Physicochemical, Sensory, and Shelf-Life Characteristics of Probiotic Cream-Filled Snakehead Fish Biscuits with Different Sugars

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ABSTRACT

The growing interest in functional foods has led to the development of novel probiotic-enriched products. Therefore, this study aims to investigate the effect of different sugar types on the viability of *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 and the overall quality of probiotic cream-filled biscuits made from snakehead fish flour. The biscuits, filled with a cream formulated from butter, sugar, vanilla paste, soy lecithin, and maltodextrin, were evaluated as a combined product. Quality assessments included probiotic cell viability, shelf-life estimation, sensory acceptability, texture, and chemical composition under storage at 25 °C, 37 °C, and 45 °C for 35 days. The results showed that cane sugar and low-calorie sugar maintained higher probiotic viability (6.06–6.24 log CFU/g at 25 °C) than coconut sugar. Biscuits containing cane sugar had the longest estimated shelf life (up to 2 months at ambient temperature). In addition, sensory evaluation revealed greater panelist preference for biscuits filled with cream containing cane sugar or low-calorie sugar, with overall acceptability scores of 5.53 and 5.26, respectively. These formulations also resulted in reduced hardness, fat, calorie, and sugar contents, while coconut sugar contributed to higher ash content. The findings suggest that cane and low-calorie sugar are suitable for enhancing the probiotic stability, sensory appeal, and nutritional profile of probiotic cream-filled fish biscuits.

Keywords: Cane sugar; coconut sugar; *Lactiplantibacillus plantarum subsp. plantarum* Dad-13; low-calories sugar

INTRODUCTION

The growing global emphasis on health and wellness has led to a marked increase in the demand for functional food products that offer physiological benefits beyond basic nutrition. Compared to conventional foods, functional foods are designed to deliver scientifically supported health-promoting, preventive, or therapeutic effects, while remaining a part of the regular daily diet (Ghorbani-HasanSaraei et al., 2019; Shahidi, 2022). Among the diverse categories, those incorporating probiotic components have gained widespread popularity in recent years (Khademi et al., 2022).

Probiotics are defined as live microorganisms that, when administered in adequate amounts, provide health benefits to the host (Hill et al., 2014). In addition, their functionality has been linked to immune modulation, suppression of pathogenic intestinal bacteria, improved

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lactose digestion, and reductions in serum cholesterol levels (Peruzzolo et al., 2025). Among the identified strains, *Lactiplantibacillus plantarum subsp. plantarum* Dad-13, originally isolated from traditional buffalo milk curd in West Sumatra, Indonesia, has demonstrated exceptional colonization ability in the human gastrointestinal tract. It also has beneficial properties such as anti-Escherichia coli activity, bacteriocin production, anti-carcinogenic effects, and vitamin B biosynthesis (Manovina et al., 2022; Rahayu et al., 2021). This native Indonesian strain has been successfully incorporated into various fermented food products, including kombucha (Majid et al., 2023), yogurt (Cahyaningati et al., 2024), milk, and ice cream (Saputro et al., 2023), where it has shown positive effects on host health and gut microbiota composition. However, the majority of these probiotic-enriched products remain limited to fermented dairy matrices, which typically require cold chain storage to preserve microbial viability. The reliance on refrigeration presents barriers to distribution, particularly in regions with limited infrastructure. This indicates the growing need to develop shelf-stable, non-fermented probiotic food systems capable of maintaining probiotic viability and functionality at ambient temperatures (Granato et al., 2010).

To overcome the limitation, selecting an appropriate food matrix is essential to ensure probiotic survival in non-refrigerated conditions. Dry, low-moisture products, such as biscuits that offer physical protection from environmental stressors, such as oxygen, dehydration, and temperature fluctuations, are particularly advantageous (Tripathi & Giri, 2014). As widely consumed, shelf-stable products with long ambient shelf lives, biscuits require no refrigeration, are easy to distribute (Ahmad & Ahmed, 2014), and offer a palatable alternative to fermented probiotic foods. Incorporating probiotics into the cream filling of biscuits also presents a novel formulation strategy that provides a dense matrix to shield probiotic cells during storage, enhancing both microbial viability and consumer acceptability. This innovative approach holds significant potential for expanding functional food options in regions with limited access to cold chain logistics.

Despite the potential, ensuring the sensory appeal of such products remains a key consideration. Sweetening agents are not only essential for improving taste and consumer acceptance but also serve functional roles in probiotic formulations (Gómez-Fernández et al., 2021). Sugar influences key physicochemical parameters, such as water activity, osmotic pressure, and membrane stability, thereby affecting the survival of probiotic cells throughout storage (Tripathi & Giri, 2014; Priour et al., 2022). Therefore, the type of sugar used can significantly impact both product quality and microbial stability.

In this study, three types of sugar were selected, including cane sugar (CS), coconut sugar (CoS), and low-calorie sugar (LCS), based on their prevalence in food applications and distinct functional characteristics. CS, primarily composed of sucrose, is widely employed in commercial formulations and serves as a conventional benchmark for comparing alternative sweeteners (García-Almeida et al., 2013). It has been reported to enhance probiotic survival due to molecular interactions with cellular biomolecules and protective effects during dehydration. CoS rich in minerals and possessing a lower glycemic index, offers not only sensory uniqueness but also potential stabilizing effects on probiotic membranes due to its complex carbohydrate structure (Saraiva et al., 2023). LCS, such as stevia, is increasingly utilized to reduce caloric load while meeting consumer health needs. However, the compatibility with probiotic systems remains underexplored (Bellmer, 2019). Despite the frequent use in functional food development, comparative data on the effects of these sugars on probiotic viability, particularly in non-fermented, shelf-stable matrices, such as cream-filled biscuits, remain limited. Therefore, this study aims to develop a functional, shelf-stable biscuit product incorporating *Lactiplantibacillus plantarum subsp. plantarum* Dad-13 in a cream filling matrix, and to evaluate the impact of CS, CoS, and LCS on the probiotic viability, physicochemical properties, and sensory characteristics of the final product. The study was carried out to address the gap in non-fermented probiotic food systems and contribute to the formulation of innovative functional snacks suitable for distribution without refrigeration.

