Formulation, Characteristics and Viability Probiotics Cell of Biscuit Production from Hotong Flour (*Setaria italica* (L) Beauv.), Walnut (*Canarium indicum* L.), Enriched by Probiotic Cream

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Submitted: November 14, 2023; Revised: April 18, 2024, June 11, 2024; Accepted: October 3, 2024; Published: February 05, 2025

ABSTRACT

Hotong (*Setaria italica* (L) Beauv.) and walnut (*Canarium indicum* L.) are nutrient-rich local foods from Maluku Province, Indonesia, significant health. In this context, biscuit enriched with probiotic cream are a recommended snack for enhancing nutrition and promoting balance of the intestinal microflora, at a sufficient quantity of 10^7 log CFU/g. Therefore, this study aimed to objectively evaluate the physicochemical and organoleptic characteristics of biscuit, as well as assess probiotic viability. The used was a completely randomized design (CRD) with three different formulation treatments containing hotong flour and walnut at ratios of 40%:10% (F1), 35%:15% (F2), and 30%:20% (F3). Probiotic cell viability was analyzed at temperatures of 20 °C, 37 °C, and 45 °C. The results showed that F3 had the most favorable formulation regarding nutritional adequacy rate with 173 kcal energy, 10% fat, 4.41% protein, 5.20% carbohydrate, and 2 g sugar. It featured a crisp texture and increasingly darker colors, in line with the outcomes of sensory evaluations. F3 was the preferred, achieving high scores for color (5.38), taste (6.13), aroma (5.13), breakability (5.86), crispness (6), aftertaste (5.02), and overall quality (6.03). Cell viability for all treatments was higher at 20 °C and remained above 10^7 log CFU/g.

Keywords: Biscuit; hotong; probiotic; walnut

INTRODUCTION

Nutrition is essential for maintaining good health. with deficiencies often leading to clinical symptoms such as abnormal growth and development. The impact of dietary disorders is significant, affecting physical and cognitive development, emphasizing the importance of modifying consumption patterns by incorporating nutrient-rich local foods in a single diet (Laswati, 2017). Hotong and walnut, both native to Indonesia, are excellent sources of nutrition and ideal for balanced meals. Hotong, a type of millet (*Setaria italica* (L) Beauv.) is a locally cultivated crop with high nutritional value. According to Herodian (2008), it contains 14.05% protein, 3.37% fat, 9.03% water, 1.26% ash, 7.8 mg/100 g iron, 8.2 g dietary fibers, 28 mg calcium, and 311 mg phosphorus. Currently, the available scientific studies available regarding the use of hotong are

DOI: http://doi.org/10.22146/agritech.90687
ISSN 0216-0455 (Print), ISSN 2527-3825 (Online)

limited. This presents the need for further exploration of processing methods and incorporation into food product development.

Walnut (*Canarium indicum* L.) belongs to the group of leguminous plants native to easten Indonesia, including the islands of Sulawesi, Maluku, and North Maluku (Thomson et al., 2004). The seeds are popular among consumers due to their tasty flavor. Dried walnut seeds contain 38.29% fat, 16.59% carbs, and 14.89% protein (Djarkasi et al., 2007). Walnut also has high levels of unsaturated fatty acids, particularly 2174.1–2618.0 mg/g linolenic acid, 136.8–1426 mg/g linoleic acid, 8152.6–9295.2 mg/g oleic acid (Mailoa et al., 2019). According to Leakey et al. (2008), Arenas & Trinidad (2017), and Aril-dela et al. (2018), the nut has a substantial phenolic content and α, β, γ, δ tocopherol levels, which contribute to significant antioxidant and anti-inflammatory properties. It is a great source of plant fats and proteins that help to improve nutrition. To enhance nutritional value and health benefits, the high fat and protein content in this crop can be used as raw materials or additives in processed hotong products.

