Characterization of High Protein and Instant Cereal Fiber Drink Enriched with Probiotic Milk Powder

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ABSTRACT

A potential functional snack for school-aged children can be formulated by fortifying instant cereal drinks with sorghum and tempeh flour, which provide additional protein and dietary fiber. Probiotic-enriched snacks are well-known, and several clinical studies have shown significant benefits to humans. This study aimed to evaluate the effects of different sorghum-to-tempeh flour ratios (5:1, 2:1, and 1:1) on the physicochemical and sensory properties of flakes, as well as the viability of *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 in an instant cereal drinks stored for 56 days at three distinct storage temperature, i.e 20, 30, and 37 °C. The formulation with a 2:1 sorghum-to-tempeh flour ratio was the most preferred by panelists. Proximate analysis revealed that all probiotic instant cereal drink formulations qualified as good sources of dietary fiber (6.4–10.6 g/100 g) and protein (14.5–16.3 g/100 g), with higher levels compared to a commercial product. In addition, the probiotic drinks maintained viable counts exceeding 6.0 log CFU/g, thereby fulfilling the FAO/WHO requirement for health-promoting effects and offering potential benefits for Indonesian school-age children.

Keywords: Physicochemical; probiotic; sensory; snack

INTRODUCTION

The protein and fiber dietary intake of most school-age children in Indonesia is below the Recommended Dietary Allowance (RDA) set by the government. Accordingly, there is a need to develop food with high levels of protein and fiber (Sandjaja et al., 2013). High-nutrient snacks must be added to the diet of Indonesian school-age children to improve nutritional condition. Instant cereal drinks are a type of ready-to-eat product consumed as an additional food consisting of cereals and milk. These beverages have grown in popularity as a snack option for school-age children due to the high source of essential nutrients (Santos et al., 2021). Previous studies showed that cereal drinks are associated with a high-quality diet rich in essential minerals, lipids, fiber, and protein (Patra et al., 2023).

Sorghum wholegrain is a gluten-free, nutrient-rich grain that contains dietary fiber, slowly digesting starches and polyphenols (Tapsell et al., 2020). With 80.42% carbohydrates and 6-8% dietary fiber, this grain is an excellent source of carbohydrates and healthy minerals for beverages (Thilakarathna et al., 2022). Adding protein-rich ingredients, such as tempeh (well-known fermented soybeans) can enhance the protein content of instant cereal drinks. A previous study showed that the *Rhizopus oligosporus* used to produce tempeh triggered enzymatic activity, which imparted superior nutritional value to fermented soybeans. Compared to non-fermented soybeans, the action of enzymes could aid in breaking protein into more soluble forms advantageous to the intestines (Ketnawa & Ogawa, 2021).

Previous studies have used different sorghum and tempeh flour ratios in preparing snack products.

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Novidahlia et al. (2020) reported that ready-to-drink cereal prepared from sorghum and tempeh flours could be used to produce a high-protein and is a source of fiber beverage, where a ratio of 20% sorghum flour and 10% tempeh was the most well-accepted among consumers. A sensory consumer study showed that cereal bars with lower percentages of tempeh flour were more readily accepted (de Melo et al., 2020). Furthermore, addition of sorghum to snack products might alter the texture and possibly decrease customer preference due to the grittiness and an unpleasant mouthfeel (Ervina et al., 2021). According to Sholichah et al. (2021), the incorporation of tempeh flour enhanced the protein content of non-gluten pasta, with the highest level observed at a 15% substitution. Addition of tempeh to snack products can also decrease consumer preference due to the intensification of the products' bitter flavor (de Melo et al., 2020).

The rise in public awareness of health issues related to diet and the increasing scientific evidence supporting probiotics has led to a surge in demand for probiotic foods (Koirala & Anal, 2021). FAO/WHO (2002) characterizes probiotics as viable microorganisms that impart health benefits to the host when ingested in adequate amounts. One such probiotic, *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13, can be utilized as an additive in milk powder-based instant cereal drinks. Notably, this strain originates from *dadih*, a traditional fermented buffalo milk produced through spontaneous fermentation. This strain is reputed for probiotic characteristics, including resistance to gastrointestinal problems and antibacterial traits. An investigation of its safety proved that the rat model's organs showed no signs of bacterial translocation (Rahayu et al., 2019).

