19 <sup>bc</sup> ±0.56 |
| SP-B   | 29.70 <sup>b</sup> ±0.14    | 2475.7 <sup>b</sup> ±235.3 | 24.66 <sup>ab</sup> ±1.78  | 11.58 <sup>ab</sup> ±0.10 | 0.69 <sup>b</sup> ±0.52 | 77.70 <sup>ab</sup> ±0.47 | 0.35 <sup>ab</sup> ±0.16 | 9.22 <sup>a</sup> ±0.18  |
| CK-B   | 28.85 <sup>c</sup> ±1.76    | 637.9 <sup>c</sup> ±96.9   | 32.73 <sup>ab</sup> ±8.63  | 7.52 <sup>b</sup> ±3.82   | 4.80 <sup>a</sup> ±0.27 | 78.66 <sup>ab</sup> ±2.17 | 0.13 <sup>b</sup> ±0.35  | 8.20 <sup>ab</sup> ±0.09 |

The values in the table are presented as mean ± SD from three replicate analyses. Different letters (a, b, ...) denote statistically significant differences ( $p \leq 0.05$ ) between vertical averages, while "ns" indicates no significant difference ( $p > 0.05$ ). The table includes data for ice cream samples with added probiotics after aging the mix (after aging), including the controlled formula (CT-A), soy protein-fortified formula (SP-A), and chicken breast protein-fortified formula (CK-A). Similarly, ice cream samples with probiotics added before aging the mix (before aging) are represented, including the controlled formula (CT-B), soy protein-fortified formula (SP-B), and chicken breast protein-fortified formula (CK-B).

alkaline nature of SPC, could potentially influence the protein–water interactions within the ice cream matrix, affecting its overall stability and melting characteristics. Moreover, the reduction in total solid contents observed in ice cream samples with higher SPC ratios may contribute to changes in texture and viscosity, influencing the rate of melting. Furthermore, insights from Roy *et al.* (2022) on the effect of whey protein isolate (WPI) supplementation on ice cream melting rate provide additional context. The study demonstrated a significant increase in melting rate with higher protein content, contradicting conventional expectations. This finding aligns with the observations regarding SP in our study, suggesting a commonality in the influence of protein content on melting behavior across different protein sources, consistent with the findings of Pon *et al.* (2015). The mechanism proposed by Roy *et al.* (2022), involving fat clumps formation and overrun, complements our understanding of how protein interactions and structural changes within the ice cream matrix impact its melting properties.

The analysis of ice cream color unveiled nuanced variations in  $L^*$  (brightness),  $a^*$  (redness/greenness), and  $b^*$  (yellowness/blueness) when comparing samples within the same formula but different production processes. This discrepancy, despite identical ingredient quantities, underscores the influence of production methods on color attributes. Interestingly, further comparison within each ice cream formula under the same production process (A and B) revealed subtle differences, particularly with the addition of chicken breast protein and SP. Notably, formulations supplemented with SP exhibited marginally lower  $L^*$  values and higher  $b^*$  values compared to the control formula. This shift toward a more yellow hue can be attributed to the inherently deeper yellow hue of SP compared to chicken breast protein, as corroborated by previous studies (Friedeck *et al.*, 2003; Akesowan, 2009). Friedeck *et al.* (2003) observed that low-fat ice cream mixes fortified with SPI exhibited significantly lower  $L^*$  values and increased  $b^*$  values compared to the control, indicating a shift toward a less white and more yellow hue with increasing SPI content. This finding resonates with our observations regarding the influence of SP supplementation on ice cream color attributes. In addition, Dervisoglu *et al.* (2005) demonstrated that the addition of strawberry flavor and color, along with increasing SPC content, led to decreases in whiteness ( $L^*$ ) and increases in redness ( $a^*$ ) values in ice cream samples. This aligns with our findings regarding the impact of formulation variations, such as the addition of chicken breast protein and SP, on ice cream color.