METHODS

In this study, probiotic cream-filled biscuits were formulated using three different types of sugar, namely cane sugar (CS), coconut sugar (CoS), and low-calorie sugar (LCS), as outlined in Table 1. Each formulation contained the same base cream matrix and probiotic concentration, with only the sugar type varied. These sugars were selected based on their distinct physicochemical properties, nutritional profiles, and prevalence in food applications. The primary variables analyzed across the groups included probiotic cell viability, shelf life stability, sensory acceptability, and chemical composition.

The ingredients used for biscuit formulation were selected based on previous studies. These included margarine (Palmia, PT Indofood CBP Sukses Makmur Tbk), powdered sugar (Bola Deli, PT Bola Keluarga), vanilla powder (Butterfly, PT Gunacipta Multirasa), low-protein wheat flour (Bogasari Kunci Biru, PT Indofood

Table 1. Experimental design and variables used in this study

Group code	Sugar type	Description	Concentration (% w/w)	Key variables evaluated
CS	Cane sugar	Commonly used sucrose-based sweetener	60%	Probiotic viability, shelf life, sensory, chemical profile
CoS	Coconut sugar	Natural sugar with higher mineral content	60%	Probiotic viability, shelf life, sensory, chemical profile
LCS	Low-calorie sugar	Stevia/erythritol-based sweetener with low caloric value	50%	Probiotic viability, shelf life, sensory, chemical profile

Sukses Makmur Tbk), cocoa powder (Windmolen, PT Ceres Indonesia), cornstarch (Mama Suka, PT Daesang Agung Indonesia), baking powder (Butterfly, PT Gunacipta Multirasa), and snakehead (Channa striata) fish flour obtained from a certified aquaculture facility in Sleman, Special Region of Yogyakarta, Indonesia. The raw materials for probiotic cream preparation comprised butter (Palmia, PT Indofood CBP Sukses Makmur Tbk), vanilla powder (Butterfly, PT Gunacipta Multirasa), cane sugar (Gulaku, PT Gula Putih Mataram), coconut sugar (Singabera, PT Intrafood Singabera Indonesia), low-calorie sugar (Tropicana Slim Stevia, PT Nutrifood Indonesia), soy lecithin (Intisari), and maltodextrin (Intisari). The probiotic strain used in this study was *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13, originally isolated from *dadih,* a traditional fermented buffalo milk from West Sumatra, by the Center for Food and Nutrition Studies (PSPG), Universitas Gadjah Mada, Yogyakarta, Indonesia. Unless otherwise specified, all analytical-grade reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA), and microbiological media were obtained from Merck (Darmstadt, Germany).

Process Making of Biscuit and Probiotic Cream

Biscuit preparation

The snakehead fish (*Channa striata*) flour biscuit was prepared by first mixing 130 g of margarine, 100 g of powdered sugar, and 1 g of vanilla powder until homogenized using a spatula. Subsequently, 150 g of low- protein wheat flour, 25 g of low-calorie starch, 10 g of cocoa powder, 20 g of skim milk powder, 60 g of snakehead fish flour, 3 g of baking powder, and 1 g of baking soda were added. The mixture was blended using a stand mixer at medium speed for 2 minutes to form a uniform dough. The dough was portioned into 7 g units using a silicone mold, arranged on a baking tray, and baked at 110 °C for 34 minutes using both top and bottom heat.

Probiotic cream preparation

The probiotic cream was prepared by first mixing 37 g of butter, 60 g of sugar, and 0.4 g of vanilla paste at medium speed for 2 minutes until a homogeneous mixture was obtained. Subsequently, 2 g of soy lecithin, 15 g of maltodextrin, and 0.6 g of *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 (10^9 CFU/g) were incorporated into the mixture. The resulting probiotic cream was used as a filling between two snakehead fish flour biscuits.

Viable cells analysis

The viability of *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 in cream-filled fish biscuits was evaluated following the method described by Majid et al. (2023). For analysis, 1 g of the probiotic cream filling (excluding the biscuit) was aseptically weighed and homogenized in 9 mL of 0.85% NaCl solution. Serial dilutions were = prepared and plated on de Man, Rogosa, and Sharpe (MRS) agar to allow selective growth of the probiotic strain. Plates were incubated at 37 °C for 48 hours, and the results were expressed as colony-forming units per gram (log CFU/g).

Water activity analysis

Water activity (Aw) refers to the availability of free water in food systems, which influences both chemical stability and microbial growth. The Aw of the samples was measured using an Aqualab PAWKIT water activity meter (Decagon Devices, Inc., Pullman, WA, USA). Biscuit samples filled with probiotic cream were homogenized and placed in a sample cup, then analyzed until a stable Aw reading was obtained (Reyes et al., 2018).

Shelf-life analysis

Shelf life determination of snakehead fish flour biscuits filled with probiotic cream using different sugar types was conducted based on the Arrhenius model and probiotic cell viability, as described by Calligaris et al. (2022). Storage temperature was used as the main variable, with 3 levels, namely 25 °C (baseline), 37 °C, and 45 °C. Samples were stored for 5 weeks, and observations were conducted weekly. Probiotic viability data from each treatment and temperature condition were subjected to linear regression analysis to evaluate the influence of temperature on product stability. The biscuits were packaged in metallized film pouches flushed with nitrogen gas to control water activity and prevent moisture absorption. This packaging strategy helped maintain product quality during storage. Accelerated shelf-life testing was performed at 37 °C and 45 °C to simulate and expedite quality degradation, while 25 °C served as a reference to represent typical ambient storage conditions and to provide a more comprehensive assessment of product stability.

