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RESEARCH

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Development and characterization of high-protein flakes made from *Spirulina platensis* in instant cereal drinks enriched with probiotic milk powder

Bintang Efrata Aprilia[1], Dwi Larasatie Nur Fibri[1*] and Endang Sutriswati Rahayu[1,2,3]

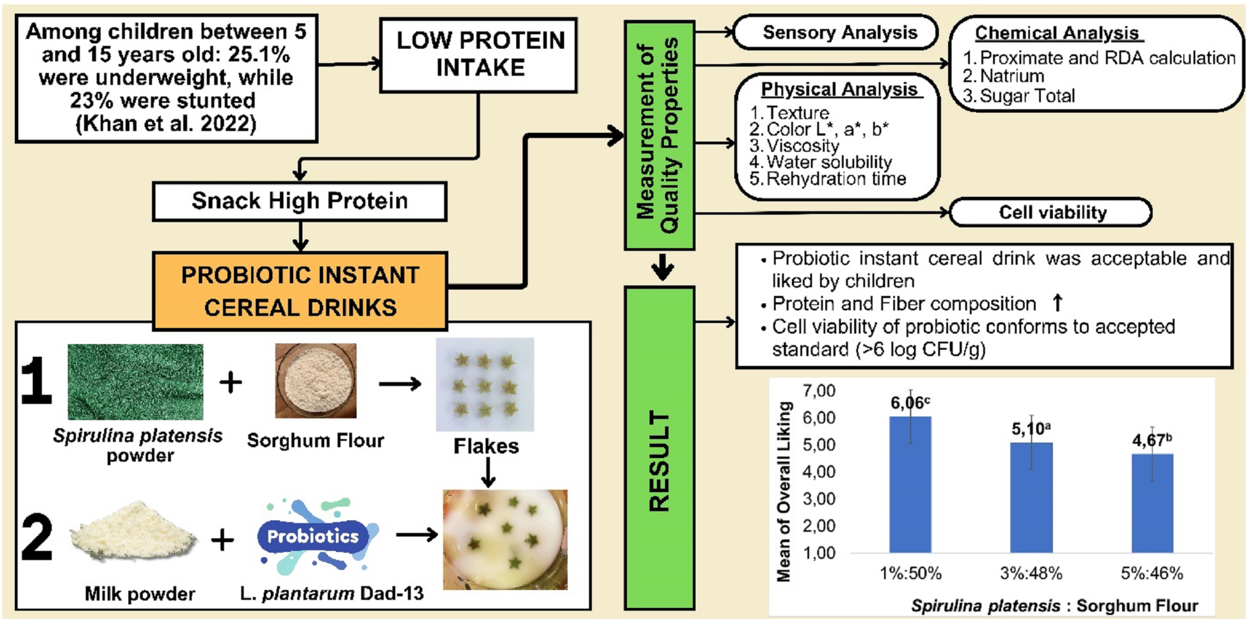
Abstract

Children aged 5 to 12 years fail to meet the necessary amount of protein requirements. This condition can lead to microbial imbalances and may interfere with the absorption of nutrients that are not optimized. There is a need for a convenient high-protein snack with probiotics. Therefore, this study developed a high protein instant cereal drink made from *Spirulina platensis* and probiotics of *Lactiplantibacillus plantarum* Dad-13. To improve the nutritional value, sorghum flour was added. This study aims to assess the physicochemical and sensory properties of a probiotic cereal drink, as well as the survival of probiotics throughout its shelf life. This study used a non-factorial completely randomized design with three replicates of experiments. The factor of the treatment was the percent ratio of flakes between *Spirulina platensis* and sorghum flour (1:50, 3:48, and 5:46%). The viability of probiotics was evaluated at three storage temperatures (20, 30, and 37 °C). Based on the results, the ratio of *Spirulina platensis* and sorghum flour resulted in significantly different levels of preference (overall liking) and physicochemical characterization ($p < 0.05$). *Spirulina* 1%:50% sorghum flour is the chosen formula because it is the most preferred. This product has the crispiest texture and the brightest color. It can be a suitable snack for children due to it can be a source of protein (12.56 g/100 g) and fiber (4.68 g/100 g). Lowering the storage temperature may slow the rate of viability loss. This study showed that children like the developed high-protein instant cereal drinks. The viability of the probiotic cells conforms to the standard, indicating the potential to provide health benefits.

Keywords Cereal drink, Probiotic, *Lactiplantibacillus Plantarum* Dad-13, Sorghum flour, *Spirulina platensis*

*Correspondence:
Dwi Larasatie Nur Fibri
dwifibri@ugm.ac.id
Full list of author information is available at the end of the article

Graphical Abstract



Introduction

Amongst children aged 5–15 years in developed countries, it is revealed that dietary intake patterns do not meet the recommended allowance. As many as 25.1% were underweight and 23% were stunted. Insufficient protein consumption is known to have an impact on children's nutritional status. Lower protein consumption has been observed in stunted children than in normal children (Khan et al., 2022; Ernalia et al., 2018). Nutrient intake can be met through the consumption of snacks, which contribute to 20% of daily nutrition. However, there is evidence of higher snack consumption in individuals with inadequate nutrition (33.8 g/day). This is due to the availability of snacks that are high in energy but poor in nutrients (Kamil et al., 2021). It follows that there is a need for a convenient high-protein snack.