The World Health Organization recommends that probiotic products contain at least 10^6 CFU per milliliter or gram to exert beneficial effects on human health. However, the survival of probiotic microorganisms is strongly influenced by storage conditions, particularly temperature and time. Previous studies have demonstrated that elevated storage temperatures significantly reduce microbial viability (Cabello-Olmo et al., 2020). For example, the viability of *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 in bovine transition milk decreased by more than one log cycle ($p < 0.05$) after 30 days at 25 °C. Nevertheless, the viable cell counts remained above the recommended threshold (>6.5 log CFU mL^{-1}) even after 50 days of storage at the same temperature (Fonseca et al., 2020).

Despite the growing interest in functional foods, probiotic-enriched instant cereal beverages and the effects of sorghum and tempeh flour incorporation on their physicochemical properties have not been extensively investigated. Therefore, the present study aimed to evaluate the influence of varying sorghum-to-tempeh flour ratios in flakes on both physicochemical and sensory characteristics. In addition, this study examined the viability of *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 in probiotic instant cereal beverages stored under different temperature conditions.

METHODS

Materials

The materials used in this study included sorghum flour sourced from the local sorghum farmers in Gunung Kidul, Yogyakarta, Indonesia. Fresh tempeh was obtained from a non-GMO local soybean tempeh producer in Attempe Factory, Yogyakarta, Indonesia. Culture powder of *Lactiplantibacillus plantarum subsp. plantarum Dad-13* was sourced from the Food and Nutrition Culture Collection (FNCC) at the Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta, Indonesia. Margarine, icing sugar, salt, skimmed milk, water, vanilla extract, and maltodextrin, which were utilized as additional ingredients in the probiotic instant cereal drink formulations, were obtained from the local market. Microbiological media were purchased from Merck (Darmstadt, Germany), while all other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA). The equipment used in this study included a cabinet dryer (Kendro, Germany), grinder (Fomac FGD-Z300, Indonesia), analytical balance (OHAUS AR3130, USA), oven (Kirin KBO-600 RA, Indonesia), hand sealer (Powerpack PCS-300 I, Indonesia), texture analyzer (LLOYD Instruments TA Plus, Ametek, UK), chroma meter (CR-400, Konica Minolta, Tokyo, Japan), and other standard laboratory equipment.

Production of Tempeh Flour

The production of tempeh flour was adapted from the method of Astawan et al. (2020). Tempeh was sliced and then blanched in hot steam at 90 °C for 15 minutes. The tempeh slices were dried for eight hours at 60 °C in a cabinet dryer, ground in a grinder, and sieved through an 80 mesh. The tempeh flour was kept at 30 °C in a plastic zipped bag with a silica gel sachet until the product was used for the experiment.

Preparation of Probiotic Instant Cereal Drink

Anandito et al. (2019) provided the method that was used to prepare the flakes for the probiotic instant cereal drink. Flakes were manufactured by weighing the ingredients on an analytical balance and blended, as shown in Table 1. All dry ingredients, such as sorghum flour, tempeh flour, icing sugar, salt, baking soda, vanilla extract, and maltodextrin were mixed. Following the addition of melted margarine, the mixture was kneaded until a smooth and homogeneous dough was formed. The dough was rolled to 1 mm thickness and subjected to oven heating at 110 °C for 60 minutes.

In the preparation of milk powder in instant probiotic cereal drinks, the ingredients in the milk powder composition including skimmed milk, icing sugar, vanilla extract powder, maltodextrin, and *Lactiplantibacillus plantarum subsp. plantarum* Dad-13 were mixed using the dry mixing method. The initial amount of probiotics mixed into the probiotic milk powder composition mixture was 10^9 CFU/g.

At the final stage of preparation, flakes (30%) were combined with milk powder (70%) to produce probiotic instant cereal drinks. The composition of each ingredient used in the formulations is summarized in Table 1. A hand sealer was used to nonvacuum seal the aluminum foil container, which measured 8 × 12 cm and had a thickness of 0.05 mm. The package contained probiotic instant cereal drinks. The size for one serving of flakes in instant cereal drinks was 35 grams, where 30% and 70% of the composition is flakes and mixed ingredients, weighing approximately 10.5 and 24.5 grams, respectively. For one serving, the instant cereal flakes was dissolved in 110 mL of water. The samples were packaged and then kept at the specified temperature for storage.

