



Original research

Probiotic gluten-free mini baguette bread: Fortified with encapsulated *Bacillus coagulans* strain and chia (*Salvia hispanica*) seeds

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ABSTRACT

The aim of the present study is to investigate the development for a functional gluten-free mini baguette bread enriched with encapsulated *Bacillus coagulans* probiotic strain and chia seeds to enhance nutritional and technological attributes during shelf life. Ten gluten-free dough formulations, based on a 50:50 rice-potato flour blend with 1-5% chia seeds and 1-3% encapsulated probiotics, were baked in triplicate. The farinograph, physicochemical, probiotic viability, sensory, scanning electron microscopy, and differential scanning calorimetry assays performed. The results illustrated that 5 % chia seed and 3 % probiotics (T9) significantly elevated water absorption (81 vs. 72 % in control), dough development time (4.6 vs. 2.0 min), stability (7.3 vs. 3.0 min), and farinograph quality number (475 vs. 361 BU), while reducing softening (74 vs. 120 BU). The bread analyses revealed that chia and probiotics improved moisture retention (31.3 to 31.6 vs. 30 % on day 10), reduced hardness (9.6 to 9.8 vs. 12 N), and maintained probiotic viability (~10 Log CFU/g). The differential scanning calorimetry indicated lower retrogradation enthalpy, confirming delayed staling, and scanning electron microscopy indicated a denser, uniform crumb structure with higher chia and probiotic levels. The sensory evaluation confirmed the higher acceptability in T9 (3.8 vs. 3.4 by day 10 of shelf life). These findings demonstrate that chia seeds and encapsulated *B. coagulans* synergistically enhance quality, and functionality of gluten-free baguettes, offering a promising approach for developing nutritious, long-lasting bakery products, and sensitive consumers.

Keywords: Baguette; Chia; Enrichment; Gluten-Free; Probiotic.

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1. Introduction

In recent years, growing public awareness of health has improved interest in functional food products tailored to specific consumer groups; among these, individuals with gluten sensitivity or celiac disease require strict adherence to a gluten-free diet (Papagianni et al., 2024). Baguette prepares traditionally from wheat flour, water, yeast, oil, and salt; despite popularity, this bread suffers from rapid staling, short shelf life, and considerable post-baking waste (Rahardjo & Sihombing et al., 2025). Hydrocolloids, high-molecular-weight hydrophilic biopolymers, are widely used in bakery formulations to enhance dough stability, delay retrogradation (ΔH), and improve shelf life as chia seeds with added nutritional value (Gao et al., 2021). Chia seeds (*Salvia hispanica*), rich in dietary fiber, omega-3 fatty acids, proteins, and antioxidants, act as

prebiotics that promote beneficial gut microbiota and gastrointestinal health (Jeewanthi et al., 2024; Salehi & Vejdaniyahid, 2025).

Among probiotic strains, *Bacillus coagulans* (*B. coagulans*) has attracted particular interest due to spore-forming ability, which enables survival under gastrointestinal stress, including stomach acidity and bile salts, thereby promoting microbial balance, inhibiting pathogens, and improving digestive health (Mohammadi et al., 2022; Pourrashidi et al., 2025). Despite these benefits, incorporating probiotics into bakery products remains challenging as high baking temperatures reduce cell viability (Papagianni et al., 2024). Encapsulation methods such as spray-drying, extrusion, freeze-drying and emulsification have been employed to protect probiotic cells, and enhance survival in order to address this issue (Ghasemi et al., 2022).

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Previous research has explored probiotic-enriched breads (Hosseini-zhad & Abedfar, 2018) and gluten-free products based on corn, potato, quinoa, tapioca, and sweet potato (Baah et al., 2022; Gao et al., 2021). Functional additions such as chia, bran, and flaxseed gels have been applied to gluten-free bread and cakes (Salem et al., 2024; Tan et al., 2025; Zdybel et al., 2019), while enzymatic strategies, including xylanase, pentosanase, and plant-derived enzymes have been shown to improve baguette dough properties (Le Loan et al., 2021; Mohammadi et al., 2022), respectively. Nevertheless, studies specifically targeting the development of gluten-free probiotic baguettes incorporating chia seeds and encapsulated probiotics remain limited; therefore, the aim of present research is to improve their nutritional value, functional attributes, and consumer acceptance.

