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Sensory Acceptability and Viability of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* in Spent Coffee Ground Candy

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Abstract

Spent coffee ground (SCG) is a residual material considered as waste that remains after brewing coffee. Conventional brewing techniques generate large amount of SGC that is rich in lignocellulose and valuable bioactive compounds that can be exploited as functional food ingredients. This study explored the development of a probiotic SCG candy using spent coffee grounds (SCG) as a sustainable and functional matrix, incorporating *Lactobacillus acidophilus* and *L. bulgaricus*. The effect of different carbohydrate sources—banana and potato—on the candy's physicochemical properties, sensory attributes, and probiotic viability during storage was evaluated. Four formulations were prepared: SCG-only (T0), SCG–banana (T1), SCG–banana/potato (T2), and SCG–potato (T3), each stored at room temperature (25±2 °C) and refrigeration (4 °C) for four weeks. Physico-chemical analyses showed moisture content ranged from 7.49% (T0) to 9.43% (T3), water activity from 0.46 to 0.54, and pH values from 6.87 to 7.61. Total soluble solids (TSS) were between 44.00 and 52.66 °Brix. These parameters remained stable throughout storage. Viable counts of *Lactobacillus* spp. remained above 8.3 log CFU/g in all samples, with SCG–banana (T1) stored at 4 °C achieving the highest count of 8.45 log CFU/g after four weeks. Sensory evaluation using a 5-point hedonic scale revealed that SCG–banana (T1) and SCG–banana/potato (T2) achieved significantly higher ratings in chewiness (4.6), mouthfeel (4.6), overall flavor (4.5), and acceptability (4.7) than the control (T0). Refrigerated storage preserved microbial viability and sensory quality better than room temperature. Overall, this study confirms that SCG can serve as a value-added matrix for probiotic delivery in confections. The inclusion of banana improved both functionality and consumer preference, supporting the development of shelf-stable, sustainable, probiotic-rich snacks.

Keywords: *Lactobacillus acidophilus, L. bulgaricus*, spent coffee ground, candy, probiotics

INTRODUCTION

Coffee is one of the most widely consumed beverages worldwide and a major agricultural product in the Philippines. From January to March 2021, the country produced approximately 19.35 thousand metric tons of dried coffee berries (PSA, 2021), with consumption reaching about 3.3 million 60-kg bags in 2020. This growing demand results in large quantities of waste, notably spent coffee grounds (SCG), estimated at six million tons annually. SCG, the solid residue left after brewing, is often discarded despite being rich in nutrients such as dietary fiber, proteins, minerals, and phenolic compounds with antioxidant activity (Cruz *et al*., 2012; Ballesteros *et al.*, 2014).

Given its bioactive composition, SCG holds potential as a functional food ingredient, especially in heat-processed products such as candy. Functional confections are gaining popularity as non-dairy carriers for probiotics, particularly for consumers seeking lactose-free options. However, maintaining the viability of probiotics during high-temperature processing is a significant challenge.

The incorporation of prebiotic-rich substrates such as potato (*Solanum tuberosum*) and Saba banana (*Musa balbisiana*)—both abundant in fermentable carbohydrates and dietary fiber—may enhance probiotic stability. Although these ingredients have been explored individually, their combined use with SCG in probiotic candy remains under-researched.

Lactobacillus acidophilus (LA-5) and *Lactobacillus bulgaricus* (LB-12) are recognized probiotic strains with proven digestive health benefits and safety status (GRAS by FDA and QPS by EFSA) (Ouwehand *et al.*, 2002; Del Campo *et al.*, 2005). This study aims to develop a functional SCG candy made from SCG enriched with potato and saba banana, and post-cooking inoculated with *L. acidophilus* and *L. bulgaricus*, as a novel non-dairy probiotic product.

MATERIALS AND METHODS

The raw materials used in this study included Saba banana (*Musa balbisiana*), potato (*Solanum tuberosum*), and spent coffee grounds (SCG). The standard candy ingredients such as white granulated sugar (commercial grade, 99.9% sucrose), glucose syrup (DE 40–42), food-grade gelatin (type B, 200 bloom strength), citric acid (anhydrous), and natural vanilla flavoring, which were all purchased from local food-grade suppliers in Indang and Tagaytay City, Cavite, Philippines.

All additives complied with local food safety standards and were used at optimized levels based on preliminary testing and literature references for fruit-based candy formulations.