Table 1. Flake formulation on the probiotic instant cereal drink production

Ingredients	Weight (g)		
Sorghum and tempeh flour ratio in flakes	F1 (5:1)	F2 (2:1)	F3 (1:1)
Flake composition on probiotic instant cereal drink			
Sorghum flour	35.71	28.57	21.42
Tempeh flour	7.14	14.28	21.42
Icing sugar	24.60	24.60	24.60
Salt	0.35	0.35	0.35
Margarine	25	25	25
Baking soda	1	1	1
Vanilla extract	1	1	1
Maltodextrin	5.20	5.20	5.20
Milk powder composition on probiotic instant cereal drink			
Skimmed milk powder	13.72	13.72	13.72
Icing sugar	8.58	8.58	8.58
Vanilla extract	0.05	0.05	0.05
Maltodextrin	1.72	1.72	1.72
Lactiplantibacillus plantarum subsp. plantarum Dad-13	0.27	0.27	0.27

Sensory Evaluation

The sensory assessment was conducted by 120 students, ages 10-12 from Johannes Bosco Primary School, Yogyakarta, Indonesia. Ethical approval for this study was obtained from the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada–Dr. Sardjito General Hospital, Indonesia, under protocol number KE/0033/01/2023. The samples were evaluated for overall liking. Three formulations of probiotic instant cereal drink flakes were presented to panelists in plastic cups, each marked with a unique three-digit label. Alongside, 3 grams of flakes mixed with 30 mL of skimmed milk, a spoon, a cup of water, crackers, and assessment sheets were provided to the panelists. To prevent appreciating things incorrectly, participants were required to pause for around two minutes and rinse the tongues with mineral water after each tasting. A seven-point hedonic scale was applied, ranging from 1 (extremely dislike) to 7 (extremely like), with intermediate scores representing 2 (dislike very much), 3 (dislike slightly), 4 (neither like nor dislike), 5 (like slightly), and 6 (like very much).

Chemical and Physical Characterization

The proximate composition of the probiotic instant cereal drink (moisture, ash, fat, protein, energy, and dietary fiber) was analyzed following the procedures described by the Association of Official Analytical Chemists (AOAC, 2000). The fat content and the moisture were evaluated using the Soxhlet and gravimetric methods, respectively, for six hours at 105 °C. The Kjeldahl method was used to determine nitrogen content, and protein values were obtained by applying a conversion factor of 6.25. Total carbohydrate content was calculated by difference according to the equation: 100 − (moisture + protein + lipid + ash + fiber). Total dietary fiber was analyzed using the multienzyme method and the calorific value was determined using a bomb calorimeter. From the instrument, a gram of the sample was palletized, placed in a crucible sample holder, and then moved to a steel capsule. All samples were analyzed in triplicate.

Flake hardness was evaluated with a Texture Analyzer (LLOYD Instruments, TA Plus, Ametek, UK). Samples were placed over a 3 mm aperture and compressed using a cylindrical probe (2 mm diameter) at a crosshead speed of 10 mm/s. Color parameters (L*, a*, b*) of the flake surface were assessed with a chroma meter (CR-400, Konica Minolta, Tokyo, Japan). The L* coordinate represents lightness (0 = dark, 100 = light), whereas a* and b* correspond to red–green and yellow–blue components, respectively.

Microbial Count

The viability of probiotic cells in the instant cereal drink (solid form) was evaluated using the total plate count (TPC) method during storage at 20, 30, and 37 °C for 56 days. Samples (1 g) were aseptically homogenized in 9 mL of 0.85% NaCl solution to obtain a 10^{-1} dilution. From this suspension, 1 mL was transferred into 9 mL of 0.85% NaCl to prepare a 10^{-2} dilution, followed by subsequent serial dilutions as required. A 1 mL sample from the final three dilutions was used to inoculate the MRS agar in petri plates. After 48 hours of incubation at 37 °C, plates with 25–250 colonies were counted. Colony counts were expressed as logarithmic colony-forming units per gram (log CFU/g). The number of viable cells was calculated according to Equation (1), following the Food and Drug Administration guidelines (2001). In this equation, S.I. stands for the total viable count, n1 and n2 are the numbers of plates counted in the lower and higher dilutions, respectively, while d is the dilution factor corresponding to the lower dilution. There are ∑C colonies total across all plates on Equation 1.