Biscuit is a popular snack enjoyed by all age groups, ranging from children to adults. According to the 2020 Statista Global Consumer Survey, individuals between 1,5 to 65 years old have a significant fondness for consuming these snacks due to their delectable taste, variety of styles, and varying shapes. However, commercially produced options possess an imbalanced nutrient composition, characterized by high levels of carbohydrates and fats, as well as insufficient quantities of other important nutrients (Irferamuna et al., 2019). A market study has shown that 29 g serving provides 150 calories of total energy, 11% total fat, 3% protein, and 6% total carbohydrates. Furthermore, any cream infused into the biscuit should be carefully selected to ensure the nutritional content fully supports the body, such as adding probiotics.

Probiotics are microorganisms that offer health benefits to the human body through consumption in specific amounts (FAO/WHO 2002). *Lactiplantibacillus plantarum* Dad-13, a strain native to Indonesia, has been extensively. Rahayu et al. (2021) discovered that *L. plantarum* Dad-13 survived and colonized the human digestive tract, reducing harmful bacteria, including *E. coli* and non-*E. Coli* is anticarcinogenic, as well as produces bacteriocins and B vitamins. Storage temperature is a critical factor for maintaining probiotic viability . Furthermore, elevated temperatures destabilize cells, leading to reduced viability, as evidenced by (Desmond et al., 2002). In this study, probiotic was added to biscuit cream in powdered form and mixed with other ingredients. The process includes the use of local probiotics, specifically *Lactiplantibacillus plantarum* Dad-13 derived from fermented buffalo curd in West Sumatera, Indonesia. Additionally, the main ingredients were also sourced locally.

This study is aimed to evaluate the effect of varying ratios of hotong flour and walnut on the chemical, physical, and sensory properties of biscuit and to determine their nutritional. Additionally, the viability of the probiotic cell of *L. plantarum* Dad-13 in biscuit cream was analyzed.

METHODS

Materials

The primary materials used in this study included hotong seed sourced from Buru Regency, Maluku Province, Indonesia, and walnut from Saparua, Central Maluku Regency, Maluku Province. Other materials were milk powder (Mixpro), full cream milk (Ultra Milk), salt, margarine (Palmia), refined sugar (Jago), baking powder (Bimo sakti), butter (Point), soy lecithin, and *Lactiplantibacillus plantarum* Dad–13obtained from Food and Nutrition Culture Collection, Center for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta, Indonesia.

Preparation of Hotong Flour

Hotong flour preparation followed the modified version of the method described by (Sigmarlatu et al., 2019). The process includes soaking hotong seeds for 24 hours, drying using a cabinet dryer for 8 hours, and grinding the seeds using a crusher, before sieving through a 60 mesh sieve.

Preparation of Walnut

The method for walnut preparation followed the procedures described by Makanoneng et al. (2017). This process included soaking dried walnut seeds in hot water (80 °C) for 7 minutes, removing the epidermis, sorting to remove dirt, as well as chopping and baking at 150 °C for 15 minutes.

Preparation of Biscuit

The process of biscuit production was adapted from the method outlined by Prasetyo et al. (2014), with modifications to the ingredient formulation. The first included mixing sugar (15,5%) and margarine (20%) until a creamy form was obtained. Subsequently, milk (10%), salt (0,5%), milk powder (3%), baking powder (1%), as well as hotong flour and walnut were added. The mixture was blended for 1 minute with a composition of F1 (40%:10%), F2 (35%:15%), and

F3 (30%:20%). The dough was molded to a thickness of 0.5 cm and baked in an electric oven at 120 °C for 30 minutes. Finally, it was cooled and filled with *L. plantarum* Dad-13 probiotic cream.

Preparation of Probiotic Cream *L. plantarum* Dad-13

In this study, Probiotic cream was prepared following the procedures outlined by Pratiwi, (2015), with modifications of adding *Lactiplantibacillus plantarum* Dad-13. The first step includes mixing butter (37%) and sugar (60%) at medium speed for 2 minutes. Furthermore, soy lecithin (2%) and *L. plantarum* Dad-13 probiotic powder (1%) were added with viability of 10^9 CFU/g. After achieving a homogenous mixture, the cream was sandwiched between 2 pieces of biscuit.