The probiotic bacteria *Lactobacillus acidophilus* (strain LA-5) and *Lactobacillus bulgaricus* (strain LB-12) were obtained as pure freeze-dried cultures from the Microbiology and Genetics Division of the Industrial Technology Development Institute (ITDI), Department of Science and Technology (DOST), Philippines. Upon revival, viable cultures were suspended in 50% (v/v) aqueous glycerol and stored at room temperature (25 °C). Identity confirmation was performed using the Analytical Profile Index (API 50 CHL) system, following manufacturer protocols.

Experimental design

This experiment used a Completely Randomized Design (CRD) wherein the samples were randomly arranged to serve as replication for analyses.

The following candies were used as treatments:

T0 – SCG candy
T1 – SCG-5% banana mix SCG candy
T2 – SCG-5% banana/potato mix SCG candy
T3 – SCG-5% potato mix SCG candy

Processing of SCG candy

The probiotic SCG candy was prepared based on the formulation of Greweling (2013), with modifications that included the addition of spent coffee grounds (SCG), lactic acid bacteria (LAB), and plant-based carbohydrate sources from banana and potato. The base formulation consisted of sugar syrup (55%), gelatin (10%), starch (10%), SCG (5%), and water (20%). In this formulation, 5% of the sugar syrup was substituted with mashed Saba banana and potato as carbohydrate fillers.

Fresh Saba bananas were procured at their mature green stage, then peeled, sliced, and boiled for 15–20 minutes to soften the starch matrix and enhance flavor. This variety is characterized by high amylose content and firm texture, which makes it suitable for starchy confections (Moorthy *et al*., 2014).

Potatoes were similarly sourced fresh, peeled, and boiled prior to use. They are rich in carbohydrates and dietary fiber, which contribute to the energy value, bulk, and satiety of the final product (Camire *et al.* 2009; Burlingame *et al.* 2009). Boiled Saba bananas and potatoes were incorporated as natural carbohydrate sources in the probiotic SCG candy formulation. On average, boiled Saba bananas contain approximately 31 g of carbohydrates per 100 g, while boiled potatoes provide around 17 g per 100 g (Camire *et al*., 2009; Moorthy *et al*., 2014; FNRI-DOST, 2020).

The SCG used in this study was collected from espresso-extracted Robusta (*Coffea canephora*) beans sourced from local coffee shops in Tagaytay City. The grounds were air-dried at ambient temperature (25 °C), then analyzed for grain size using standard mesh sieving and pH with a calibrated digital pH meter (PAL-pH, Atago Co., Ltd., Tokyo, Japan).

To preserve the viability of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus*, a post-cooking inoculation technique was employed. After cooking the candy base—composed of sugar syrup, gelatin, SCG, boiled mashed potato, and banana at approximately 115 °C to reach the desired consistency, the mixture was allowed to cool to below 40 °C. At this temperature, safe for probiotic survival, the freeze-dried probiotic cultures (reconstituted in sterile water) were aseptically incorporated and thoroughly mixed into the candy mass to ensure even distribution. The inoculated mixture was then poured into sterile silicone molds, allowed to set at ambient temperature, individually wrapped in moisture-resistant food-grade packaging, and stored at 4 °C to maintain probiotic viability.

Inoculation of lactic acid bacteria into SCG candy

Freeze-dried starter cultures containing strains of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* were used as probiotic inoculants for the SCG candy. The lactic acid bacteria were activated by culturing twice in de Man, Rogosa and Sharpe (MRS) broth at 37 °C for 18 hours (overnight incubation). After incubation, the bacterial cells were harvested by centrifugation at medium speed (approximately 4000 rpm) for 10 minutes. The resulting pellets were washed twice with 1.0% (w/v) normal saline solution (NSS) to remove residual medium components, and then concentrated tenfold using the same diluent. A 1.0% (v/v) inoculum of each probiotic culture was aseptically added to the SCG soft candy mixture after it had cooled to below 40 °C, in order to maintain probiotic viability. This process was adapted to achieve a final probiotic concentration of approximately 10v CFU/g, following the method described by Velando *et al*. (2013).