$$S.I = \frac{\Sigma C}{(1\ x\ n1)+(0{,}1\ x\ n2)xd} \quad (1)$$

Statistical Analysis

The means ± standard deviation (SD) of each data set were presented in triplicate. All statistical analyses were conducted with IBM SPSS Statistics software, version 26.0 (IBM Corp., Armonk, NY, USA). The sensory evaluation used the Kruskal Wallis and Mann-Whitney tests to determine the relationship between the probiotic instant cereal drink formulations and the degree of preference by the panelists. Data from physicochemical and viability analyses were subjected to one-way ANOVA. When significant differences were observed, Duncan's multiple range test was applied to separate means. A probability value of $p < 0.05$ was regarded as statistically significant.

RESULTS AND DISCUSSION

Sensory Evaluation

Sensory attributes of the probiotic instant cereal drink were evaluated using a hedonic test to assess panelists' preferences. Figure 1 shows the results of the relevant hedonic test and the sensory assessment of the probiotic instant cereal drink. Analysis of overall acceptance revealed that the formulations exhibited significant differences ($p < 0.05$) depending on the sorghum-to-tempeh flour ratio used in the flakes. The panelists favored the F2 formulation, consisting of a sorghum and tempeh flour ratio of 2:1 over F1 (5:1) and F3 (1:1) based on sensory evaluation. In this study, the hedonic test was only carried out based on the "Overall" parameter because children still have limited memory ability in remembering the flavor of the product in the sensory test and minimal linguistic abilities. The mean score of 6.28 showed a strong preference for the F2 formulation. Furthermore, judges' endorsement of the cereal diminished as the tempeh flour added rose due to the more astringent flavor at higher concentrations. These results align with several studies that have examined the influence of tempeh flour addition on the quality attributes of snack foods. The lower scores were attributed to the flavor attribute of tempeh flour-added cereal bars, due to the bitter taste (de Melo et al., 2020). Isoflavones release a bitter flavor in food, which stems from alterations in isoflavone glycoside compounds to aglycones during tempeh drying. When higher tempeh flour was added to the flakes, the color became deeper, thereby reducing panelists' tolerance for color characteristics. According to Purnasari et al. (2022), panelists favored tempeh probiotic snack bars

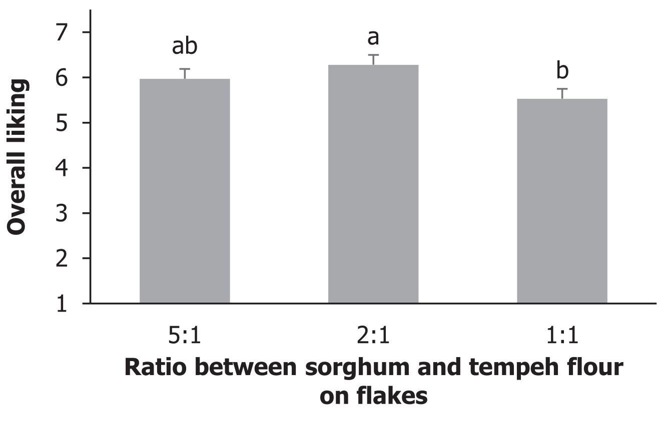

Figure 1. Total acceptability of probiotic instant cereal drink with variation flakes ratio between sorghum and tempeh flour. Different superscript letters (a, b) represent significant differences ($p < 0.05$).

in light brown hues, which stem from lower amounts of tempeh flour. As more tempeh flour was incorporated, the shade darkened. Heating causes non-enzymatic browning, leading to colour variations

Ervina et al. (2021) demonstrated a decline in liking scores, from 6.80 at 0% sorghum substitution to 5.54 at 100%, indicating that higher levels of sorghum flour reduced consumer acceptance. This result showed that adding sorghum to biscuit dough had a significant impact on customer approval. Additionally, sorghum flour can alter the textural characteristics of biscuits and potentially reduce customer preference due to its gritty and harsh mouthfeel. The formulation adopted in this study was developed with reference to the method of Novidahlia et al. (2020). The beverage made from sorghum and tempeh flour comprised 20% sorghum and 10% tempeh flour, respectively.