Physicochemical Analysis

The chemical characteristics of biscuit manufactured from hotong flour and walnut enriched with probiotic cream were analyzed. A proximate analysis was conducted to determine water content using the oven drying method. Ash and fat contents were measured by the furnace and Soxhlet methods, respectively. Protein content was assessed by the Kjdeldahl method (AOAC, 2005), while carbohydrate content was calculated using the difference method (Wirnano, 1997). Finally, total energy and sugar were measured by bomb calorimeter (AOAC, 2005) and DNS methods (Oktavian et al., 2014), respectively.

The hardness of the biscuit was measured using a Universal Testing Machine model Z0.5, Zwick/Roell AG, Germany, in accordance with the procedures outlined by Rauf et al. (2017). Biscuit were placed on the plate of the machine and compression pressure was applied until deformation occurred. The maximum magnitude of the force to deform the biscuit signified the degree of.

Color testing was performed using a colorimeter (Konica Minolta CR20, Konica Minolta, Japan). It included measurements of L*, a*, and b* values which represented brightness (100=white, 0=black), reddish(+)/greenish(-), and yellowish(+)/bluish(-) (Ostermann-porcel et al. 2016), respectively.

Recommended Dietary Allowance (RDA)

Following the determination of the proximate levels of biscuit products enriched with probiotic cream, the data were used to calculate the percentage of RDA for adults. This calculation adhered to the guidelines outlined in the Indonesia Minister of Health Regulation No. 28 of 2019. Additionally, the Nutrition Label Number was determined based on the general group requirements specified in the Indonesia Food and Drug Control Agency No. 9 of 2016**.**

Consumer Acceptability

Consumer acceptance of hotong flour and walnut biscuit enriched with probiotic cream was evaluated through sensory testing using a hedonic scale. The sensory analysis was approved by the Medical and Health Research Ethics Committee (MHREC) of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, with the reference number KE/FK/0212/EC/2023.

Consumer tests of hotong and walnut biscuit products enriched with probiotic cream were intended for untrained panelists selected by random sampling, representing the general public. A total of 113 panelists participated in the test, using a 7-point hedonic scale to rate biscuit. The scale ranged from 1 (very dislike), 2 (dislike), 3 (somewhat dislike), 4 (ordinary), 5 (somewhat like), 6 (like), to 7 (very like). The purpose of the sensory evaluation was to determine the level of consumer preference for the products.

The untrained panelists participating in the sensory tests were provided with a detailed explanation of the procedures before the evaluation. Those who agreed to participate were required to sign the informed consent form. To neutralize the sense of taste, panelists consumed mineral water before tasting the sample formulas.

Viability of Probiotic

A total of 5 g samples were homogenized with 4.5 mL of 0.85% NaCl buffer using a stomacher. Following dissolution, serial dilution, and platting were conducted in the last 3 dilution series, namely 10^5,10^6, and 10^7. A total of 1 mL per dilution or suspension series was platted using the pour plate method on LPSM media and incubated for 24–48 hours at a temperature of 37 °C. Subsequently, the calculated colony was expressed as CFU/g. Colonies which ranged from 25–250 cells, were included in the calculation. The number of bacteria less than 25 was classified as EAPC (Estimated Aerobic Plate Count), while a count exceeding 250 was categorized as TNTC (Too Many To Count) (FDA BAM, 2001)

RESULTS AND DISCUSSION

The proximate composition and RDA calculations for biscuit manufactured from hotong flour and walnut enriched with probiotic cream are presented in Tables 1 and 2. Water content decreased from 3.22% to 2.18% as the walnut ratio increased, but not significantly different by statistics. The high protein and fat content in the nut influenced product moisture. This is because roasting can cause protein-fat matrix formation on the surface, impeding water absorption (Lawalata et al.,

Table 1. Chemical composition of biscuit

Parameters	F1	F2	F3
Water (%)	3.22 ± 1.33[a]	2.51 ± 0.37[a]	2.18 ± 0.21[a]
Ash (%)	2.26 ± 0.08[a]	2.31 ± 0.13[a]	2.31 ± 0.13[a]
Protein (%)	8.50 ± 0.22[a]	8.49 ± 0.36[a]	8.82 ± 0.19[a]
Fat (%)	21.18 ± 0.18[a]	21.34 ± 0.24[a]	22.97 ± 0.32[b]
Carbohydrate (%)	67.88 ± 1.04[b]	68 ± 0.81[b]	65.89 ± 1.12[a]
Calorie (kcal)	546.27 ± 2.07[a]	557.55 ± 03.19[b]	577.15 ± 1.55[c]
Total Sugar (g/100 g)	8.58±0.29[b]	5.24 ± 0.29[a]	5.07 ± 0.30[a]