Effect of storage conditions on the sensory and viability of lactic acid bacteria in SCG candy

Forty grams of inoculated probiotic SCG candy were placed in sterile plastic containers (Axygen®, Axygen Inc., USA). The samples were shaken vigorously using a vortex mixer for 5 minutes to ensure homogeneity, then sealed and placed inside airtight secondary containers. The samples were stored under two different conditions: room temperature (25±2 °C) and refrigerated temperature

(4 °C). These conditions were maintained throughout the storage period (4 weeks) to evaluate the effect of temperature on the sensory and viability of lactic acid bacteria (LAB) and product stability.

Physicochemical analyses of probiotic SCG candy

Moisture content analysis. Oven drying was used to measure the moisture content of the probiotic SCG candy following the AOAC official method 925.10 (AOAC International, 2005). Five grams of the probiotic SCG candy was placed in a petri dish and dried in an oven for three hours at 105 °C. It was cooled in the desiccator and weighed.

Water activity. The water activity of the probiotic SCG candy was determined using a water activity meter (AquaLab Series 4TE, Meter Group Inc., Pullman, WA, USA). Measurements were conducted at 25±1 °C according to the manufacturer's instructions and AOAC Official Method 978.18.

Total soluble solid analysis. Total soluble solid (TSS) of the probiotic SCG candy was determined using a hand-held refractometer (Naidu *et al*., 2008).

pH. The pH of the probiotic SCG candy was determined using a pH meter (PAL-pH, Atago Co., Ltd., Tokyo, Japan).

Sensory evaluation of probiotic SCG candy

Ten laboratory panelists evaluated the probiotic SCG candy samples at two different storage points: initially (day 0) and after one month of storage. The sensory evaluation focused on the following attributes: color, aroma, chewiness, sweetness, coffee flavor, overall flavor, off-flavor, mouthfeel, and overall acceptability. A 5-point hedonic scale was used for the sensory test, where 1 = "extremely disliked" and 5 = "extremely liked", indicating that the test was based on hedonic analysis to assess the panelists' degree of liking for each sensory attribute.

Determination of viability of LAB

This method follows established microbiological procedures for LAB viability in fermented or functional food products (U.S. FDA, 2001). The viability of lactic acid bacteria (LAB) in the probiotic SCG candy was determined using the standard plate count method. A 10 g portion of the candy sample was aseptically transferred into a sterile stomacher bag containing 90 mL of sterile 1.0% (w/v) normal saline solution (NSS). The mixture was homogenized for 2 minutes using a stomacher (BagMixer 400, Interscience, France) to obtain a 10^{-1} dilution.

Subsequent serial tenfold dilutions were prepared in NSS. From each appropriate dilution, 1 mL was poured plated in duplicate onto ROGOSA SL agar (Difco™, USA). Plates were incubated at 37 °C for 48 hours under aerobic conditions. Following incubation, colonies exhibiting typical LAB morphology were counted. The results were expressed as colony forming units per gram (cfu/g).

Statistical analysis

All experiments were performed in triplicate and results are expressed as means. Paired t-tests and Repeated Measures Analysis of Variance (RMANOVA) were used to compare the mean of each lactobacillus across time. All the statistical tests used SPSS 17.0 and p-values of <0.05 indicate significant differences.

RESULTS AND DISCUSSION

Physico-chemical properties of probiotic SCG candy

The physico-chemical properties of the probiotic SCG candy formulations varied depending on the incorporation of banana and/or potato alongside spent coffee grounds (SCG) and are presented in Table 1.

Moisture content was significantly influenced by the added ingredients, with T3 (SCG + potato) exhibiting the highest moisture levels at both Week 1 (9.43%) and Week 4 (9.35%), followed by T2 (SCG + banana/potato) and T1 (SCG + banana). The control sample (T0, SCG only) maintained the lowest moisture content across both time points (7.49–7.44%) and showed no significant change during storage. These results suggest that starch-rich additives such as banana and potato contribute to higher moisture retention in the SCG candy matrix. This finding is supported by the inherent water-binding properties of banana and potato starches. Banana starch has been reported to contain moisture levels around 9–10%, demonstrating its capacity to retain water in food matrices (Sundarram & Murthy, 2014). Similarly, potato starch is recognized for its high starch granule content and excellent water-holding capacity, which enhances moisture retention in composite formulations (Nawaz *et al*., 2020). These attributes likely contributed to the elevated moisture content observed in the treatments containing these ingredients, particularly T3.