Sensory evaluation

The sensory evaluation was conducted with 100 untrained panelists, whose number was determined using the Raosoft sample size calculator (Raosoft, 2004), based on the total population of the Special Region of Yogyakarta. Ethical approval for the sensory 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, under protocol number KE/0034/01/2023.

Sample preparation was performed one day before the evaluation. Biscuits produced from snakehead fish flour and filled with probiotic cream containing different types of sugar were individually placed in coded paper cups. Each panelist received tissue paper, plain crackers, and mineral water to cleanse the palate between samples. The evaluation was conducted using a 7-point hedonic scale, where 1 indicated “strongly dislike,” 4 represented “neither like nor dislike,” and 7 corresponded to “strongly like.” Panelists were asked to assess the products based on appearance, aroma, taste, texture, and overall acceptability.

Chemical composition analysis

The proximate composition of snakehead fish flour biscuits filled with probiotic cream, including protein, fat, ash, and moisture content, was determined following the AOAC Official Method 925.10 (Horwitz, 2005), which used the air oven method for quantifying total solids and moisture in flour-based products. Total sugar content was measured using the Nelson–Somogyi method, a standard colorimetric assay for reducing sugars (Putri et al., 2022). Caloric content was assessed using a bomb calorimeter (Shehab et al., 2022), in which the sample undergoes complete combustion in an oxygen-rich, sealed chamber to quantify the total energy released, providing an accurate estimation of the product’s caloric value.

Statistical analysis

All experiments were performed in triplicate, and the data obtained were analyzed using Minitab software (version 19.2020.1). Normality of the data distribution was assessed using the Kolmogorov–Smirnov test. For normally distributed data, one-way analysis of variance (ANOVA) followed by Duncan’s Multiple Range Test (DMRT) was used to evaluate significant differences at the 5% level ($p < 0.05$). For non-normally distributed data, the Kruskal–Wallis test was used to determine group differences, followed by pairwise comparisons using the Mann–Whitney U test when significance was observed ($p < 0.05$).

RESULTS AND DISCUSSIONS

Changes in Probiotic Viability During Storage

The viability of probiotic microorganisms is a critical determinant of the functional efficacy of probiotic-enriched food products. Fig. 1 shows the changes in viable cell counts (log CFU/g) of *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 in snakehead fish flour biscuits filled with probiotic cream formulated with different sugar types, cane sugar (CS), coconut sugar (CoS), and low-calorie sugar (LCS) during 35 days of storage at 25 °C, 37 °C, and 45 °C.

At 25 °C, the decline in probiotic viability was relatively moderate, with final counts ranging from 6.06 to 6.24 log CFU/g by day 35 still exceeding the minimum therapeutic threshold of 6 log CFU/g. However, elevated storage temperatures resulted in a significantly sharper decline. At 37 °C, cell viability dropped to approximately 4.90 log CFU/g, while at 45 °C, it fell further to 4.05 log CFU/g, corresponding to a reduction of 2.1 and 2.8 log units, respectively. This temperature-dependent decrease is consistent with previous findings by Mohammadi et al. (2012), who reported that higher storage temperatures accelerate probiotic degradation due to increased metabolic activity and structural instability of the cells. These results were compared with previous work by Muzzafar and Sharma (2018), in which microencapsulated probiotics retained a viability of 9.28 log CFU/g after 35 days at 25 °C. However, this study used free (non-encapsulated) probiotic cells, which contributed to the more rapid