Chemical properties

The physicochemical characteristics of probiotic instant cereal drinks are shown in Table 2. These characteristics were examined to ascertain the effect of the ratio of sorghum and tempeh flours in flakes on the qualities of probiotic instant cereal drinks.

The chemical characterization was conducted to determine water, ashes, protein, lipid, total carbohydrate, total dietary fiber, and total energy. The result showed that the proximate values meet the Indonesian National Standard i.e maximum of 3% water content, 4% ashes, and at least 5% protein, 7% lipid, and 60% carbohydrate.

Based on Table 2, the protein content of probiotic instant cereal beverages obtained in this study ranged from 14.51% to 16.35%. The higher protein level was attributed to the incorporation of tempeh flour, which is rich in protein, and was comparable to the findings of Novidahlia et al. (2020), who reported values between 11.34% and 20.28% depending on the sorghum–tempeh flour ratio ($p < 0.05$). Importantly, the protein content of the developed beverages exceeded the minimum requirement for cereal milk set by the Indonesian National Standard (SNI), which is 5%. Moreover, the product qualified as a protein source according to both Indonesian Food and Drug Authority regulations (≥12

Table 2. Physical properties, proximate composition, and energy values of probiotic instant cereal drinks produced with different sorghum and tempeh flour ratios on flakes

Parameter	Flake formulation on probiotic instant cereal drink		
	F1 (5:1)	F2 (2:1)	F3 (1:1)
Physical properties			
Color L*	46.37 ± 0.59[a]	41.22 ± 0.33[b]	30.94 ± 0.50[c]
Color a*	4.32 ± 0.65[a]	4.98 ± 0.17[a]	6.90 ± 0.91[b]
Color b*	22.88 ± 0.52[a]	25.66 ± 0.49[b]	27.34 ± 0.70[c]
Hardness (N)	245.97 ± 18.79[a]	170.30 ± 13.26[b]	126.66 ± 13.57[c]
Milk absorption (%)	29.52[a]	35.83[b]	40.83[c]
Chemical properties and energy values			
Moisture (%)	2.96 ± 0.02[a]	2.78 ± 0.03[b]	2.64 ± 0.02[c]
Ash (%)	2.63 ± 0.01[a]	2.86 ± 0.08[b]	2.99 ± 0.10[b]
Protein (%)	14.51 ± 0.13[a]	15.63 ± 0.11[b]	16.35 ± 0.05[c]
Fat (%)	8.27 ± 0.03[a]	7.71 ± 0.03[b]	7.37 ± 0.01[c]
Carbohydrate (%)	71.37± 0.26[a]	70.99 ± 0.22[ab]	70.87 ± 0.07[b]
Fiber (%)	10.65 ± 0.06[a]	8.82 ± 0.02[b]	6.48 ± 0.05[c]
Energy (kcal/100 g)	413.84[a]	414.55[b]	432.01[b]
Total sugar (%)	58.64 ± 0.14[a]	57.31 ± 0.17[b]	56.49 ± 0.25[c]
Sodium (mg/100 g)	184.38 ± 1.22[a]	186.23 ± 0.12[b]	188.00 ± 0.47[c]

* The results of the proximate composition were the average of three repetitions and were presented in mean ± SD (standard error). Means with different letters (a, b, c) within a row are significantly different ($p > 0.05$).

g/100 g protein) and Codex Alimentarius standards (≥5 g/100 g protein) (BPOM, 2016; Lewis, 2019). This outcome is favorable given the critical role of dietary protein in supporting structural and regulatory functions of the body.