To establish a comprehensive understanding of the relationship between physical properties and probiotic supplementation, it is essential to explore how the incorporation of probiotics influences the texture, melting

rate, and color attributes of low-fat Sao Hai ice cream. Previous studies have highlighted the significant impact of probiotic supplementation on the physicochemical properties of dairy products (Homayouni and Norouzi, 2016; Afzaal *et al.*, 2019). Considering the findings from our study and existing literature, the addition of probiotics could potentially induce changes in ice cream texture by altering protein–water interactions within the matrix. Specifically, the encapsulation of probiotics might contribute to the formation of a protein network that affects the viscosity and overrun of ice cream, as observed in formulations containing SP and chicken breast protein (O' Flynn *et al.*, 2021; Taha *et al.*, 2019). Furthermore, the presence of probiotics may influence the melting rate of ice cream through interactions with other ingredients, such as SPC, affecting pH and total solid contents, as demonstrated by Dervisoglu *et al.* (2005) and Roy *et al.* (2022). In addition, variations in ice cream color attributes, particularly yellowness ( $b^*$ ), observed in formulations supplemented with probiotics could be attributed to the inherent characteristics of probiotic strains or the encapsulation materials used (Friedeck *et al.*, 2003; Akesowan, 2009). By integrating these insights, we establish a connection between the incorporation of probiotics and the observed changes in physical properties, contributing to a deeper understanding of the multifaceted effects of probiotic supplementation on ice cream formulations.

### Effect of protein source on pH and stability in fortified ice cream

The evaluation of pH values in the ice cream formulations, as depicted in Table 7, revealed notable differences ( $p \leq 0.05$ ) among various formulas, offering insights into quality variations. Chicken breast protein-enriched ice cream exhibited the lowest pH, a phenomenon possibly attributed to the inherent acidity of chicken breast as a raw protein source, influenced by postmortem lactic acid accumulation (Eady *et al.*, 2014). This finding is consistent with Mohammadi *et al.* (2011), who highlighted the significance of pH in determining the survival of probiotic bacteria in fermented ice cream. Interestingly, pH levels increased significantly ( $p \leq 0.05$ ) across all formulations during the 21-day storage period, contrary to findings from studies on probiotic-fortified ice cream, which reported increases in acid content (Ahmadi *et al.*, 2014). This observation suggests potential interactions between proteins and ice cream components, potentially leading to structural breakdown or amino acid release, contributing to the observed pH rise. Cruz *et al.* (2009) further elaborate on the challenges of incorporating probiotic cultures into ice cream formulations, emphasizing the importance of optimizing processing steps to maintain product quality, including pH control during

**Table 7.** pH of low-fat Sao Hai rice ice cream fortified with synbiotics during making process and storage.

| Sample | Before churning          | After churning             | Storage (Days)            |                           |                           |                           |
|--------|--------------------------|----------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
|        |                          |                            | 0                         | 7                         | 14                        | 21                        |
| CT-A   | 6.89 <sup>bc</sup> ±0.09 | 6.92 <sup>bc</sup> ±0.08   | 6.92 <sup>bc</sup> ±0.01  | 7.27 <sup>caB</sup> ±0.03 | 7.42 <sup>bcA</sup> ±0.07 | 7.42 <sup>aA</sup> ±0.04  |
| SP-A   | 7.27 <sup>aC</sup> ±0.06 | 7.27 <sup>aBC</sup> ±0.07  | 7.27 <sup>aC</sup> ±0.09  | 7.52 <sup>aAB</sup> ±0.06 | 7.62 <sup>aA</sup> ±0.08  | 7.53 <sup>aA</sup> ±0.01  |
| CK-A   | 6.71 <sup>cC</sup> ±0.02 | 6.74 <sup>cABC</sup> ±0.04 | 6.74 <sup>bBC</sup> ±0.00 | 6.99 <sup>dAB</sup> ±0.05 | 6.99 <sup>dAB</sup> ±0.05 | 7.11 <sup>bcA</sup> ±0.02 |
| CT-B   | 6.97 <sup>bc</sup> ±0.07 | 7.25 <sup>bAB</sup> ±0.02  | 7.17 <sup>aB</sup> ±0.01  | 7.34 <sup>±bcA</sup> 0.02 | 7.29 <sup>cAB</sup> ±0.06 | 7.37 <sup>abA</sup> ±0.09 |
| SP-B   | 7.31 <sup>aB</sup> ±0.07 | 7.31 <sup>abB</sup> ±0.06  | 7.31 <sup>aB</sup> ±0.06  | 7.40 <sup>bb</sup> ±0.09  | 7.55 <sup>abA</sup> ±0.03 | 7.62 <sup>abA</sup> ±0.05 |
| CK-B   | 6.60 <sup>c</sup> ±0.02  | 6.73 <sup>c</sup> ±0.08    | 6.74 <sup>b</sup> ±0.11   | 6.81 <sup>e</sup> ±0.04   | 6.95 <sup>d</sup> ±0.06   | 6.92 <sup>c</sup> ±0.05   |