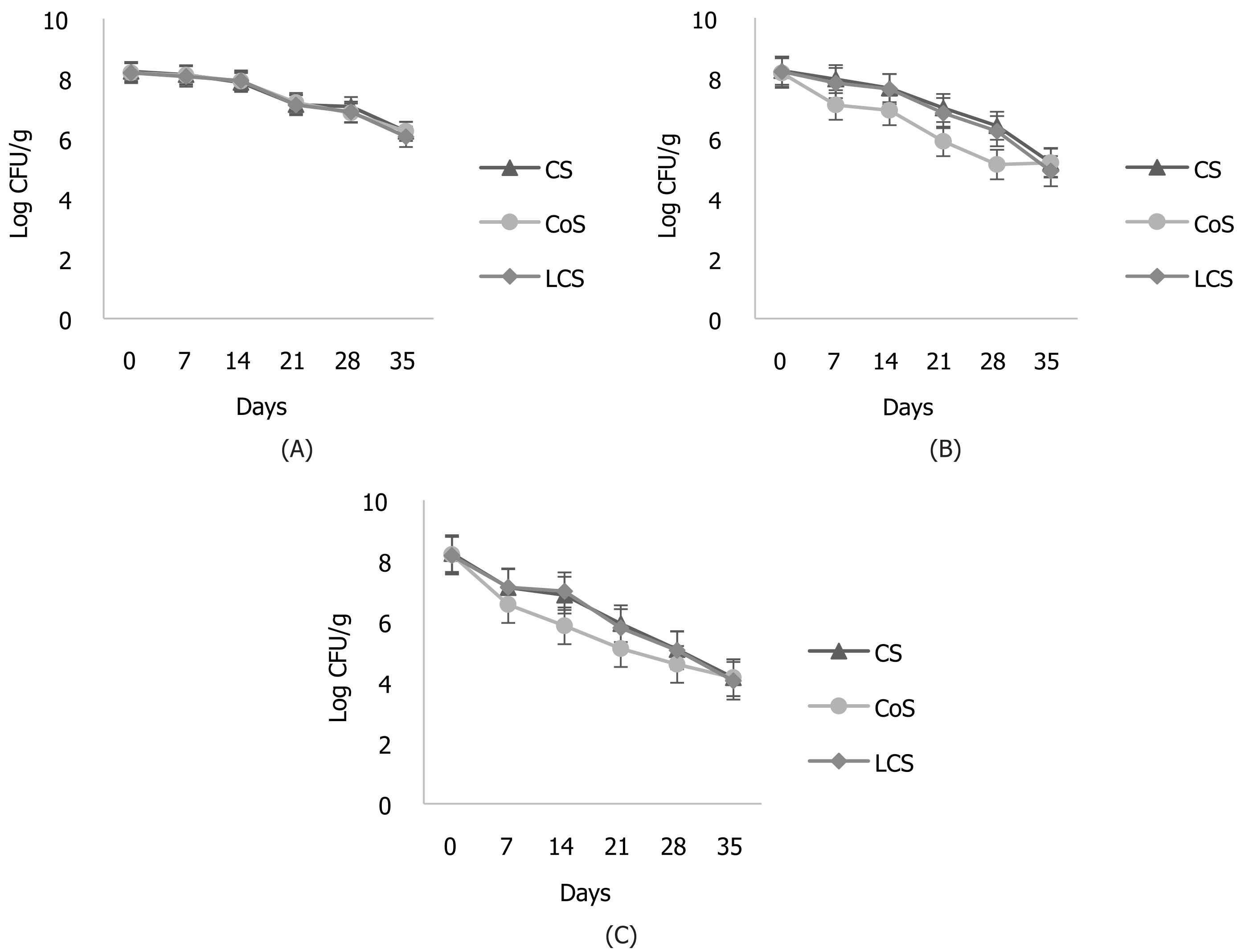

Figure 1. Changes in cell viability of snakehead fish flour biscuits with probiotic cream filling using different types of sugar (CS = Cane Sugar, CoS = Coconut Sugar, and LCS = Low-Calorie Sugar) for 35 days at different storage temperatures. Changes in probiotic cell viability at 25 °C (A), 37 °C (B) and 45 °C (C)

decline in viability observed. Without encapsulation, probiotic cells are more vulnerable to environmental stressors such as oxidation, osmotic imbalance, and thermal damage, especially during extended storage. Beyond encapsulation, the surrounding food matrix was another crucial factor influencing probiotic stability. In this formulation, the probiotic cream rich in fats and sugars acted as a semi-protective microenvironment that may have partially buffered the cells from thermal and oxidative stress (Matouskova et al., 2021). In addition, the biscuit shell provided a secondary barrier against external factors such as moisture ingress and oxygen exposure. This dual-layer structural arrangement was consistent with the concept proposed by Rysavka et al. (2020), which emphasized the role of complex food matrices in enhancing the survivability of probiotic bacteria under adverse conditions.

In addition to storage temperature and duration, the type of sugar incorporated into the probiotic cream was found to significantly influence the viability of *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13. Among the tested formulations, those containing cane sugar (sucrose-based) consistently demonstrated the highest probiotic survival rates throughout the storage period. Sucrose, the primary constituent of cane sugar, is a non-reducing disaccharide that offers multiple physicochemical advantages for preserving probiotic stability. Its ability to form hydrogen bonds with cellular components, such as membrane phospholipids and cytosolic proteins, maintains membrane integrity during stress conditions (Arepally et al., 2022; Ozcan et al., 2017). In addition, sucrose functioned as a cryoprotectant, reducing water activity and limiting oxidative degradation within the food matrix (Oluwatosin et al., 2021). These properties collectively reduced cellular stress and enhanced the resilience of probiotics throughout storage.

Low-calorie sweeteners such as stevia also exhibited favorable effects on cell viability, comparable to those observed in the sucrose-based formulation. Unlike conventional sugars, stevia-based sweeteners do not serve as fermentable substrates for probiotics, thereby minimizing osmotic pressure and metabolic overactivity, which leads to premature cell exhaustion in closed systems (Mekdade et al., 2025). In addition, stevia is frequently blended with erythritol, a polyol that has been shown to act as both an osmoprotectant and antioxidant, stabilizing bacterial cells by preserving membrane integrity and neutralizing reactive oxygen species (Saraiva et al., 2023). These synergistic mechanisms likely contributed to the sustained viability observed in the stevia-containing samples.

Formulations using coconut sugar resulted in notably lower probiotic viability over the storage period. Coconut sugar contained a higher proportion of reducing monosaccharides, particularly glucose and fructose, which are more osmotically active and chemically reactive than disaccharides. These sugars increased osmotic stress on cells by drawing water out of the cytoplasm, potentially leading to plasmolysis and cell death (Zhu et al., 2006). Moreover, glucose and fructose were prone to initiating Maillard reactions during storage, generating reactive carbonyl intermediates that could damage cell membranes and proteins, ultimately compromising probiotic functionality (Tymczyszyn et al., 2007). The absence of structural and antioxidative