Based on the Indonesian Food and Drug Authority regulation, products containing ≥3 g dietary fiber per 100 g qualify as a fiber source (BPOM, 2016). With 6.48-10.65% dietary fiber per 100 g, all formulations of probiotic instant cereal drinks were outstanding fiber sources, as shown in Table 2. The high fiber content in all formulations results from sorghum flour, which was used to produce the cereal flakes. This value is similar to the statement of Devi et al. (2013) that a high-protein extruded snack made from sorghum flour fiber delivers a dietary fiber range of 7.01 to 13.91. The probiotic instant cereal drink represents a nutrient-dense product, with its high fiber content playing a beneficial role in gastrointestinal health.

Table 3. Nutrient profile of selected formulation of probiotic instant cereal drink and commercial instant cereal drink

Nutrient	Product	
	Selected formulation of probiotic instant cereal drink	Commercial instant cereal drink
Protein		
(g)	5	1-3
(%RDA)	9	1-5
Total dietary fiber		
(g)	3	1-3
(%RDA)	10	3-6
Carbohydrate		
(g)	24	23-27
(%RDA)	8	7-8
Fat		
(g)	2	1-3.5
(%RDA)	4	1-5
Energy		
(kkal)	145	120-150
(%RDA)	7	6-7
Total sugar		
(g)	20	8-20
(%RDA)		
Sodium		
(g)	66	40-160
(%RDA)	4	1-10

Based on the organoleptic and proximate analyses, the optimal formulation of probiotic instant cereal drink was obtained from the F2 treatment, which consisted of a sorghum-to-tempeh flour ratio of 2:1. The resulting cereal flakes were designed to meet approximately 10% of the daily nutritional requirements of primary school children. A 35 g serving provides 5.47 g protein, 2.69 g fat, 24.84 g carbohydrates, 3.08 g dietary fiber, and 145 kcal. Table 3 compares the nutrient profiles of the commercial and probiotic quick cereal drinks to the Indonesian RDA, following the nutritional labeling rules of The National Agency for Drug and Food Control. The RDA reference requirement values were computed based on a diet of 2,150 calories. The commercial instant cereal and the probiotic instant cereal drink could contribute 7% of the required daily energy intake, owing to the equivalent calorie contents. As shown in Table 3, the probiotic instant cereal drink contained 4% higher protein and 10% higher total dietary fiber than the commercial.

Physical Characterization

The color of the flakes is a key element that customers consider when evaluating the final quality of the product. According to the result in Figure 2, the flakes with the greatest concentration of tempeh flour (formula F3 (1:1)), received the lowest sensory test score. This was due to the Maillard reaction during baking, resulting in darker flakes as the concentration of tempeh flour increased. The cooking process influences the Maillard reaction and affects both sensory and nutritional aspects. This study revealed significant differences in the L*, a*, and b* values of the flakes in probiotic instant cereal drinks ($p < 0.05$) (Table 2). These variations can be attributed to the relatively brighter color of sorghum flour compared to tempeh flour, resulting in a lighter appearance with higher proportions of sorghum flour. This finding is in line with Nicole et al. (2021), who reported that tempeh-based crackers undergo more pronounced darkening due to Maillard reactions associated with their higher protein content.

The most important quality factor that determines the tenderness of the product is its hardness. According to the data in Table 2, the hardness of flakes increased by adding more sorghum flour to the flakes. Products

(a) (b) (c)

Figure 2. Effect of the ratio of sorghum and tempeh flour on the color of flakes (a) F1 = 5:1, (b) F2 = 2:1, and (c) F3 = 1:1 sorghum:tempeh flour.

Table 4. Changes in probiotic *Lactiplantibacillus plantarum subsp. plantarum* Dad-13 viability of probiotic instant cereal drink during storage at different temperatures for 56 days

Storage time (days)	Viability (Log CFU/g)		
	37 °C	30 °C	20 °C
0	9.55 ± 0.01^{Af}	9.55 ± 0.01^{Ag}	9.55 ± 0.01^{Af}
7	8.89 ± 0.29^{Ae}	9.13 ± 0.10^{Af}	9.2 ± 0.04^{Ae}
14	8.50 ± 0.15^{Ad}	8.59 ± 0.11^{Ae}	8.79 ± 0.21^{Ad}
21	8.07 ± 0.11^{Bc}	8.54 ± 0.05^{Ae}	8.63 ± 0.09^{Ad}
28	7.77 ± 0.05^{Bb}	8.49 ± 0.02^{Ae}	8.58 ± 0.04^{Ad}
35	7.69 ± 0.04^{Cb}	8.22 ± 0.17^{Bd}	8.45 ± 0.08^{Ad}
42	7.66 ± 0.04^{Cb}	8.02 ± 0.03^{Bc}	8.45 ± 0.01^{Ac}
49	6.56 ± 0.09^{Ca}	7.87 ± 0.02^{Bb}	8.18 ± 0.03^{Ab}
56	6.47 ± 0.08^{Ca}	7.53 ± 0.03^{Ba}	7.81 ± 0.04^{Aa}