Water activity (A_w) values for all formulations remained below/ 0.60, indicating microbiological stability and a low risk of spoilage throughout the storage period. No significant differences were observed in A_w among treatments or over time, with values ranging from 0.46 to 0.54. According to Razak *et al*. (2020), sugar-rich products such as confectionery generally exhibit microbial stability when water activity is maintained below 0.60, as most bacteria, yeasts, and molds are unable to grow at this level.

Similarly, pH values remained relatively stable across treatments and storage duration, ranging from 6.87 to 7.61, and showed no statistically significant changes. This suggests that the incorporation of fruit or tuber components did not notably alter the acidity of the product. Banana and potato starches are known to have near-neutral pH values, typically between 6.4 and 6.9, which contributes to the buffering effect in food systems (Hanif *et al*., 2023; Fawale *et al*., 2021). This pH stability plays a key role in preserving the physicochemical and microbial integrity of the final candy product. The pH of SCG candies ranges from 6.87–7.61, which is considered neutral. For the fresh probiotic SCG candy, the pH is not significantly different from probiotic SCG candy without a carbohydrate source. The standard

Table 1. Physico-chemical properties of probiotic (*L. acidophilus* and *L. bulgaricus*) SCG candy

Physicochemical properties	Treatments							
	SCG	(T0)	SCG-banana (T1)		SCG-banana/ potato, (T2)		SCG-potato (T2)	
	Wk1	Wk4	Wk1	Wk4	Wk1	Wk4	Wk1	Wk4
Moisture, %*	7.49 a	7.44 a	8.41 b	8.39 b	8.70 b	8.64 b	9.43 c	9.35 c
Water activity, ns	0.46	0.48	0.54	0.52	0.53	0.54	0.52	0.51
pH, ns	6.87	6.97	7.61	7.54	7.33	7.29	7.32	7.42
Total soluble solid, °Brix, ns	51.57	52.66	44.00	45.23	47.67	46.73	50.57	49.65

*Means followed by a common letter are not significant at p<0.05,
* - significant; ns = no significant difference at p<0.05, Wk = week.

pH values in SCG candy are in the range of 2–8 (Fontana, 2005), therefore the pH values obtained for all the treatments were accepted based on the standards.

Total soluble solids (°Brix) were highest in the control (T0), with a slight increase from Week 1 (51.57 °Brix) to Week 4 (52.66 °Brix). In contrast, the banana-containing formulation (T1) had the lowest °Brix values, which may be attributed to the dilution of sugars due to the moisture-rich nature of banana pulp. This trend aligns with previous findings that incorporation of high-moisture fruits, such as banana, can reduce °Brix content in sugar-based products by increasing water content and diluting soluble solids (Singh *et al*., 2019). Across all treatments, °Brix values remained statistically unchanged over the storage period, indicating a stable sugar profile. This stability, along with consistent water activity and pH values, suggests that the formulated probiotic SCG candies remained physically and chemically stable over four weeks.

Furthermore, ingredient selection—particularly the inclusion of starch- and moisture-rich components—significantly influenced moisture retention and soluble solids content without adversely affecting microbial safety or acid-base balance (Shrestha *et al*., 2021; Razak *et al*., 2020).

Changes in physicochemical properties of SCG candy during storage

A decreasing trend in pH was observed after one month of storage under both refrigerated and room temperature conditions. The decline in pH, particularly under refrigeration, can be attributed to the continued metabolic activity of lactic acid bacteria (LAB), which break down sugars and produce organic acids, such as lactic acid, contributing to increased acidity. Remarkably, despite this acid production, the probiotic SCG candy did not exhibit a sour taste, likely due to the buffering effect of the candy matrix. *Lactobacillus* species, including *L. acidophilus* and *L. bulgaricus*, are known to thrive in mildly acidic conditions, typically between pH 3.5–6.8, due to their acid tolerance mechanisms (Hayek & Ibrahim, 2013).

The initial increase in alkalinity observed in the probiotic SCG candy formulations can be attributed to the incorporation of starch-rich carbohydrate sources such as banana and potato, which may have contributed to buffering effects or reduced acidity at baseline (Hanif *et al*., 2023). After storage, however, both storage conditions led to a pH reduction, with a more pronounced effect in the refrigerated group, likely due to suppressed microbial degradation processes at room temperature that delay acidification (Shrestha *et al.,* 2021).