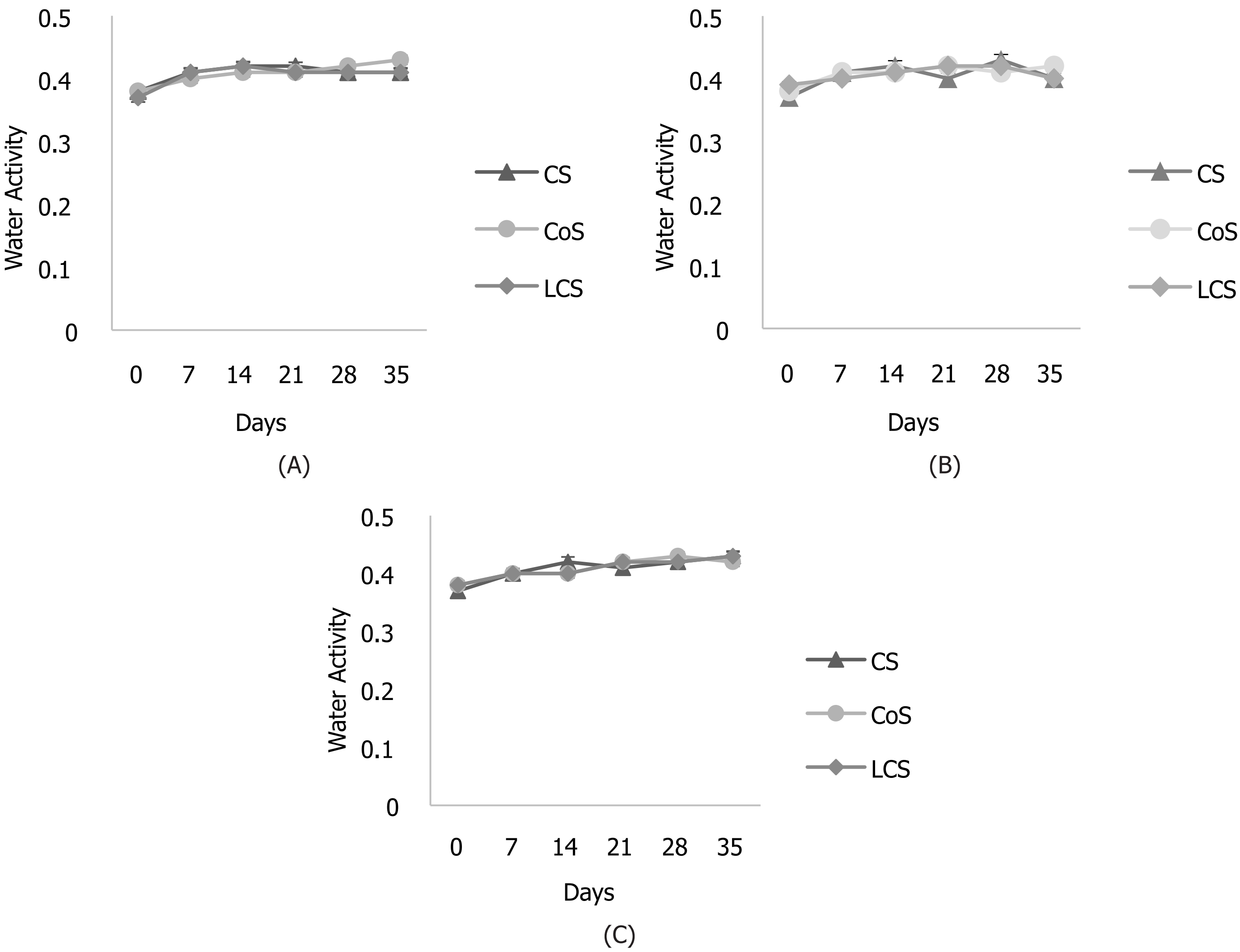

Figure 2. Changes in Aw of snakehead fish flour biscuits with probiotic cream filling using different types of sugar (CS = Cane Sugar, CoS = Coconut Sugar, and LCS = Low-Calorie Sugar) for 35 days at different storage temperatures. Changes in Aw at 25 °C (A), 37 °C (B), and 45 °C (C) temperatures.

protection mechanisms, such as those found in sucrose or erythritol, further exacerbated cell instability in coconut sugar matrices. These results showed that the survival of probiotic bacteria in cream-filled biscuit matrices was not solely dependent on thermal conditions, but was also profoundly affected by the physicochemical properties of the sweetening agents used. The superior performance of cane sugar and low-calorie sweeteners emphasized the importance of selecting sugars that not only met sensory and nutritional goals but also contributed to the structural and functional preservation of probiotic cells during storage.

Changes in Water Activity During Storage

The water activity (Aw) of snakehead fish flour biscuits filled with probiotic cream using different types of sugar was monitored over a 35-day storage period at 3 temperature conditions. The results are presented in Figure. 2.

Overall, Aw values remained relatively stable throughout the storage period across all treatments and temperatures, with an average Aw of approximately 0.41. These values were within the optimal range for dry food products, where microbial growth was generally inhibited. According to Chowdhury et al. (2012), dehydrated products typically exhibited Aw values near 0.30, while fresh, moist foods reached values close to 0.99. The stability observed in this study showed that the product was sufficiently dry to prevent microbial proliferation, thereby contributing to extended shelf life and improved safety. The difference in Aw values compared to biscuit-based products with honey cream filling, which exhibited higher average Aw values (~0.60), as reported by Patrignani et al. (2022), was attributed to differences in formulation, ingredient hygroscopicity, and packaging systems. In this study, metallized film packaging filled with nitrogen gas was used to protect the biscuits from moisture uptake. Nitrogen, being an inert gas, displaced oxygen inside the packaging, thereby minimizing oxidative reactions and limiting water vapor transfer (Hempel et al., 2013). This packaging strategy contributed to the stability of Aw across the storage period.