Note: Data are the mean of three replications ± standard deviation. Data followed by different lowercase letters in the same column indicates significantly different data at the 0.05 significance level relative to storage time at the same storage temperature. Data followed by different capital letters on the same line indicates significantly different data at the 0.05 significance level regarding temperature treatment at the same storage time.

with non-gluten flour substitutes, such as sorghum flour will produce a texture that is not hollow and not too fluffy, ensuring less crunchiness. This leads to a decrease in the fracturability value of the flakes. The non-gluten flour will produce food products with a higher hardness level. Similar results were reported by Brites et al. (2019), indicating that increasing the proportion of high amylose millet flour in cookies led to higher hardness values. Moreover, higher millet flour levels were related to the hardness values of cookies due to the dough spreads during cooking. Similarly, sensory analysis conducted by elementary school-age children so that panelists did not like flakes with high level of hardness.

Probiotic Cell Viability

Elementary school-aged children preferred the probiotic instant cereal drink formulation (F2; 2:1), which were used for microbiological testing every seven days stored at 20, 30, and 37 °C for 56 days. The viability of *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 was evaluated during storage (Table 4).

In the probiotic instant cereal drink, *Lactiplantibacillus plantarum subsp. plantarum Dad-13* showed an initial cell count of 9.55 log CFU/g, as confirmed by the viability test. Storage conditions significantly affected ($p < 0.05$) the survival of cells over 56 days (Table 4). A gradual decline in viable counts was observed under all conditions, with the highest viability (7.81 log CFU/g) maintained at 20 °C compared to 30 °C and 37 °C. These findings are consistent with Fonseca et al. (2020), who reported that fermented transition milk stored at 25 °C preserved the viability of *L. plantarum Dad-13* for up to 50 days. Similarly, Borges et al. (2016) observed a decline in the viability of *L. plantarum* 299v during three months of storage in fruit-based powders. Despite the reduction, the viable cell counts in the current study remained above the minimum probiotic threshold when stored at room temperature.

The viability of probiotic powder decreases with the increase storage temperature. Elevated storage temperature accelerates the metabolism rate of lactic acid bacteria, causing a lag, an exponential, stationary phase, and then entering the death phase during storage (Panuh & Sakulrat, 2022). Bacteria cell dehydration occurred during heat storage, inflicting osmotic shock and lysis. High temperatures damage various structures within cells, including cell membranes, ribosomes, DNA, RNA, and enzymes (Raza et al., 2021). In this study, factors related to storage temperature were responsible for the decline in probiotic cell viability. Storage conditions are important for dried cells to remain stable. A previous study suggested that dried cultures should be kept in dark environments, with controlled water activity, or under vacuum. After 56 days of storage at 20, 30, and 37 °C, probiotic cell viability remained above 6 log CFU/g at all temperatures. However, the cell viability remained at the recommended level by FAO/WHO (2002) (>10^7 CFU/g) to have beneficial effects as a probiotic.

CONCLUSION

In conclusion, the various ratios of sorghum and Tempeh flour in flakes in probiotic instant cereal beverages affected sensory and physicochemical characteristics. Panelists preferred flakes with sorghum:tempeh flour ratio of 2:1 (F2). Compared to conventional instant cereal drinks, the flakes with the selected sorghum-to-tempeh flour ratio exhibited higher protein and fiber contents. Moreover, the probiotic instant cereal drink maintained cell viability above 6 log CFU/g at all storage temperatures after 56 days, thereby meeting the FAO/WHO recommended threshold for conferring probiotic health benefits.

CONFLICT OF INTEREST

The authors declare that the results are original and have not been published before, and no conflicts of interest were declared.

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