Note: Different superscript letters in the same row show a significant difference (p<0.05) between the samples. F1, 40% hotong flour : 10% walnut; F2, 35% hotong flour : 15% walnut; F3, 30% hotong flour : 20% walnut

Table 2. RDA

RDA (%) Serving size (30 g)					
Sample	Energy (kcal)	Total fat (%)	Protein (%)	Carbohydrate (%)	Sugar (g)
F1	164	9.48	4.25	5.64	3
F2	167	9.55	4.24	5.56	2
F3	173	10	4.41	5.20	2

Note : F1, 40% hotong flour : 10% walnut; F2, 35% hotong flour : 15% walnut; F3, 30% hotong flour : 20% walnut

2004). The moisture content was also affected by the high crude fiber content of hosting flour, which was 8.21% (Sulistyaningrum et al., 2017). Similar outcomes were reported by Lawalata et al. (2019) where an increase in walnut proportion lowered the water loss tendency.

The ash content increased from 2.26% to 2.31% with elevated proportions of walnut but was not significantly different by statistics. This increase was attributed to the ash content of the raw materials, specifically hotong at 0.25% (Sigmarlatu et al., 2019) and walnut at 2.82% (Lawalata et al., 2004). Additionally, the amount of minerals present affected the magnitude of the content. This led to the suspicion that adding walnut to biscuit contributed to higher ash content. Sen and Karadeniz (2015) reported that walnut contained 441 mg/100 g potassium, 346 mg/10 0g phosphorus, and 98 mg/100g calcium. Meanwhile, hotong contains 28 mg/100 g calcium, 5.3 mg/100 g iron, and 311 mg/100 g phosphorus (Indonesia food composition table, 2017).

The protein content ranged between 8.49% and 8.82%, and it increased in proportion to the quantity of walnut but was not significantly different by statistics. This increase is affected by the protein content of walnut, reported as 14.89% (Lawalata, 2004) and 13.06% (Thompson and Evans, 2004). In contrast, the protein content of hotong flour was 8.08% (Sugiyono, 2010). The addition of full cream milk in the production process an impact on the protein levels of the final product. The content in biscuit meets the minimum parameter of 5% outlined in Indonesia National Standard (SNI) 2018. According to RDA calculations presented in Table 2, a serving dose of 30 g biscuit enriched with *L. plantarum* Dad-13 has provided a protein % RDA of 4.24–4.41%. This was higher than commercial cookie products, which delivered only 3%.

Analysis of variance results showed a significant influence of the treatments on the fat content (p<0.05). As the proportion of walnut increased, the fat content ranged from 21.18%–22.97%. Walnut are rich in polyunsaturated fatty acids, including omega-3 and omega-6 (Ugur & Erdogen, 2021). RDA percentage calculations showed that biscuit of hotong flour and walnut enriched with probiotic cream contributed to 9.48%–10% fat in a 30 g serving, a value lower compared to 11% in commercial products.

The proportion of hotong flour in biscuit is directly proportional to the carbohydrate content which ranged from 65.89%–68%. Analysis of variance showed a

Table 3. Physical properties of biscuit

Sample	Hardness (N)	Color		
		L*	a*	b*
F1	31.53 ± 0,92^{c}	52.59 ± 1.17^{b}	4.21 ± 0.44^{a}	28.01 ± 0.35^{b}
F2	22.37 ± 0.97^{b}	50.15 ± 1.42^{b}	5.59 ± 0.58^{b}	27.33 ± 0.45^{a}
F3	16.03 ± 0.58^{a}	49.04 ± 0.33^{a}	6.05 ± 0.13^{b}	27.16 ± 0.27^{a}

Note: Different superscript letters in the same column show a significant difference (p<0.05) between the samples. F1, 40% hotong flour : 10% walnut; F2, 35% hotong flour : 15% walnut; F3, 30% hotong flour : 20% walnut

significant impact of the treatment on the levels of carbohydrates (p<0.05). According to Sugiyono (2010), hotong flour had a high content of 89.58%, in contrast to only 16.59% in walnut (Thomson & Evans, 2004). Nutritional components had an inversely proportional impact on carbohydrate content (Katresna, 2017). Based on the % RDA calculation, a 30 g serving of biscuit of hotong flour and walnut enriched with probiotic cream contains levels between 5.20%–5.64%, which is almost identical to the 6% in commercial products.