One snack that is currently being developed is an instant cereal drink. Cereal consumption in developing nations has risen annually from 180 to 800 million tons and it is projected to persist with an upward trend until 2030 (Nakuja, 2016; OECD, FAO 2021). Sorghum, a type of cereal that is gluten-free and it is nutritionally similar to wheat. It contains higher levels of fiber and ash compared to wheat (Jocelyn et al., 2020). However, cereal is acknowledged to be rich in carbohydrates with low quality protein due to limitations in the amounts of essential amino acids, notably, lysine (Shewry, 2007). This insufficiency can be supplemented by adding other ingredients, which are also protein-rich with a considerable amount of essential amino acids (Mbaeyi-Nwaoha & Onweluzo, 2013).

Another protein source that can be added is microalgae. *Spirulina platensis* is one of the microalgae that can provide protein because it has the highest protein content (55–70%) and contains all essential amino acids (Jung et al., 2019). Several studies have incorporated *Spirulina platensis* into food products such as chocolate, cookies, and noodles due to its potential benefits (Sahin, 2019; Ersyah et al., 2022). However, *Spirulina platensis* has an unpleasant flavor and color that can affect the sensory and consumer acceptance of the product. Incorporation of *Spirulina platensis* above 5% in instant noodle products results in sensory attributes such as flavor and color that are less preferred by panelists due to their unpleasant sensory attributes (Ersyah et al., 2022). Therefore, it is important to formulate *Spirulina platensis* in instant cereal drink snack products to determine the sensory preferences of children.

High protein in food must be accompanied by a healthy digestive system to optimize nutrient absorption, because nutritional imbalances can lead to gastrointestinal. Therefore, improving the digestive tract with probiotics is essential. Probiotics are living microorganisms that can provide health benefits to the host when consumed in sufficient quantities (> 10^6 CFU/g) (FAO/WHO, 2002). *Lactiplantibacillus plantarum* Dad-13 is a local probiotic isolated from spontaneous fermentation of buffalo's milk (dadih) from West Sumatra. This probiotic is safe because it has gone through safety tests and found no bacterial translocation in rat organs (Rahayu et al., 2019). A previous study has shown that giving *L. plantarum* Dad-13 to food can provide better nutritional status (Kamil et al., 2022). Furthermore, probiotic survival depends on storage conditions, such as time and temperature. Overall, high temperatures have a significant impact on the viability of probiotics (Jannah et al., 2022).

No studies have been conducted on the formulation of probiotic instant cereal drink made from sorghum flour and *Spirulina platensis* enriched milk probiotic. Therefore, the aims of this study were to evaluate the probiotic instant cereal drink formula based on sorghum flour with *Spirulina platensis* which is the most preferred by children, evaluate the nutritional value of the product, and evaluate the viability of probiotic L. *plantarum* Dad-13 during storage at various temperatures.

Materials and methods

Raw materials

Sorghum flour is manufactured by Abah Sorghum, which is situated in West Java, Indonesia. *Spirulina platensis* was acquired from Algaepark Indonesia Mandiri, Co. (Yogyakarta, Indonesia), which possesses a Certificate of Analysis bearing the number 31,722,327. Additional components utilized include icing sugar, sodium chloride, margarine (Blue Band), cocoa powder (Windmolen), liquid milk (Indomilk chocolate variant), a citric acid solution (0.83%) from Cap brand, maltodextrin (food grade), skim milk powder (Lactona), vanilla extract (Koepoe-Koepoe), and sodium bicarbonate (Koepoe-Koepoe). The microbial strain employed was Lactiplantibacillus plantarum Dad-13, sourced from the Food and Nutrition Culture Collection (FNCC) at the Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta. The microbiological assessment was conducted utilizing De Man Rogosa and Sharpe (MRS) agar obtained from Merck (Darmstadt, Germany).