From a mechanistic perspective, the interaction between sugar and water played a significant role in modulating water activity and probiotic cell survival. Sugars are known to bind water molecules through hydrogen bonding with their hydroxyl groups, effectively reducing the amount of free water available for microbial growth (Jamaluddin et al., 2014). This phenomenon was particularly relevant in systems with high sugar content, such as cream fillings, where sugars help control Aw and protect cellular structures from osmotic stress (Tymczyszyn et al., 2007). However, the influence of water activity extended beyond microbial inhibition. Osmosis, as a key physiological process, governs water movement across the cell membrane of probiotics. In high-Aw environments, excess water influx could lead to cell swelling or lysis, while low-Aw conditions, induced by sugar binding, created a protective effect by reducing water availability and stabilizing the cellular membrane (Rimadhini et al., 2020). These results showed that maintaining low and stable water activity was essential

Table 1. The results of plotting the data on cell reduction against storage time

Treatment	Temperature (°C)	R^2	
		Orde 0	Orde 1
CS	25	0,9308	0,9168
	37	0,9305	0,8944
	45	0,9846	0,9674
CoS	25	0,9263	0,9097
	37	0,9804	0,9599
	45	0,9691	0,9518
LCS	25	0,9224	0,9076
	37	0,9272	0,8877
	45	0,9775	0,8877

CS = Cane Sugar, CoS = Coconut Sugar, and LCS = Low-Calorie Sugar.

Table 2. The equation, intercept, and slope of the reduction in probiotic cell viability over 35 days of storage at zero order

Treatment	Temperature (°C)	Equation	a (*intercept*)	b (*slope*)	R^2
CS	25	y = -0,3977x + 8,441	8,441	0,3977	0,9308
	37	y = -0,5889x + 8,5471	8,5471	0,5889	0,9305
	45	y = -0,786x + 8,1967	8,1967	0,786	0,9846
CoS	25	y = -0,4369x + 8,4738	8,4738	0,4369	0,9263
	37	y = -0,7763x + 8,159	8,159	0,7763	0,9804
	45	y = -0,9131x + 7,8595	7,8595	0,9131	0,9691
LCS	25	y = -0,4286x + 8,4481	8,4481	0,4286	0,9224
	37	y = -0,6329x + 8,5205	8,5205	0,6329	0,9272
	45	y = -0,8014x + 8,2019	8,2019	0,8014	0,9775

CS = Cane Sugar, CoS = Coconut Sugar, and LCS = Low-Calorie Sugar.

Table 3. The calculation of the Arrhenius equation based on probiotic cell viability during storage

Treatment	Temperature (K)	1/T (K)	b (*slope*)= k0	ln b = ln k0
CS	298	0,003355705	0,3977	-0,922057327
	310	0,003225806	0,5889	-0,529498889
	318	0,003144654	0,786	-0,240798487
CoS	298	0,003355705	0,4369	-0,828050943
	310	0,003225806	0,7763	-0,253216236
	318	0,003144654	0,9131	-0,090909875
LCS	298	0,003355705	0,4286	-0,847231196
	310	0,003225806	0,6329	-0,457442847
	318	0,003144654	0,8014	-0,221395081

CS = Cane Sugar, CoS = Coconut Sugar, and LCS = Low-Calorie Sugar.

for preserving probiotic viability and product shelf life. The combination of low-Aw formulation, protective packaging, and sugar interactions contributes to the microbiological and physicochemical stability of the probiotic biscuit product.

Determining The Shelf Life of Products

To determine the shelf life of snakehead fish flour biscuits filled with probiotic cream, the viability data of *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 were modeled using zero- and first-order reaction kinetics. As shown in Table 1, the coefficient of determination (R^2) values for the zero-order model were consistently higher than those for the first-order model across all sugar types and temperatures, showing that the reduction in probiotic cell counts followed a zero-order degradation model. This result is further supported by linear regression equations with strong correlation coefficients ($R^2 > 0.92$), presented in Table 2. In the zero-order kinetic model, the rate of probiotic loss remained constant over time, with slope values (k_0) increasing as storage temperature increased (Table 2). For instance, in the CS formulation, the rate of decline rose from 0.3977 at 25 °C to 0.7860 at 45 °C. These kinetic parameters were used to calculate the Arrhenius plots, allowing estimation of the activation energy (Ea) for each treatment (Table 3). A strong temperature dependence was observed, consistent

Table 4. Shelf determination of snakehead fish flour biscuits with probiotic cream filling

Treatment	Temperature (°C)	Temperature (K)	ln (k)	k	Ts	Shelf life (Weeks)
CS	25	298	-9.2620	0.3960	9.14	9
	37	310	-5.0949	0.6008	6.02	6
	45	318	-2.4915	0.7794	4.64	5
CoS	25	298	-7.9607	0.4510	8.02	8
	37	310	-3.3116	0.7180	5.04	5
	45	318	-4.0717	0.9601	3.77	4
LCS	25	298	-8.4393	0.4300	8.42	8
	37	310	-4.5839	0.6322	5.73	6
	45	318	-2.1753	0.8045	4.50	4

CS = Cane Sugar, CoS = Coconut Sugar, and LCS = Low-Calorie Sugar.