2. Materials and Methods

2.1. Materials

B. coagulans purchased from the collection center of industrial microorganisms (Karaj, Iran). Chia seed, potato, and rice flour and improver obtained from the local market and Fariman Company (Tehran, Iran), respectively. The media and chemicals used to perform the assays based on analytical grade and purchased from Merck Company (Darmstadt, Germany).

2.2. Encapsulation of *B. coagulans* bacteria

In the present research, *B. coagulans* bacteria were encapsulated using an emulsion method with calcium alginate and chloride. The prepared 2% (w/v) sodium alginate solution was sterilized, fully hydrated, and subsequently combined in a 1:1 ratio with a 2% (w/v) calcium chloride solution. The bacterial suspension (1 % v/v) was added under sterile conditions, and the mixture was then blended dropwise into sunflower oil containing Tween 80 under stirring to form a stable emulsion. Calcium chloride added to induce gelation, forming alginate microcapsules, which were collected after 30 min and stored at 4 °C for 12 h to complete solidification (Mirzaei et al., 2011).

2.3. Gluten-free baguette preparation

Gluten-free baguettes were made using a 50:50 brown rice and potato flour mixture with optional additions of 1 to 5 % chia seeds and 1 to 3 % encapsulated *B. coagulans*. The dry ingredients were mixed to form a dough, followed by the gradual addition of water and oil. The dough underwent primary fermentation, degassing, portioning, shaping, and intermediate fermentation, followed by final proofing under controlled temperature and humidity with steam. Baking was performed in a preheated oven, and the loaves were cooled for 1.5 h, then packaged and stored for analysis. All 10 formulations were baked in triplicate and contained 2 % yeast, 1.5 % salt, 1 % sugar, and 2.5 % sunflower oil, with the water content optimized empirically, as outlined in Table 1 (Mohammadi et al., 2022).

2.4. Dough and bread analyses

Dough farinograph analysis performed according to the standard method AACC 54-21 (2009) using a Brabender Farinograph (Brabender OHG, Germany). The parameters evaluated included water absorption, dough development time (DDT), softness, stability time, and farinograph number (Papagianni et al., 2024).

2.5. Physicochemical measurements (pH, moisture, specific volume and hardness)

pH for supernatant was determined directly using a digital pH meter (Metrohm 827, Switzerland) without filtration. Each freshly baked bread (5 g) was dried in an oven (CTO-10AS, Shimadzu, Japan) at 105 ± 3 °C until constant weight. Moisture content was calculated as the loss percentage during drying, following the AACC (2009) standard method.

The specific volume of whole bread loaves is calculated as cm³ divided by weight and expressed in cm³/g. After final proofing, the dough was flattened, and the height of specific sections was measured during shaping. Then, baking was performed, and the bread height was measured again to calculate the final specific volume (Huerta et al., 2018). Bread hardness is measured after baking and during storage using a TA.XT Plus Texture Analyzer (Stable Micro Systems, Godalming, UK). Rectangular cubes (60 × 40 × 10 mm) were cut from center of each loaf and the force required to compress sample by 50 % original thickness recorded as bread hardness (N). The probe speed was 60 mm/min with a 50 g starting force (Ghasemi et al., 2022).

2.6. Probiotic bacteria viability

The loaves were first chopped, and each sample was then homogenized under sterile conditions in 9 volumes of 0.1 % (w/w) sterile peptone water using a Stomacher 400 (Seward, USA) to assess the survival of probiotic bacteria in the bread samples. Serial dilutions prepared in sterile peptone water, and 100 µL of each dilution was plated on De Man, Rogosa, and Sharpe agar containing 200 mg/L nystatin to inhibit yeast growth without affecting probiotic bacteria. Plates incubated at 37 °C for 48 h and results expressed as colony-forming units per gram (Log CFU/g) and probiotic viability measured on days 1, 2, 5, 7, and 10 of shelf life (Bustamante et al., 2020).

2.7. Sensory trait evaluation

Sensory characteristics for baguette samples were assessed in terms of taste, aroma, appearance, and overall acceptability. The panelists (equally male and female) investigated all samples during the shelf life, and evaluation conducted on a 5-point hedonic scale including 5 = excellent to 1 = very poor quality (Salem et al., 2024).