Production of probiotic instant cereal drinks

The methodology employed in the fabrication of instant cereal drinks encompasses the refinement of various antecedent research techniques, as evidenced by the work of Letras et al. (2022) and Batista et al. (2017). The preliminary phase involves the creation of cereal flakes, wherein a combination of sorghum flour (50, 48, 46%) and *Spirulina platensis* (1, 3, and 5%) is amalgamated with additional dry constituents: icing sugar (21%), sodium chloride (0.2%), vanilla extract (0.1%), and sodium bicarbonate (0.2%). Subsequently, margarine (16%), a citric acid solution (4.5%), and liquid milk (7%) are incorporated into the blend, which is agitated until a homogeneous mixture is achieved. The resulting dough is then compressed using a roller to attain a thickness of 0.03 cm, shaped with a plastic mold, and subjected to baking in an oven (Kirin, model KBO-200RAB, Indonesia) at a temperature of 110 °C for 35 min. Subsequently, the flakes are permitted to cool at room temperature for a period of 5 min. The subsequent phase is dedicated to the synthesis of powdered milk. A dry mixture is prepared comprising skim milk (42.42%), sucrose (35%), maltodextrin (8.00%), sodium chloride (0.2%), vanilla (0.10%), and *Lactiplantibacillus plantarum* Dad-13 powder (10^9 CFU/g) (14.28%). Subsequently, the flakes and powdered milk are combined in a ratio of 30:70. A single serving of the instant cereal beverage is quantified at 32 g. To prepare one serving, it is required to combine 32 g of the product with 110 mL of cold water (25 °C).

Sensory analysis

Prior to the commencement of the research, approval was obtained from the Ethics Commission Committee within the Faculty of Medicine, Public Health, and Nursing at Universitas Gadjah Mada. The ethical clearance protocol is designated as KE/0033/01/2023 and is in alignment with the principles outlined in the Declaration of Helsinki. The institutional approval number was KE/FK/0211/EC/2023. The consumer evaluation is designed for untrained participants between the ages of 10 and 12, specifically students from SDN Bhayangkara in Yogyakarta. A total of 120 participants were recruited for this study, comprising 61 males and 59 females. Subsequently, the panelists will provide their evaluations via a form incorporating an image scale. The image scale comprises seven distinct levels, as outlined below: The scale employs the following categories: 1 (extremely dislike), 2 (dislike very much), 3 (dislike slightly), 4 (neither like nor dislike), 5 (like slightly), 6 (like very much), and 7 (extremely like). The evaluation pertains to the overall preference for the instant cereal drinks under investigation, which consist of flakes and probiotic milk powder dissolved in water. In order to be eligible for inclusion in the study, participants must be between the ages of 10 and 12, reside in Yogyakarta City, possess good physical and mental health, be able to consume milk-based products, and obtain parental consent along with signed informed consent to participate in the study. Individuals with impaired taste perception will be excluded from the study.

Chemical analysis
Chemical analyses, including proximate composition, sodium content, and total sugars, were conducted on both the flakes and the probiotic milk powder. The proximate analysis was executed in accordance with the guidelines established by the Association of Official Analytical Chemists (AOAC, 2005). This particular analysis pertains to probiotic instant cereal drink, encompassing both flakes and probiotic milk powder. The moisture content was assessed using the gravimetric method, while protein content was determined via the Kjeldahl method (which involves measuring nitrogen content and multiplying by a factor of 6.25). Fat content was evaluated through the Soxhlet extraction method, and dietary fiber along with total caloric content was measured utilizing a bomb calorimeter. The carbohydrate content was calculated by difference (100 – ∑other components). Sodium concentration was determined through atomic absorption spectrophotometry ($\lambda = 589$ nm). The total sugar content was analyzed using the DNS method as outlined by Rezaei et al. (2020).

Recommended dietary allowance (RDA) calculation
Upon determining the proximate composition and sodium content of probiotic instant cereal drinks, the resultant data is employed to compute the percentage of the Recommended Dietary Allowance (RDA) in children aged 10 to 12 years, in accordance with the Nutrition Label Number established by the Indonesian Food and Drug Monitoring Agency Number 9 (BPOM, 2016). The percentage RDA for the demographic group aged 7 to 12 years pertains to the general classification category. The daily nutritional requirements for each individual are quantified as 2150 kCal of energy, 60 g of protein, 67 g of fat, 325 g of carbohydrates, 30 g of dietary fiber, and 1500 mg of sodium.

Physical analysis
The research undertaken involved numerous physical analyses, which were categorized into distinct groups. Analyses pertaining to color and texture were exclusively executed on the flake samples. Color metrics (L*, a*, b*) were evaluated utilizing a Chromameter (CR-400, Konica Minolta, Tokyo, Japan). The lightness is denoted by the (L*) metric, which spans from dark to light (0-100). The intensity of the red-green chromaticity is represented by the (a*) metric. The intensity of the yellow-blue chromaticity is represented by the (b*) metric. The texture analysis was conducted employing a texture analyzer (LLOYD Instruments, TA plus AMETEK, UK), calibrated to a testing velocity of 0.5 mm/s, compressing the sample to 50% of its height and wait time 0.5 s. Tests regarding water solubility and rehydration duration were executed on the probiotic milk powder specimens. The water solubility assessment for probiotic milk powder was conducted in accordance with the protocols established by the Association of Official Analytical Chemists (AOAC, 1995). The rehydration duration was ascertained by mixing the sample with 110 mL of water at 25 °C. The rehydration duration was recorded from the moment the water was introduced until the mixture transformed into a probiotic cereal beverage. This evaluation is intended to categorize the product as either an instant serving (Novidahlia et al., 2020). The viscosity assessment was performed on the probiotic milk powder specimens subsequent to their dissolution in water. The viscosity evaluation employed a Brookfield DV2T viscometer (60 rpm, temperature 25 °C), with spindle Lv-1 (61).