The values in the table are presented as mean ± SD from three replicate analyses. Different lowercase letters (a, b, ...) denote statistically significant differences ( $p \leq 0.05$ ) between vertical averages. Different uppercase letters (A, B, ...) denote statistically significant differences ( $p \leq 0.05$ ) between horizontal averages. The table includes data for ice cream samples with added probiotics after aging the mix (after aging), namely, the controlled formula (CT-A), soy protein-fortified formula (SP-A), and chicken breast protein-fortified formula (CK-A). Similarly, ice cream samples with probiotics added before aging the mix (before aging) are presented, namely, the controlled formula (CT-B), soy protein-fortified formula (SP-B), and chicken breast protein-fortified formula (CK-B).

fermentation. Surprisingly, no significant difference ( $p > 0.05$ ) in pH was observed between ice cream formulations produced using different processes, suggesting that the encapsulation of probiotic cultures may not exert a discernible influence on ice cream pH. This finding is consistent with previous research on ice cream pH dynamics, highlighting the intricate interplay between formulation methods and pH stability, components and storage conditions in shaping ice cream quality attributes. By incorporating insights from Mohammadi *et al.* (2011) and Cruz *et al.* (2009), we deepen our understanding of the factors influencing pH variations in ice cream formulations, providing valuable context for the discussion of quality changes during storage and the challenges of probiotic incorporation.

### Probiotic survival rate in low-fat Sao Hai rice ice cream

The survival analysis of *Lactobacillus acidophilus* LA5 in encapsulated form, as depicted in Figure 1, aligns with previous research by Homayouni and Norouzi (2016) on probiotic survival in fermented soy-based ice cream. Both studies demonstrate the robustness of encapsulation in shielding probiotic cells from mechanical stress and environmental factors during ice cream production and storage. Previous study observed a significant retention of probiotic viability in fermented soy ice cream even after freezing and extended storage, reinforcing the efficacy of encapsulation in preserving probiotic viability in challenging environments. This convergence of findings underscores the critical role of encapsulation in maintaining probiotic viability and highlights its potential applicability across different frozen dairy product formulations (Wattanaprasert *et al.*, 2017; Talwalkar and Kailasapathy, 2004; Akalin and Erişir, 2008; Afzaal *et al.*, 2020).

Furthermore, while our study primarily focused on probiotic survival, it is essential to consider its broader implications on physicochemical properties, as emphasized by Cruz *et al.* (2009). The significant increase in acidity and overrun observed in the fermented soy ice cream compared to the control sample underscores the impact of probiotic fermentation on these properties. This parallels the discussion by Cruz *et al.* (2009) regarding the importance of physicochemical properties in determining the overall quality and consumer acceptance of ice cream products. Future research endeavors should thus aim to explore the effects of encapsulation on these properties in greater detail, drawing insights from studies such as Cruz *et al.* (2009) and Homayouni and Norouzi (2016), to optimize product formulations and enhance consumer satisfaction.

Moreover, Afzaal *et al.* (2019) and Mohammadi *et al.* (2011) provide valuable insights into the survival and physicochemical properties of encapsulated probiotics in ice cream formulations. Afzaal *et al.* (2019) demonstrated similarly high survival rates of encapsulated probiotics in ice cream, corroborating our findings and reinforcing the effectiveness of encapsulation as a strategy for preserving probiotic viability. Mohammadi *et al.* (2011) explored the rheological properties of ice cream mixtures, shedding light on the complex interplay between probiotic fermentation and viscosity. By integrating these findings into our discussion, we gain a comprehensive understanding of the multifaceted effects of encapsulated probiotics on ice cream quality and consumer acceptance (Afzaal *et al.*, 2019; Mohammadi *et al.*, 2011; Cruz *et al.*, 2009).