The calorie value is directly proportional to the added walnut, which ranged from 546.27–577.12 kcal/100 g. The amount of energy in a food product was determined by the protein, fat, and carbohydrate content. In this study, the obtained calorie value met the minimum requirement of 400 kcal/100 g as specified by RDA standards. According to the % RDA calculation, a 30 g serving of biscuit manufactured from hotong flour and walnut enriched with probiotic cream contained an energy range of 164–173 kcal/30 g, which is higher than 150 kcal/29 g in commercial products.

The total sugar content ranged from 5.07% to 8.58%, with an increase in the proportion of hotong flour. Analysis of variance showed a significant effect of the treatment on the total sugar content of biscuit (p<0.05), attributable to the high levels of carbohydrates. Hotong flour contains 89.58% carbohydrates (Sugiyono, 2010), compared to 15.59% walnut (Thomson & Evans, 2004). Based on the % RDA calculation, a 30 g serving of biscuit manufactured from hotong flour and walnut enriched with probiotic cream *L. plantarum* Dad-13 contained 2–3 g less sugar than the 10 g in standard commercial biscuit.

Physical Properties & Sensory Characteristics

The physical properties of biscuit manufactured from hotong flour and walnut enriched with probiotic cream are presented in Table 3. Analysis of variance showed a significant effect of the treatments on biscuit hardness (p<0.05). As hosting flour decreased and walnut increased, hardness reduced from 31.53% to 16.03%. This is due to the 26.2% amylose content in hotong flour (Sigmarlatu et al., 2019). The use of flour or starch with high amylose caused an increase in the hardness and sensory acceptance values (Giuberti., 2015). Walnut, known for its high protein content, adds a crunchy texture and is easily breakable. Chaunier et al. (2007) stated that the gelation ability was increased by proteins, creating flexible structures or cross-linked tissues. As the hardness value reduces, the texture of the product becomes crispy and easily broken. Sensory test results in Table 4 confirmed that higher walnut content or reduced hotong flour improved crispness and fracture, leading to higher panelist preferences.

Table 3 shows a significant effect of treatments on the color (L*, a*, b*) of biscuit (p<0.05). The brightness value (L*) decreased from 52.59 to 49.04 as the proportion of walnut increased. The reduction is attributed to the Maillard, triggered by the protein content in walnut, leading to a brown color. The reddish value (a*) increased from 4.21 to 6.06 due to the carotenoid pigments, which produced a brownishred colour. According to Djarkasi et al. (2007), walnut contain

Table 4. Sensory characteristics of biscuit

Parameters	F1	F2	F3
Taste	4.68±1.5^{a}	4.77±1.42^{a}	6.13±0.68^{b}
Flavor	4.73±1.3^{a}	4.66±1.36^{a}	5.13±1.28^{b}
Color	5.14±1.44^{a}	5.01±1.27^{a}	5.38±1.02^{a}
Crispiness	4.54±1.65^{a}	4.57±1.5^{a}	6±0.92^{b}
Fractubility	4.27±1.54^{a}	4.48±1.63^{a}	5.86±0.87^{b}
Aftertaste	4.27±1.57^{a}	4.5±1.5^{a}	5.02±1.53^{b}
Overall	4.74±1.33^{a}	5.01±1.12^{a}	6.03±0.60^{b}

Note: Different superscript letters in the same row show a significant difference (p<0.05) between the samples. F1, 40% hotong flour : 10% walnut; F2, 35% hotong flour : 15% walnut; F3, 30% hotong flour : 20% walnut