Cell viability
Cell viability test refers to Jannah et al. (2022) with modifications of temperatures and storage time. The cell viability of the probiotic instant cereal drink was assessed using the total plate count method during storage at varying temperatures of 20 °C, 30 °C, and 37 °C for 63 days. The probiotic instant cereal drink samples were serially diluted at a concentration of 0.5 gram into 4.5 mL of 0.85% NaCl, resulting in a 10^1 dilution, then dilute to 10^8. Using the pour plate technique, 0.1 mL from the last three dilutions was inoculated in MRS agar. Following this, the medium was incubated at 37 °C for 48 h, Colonies that can be included in the calculation are colonies that are in the number of 25–250 colonies. Colonies that were counted were represented as log CFU/g.

Statistical analysis
The resulting product data is processed using the Statistical Product and Service Solution (SPSS) program 22. The statistical test used for sensory analysis was by using Kruskal-Wallis, while for chemical, physical and viability analysis using a test of variance (ANOVA) to determine whether the treatment used in the study had a significant effect. If the value of $p < 0.05$, the treatment had a significant effect and continued with the *Mann-Whitney* test for sensory analysis and *Duncan* for chemical, physical and viability analysis at 95% confidence interval ($\alpha = 0.05$).

Results and discussion
Sensory analysis
The attribute used in the sensory analysis is the overall acceptance of the product by the panelists. The reason for choosing a single attribute is the low sensitivity in children and their cognitive abilities are limited, restricting to focus on one aspect at a time (Guinard, 2001). Figure 1 shows that the ratio of *S. platensis* and sorghum flour resulted in significantly different levels of preference

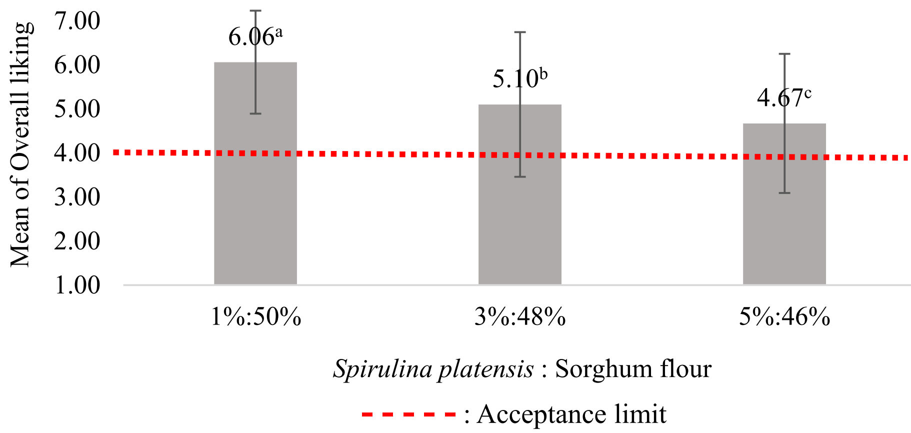


Fig. 1 The preference of instant cereal drink. Different letters mean significantly differences ($p < 0.05$)

($p < 0.05$). Increasing the amount of *S. platensis* while decreasing the amount of sorghum flour resulted in a product that was less preferred by children. However, the three formulas have an average value of overall liking above the acceptance limit value (>4).

The acceptance level of a product is influenced by taste, appearance (color), and texture (Murray, 2003). According to Hoffman et al. (2016), children have a preference for the sweet taste of food. This preference is particularly high among 8-11-year-olds. Increasing the amount of *Spirulina platensis* in the product may increase the bitterness and mask the sweetness of the product, this can potentially affect its acceptability (Ghaly et al., 2015). The bitter taste is due to several bitter amino acids (BAAs) present in *S. platensis*, such as arginine, histidine, methionine, and tryptophan (Siahbalaei et al., 2021). Therefore, increasing the amount of *S. platensis* results in higher levels of these BAAs, resulting in a more bitter flake. It was suspected that there was a decrease in children's acceptance of probiotic instant cereal drinks.

The higher amount of *Spirulina platensis* along with the reduction of sorghum flour could enhance the green hue of the flakes, resulting the flakes having a darker shade (Fig. 2). This was evident from a physical analysis of color (Table 1), which the addition of *Spirulina platensis* resulted in an increase in greenness (-a*) and a decrease in lightness (L*) values.