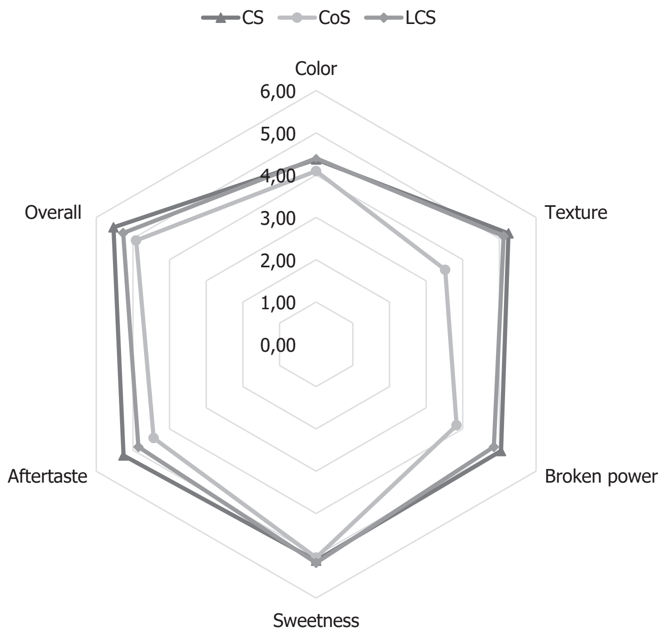

Fig 3. Sensory evaluation scores (hedonic scale: 1 = very much dislike; 7 = very much like) for snakehead fish flour biscuits with probiotic cream filling using different sugar types. CS = Cane Sugar, CoS = Coconut Sugar, and LCS = Low-Calorie Sugar. Sensory parameters include appearance, texture, breaking strength, sweetness, aftertaste, and overall acceptability. Graph lines are differentiated using distinct patterns for readability.

with the Arrhenius model of thermal degradation in biological systems.

Shelf life estimation was conducted based on the time required for viable probiotic counts to drop below the FAO/WHO minimum threshold of 6 log CFU/g, a widely accepted benchmark for functional probiotic foods. As shown in Table 4, the shelf life of products decreased with increasing temperature. For instance, biscuits containing cane sugar had an estimated shelf life of 9 weeks at 25 °C, compared to only 5 weeks at 45 °C. Among all treatments, formulations with cane sugar and low-calorie sugar exhibited longer shelf life, which correlated with their higher probiotic viability and more stable matrix conditions. The observed differences in shelf life can be attributed to the protective roles of sugar types and food matrix characteristics. Sucrose (in cane sugar) and stevia (in low-calorie sugar) are less reactive and provide osmoprotective and antioxidative effects, helping to preserve cell membrane integrity and mitigate oxidative damage (Arepally et al., 2022; Ozcan et al., 2017). However, coconut sugar, which is high in reducing sugars such as glucose and fructose, can accelerate probiotic deterioration through Maillard reactions and osmotic stress (Tymczyszyn et al., 2007; Zhu et al., 2006). The shelf life of probiotic-enriched biscuits is significantly influenced by storage temperature and sugar composition. The use of zero-order kinetic modeling and the Arrhenius equation provided accurate estimations, confirming that storage at lower temperatures (e.g., 4 °C) could extend the product's shelf life up to approximately one year, while higher ambient temperatures (e.g., 37 °C) limited shelf life to around one month. These insights are crucial for ensuring product quality and functionality during distribution and storage (Jannah et al., 2022; Dinkçi et al., 2019).

Sensorial properties of products

The sensory evaluation of snakehead fish flour biscuits with probiotic cream filling, presented in Fig. 3, showed that the type of sugar significantly influenced several sensory attributes. Statistical analysis indicated significant differences ($p < 0.05$) in appearance, texture, breaking strength, aftertaste, and overall acceptance, while sweetness intensity did not differ significantly among the treatments ($p > 0.05$).

Differences in visual appearance across treatments were largely attributed to the physicochemical properties of the sugar types used in the cream formulation. Biscuits prepared with CS and LCS exhibited a lighter, more uniform cream color, while those containing CoS presented a noticeably darker, brown hue. This variation arises from the inherent composition and processing characteristics of coconut sugar, which contains a higher concentration of reducing sugars and undergoes minimal refinement. Such properties promote caramelization and pigment development during processing, contributing to the darker appearance (Basuki et al., 2021). Color is a major sensory attribute that shapes consumer perception and preference, often influencing initial judgments about product quality and appeal (Martínez et al., 2021). The lighter color associated with CS and LCS enhanced visual acceptability among panelists, while the darker tone of CoS has been perceived as less attractive in comparison.

Texture evaluations showed that biscuits formulated with CS and LCS received significantly higher scores compared to those made with CoS. The finer refinement and lower moisture content of CS and LCS likely contributed to the development of a smoother and more homogenous cream texture. However, CoS produced a cream with a coarser and more irregular texture, attributable to its higher water content and larger, less uniform particle size (Hebbar et al., 2022). These textural differences also affected the mechanical properties of the final product, particularly breaking strength. Biscuits containing CoS exhibited increased resistance to fracture, suggesting the formation of a denser and more cohesive cream matrix. According to Panpipat et al. (2021), such firmness resulted from intensified interactions between lecithin and water, especially in formulations with higher moisture levels. Shen et al. (2020) noted that elevated water activity could destabilize emulsions, leading to reduced structural integrity and increased textural rigidity.

Regarding aftertaste, the panelists rated CoS the lowest. This could be attributed to its distinctive caramel and earthy flavor, which lingered after consumption and was not universally preferred. However, CS and LCS provided a cleaner, sweeter aftertaste. According to Ross (2021), aftertaste is a crucial factor in consumer preference, as it represents the residual flavor impression post-consumption. The caramelized notes in CoS stem from the traditional cooking and concentration processes used during coconut sugar production (Urbanus et al., 2014; Basuki et al., 2021), which accounts for its lower acceptability in this sensory parameter. Overall acceptability scores showed that CS and LCS received the highest ratings from the panelists, with average scores of 5.53 and 5.26, respectively. Based on the 7-point hedonic scale (1 = very much dislike; 7 = very much like), these scores indicated that the panelists generally favored the products. However, CoS lagged with a mean score of 4.91, corresponding to a neutral to slightly liked perception. This suggested that lighter-colored, cleaner-tasting sugars such as sucrose and stevia were more suitable for probiotic cream fillings in biscuit products. Although coconut sugar contributed a unique flavor profile, its textural and visual properties appeared to be less favored. Ultimately, CS emerged as the most preferred treatment, combining favorable appearance, texture, and overall sensory appeal in the eyes of the panelists.