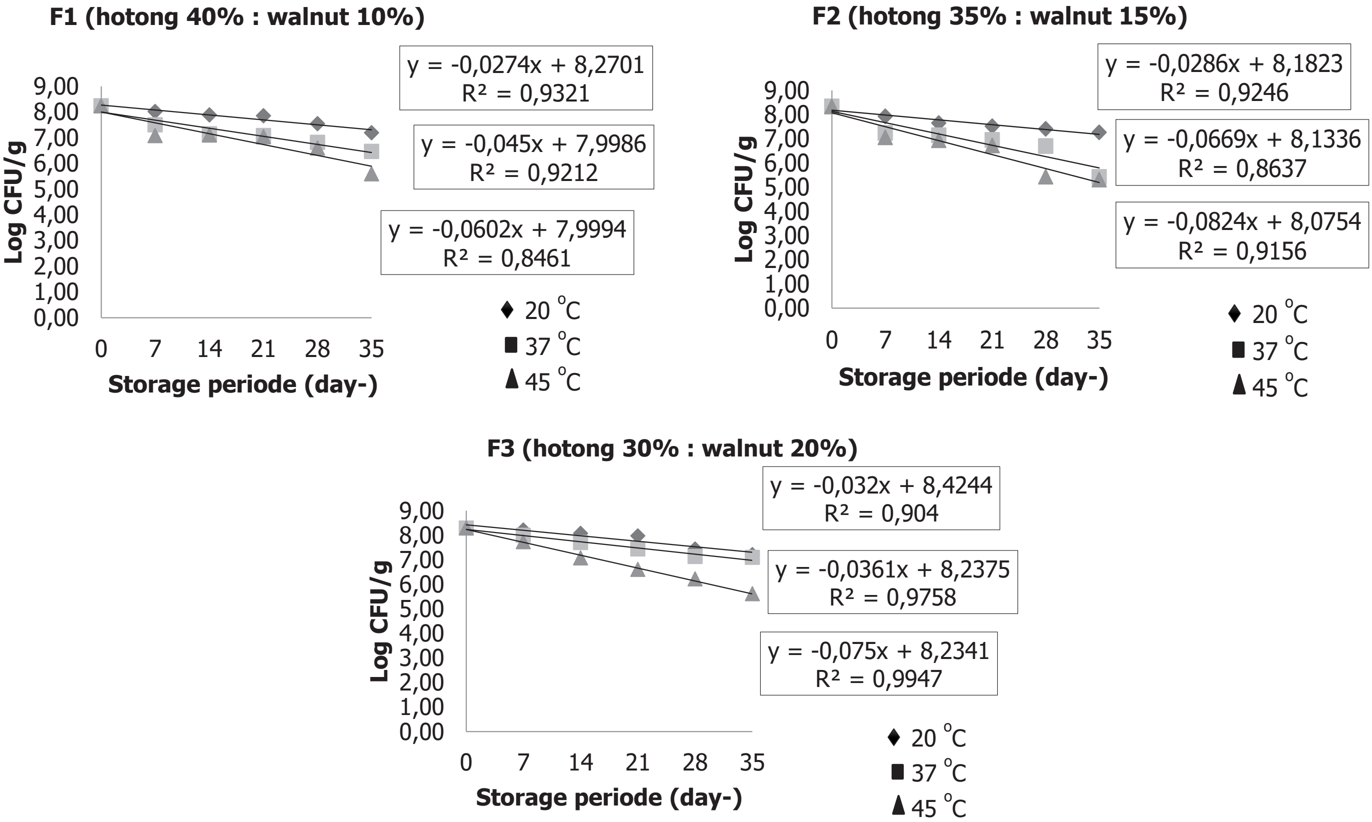

Figure 1. Cell viability of probiotic *L. plantarum* Dad-13 on cream biscuit of hotong flour and walnut at 20 °C, 37 °C, and 45 °C for 35 days

phenolic compounds, carotenoids, and tocopherol. The yellowness value (b*) decreased from 28.01 to 27.16 with a reduced proportion of hotong flour. Sugiyono et al. (2010) stated that the basic color of hotong was yellow. Therefore, the quantity of flour is proportional to the yellowness value (b*). The colour changes influenced the assessment of panelists, as shown in Table 4. The higher the proportion of walnut added, the more the preference value of biscuit.