According to Koleoso et al. (2014), children aged 9–12 years prefer bright colors to dark colors in food. Bright colors can create favorable perceptions and positive attributes in food, while dark colors suggest undesirable product characteristics. The unsightly, dark hue of the flakes is thought to adversely affect the attractiveness of the instant cereal probiotic drink aimed at children. *Spirulina platensis* contains several pigments including phycocyanin, which produces a blue-green color (14%), and chlorophyll (1%), which contributes to green color (Jung et al., 2019). Higher amounts of *Spirulina platensis* may enhance the concentration of chlorophyll pigments, thus darkening the green hue of the flakes (Nakib et al., 2019). These findings align with Ziena et al. (2020) that increasing the amount of *S. platensis* in biscuits will decrease the values of (L*) and (b*),but increase the value of (a*). Due to the high levels of sorghum flour present, the green color of the flakes is likely to fade and be replaced by a yellowish cream hue (b*).

Table 1 The average color value and hardness-crispness of flakes in instant cereal drinks

Parameter	S. platensis 1%: SF 50%	S. platensis 3%: SF 48%	S. platensis 5%: SF 46%
L*	36.68 ± 1.60^c	26.33 ± 1.41^b	21.04 ± 0.42^a
a*	-3.03 ± 0.53^b	-3.64 ± 0.37^b	-4.69 ± 0.68^a
b*	16.76 ± 0.82^b	10.22 ± 0.90^a	8.73 ± 1.84^a
Hardness (N)	134.17 ± 6.44^a	213.54 ± 2.38^b	330.04 ± 5.06^c
Crispness	969.74 ± 16.38^a	671.14 ± 16.79^b	356.80 ± 20.78^c

The values are average ± standard deviation ($n = 3$). Different superscript letters (a, b, c) within a row represent significantly different (p<0.05). L*: Lightness, a*: greeness, and b*: yellowness

SF Sorghum flour

Food texture can also influence children's decisions when choosing food. Children aged 9–12 years prefer foods with a crunchy texture. This crunchy texture has a strong influence on the acceptance of food categories such as cereals (Laureati et al., 2020; Chow et al., 2022). Increasing the amount of *S. platensis* while decreasing the amount of sorghum resulted in a harder texture. This result was supported by texture analysis (Table 1), which showed an increase in hardness value and a decrease in crispness in the flakes. According to Supriyadi (2012), the harder a product is, the less crispy

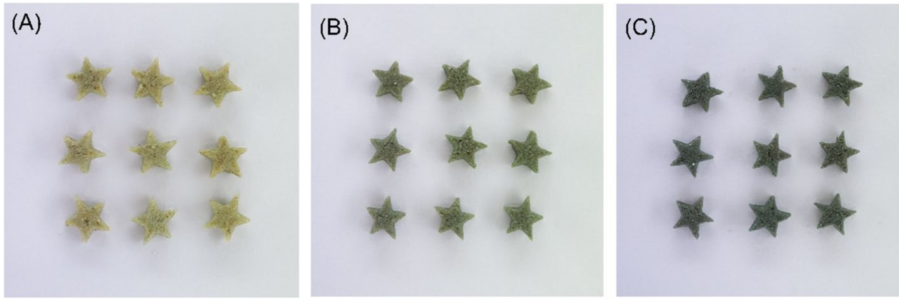


Fig. 2 Percent ratio of flakes *S. platensis*: Sorghum Flour (**A**) 1:50; **B** 2:48; and **C** 5:46%

it will be. These results are in line with the research of Onacik-Gür et al. (2018), that a higher amount of *S. platensis* substituted into cookies will produce a greater hardness and a decrease in the level of liking. Therefore, it is suspected that there is a decrease in the level of acceptance due to the increasingly less favorable texture.

Crispness in dry foods is caused by the formation of air cavities during the expansion process during heating. Starch content, the presence of water, and the gelatinization process play a role in the formation of these cavities. Sorghum flour has a starch content of 70%. Starch gelatinizes during the heating process, weakening the hydrogen bonds that play a role in regulating the structural integrity of the starch granules. The water content is absorbed, causing the starch granules to expand. The perfect gelatinization process will result in optimal swelling and then reduce the level of hardness (Kyaw et al., 2001). *S. platensis* contains hydrophilic proteins. Therefore, as more *S. platensis* is added, the amount of water bound by the protein increases, reducing the amount of water available for the starch to expand during heating. This results in smaller pores in the dough and affects the harder texture (Nuraenah et al., 2022).

Proximate analysis and RDA calculation

The proximate composition of probiotic instant cereal drink is reported in Table 2. The ratio of *Spirulina platensis* and sorghum flour results significant different ($p < 0.05$) for all chemical compositions. The purpose of chemical analysis is to determine the amount of nutrient content of each formula. There are nine chemical compositions.