Chemical composition of the product

A chemical composition analysis was carried out on snakehead fish flour biscuits with probiotic cream filling using different types of sugar, and the chemical composition was summarized in Table 2.

The compositional analysis of snakehead fish flour biscuits with probiotic cream filling showed that the sugar

Table 2. The chemical composition of snakehead fish flour biscuits with probiotic cream filling uses different types of sugar.

Treatment	Protein (%)	Fat (%)	Ash (%)	Moisture (%)	Calories (kcal)	Total sugar (%)
CS	11.22 ± 0.11[a]	28.47 ± 0.62[a]	2.01 ± 0.07[a]	4.17 ± 0.15[a]	79,05 ± 0,11[a]	33.54 ± 1.59[a]
CoS	11.25 ± 0.15[a]	28.38 ± 0.79[a]	2.37 ± 0.10[b]	5.20 ± 0.19[b]	79,95 ± 0,07[a]	34.10 ± 2.46[a]
LCS	11.11 ± 0.35[a]	34.29 ± 0.5[b]	2.04 ± 0.07[a]	4.31 ± 0.28[a]	66 ± 0,10[b]	22.96 ± 1.50[b]

Notes: Different superscript letters in the same row are significantly different based on one-way ANOVA with DMRT ($p < 0.05$). (CS = Cane Sugar, CoS = Coconut Sugar, and LCS = Low-Calorie Sugar) type used influenced several key nutritional attributes (Table 2). Among the formulations, the LCS treatment demonstrated a distinct profile, notably with lower total sugar and caloric content, consistent with its intended formulation purpose. However, biscuits containing coconut sugar (CoS) exhibited the highest ash and moisture contents, reflecting the less refined nature of this sugar type (Norhayati et al., 2015). The protein content did not significantly differ across treatments, ranging from 11.11% to 11.25%, suggesting that the sugar type had minimal influence on protein levels. This was consistent with the compositional characteristics of the sugars used, as neither cane sugar, coconut sugar, nor commercial low-calorie sweeteners contribute appreciable protein content (Beegum et al., 2022). The slightly lower protein content compared to previous formulations with soy protein isolates (23.08%; Adi et al., 2022) was attributed to the absence of such protein fortification in this study.

In terms of lipid content, the LCS formulation displayed a significantly higher fat concentration (34.29%) than the other treatments. This increase stems from formulation factors, particularly the fat content of the cream filling, which uses butter as the primary fat source (Kala, 2014). Similar patterns were observed in the energy content, where LCS biscuits yielded the lowest caloric values (66 kcal), compared to the higher energy content of CS and CoS treatments (approximately 79 kcal). This difference reflected the nature of the sweeteners used, as commercial LCS products, such as Tropicana Slim, typically contributed negligible calories (Srikaeo, 2015). The moisture content was significantly higher in the CoS formulation (5.20%) compared to CS (4.17%) and LCS (4.31%). This could be attributed to the intrinsic water content of coconut sugar (12–20%) and the absence of extensive crystallization processes during its production (Basuki et al., 2021). However, CS and LCS undergo more rigorous refining steps such as boiling, pressing, and crystallization that reduce moisture retention (Duraisam et al., 2017).

Regarding ash content, which reflected the mineral composition, CoS again stood out with a significantly higher value (2.37%). This supported the results by Sampaio et al. (2023), who noted that minimally refined sugars, such as coconut sugar, retain more minerals due to the absence of crystal purification steps. Meanwhile, refined sugars, including cane sugar and low-calorie formulations, experience mineral loss during crystallization, resulting in lower ash levels (Farahbod, 2019). The total sugar content was highest in the CoS (34.10%) and CS (33.54%) treatments, and significantly lower in the LCS sample (22.96%). This trend was consistent with the known composition of cane sugar, which consisted almost entirely of sucrose, a non-reducing disaccharide (Kumari & Asthir, 2016). Coconut sugar contained a mixture of sucrose, glucose, and fructose, with total sugar contents reported up to 91% (Haryanti & Sulistyo, 2022). Meanwhile, LCS formulations were composed primarily of sugar alcohols such as sorbitol and aspartame, which provided sweetness without contributing to sugar content (Mikulic-Petkovsek et al., 2012). Therefore, the choice of sugar significantly affected the nutritional profile of probiotic cream-filled biscuits, with LCS offering a lower-calorie alternative, while coconut sugar contributed greater mineral and moisture content but could compromise shelf stability due to its hygroscopic nature.

CONCLUSION

In conclusion, cane sugar cream biscuits demonstrate superior ability to maintain probiotic cell viability during storage, with an estimated shelf life of approximately 9 weeks at 25°C, based on the deterioration model and Arrhenius equation ($y = -0.3977x + 8.441$). The recommended storage temperature for optimal stability is 20–25°C, at which the product is estimated to maintain acceptable quality for approximately 8 to 9 weeks. Sensory evaluation shows that panelists preferred biscuits with cane sugar and low-calorie sugar creams, both showing comparable acceptability ($p > 0.05$). Biscuits formulated with coconut sugar cream exhibited higher mineral content, as reflected in elevated ash levels, suggesting a richer chemical composition compared to other treatments. However, their texture and probiotic stability are less favorable. The Arrhenius equations obtained for CoS and LCS are $y = -0.4369x + 8.4738$ and $y = -0.4286x + 8.4481$, respectively, indicating a slightly faster degradation rate compared to cane sugar cream. This supports the conclusion that cane sugar cream provides better stability for maintaining probiotic viability during storage.

CONFLICT OF INTEREST

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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