Regarding moisture content, probiotic SCG candies stored under refrigerated conditions had relatively lower values compared to those stored at room temperature. This difference may be explained by reduced molecular mobility and evaporation at low temperatures, leading to better moisture retention. In contrast, room temperature conditions can facilitate water migration within the matrix and slight absorption of environmental humidity, especially in semi-permeable packaging systems (Chávez *et al*., 2020). Moreover, ongoing carbohydrate respiration and water release may be less active under cooler conditions, contributing to the lower moisture content observed in refrigerated samples.

Water activity (aw) across all treatments remained within the acceptable stability range of 0.40–0.60 (Fontana, 2005), ensuring microbiological safety. Notably, refrigerated samples had lower aw values than those stored at room temperature. This is consistent with the findings of Troller & Stinson (2015), who reported that water activity generally increases with temperature due to higher vapor pressure and molecular motion. The relatively higher A_w under room temperature conditions may present a slightly elevated risk for quality degradation if storage exceeds one month, particularly in humid climates.

A decrease in total soluble solids (TSS), measured in °Brix, was noted in both storage conditions after one month. However, the reduction in °Brix was more pronounced in refrigerated samples. This trend may be linked to sugar degradation and metabolic conversion to acids under refrigeration, whereas room temperature samples exhibited a slower reduction in TSS, possibly due to decreased bacterial activity or higher evaporation rates concentrating sugars (Singh *et al.*, 2019). Studies on fruit-based and starch-enhanced confectionery products have reported similar TSS stability patterns during short-term storage (Shrestha *et al.*, 2021).

Sensory properties of probiotic SCG candy

The probiotic SCG candy was evaluated after production by ten laboratory panelists using a five-point hedonic scale, based on quality attributes such as color, aroma, chewiness, sweetness, coffee flavor, overall flavor, off-flavor, mouthfeel, and overall acceptability.

Table 2 presents the sensory evaluation results of probiotic SCG candies formulated with spent coffee grounds (SCG) and various carbohydrate sources. Sensory scores were evaluated based on a 5-point hedonic scale (1 = extremely dislike, 5 = extremely like) over a four-week storage period.

Across all samples, color, aroma, sweetness, and coffee flavor did not differ significantly ($p > 0.05$), regardless of treatment or storage time. Color ratings ranged from 4.3 to 4.7, with the control (T0) scoring 4.7 in Week 1 and 4.6 in Week 4. Treatments T1 and T3 had slightly lower color ratings after four weeks (4.3 and 4.5, respectively), though differences were not statistically significant. The dominance of SCG's natural dark pigmentation likely masked any visual variation introduced by banana or potato. Similar observations were reported by Singh & Wani (2015), who found that the base matrix color overpowered added fruit pulp color in functional candies.

Aroma scores were consistently high across all treatments (4.3–4.9), indicating that the addition of banana or potato did not negatively impact the aroma profile. Sweetness scores ranged from 3.1 to 3.9, with the highest sweetness noted in T1 (banana) at Week 1 (3.9), while the lowest was in T3 (potato) at week 4 (3.1). This slight difference

Table 2. Sensory properties of probiotic SCG candy

Physicochemical properties	Probiotic (*L acidophilus* and *L. bulgaricus*) SCG candy mixes							
	SCG (T0)		SCG-banana (T1)		SCG-banana/ potato, (T2)		SCG-potato (T3)	
	Wk1	Wk4	Wk1	Wk4	Wk1	Wk4	Wk1	Wk4
Color^{ns}	4.7	4.6	4.5	4.3	4.7	4.4	4.7	4.5
Aroma^{ns}	4.6	4.3	4.7	4.5	4.7	4.5	4.9	4.6
Sweetness^{ns}	3.5	3.2	3.9	3.8	3.7	3.5	3.3	3.1
Coffee flavor^{ns}	4.3	4.1	4.5	4.6	4.2	4.3	4.1	4.0
Off flavor*	3.5 ^{a}	3.2 ^{a}	4.0 ^{b}	4.3 ^{b}	4.2 ^{b}	4.1 ^{b}	3.8 ^{b}	3.6^{b}
Overall flavor*	3.8 ^{a}	3.4 ^{a}	4.3 ^{b}	4.4 ^{b}	4.4 ^{b}	4.5 ^{b}	4.7 ^{b}	4.6^{b}
Chewiness*	4.0 ^{a}	3.9 ^{a}	4.6 ^{b}	4.4 ^{b}	4.5 ^{b}	4.6 ^{b}	3.9 ^{a}	3.8^{a}
Mouthfeel *	4.1 ^{a}	3.8 ^{a}	4.6 ^{b}	4.5 ^{b}	4.5 ^{b}	4.6 ^{b}	4.3 ^{a}	4.2^{a}
Overall acceptability *	4.1 ^{a}	3.7 ^{a}	4.7 ^{b}	4.6 ^{b}	4.5 ^{b}	4.7 ^{b}	4.2 ^{a}	4.0^{a}