### Proximate analysis of low-fat Sao Hai rice ice cream

In analyzing the proximate analysis results of the low-fat Sao Hai rice ice cream fortified with synbiotics, our

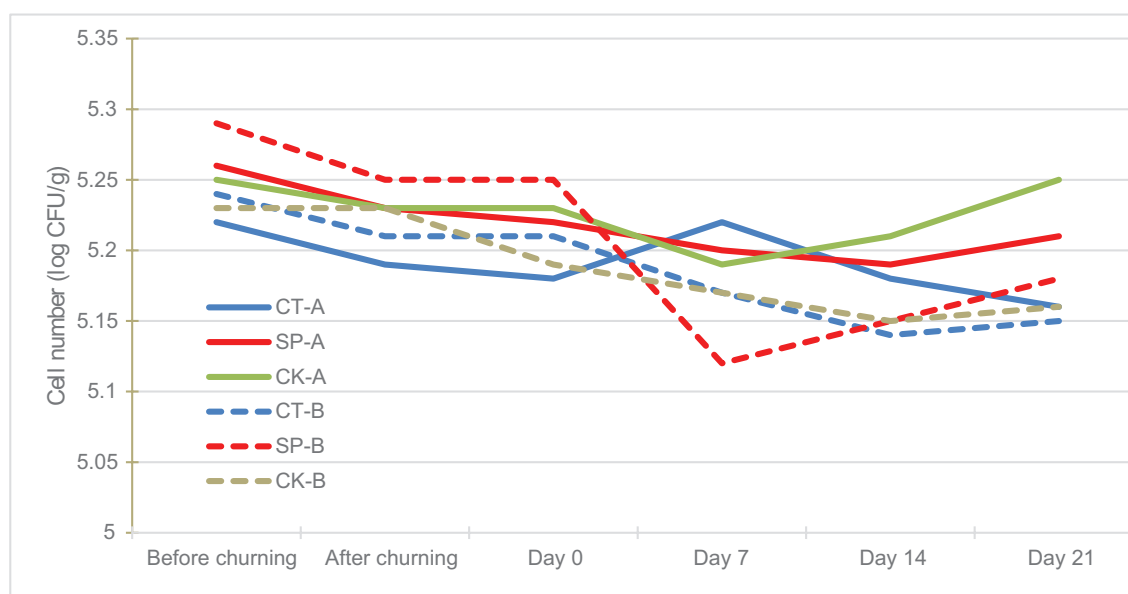


Figure 1. Survival rate of encapsulated *Lactobacillus acidophilus* LA5 in Sao Hai ice cream.

study observed significant variations in the nutritional composition among the control (CT), chicken breast protein-fortified (CK), and SP-fortified formulations, as evidenced by the data presented in Table 8. CK displayed the highest total calories and protein content, while CT exhibited the lowest total fat and crude fiber content. Interestingly, SP's nutritional profile fell between CT and CK, indicating the substantial impact of protein source selection on ice cream composition. These findings are consistent with recent research by Asres *et al.* (2022), where alternative protein sources like soy milk were found to have higher protein content compared to cow's milk, thereby influencing the nutritional profile of ice cream formulations. Moreover, Bisla *et al.* (2012) highlighted variations in moisture and ash content among different milk types, further supporting our observations regarding the influence of protein sources on ice cream composition.

Furthermore, our results establish a comprehensive link between nutritional composition and other key parameters such as pH, sensory attributes, and amino acid profile. Previous studies have shown that variations in protein content and composition can affect ice cream pH and texture, as well as sensory attributes such as taste and mouthfeel. For instance, the amino acid profile analysis revealed distinct differences between SPI and chicken breast powder, indicating their complementary roles in enhancing the nutritional richness and functional properties of ice cream (Matthews, 2020; Kaspy *et al.*, 2023). In addition, integrating these amino acid profiles with the proximate analysis results from Table 8 sheds light on the direct impact of protein sources on the total protein content observed in the ice cream formulations.