2.8. Scanning electron microscopy (SEM) assay

Bread samples (~5 × 5 mm) were dehydrated through a graded ethanol series, air-dried, and mounted on aluminum stubs with carbon tape. Samples coated with ~10 nm gold to improve

Table 1. Farinograph results of gluten-free baguette dough containing chia seeds and encapsulated probiotics.

Sample	Rice flour (%)	Potato flour (%)	Chia seed (%)	Encapsulated probiotic (%)	Water absorption (%)	Dough development time (min)	Softening Degree (BU)	Stability Time (min)	Farinograph Number (BU)
C	50	50	0	0	72.0 ± 0.2 ^h	2.0 ± 0.1 ^e	120 ± 2 ^a	3.0 ± 0.1 ^d	361 ± 3 ^f
T1	49.5	49.5	1	1	73.2 ± 0.2 ^g	3.1 ± 0.1 ^d	110 ± 2 ^b	3.9 ± 0.1 ^c	385 ± 3 ^e
T2	49.5	49.5	1	2	73.9 ± 0.2 ^f	3.3 ± 0.1 ^c	108 ± 2 ^b	4.0 ± 0.1 ^c	392 ± 3 ^d
T3	49.5	49.5	1	3	74.1 ± 0.2 ^e	3.4 ± 0.1 ^c	106 ± 2 ^b	4.1 ± 0.2 ^c	395 ± 3 ^d
T4	48.5	48.5	3	1	76.7 ± 0.2 ^{cd}	3.8 ± 0.1 ^b	94 ± 2 ^c	5.3 ± 0.2 ^b	424 ± 3 ^c
T5	48.5	48.5	3	2	76.8 ± 0.2 ^{cd}	3.9 ± 0.2 ^b	92 ± 2 ^c	5.5 ± 0.1 ^b	432 ± 3 ^b
T6	48.5	48.5	3	3	77.3 ± 0.2 ^c	4.0 ± 0.2 ^b	91 ± 2 ^c	5.7 ± 0.1 ^b	435 ± 3 ^b
T7	47.5	47.5	5	1	79.7 ± 0.1 ^b	4.4 ± 0.1 ^a	78 ± 2 ^d	7.1 ± 0.1 ^a	466 ± 3 ^a
T8	47.5	47.5	5	2	80.9 ± 0.1 ^a	4.5 ± 0.2 ^a	75 ± 2 ^d	7.2 ± 0.1 ^a	471 ± 3 ^a
T9	47.5	47.5	5	3	81.2 ± 0.2 ^a	4.6 ± 0.2 ^a	74 ± 2 ^d	7.3 ± 0.1 ^a	475 ± 3 ^a

Different letters in each column indicate significant differences at $p < 0.05$.

conductivity, and microstructure, and porosity was examined using a JSM-7800F SEM at 5 to 20 kV (Hargreaves & Zandonadi, 2018).

2.9. Differential scanning calorimetry (DSC) analysis

Breadcrumb samples (10 to 15 mg) were sealed in aluminum pans and heated from 25 to 100 °C at 10 °C/min under nitrogen to determine amylopectin ΔH (J/g) from endothermic peak and measurements were performed in triplicate. ΔH values fitted to logistic curves to evaluate kinetics (amplitude A, slope k, inflection point x_0) factors (Iglesias-Puig & Haros, 2013).

3. Results and Discussion

3.1. Farinograph number assessment

Based on Table 1, control (C) illustrates the lowest water absorption (72.0 ± 0.2 %), while T9 exhibits the highest value (81.0 ± 2.0 %). The water absorption trend gradually increased T7, T8, and T9 with the maximum and no statistically significant differences among them. C indicated 72 % water absorption, and adding chia seeds significantly improved this factor by the highest levels (79.7 to 81.0 %) observed in treatments containing 5 % chia (T7 to T9). The inclusion of encapsulated probiotics had a mild enhancing effect, as water absorption was slightly promoted by higher population levels.

C has the shortest DDT (2.0 ± 0.1 min), whereas T9 exhibits the longest (4.6 ± 0.1 min), as depicted in Table 1. DDT was elevated progressively T7, T8, and T9 that recorded the highest values with no significant differences among them. The encapsulated probiotics and addition of chia content prolonged DDT, reaching 4.6 min in T9.