The amount of *Spirulina platensis* that was increasing, resulted in an increase in the moisture content. This is influenced by the surface of Spirulina powder which has a highly hydrophilic side that binds to water (Cheng et al. 2023). However, the moisture content of all formulas has met the prescribed limit (maximum of 5% w/w) (FAO, 2011). According to Akther et al. (2020) the moisture content in dry products below 8% can be considered ideal because it can prevent the growth of microorganisms in food. The amount of *Spirulina platensis* that getting increased, also resulted in an increase in the ash content. It could be associated that *Spirulina platensis* has a high total mineral content, mainly composed of potassium

Table 2 Chemical composition of instant cereal drinks

Chemical composition	S. platensis 1% : SF 50%	S. platensis 3% : SF 48%	S. platensis 5%: SF 46%
Moisture content (%)	3.14 ± 0.10^a	3.90 ± 0.13^b	4.01 ± 0.14^b
Ash content (%)	2.85 ± 0.04^a	2.99 ± 0.01^b	3.04 ± 0.02^c
Protein content (%)	12.56 ± 0.06^a	14.14 ± 0.15^b	14.99 ± 0.34^c
Lipid content (%)	4.98 ± 0.48^a	5.48 ± 0.15^b	5.51 ± 0.16^b
Carbohydrate (%)*	79.65 ± 0.53^c	77.35 ± 0.21^b	76.45 ± 0.47^a
Dietary fiber (%)	4.68 ± 0.04^a	6.71 ± 0.06^b	8.48 ± 0.04^c
Total energy (kCal)	407.01 ± 5.42^a	403.75 ± 1.76^a	400.28 ± 8.90^a
Sodium (mg/100 g)	530.74 ± 10.94^a	579.46 ± 6.53^b	674.64 ± 62^c
Sugar total (g/100 g)	49.22 ± 2.08^a	59.23 ± 1.07^b	59.78 ± 2.33^b

The values are average ± standard deviation (n=3) and expressed on a dry basis except for moisture content. Carbohydrates are calculated by difference. Different superscript letters (a, b, c) within a row represent significantly different (p<0.05) and calcium (Saharan & Jood, 2017). The ash content has met the requirement of Indonesian National Standard (SNI 01-4270-1996, 1996) maximum of 4% (w/w).

Based on Table 2, it was discovered that the probiotic instant cereal drinks obtained in this study have a protein content ranging from 12.56 to 14.99%. Increasing the amount of *Spirulina platensis* resulted in a greater protein content. This result is similar to that reported by Letras et al. (2022), the incorporation of *Spirulina platensis* up to 5% into cereal products is known to enhance the protein content, with an upward push of 9.87%. This is influenced by *Spirulina platensis* which has high protein content up to 60.4% (CoA number 31722327). The protein content of the three probiotic instant cereal drink formulas has met the minimum standard of 5% and qualified as a protein source food, which is at least 5 g/100 g (Codex, 1997). Furthermore, all three formulas could provide 6–8% of the recommended daily allowance of protein. In contrast, other commercial products only offer a 4% contribution (Table 3). This is a highly favorable outcome because protein plays in the body's fundamental building blocks, regulatory functions.

The addition of *Spirulina platensis* may increase the fat content, but the three formulas have a relatively low-fat content of between 1.5 and 2 g per serving per package. This amount is less than the commercial product that contains 5.4 g of fat in the same number of servings (Table 3). This could be influenced by the use of low-fat ingredients in the instant cereal drink, such as sorghum (5.14–5.46%) (Centeno et al., 2021) and skim milk (Wu et al., 2021). Furthermore, it is acknowledged that the incorporation of milk fat in commercials may have an impact on this. However, the probiotic cereal drink may provide a lower fat snack option.

The carbohydrates are quite high due to the presence of sorghum flour. The main composition of sorghum was carbohydrates (70.07-73.17%) (Pontieri et al., 2022). The carbohydrate content in all formulas is similar to the commercial product and has met the minimum requirement of 60% (SNI 01-4270-1996, 1996) and can provide up to 7–8% RDA (Table 3). As the amount of *Spirulina platensis* increased resulted in greater dietary fiber. This is influenced by the role of *Spirulina platensis* powder, which contains a high dietary fiber content of 13%. The dietary fiber of all formulas is higher than commercial and qualify as dietary fiber source, which are > 3 g/100 g (BPOM, 2022). The fiber content of the probiotic instant cereal drink makes it a nutritious addition to one's diet as it promotes better gastrointestinal function.

The Indonesian Ministerial Regulation on Health (2013) states that sugar consumption in one day is limited to 50 g/person and sodium to 1500–2000 mg/person. The sugar content of all formulas was 15.75–19.13 g/32 g. The probiotic instant cereal drink contains a similar amount of total sugar to commercial products. The total sugar in probiotic instant cereal drink comes from the added sucrose in the formula. This addition is made to enhance the sensory appeal of the product, particularly for children who have a preference for sweet-tasting milk, as noted by Quann and Adams (2013). Additionally, the sugar content is also contributed by the lactose present in skim milk, which accounts for 42.42% of the total sugar content. Based on the suggestion that snacks contribute as much as 20% of the RDA (sodium is around 400 mg/day), so the probiotic instant cereal drink is recommended to be consumed 2 times the serving size.