Table 8. Proximate analysis result of low-fat Sao Hai rice ice cream fortified with synbiotics.

| Amount/100 g       | CT          | SP          | CK          |
|--------------------|-------------|-------------|-------------|
| Total calories     | 149.34 kcal | 155.16 kcal | 159.06 kcal |
| Total carbohydrate | 27.45 g     | 27.40 g     | 26.61 g     |
| Moisture           | 67.08 g     | 64.94 g     | 64.81 g     |
| Ash                | 0.31 g      | 0.42 g      | 0.40 g      |
| Total fat          | 3.78 g      | 3.32 g      | 3.98 g      |
| Protein            | 1.38 g      | 3.92 g      | 4.20 g      |
| Crude fiber        | 0.14 g      | 0.20 g      | 0.30 g      |

The table includes data for ice cream samples with added probiotics after aging the mix (after aging), namely, the controlled formula (CT), SP-fortified formula, and chicken breast protein-fortified formula (CK).

### Sensory analysis of low-fat Sao Hai rice ice cream fortified with synbiotics

In our comprehensive sensory evaluation using a nine-point hedonic scale with a panel of 30 participants (refer to Table 9), we observed a consistent preference for the control ice cream formula across all evaluated aspects compared to the other formulations. Notably, the control formula garnered higher scores for color, flavor, taste, texture, and overall acceptance, registering impressive scores of 7.70, 6.80, 7.27, 7.43, and 7.33, respectively. This unanimous preference can be attributed primarily to the vibrant creamy yellow color of the control formula, a stark contrast to the darker hues resulting from including SP and chicken breast protein additives (as depicted in Figure 2). These findings corroborate previous studies

Table 9. Sensory evaluation of low-fat Sao Hai rice ice cream fortified with synbiotics.

| Sample | Color                 | Smell                 | Taste                 | Texture               | Overall acceptance    |
|--------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| CT     | 7.7 <sup>b</sup> ±1.0 | 6.8 <sup>a</sup> ±1.5 | 7.3 <sup>a</sup> ±1.1 | 7.4 <sup>c</sup> ±1.3 | 7.3 <sup>c</sup> ±1.3 |
| SP     | 6.9 <sup>a</sup> ±1.1 | 6.4 <sup>a</sup> ±1.4 | 6.6 <sup>a</sup> ±1.4 | 6.0 <sup>b</sup> ±1.7 | 6.3 <sup>b</sup> ±1.5 |
| CK     | 6.9 <sup>a</sup> ±1.2 | 4.6 <sup>b</sup> ±1.7 | 4.2 <sup>b</sup> ±1.9 | 3.7 <sup>a</sup> ±1.6 | 4.4 <sup>a</sup> ±1.8 |

The values in the table are presented as mean ± SD from three replicate analyses. Different letters (a, b, ...) denote statistically significant differences ( $p \leq 0.05$ ) between vertical averages. The table includes data for ice cream samples with added probiotics after aging the mix (after aging), namely, the controlled formula (CT), SP -fortified formula, and chicken breast protein-fortified formula (CK).