The highest softening degree was observed in C (BU 120), indicating weak mechanical stability for the dough. The softening degree steadily declined with addition of chia, reaching the minimum level (BU 74) in T9 (Table 1). These suggested that dough stability and resistance to mechanical stress improved with further chia content.

The stability time for C is only 3 min, and chia addition significantly improves this parameter by T9 showing the most (7.3 min) levels in Table 1. The chia mucilage was enhanced dough

viscosity and resistance to mixing and encapsulated probiotics had a minor positive effect on stability.

C had 361 BU farinograph number that was significantly elevated with further chia levels and probiotics, reaching 475 BU in T9 (Table 1). The overall improvement was indicated in rheological attributes for dough.

Chia seeds, especially at 5 % had the most significant positive effects on farinograph characteristics, including improved water absorption, longer DDT, stability, and reduced softening. Encapsulated probiotics had a mild reinforcing influence, mainly increasing water absorption and farinograph values. The combination of 5 % chia and 3 % probiotics (T9) illustrated the best overall rheological performance. These findings are consistent with previous studies, indicating that fiber and probiotic additives can largely compensate for weaknesses of gluten-free dough (Papagianni et al., 2024; Pires et al., 2023). In a previous study, higher gluten-free doughs with xanthan gum significantly enhanced water absorption (Anwar et al., 2024), which aligned to the present research reaching 81 % in T9 with chia addition. DDT was elevated to 4.6 min in T9, consistent with a previous study indicating the variable effects of non-gluten ingredients (Nouri, 2025). The softening attributes decreased, reflecting an improvement in mechanical stability (Pires et al., 2023). Farinograph units promoted from 360 in C to 475 for T9, which was in line with past research that indicated further rheological properties (Papagianni et al., 2024). Probiotics and functional ingredients such as chia significantly enhanced water absorption, DDT, stability, and overall dough quality, consistent with multiple studies (Anwar et al., 2024; Majzoobi et al., 2019).

3.2. Determination of pH changes

The pH results for mini baguette samples are presented in Fig. 1 and on the 1st day range between 6.0 and 6.05, gradually declining over the shelf life. By day 10, the minimum pH was observed in T7 to T9 (5.43, 5.45, and 5.46, respectively), while C exhibited the maximum (5.50). The reduction in pH was attributed to microbial activity, residual fermentation and organic acid production over time. Samples containing chia and probiotics were represented a lower level of pH due to active probiotics and their acidic metabolites. The lowest pH corresponded to samples with more levels of chia and probiotics (T7 to T9). All samples demonstrated a