Physical analysis

Viscosity, solubility and rehydration time

The viscosity of milk in instant probiotic cereal drinks is 6.2000 ± 0.2757 cP at 25 °C. Similarly, commercial skim milk products exhibit a viscosity of 1.5 cP (Bakshi & Smith, 1984). The differences between the results are thought to be influenced by the different fat content between instant cereal drinks and commercial products. According to Bakshi and Smith (1984), skim milk has

Table 3 Nutrition value of probiotic instant cereal drinks (1 serving size = 32 g)

Nutrition	**A**		**B**		**C**		**Commercial**	
Total Energy	**130 kCal**		**129 kCal**		**128 kCal**		**160 kCal**	
	G	**%RDA**	**g**	**%RDA**	**g**	**%RDA**	**g**	**%RDA**
Total fat	1.5	2	2	3	2	3	5.4	8
Protein	4	6	5	8	5	8	2.9	4
Total Carbohydrate	25	8	24	7	23	7	22	7
Dietary fiber	2	5	2	7	3	9	1.9	6
Sugar	15.75		18.95		19.13		19	
Sodium	170 mg	11	185 mg	12	216 mg	14	128 mg	9

A)*S. platensis* 1%:SF 50%; (B)*S. platensis* 2%:SF 48%; and (C)*S. platensis* 5%:SF46%

lower viscosity than whole milk at the same temperature. Increased fat content leads to higher total solids in the solution, which in turn affects the increase in viscosity of the solution. In order to produce better viscosity, these results need to be improved in future studies.

Increased solubility is directly related to improved quality in instant drink products. The probiotic instant cereal drink had 9.43% solubility in cold water with a short rehydration time of 2.73 ± 0.86 s. The solubility and rehydration time of milk in cold water is significantly affected by the fat content of the milk. The greater the fat content in milk, the more challenging it is for the milk to dissolve in cold water. Powdered skimmed milk has the ability to rehydrate within 22 s of wetting, while other milk types take 20 min. It is because skim milk is more comfortable to moisten compared to other milk types because its surface particles have less than 0.03 g/100 g of fat, while in regular milk, it can reach 0.5–3 g/100 g (Wu et al., 2021). With the presence of fat, the surface hydrophobicity of the milk powder product increases significantly. The solubility of the probiotic instant cereal drink is comparable to the commercial product, which has a solubility of 89.96% and meets the reference of ± 85% (Tamime, 2009). Rewthong et al. (2011) report a maximum rehydration time limit of 5 min for instant products. Thus, the probiotic instant cereal drink has convenient presentation with high solubility.

Cell viability

Spirulina platensis 1%:50% sorghum flour was chosen as the preferred instant cereal drink formula. Dana et al. (2021) reported that sensory is the most important factor influencing consumers when choosing food. Cell viability plays a crucial role in determining whether probiotic-based food products can be classified as such FAO/WHO (2002) recommends a minimum of 10^7 CFU/g of probiotics for consumption. Probiotics must be consumed in sufficient quantities to survive in the upper gastrointestinal tract to reach the intestine for the probiotic to have a health effect on its host. Probiotic products are very susceptible to a decrease in cell count during storage, as there are several environmental factors that can affect such as oxygen levels, temperature, water activity, and packaging materials (Palanivelu et al., 2022).

The probiotic viability test in the selected instant cereal drink formula was conducted at three different storage temperatures. This test was conducted to determine the effect of temperature and length of storage on the viability of *L. plantarum* Dad-13 in probiotic instant cereal drinks. The viability of *L. plantarum* Dad-13 cells in probiotic instant cereal beverage products at the end of the storage period at three different temperatures decreased compared to day 0 (Fig. 3; Table 4).

Viability declined the least at a storage temperature of 20 °C, specifically by 0.86 log CFU/g. Conversely, storage at 37°C resulted in a decrease of approximately 1.96 log CFU/g. These findings confirm the assertion by Strasser et al. (2009) that higher storage temperatures can lead to greater reduction in the number of *L. plantarum* cells. Jannah et al. (2022) also explained that the viability of *Lactiplantibacillus plantarum* Dad-13 in instant coffee drinks stored at 37°C showed the greatest decrease at the end of the shelf life. The decrease in cell viability