*Means followed by a common letter are not significant at $p<0.05$,
* = significant, ns = no significant difference at $p<0.05$, Wk= week.

may be attributed to the natural sugars present in banana, enhancing the perceived sweetness, as previously reported by Yadav *et al.* (2018).

Coffee flavor was retained well across formulations, with values ranging from 4.0 to 4.6. T1 (banana) showed the highest coffee flavor score at week 4 (4.6), suggesting that banana's mild flavor complemented rather than masked the coffee taste. This supports findings from Khurana *et al.* (2020), who demonstrated that compatible ingredient pairing can enhance core flavors in functional confectionery.

Significant differences ($p < 0.05$) were found in off-flavor, overall flavor, chewiness, mouthfeel, and overall acceptability. Off-flavor was lowest in the control (T0) with scores of 3.5 (week 1) and 3.2 (week 4), indicating higher perceived off-notes in T1 –T3 , particularly due to identifiable flavors from added ingredients. However, T1 (4.0–4.3), T2 (4.2–4.1), and T3 (3.8–3.6) were statistically higher than T0 , with panelists noting that the potato in T3 contributed an identifiable off-flavor. This aligns with findings from Irwandi *et al.* (2016), who reported that starchy root additions may impart undesirable aftertastes in candies.

Overall flavor scores were significantly improved with carbohydrate addition. T3 (SCG + potato) had the highest score at Week 4 (4.6), followed closely by T2 (4.5) and T1 (4.4), compared to the control's lower rating of 3.4. These enhancements suggest a synergistic effect between SCG and added carbohydrates in improving flavor complexity.

The chewiness of the candies was also positively impacted by banana and banana/potato combinations. T1 and T2 had the highest chewiness scores, reaching 4.6 and 4.5 at week 1, respectively, while T0 and T3 had lower and statistically similar scores (3.8–4.0). Banana's fiber and pectin content likely contributed to a more elastic, cohesive texture (Sun-Waterhouse *et al.*, 2013).

Mouthfeel ratings followed a similar trend: T1 and T2 scored significantly higher (4.6–4.5) than T0 (3.8) and T3 (4.2), with panelists describing the potato-containing candy as having a somewhat mushy texture. These results support prior findings that fruit-derived fibers enhance texture perception, while starches alone can lead to less favorable consistency (Khurana *et al.*, 2020).

Overall acceptability was highest in T1 and T2, reaching 4.7 in week 1 and maintaining high scores in week 4 (4.6–4132132.7). In contrast, the control and potato-only samples scored significantly lower (T0 : 3.7; T3 : 4.0), emphasizing the role of fruit-based carbohydrate additions in improving product appeal. These findings are consistent with Singh & Wani (2015), who observed that candies enriched with fruit pulp had higher sensory acceptability due to improved sweetness, flavor, and textural attributes.

Changes in sensory properties of SCG candy during storage

To assess the impact of storage conditions on sensory quality, probiotic SCG candies were stored for one month under two conditions: room temperature (25±2 °C) and refrigerated temperature (4 °C). Sensory evaluation was then conducted and compared to freshly produced samples.

After one month of storage, no significant differences ($p > 0.05$) were observed in color, aroma, sweetness, or coffee flavor across all treatments, regardless of storage condition. These results indicate that the visual and aromatic attributes, as well as the basic flavor profile of the SCG candies, remained stable during storage. The retention of these qualities suggests effective formulation stability, supported by the low water activity and consistent pH levels previously measured. Similar findings were reported by Sun-Waterhouse et al. (2013), who noted minimal changes in basic sensory traits of functional confections stored under controlled temperature and humidity conditions.