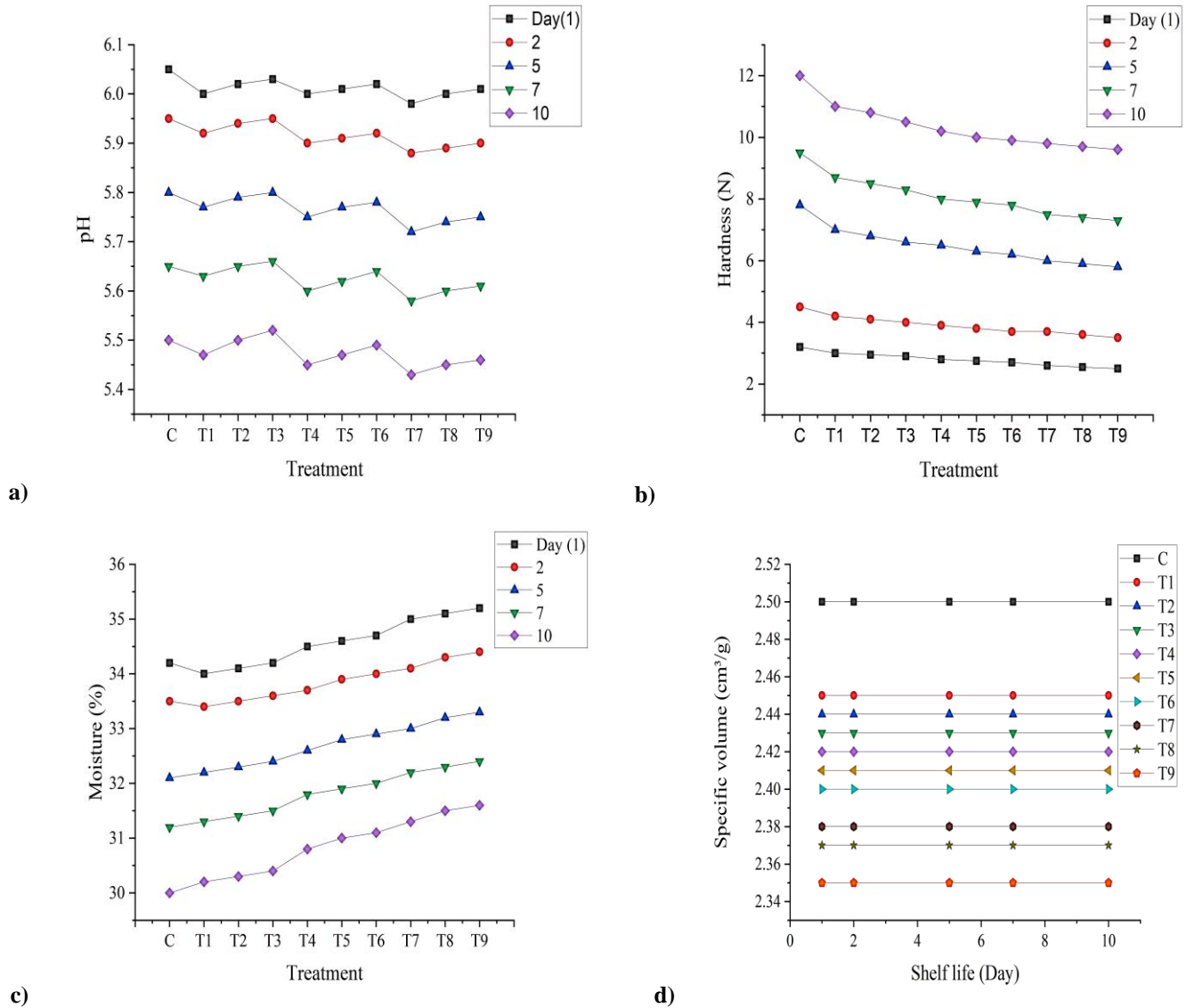


Fig. 1. Changes in a) pH, b) hardness (N), c) moisture content (%) and d) specific volume (cm³/g) during 10 days of shelf life under different treatments (C and T1 to T9). Values are expressed as mean ± standard deviation (n=3) and distinct superscript letters within the same column present significant differences among treatments at p < 0.05.

pH reduction during shelf life due to microbial activity and organic acid production, which was intensified by probiotics and chia (Salem et al., 2024; Tan et al., 2025).

3.3. Hardness indicator attribute

Fig. 1 illustrates the changes in bread hardness during storage, and on the day 1, hardness ranged from 2.5 to 3.2 N; thereby, elevating significantly over time. By day 10, the hardness of sample C reached 12 N, whereas breads containing chia and probiotics, particularly T7 to T9, were softer with values ranging from 9.6 to 9.8 N. Chia and probiotics influenced texture through water absorption and also gel formation, slowing starch ΔH and delaying staling (Iglesias-Puig & Haros, 2013; Sadeghi et al., 2024). Bread hardness reinforced during shelf life due to starch ΔH and chia and

probiotics slowed this process, resulting in softer textures over time (Romankiewicz et al., 2017). The mechanisms include water retention, gel formation by chia, fermentation, gas production, and rheology modification by probiotics, which collectively improve texture and shelf life (Sadeghi et al., 2024).

3.4. Moisture content range

The initial moisture content of the samples ranged from 34 to 35.2% and significantly decreased during shelf life (Fig. 1). By day 10, the moisture content of sample C dropped to 30%, whereas samples containing chia, particularly T7 to T9, retained higher levels, ranging from 31.3 to 31.6%. Moisture retention was significantly improved in samples containing chia, particularly T7 to