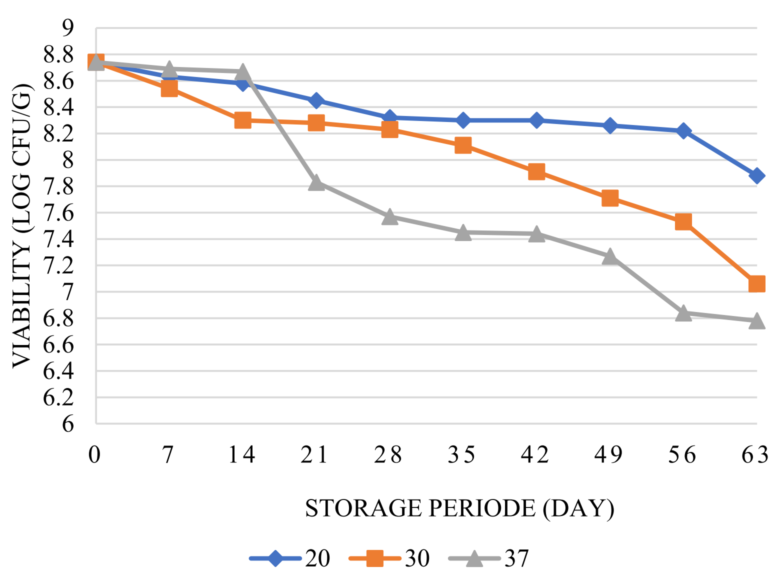


Fig. 3 Viability of probiotic in cereal instant drink after storage

Table 4 Probiotic cereal instant drink viability after storage

Storage Period (day)	Viability (Log CFU/g)		
	20^{o}C	30^{o}C	37^{o}C
0	8.74 ± 0.21^{Ag}	8.74 ± 0.21^{Ag}	8.74 ± 0.21^{Ag}
7	8.63 ± 0.09^{Afg}	8.54 ± 0.12^{Bfg}	8.69 ± 0.18^{Cfg}
14	8.58 ± 0.25^{Af}	8.30 ± 0.02^{Bf}	8.67 ± 0.27^{Cf}
21	8.45 ± 0.15^{Ae}	8.28 ± 0.02^{Be}	7.83 ± 0.04^{Ce}
28	8.32 ± 0.10^{Ade}	8.23 ± 0.22^{Bde}	7.57 ± 0.29^{Cde}
35	8.30 ± 0.09^{Ad}	8.11 ± 0.07^{Bd}	7.45 ± 0.00^{Cd}
42	8.30 ± 0.06^{Acd}	7.91 ± 0.56^{Bcd}	7.44 ± 0.03^{Ccd}
49	8.26 ± 0.11^{Ac}	7.71 ± 0.18^{Bc}	7.27 ± 0.03^{Cc}
56	8.22 ± 0.11^{Ab}	7.53 ± 0.47^{Bb}	6.84 ± 0.01^{Cb}
63	7.88 ± 0.00^{Aa}	7.06 ± 0.00^{Ba}	6.78 ± 0.06^{Ca}

The values are average ± standard deviation ($n = 3$). Different capital superscript letters (A, B, C) represent significant differences ($p < 0.05$) to temperature treatment at the same storage time. Different lowercase superscript letters (a, b, c, d, e, f, g) represent significant differences ($p < 0.05$) to storage time at the same temperature

also occurred in the length of storage time at each storage temperature (Table 4). However, the viability at 20ºC and 30ºC at the end of the storage period was still above 7 log CFU/g.

The presence of oxygen can potentially decrease viability (Jannah et al., 2022). According to Siracusa (2012), increased temperature can lead to the diffusion of oxygen from the environment into the packaging due to increased packaging permeability, thus resulting in an increase in the amount of oxygen within the packaging. *L. plantarum* Dad-13 is a bacterium that grows under microaerophilic conditions and can tolerate low levels of oxygen. Excessive oxygen levels can be harmful, causing cellular damage and eventual death. This is in line with Jannah et al. (2022) findings that vacuum-sealed packaging results in higher viability of probiotic coffee *L. plantarum* Dad-13 compared to non-vacuum packaging after the storage period.

Conclusion

The probiotic instant cereal drink formula that children liked the most was *Spirulina platensis* 1%: 50% sorghum flour with an average preference of 6.06. The nutritional content of the selected formula is 12.56% protein, 4.98% fat, 79.65% carbohydrates and 4.68% dietary fiber. The percentage of RDA for each one serving (32 g) is 4% protein, 2% fat, 8% carbohydrates, 5% dietary fiber, and 11% sodium. The total energy is 130 kCal with a total sugar content of 15.75 g. This instant cereal drink meets the requirements as a source of protein and fiber. *L. plantarum* Dad-13 in instant cereal drinks had viability which was still above 7 log CFU/g at 20^{o}C and 30^{o}C storage, while above 6 log CFU/g at 37^{o}C storage after 63 days of storage.

Abbreviations

RDA	Recommended Dietary Allowance
CFU	Colony-Forming Units
BPOM	The Indonesian Food and Drug Authority
SNI	Indonesian National Standard
SF	Sorghum Flour

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Authors' contributions
The authors performed the following responsibilities. Bintang Efrata Aprilia: Physiochemical, sensorial, viability and statistical analysis, writing original manuscript draft. Dwi Larasatie Nur Fibri: Supervision, proofreading. Endang Sutriswati Rahayu: Supervision, proofreading.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
All procedures performed in studies involving human participants have been approved by the from the Ethics Commission Committee, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, written consent was sought from the panelists partaking in the sensory evaluation of the probiotic instant cereal drink. The panelists were informed that participation was completely voluntary and any participant can decide to withdraw from the research at any time without giving reason for the action.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia. [2]University Center of Excellence for Integrated Research and Application for Probiotic Industry, Universitas Gadjah Mada, Yogyakarta, Indonesia. [3]Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta, Indonesia.

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