However, significant differences ($p < 0.05$) were found in chewiness, overall flavor, off-flavor, mouthfeel, and overall acceptability between the two storage conditions. SCG candies stored at refrigerated temperature (4°C) maintained significantly better scores in these sensory attributes compared to those stored at room temperature. Refrigerated storage appeared to help preserve the textural integrity of the candy, likely minimizing starch retrogradation and moisture migration, which may occur more rapidly at higher temperatures. This is consistent with findings by Tripathi and Giri (2014), who reported improved sensory and probiotic stability in refrigerated functional foods.

Panelists noted that candies stored at room temperature developed slightly firmer textures and marginally increased off-flavors, likely due to slow degradation of ingredients or flavor migration during storage. These subtle deteriorations negatively affected mouthfeel and acceptability. On the other hand, refrigerated storage helped maintain the product's softness and flavor uniformity, contributing to higher ratings for chewiness (e.g., 4.4–4.6 vs. 3.8–3.9), mouthfeel (4.5–4.6 vs. 3.8–4.2), and overall acceptability (4.6–4.7 vs. 3.7–4.0).

These results emphasize the importance of cold storage for preserving the quality of functional candies containing heat-sensitive or flavor-rich ingredients, such as probiotics, SCG, and fruit-based components. Cold storage appears to extend sensory shelf life by maintaining favorable organoleptic attributes, consistent with other studies on probiotic confections and dairy-free snacks (Khurana *et al*., 2020; Yadav *et al.*, 2018).

Viability of lactobacillus (*L. acidophilus/ L. bulgaricus*) during storage

The number of lactobacillus strains in probiotic SCG candy was counted for both storage conditions weekly until one month of storage to assess the viability of probiotics. The viability of lactobacillus strains in probiotic SCG candy is shown in Table 3.

The viability of probiotic lactic acid bacteria (*Lactobacillus acidophilus* and *L. bulgaricus*) in the SCG-based candy matrix was monitored over four weeks under refrigerated (4 °C) and room temperature (25±2 °C) storage conditions (Table 3). The initial population counts at week 1 ranged from 8.33 to 8.40 log CFU/g across all treatments, indicating successful incorporation of viable probiotic cells following production.

Table 3. Lactobacillus (*L. acidophilus/L. bulgaricus*) count during storage

Storage temperature	Probiotic SCG candy (Treatments)	Populations of probiotics (log cfu/g) Week 1*	Week 4*
Refrigerated (~4 °C)*	SCG (T0)	8.34 a	8.39 a
	SCG-Banana (T1)	8.38 a	8.45 b
	SCG-Banana/potato (T2)	8.34 a	8.43 b
	SCG-Potato (T3)	8.39 a	8.44 b
Room temperature (~25 ±2°C)*	SCG (T0)	8.33 a	8.30 a
	SCG-Banana (T1)	8.35 a	8.33 a
	SCG-Banana/potato (T2)	8.40 a	8.36 b
	SCG-Potato (T3)	8.36 a	8.37 b

Note: *Means for n = 3, the test isolates that have no common superscript are significantly different ($p<0.05$) according to Duncan Multiple range test.

At refrigerated storage (4 °C), viable counts increased slightly over time. For example, the SCG–banana (T1) treatment increased from 8.38 to 8.45 log CFU/g, while the SCG–banana/potato (T2) sample rose from 8.34 to 8.43 log CFU/g. A similar trend was observed for T3 (SCG + potato), which increased from 8.39 to 8.44 log CFU/g. These increases were statistically significant ($p < 0.05$) for T2, T2, and T3, likely due to the protective effect of added carbohydrates, which may provide fermentable substrates or improve microenvironmental stability within the candy matrix. This observation aligns with findings by Ranadheera *et al.* (2010), who reported that plant-derived ingredients such as fruit or tuber starches can enhance probiotic survival by offering prebiotic effects and buffering action.

In contrast, under room temperature storage, viable counts were generally stable or slightly declined. The SCG–banana (T1) decreased marginally from 8.35 to 8.33 log CFU/g, while T0 (control) dropped from 8.33 to 8.30 log CFU/g. These changes were not statistically significant ($p > 0.05$). However, the SCG–banana/potato (T2) and SCG–potato (T3) treatments retained relatively stable counts, with T2 showing a slight but statistically significant decline (from 8.40 to 8.36 log CFU/g). These reductions may be attributed to greater environmental stress under ambient storage, such as oxygen exposure, moisture loss, or metabolite accumulation, which can reduce probiotic viability in non-refrigerated functional food systems (Tripathi & Giri, 2014).