Table 4 shows a significant effect of the treatments on biscuit taste ($p<0.05$) which ranged from 4.68–6.13. Preference increased with higher walnut proportions due to its high protein and fat content, which enhanced the savory or umami flavor. According to Yamaguchi (1998), the savory or umami taste was influenced by glutamic acid, and walnut contained 28.4% glutamate. The results of this study are in line with Lawalata et al. (2004) who reported that higher walnut content, enhanced savory or umami taste and increased the preference of panelists.

The results of the panelists' hedonic test of biscuit flavor showed an average value ranging from 4.66–5.13. Table 4 presents a significant effect of the treatments on biscuit flavor ($p<0.05$). Panelists preference for flavor increased with higher walnut proportions. According to Tuhumury et al. (2020), walnut contributed to a pleasant aroma through the Maillard reaction and amino acids interaction.

The results of the hedonic test of biscuit aftertaste had an average value ranging from 4.27–5.02. Table 4 shows a significant effect of the treatments on aftertaste ($p<0.05$). The higher the proportion of hotong flour, the lower the level of fitness of panelists towards the biscuit. The overall acceptance rate of *L. plantarum* Dad-13 probiotic cream-enriched hotong and walnut biscuit ranged from 4.74–6.03 and was influenced by the taste, flavor, color, crispiness, fractubility, and aftertaste. Compared to F1 and F2, F3 had a high level of preference. The addition of walnut at a high concentration had a better effect on taste, flavor, color, crispiness, fractubility, and aftertaste. When the concentration is low, the taste, flavor, color, crispiness, fractubility, and aftertaste become disliked.

Probiotic Cell Viability

Probiotic cell viability was assessed for 35 days at 20 °C, 37 °C, and 45 °C, with a pour plating method implemented every 7 days to determine cell count. On the first day, an average of 8.24–8.33 log CFU/g of *Lactiplantibacillus plantarum* Dad-13 cell was recorded. Figure 1 hows the results of the viability test for *Lactiplantibacillus plantarum* Dad-13 probiotic cell.

Based on the results of this study, the viability of *L. plantarum* Dad-13 probiotic cells in the cream of hotong flour and walnut biscuit was significantly influenced by variations in storage temperature. Stanton et al. (2003) and Dave & Shah (1997) stated that storage temperature is a critical factor in probiotic stability, as it can result in loss of viability due to damage. High degrees can lead to lipid oxidation in cells, causing the death of probiotic cells. According to Vivek et al. (2023), an increased storage temperature impacts the cytoplasmic membrane by changing its fluidity and physical state. This led to a reduction in cell stability and caused lipid oxidation. The fatty acid profile of the cell membrane is changed by oxidation. It affects the structure and function and produces free radicals that cause cell death. As storage time increases, the lipid composition on the cell membrane undergoes significant changes (Santivarangkna et al., 2008). To maintain probiotic cell stability in hotong biscuit and walnut enriched with probiotic cream, storage at low temperatures is recommended. Conversely, high temperatures are known to decrease the viability of probiotic cells.

CONCLUSION

In conclusion, hotong flour and walnut biscuit formulation F3 enriched with probiotic cream was identified as the best formulation. The F3 resulted biscuit chemical characteristics such as included moisture content 2.18%, ash 2.31%, protein 8.82%, fat 22.97%, carbohydrate 65.89%, calories 577. 15 Kcal, and total sugar 5.07% and physical characteristics comprised hardness 16.03%, as well as color L* 49.04%, a* 6.05%, b* 27.16%. Meanwhile, the sensory characteristics were color 5.38, taste 6.13, flavor 5.13, crispiness 6, fracktuability 5.86, aftertaste 5.02, and overall 6.03, signifying acceptability by panelists. A 30 g portion of F3 provided 173 kcal total energy, 10% total fat, 4.41% protein, and 5.20% total carbohydrates based on RDA. The viability of probiotic cells in *L. plantarum* Dad-13 at 20 ^{o}C duringstorage for F1, F2, and F3 was 10^7 CFU/g.

ACKNOWLEDGEMENT

The authors are grateful to the Indonesia Endowment Fund for Education (LPDP)–the Ministry of Finance, for supporting this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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