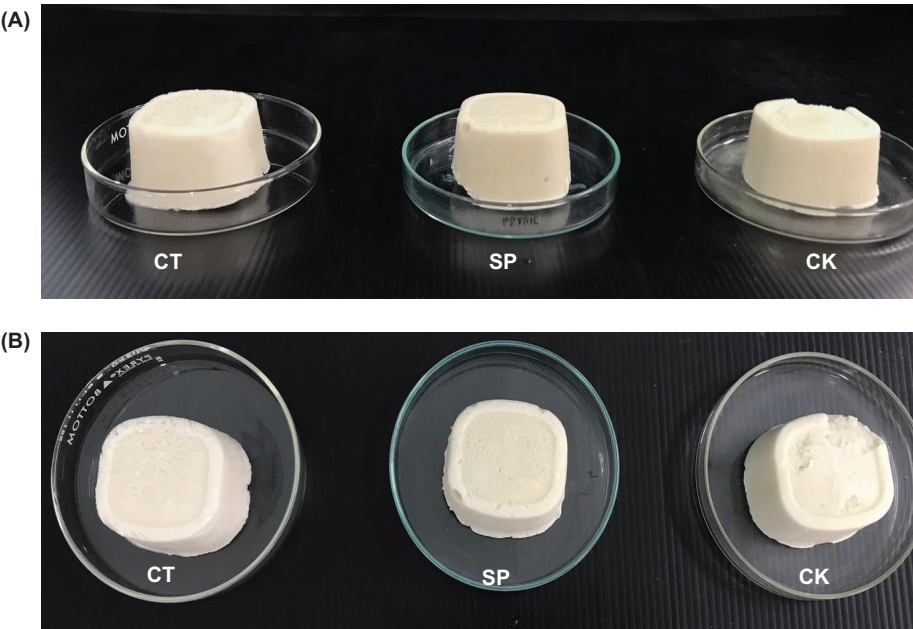


Figure 2. The appearance of low-fat Khao Sao Hai ice cream fortified with synbiotics, showcasing side (A) and top (B) views for the control formula (CT), soy protein–fortified formula (SP), and chicken breast protein-fortified formula (CK).

by Friedeck *et al.* (2003), which underscore the substantial impact of protein additives on ice cream coloration.

Moreover, our evaluation revealed noteworthy texture improvements in the control formula, aligning with the research by Taha *et al.* (2019), who demonstrated that increased viscosity resulting from added protein contributes significantly to enhanced texture. However, it is worth mentioning that while protein supplementation had a positive effect on texture, the chicken breast protein formula received lower scores due to its distinctively noticeable smell, as reported by Dervisoglu *et al.* (2005). This highlights the critical importance of protein source selection in optimizing sensory attributes, as discussed in the study by Cruz *et al.* (2009), which emphasizes the need for careful consideration when choosing protein additives to avoid sensory drawbacks.

Despite SP demonstrating similarities in smell and taste compared to the control, it exhibited slightly lower

scores in color, texture, and overall preference, consistent with the findings of Asres *et al.* (2022). Similarly, the recent study by Roy *et al.* (2022) shed light on the significant impact of protein concentration on flavor, texture, and meltdown characteristics, further accentuating the intricate relationship between protein supplementation and sensory attributes in ice cream formulations. These findings collectively underscore the multifaceted influence of protein supplementation on sensory attributes and consumer preference, emphasizing the importance of meticulous protein source selection and concentration adjustment in optimizing ice cream formulations to meet consumer expectations and preferences.

### Conclusions

In conclusion, the study began by formulating an ice cream recipe primarily using 75% Sao Hai rice milk,

serving as the base for developing protein-enriched variants. The addition of soy and chicken breast protein led to notable changes in various physical and chemical properties. Proximate analysis revealed increased energy and protein content in soy and chicken breast protein-fortified ice cream compared to the control, with soy-fortified ice cream containing the highest protein content of 3.92g and chicken breast protein-fortified ice cream showing a significant increase in energy content of 159.06 kcal. The amino acid profile analysis highlighted distinct differences between SPI and chicken breast powder, with SP rich in lysine, methionine, and phenylalanine. In contrast, chicken breast powder contained elevated leucine, isoleucine, and valine levels. These findings underscored the complementary nature of soy and chicken proteins, suggesting their potential to enhance ice cream nutritional profiles. Sensory evaluations supported these findings, with the control formula preferred over SP-fortified ice cream, while chicken breast protein-fortified ice cream received the least favorable response. Encapsulation of probiotics with Konjac glucomannan and SPI showed promising results, ensuring a survival rate exceeding 98% during storage at  $-20^{\circ}\text{C}$  for 21 days. The study acknowledged limitations such as the impact of the drying process on probiotic survival and potential for further enhancement of protein and fat content in future experiments. In this research, the potential of soy and chicken breast proteins to enhance the nutritional profile of ice cream formulations is demonstrated, with soy-fortified ice cream containing the highest protein content and chicken breast protein-fortified ice cream showing increased energy content. In addition, high probiotic viability is ensured through the encapsulation of probiotics with Konjac glucomannan and SPI, offering promising avenues for the development of healthier ice cream alternatives with improved nutritional value and consumer acceptance.

## Author Contributions

The study was conceptualized by P.P. and C.B.; methodology was formulated by K.T., N.I., and N.V.; validation was done by P.P. and C.B.P.P. prepared the original draft; C.B. reviewed and edited the manuscript; and supervision was done by C.B. All authors have read and agreed to the published version of the manuscript.

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## Conflicts of Interest

The authors declare no conflicts of interest.

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