Despite minor fluctuations, all samples across both storage conditions retained counts above 8.3 log CFU/g throughout the storage period. These levels exceed the minimum threshold of 6–7 log CFU/g generally recommended to confer probiotic health benefits (Champagne *et al.*, 2018). Thus, the probiotic SCG candies, particularly those stored at refrigerated temperature, maintained functional viability over one month of storage.

These results demonstrate that the use of banana and/or potato as carbohydrate sources not only improved sensory acceptability (as previously discussed) but also supported greater stability and survivability of *Lactobacillus* spp. over time. The beneficial effects of refrigerated storage further highlight the importance of cold chain maintenance in extending the shelf life of non-dairy probiotic products (Ranadheera *et al.*, 2010; Tripathi & Giri, 2014).

Figure 1 illustrates the growth trend of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* incorporated into SCG candy formulations during storage. Viable cell counts (log cfu/g) were measured over 4-weeks at two storage conditions: refrigerated (~4 °C) and room temperature (~25±2 °C). The growth curve shows initial stabilization of probiotic populations in week 1, followed by slight fluctuations depending on the formulation and storage temperature. Overall, all SCG candy variants maintained viable counts above the recommended minimum threshold (10 cfu/g), indicating effective probiotic retention in the matrix throughout the storage period. Refrigerated samples generally showed better stability and slightly enhanced survival compared to those stored at room temperature.

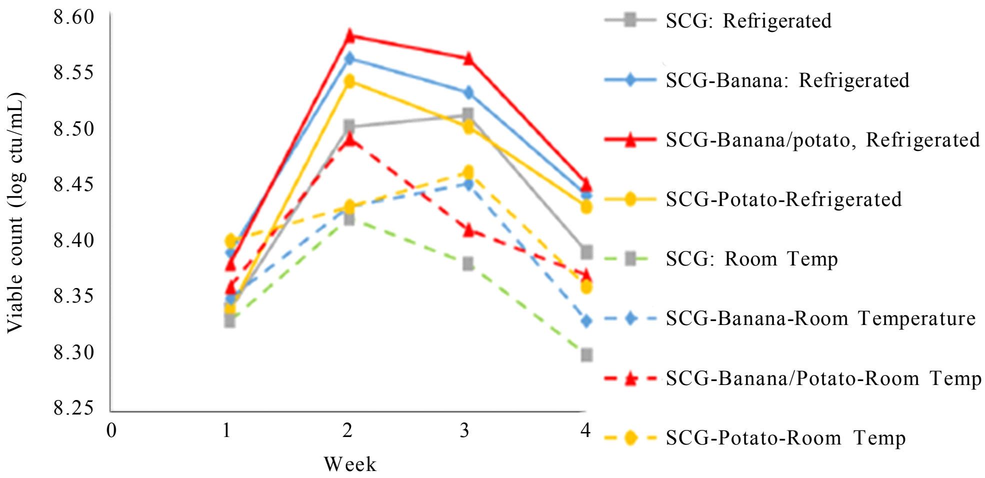

Figure 1. Growth curve of *Lactobacillus* in probiotic SCG candy

CONCLUSION

Physicochemical analysis revealed that the inclusion of fruit- or root-based carbohydrates slightly increased moisture content and total soluble solids, but had no significant impact on water activity or pH. Sensory evaluation indicated that probiotic SCG candies enriched with banana and banana/potato blends (T1 and T2) achieved significantly higher ratings in chewiness, mouthfeel, overall flavor, and acceptability compared to the control and potato-only variants, with scores retained after four weeks of storage. Storage trials demonstrated that refrigeration (4 °C) effectively preserved probiotic viability, sensory quality, and overall acceptability over one month, with *Lactobacillus* counts maintained above 8.3 log CFU/g, exceeding the minimum therapeutic threshold. In contrast, room temperature storage resulted in slight sensory and microbial declines, although all formulations remained within acceptable probiotic levels. The addition of banana and banana/potato not only enhanced sensory attributes but also contributed to the improved stability of probiotic populations. The integration of SCG as a sustainable, fiber-rich matrix supports the valorization of food waste into health-oriented confectionery products. Overall, the developed probiotic SCG candy demonstrates strong potential as a novel, shelf-stable functional food product with favorable sensory and microbial stability under refrigerated storage. Probiotic SCG candy may also serve as a raw material for lactic acid fermentation, and the product could be a nutritious and healthy functional food.

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