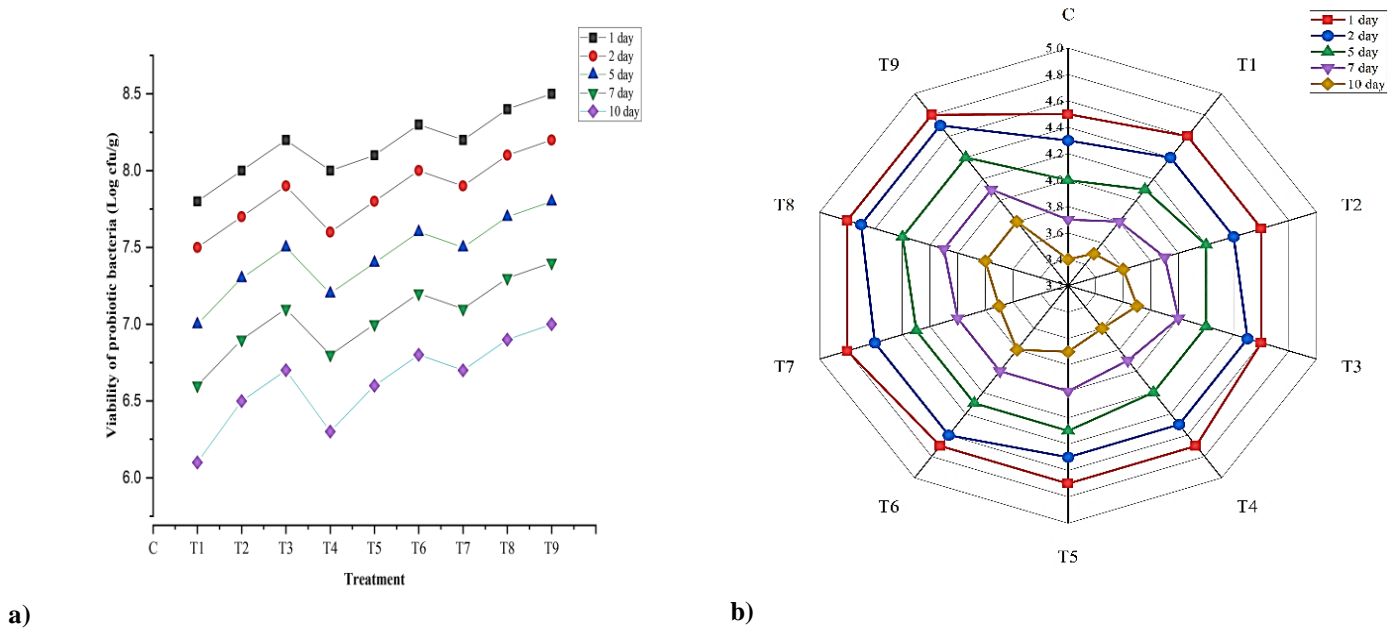


Fig. 2. a) Probiotic bacterial survival and b) sensory evaluation in mini baguette samples during the shelf life (days 1, 2, 5, 7, and 10).

T9, due to their water-binding capacity and mucilage content (Alpers et al., 2021). Encapsulated probiotics further enhanced moisture retention through the exopolysaccharide production, contributing to prolonged freshness and quality (Ghasemi et al., 2022).

3.5. Specific volume ratio

Specific volumes (cm^3/g) for breads did not vastly change during shelf life and stayed close to initial values (Fig. 1). The highest specific volume was observed in C ($2.5 \text{ cm}^3/\text{g}$), while the lowest was in T9 ($2.35 \text{ cm}^3/\text{g}$).

The slight reduction observed in samples containing chia and probiotics is likely due to a decline in gluten network strength and an increase in dough density. Specific volume slightly declined with addition of chia due to higher dough density and gas retention, but remained within acceptable ranges for gluten-free breads (Huerta et al., 2018; Rahardjo & Sihombing, 2025).

3.6. Probiotic viability influence

The probiotic viability is depicted during shelf life in Fig. 2, and on day 1, counts range from 7.8 to 8.8 Log CFU/g, and gradually reduced over 10 days. Samples with higher probiotic levels and chia content (T7 to T9) exhibited the maximum survival rates, maintaining approximately 10 Log CFU/g, which exceeded the minimum recommended level of 6 Log CFU/g for health benefits. Samples with further probiotic levels and chia (T7 to T9) maintained the most counts (~ 10 Log CFU/g), exceeding the recommended threshold (6 Log CFU/g) for health benefits (Zdybel et al., 2019). The presence of chia likely provided favorable moisture and environmental conditions for probiotic survival, while encapsulation enhanced resistance to baking and storage stresses. Chia improved environmental moisture for survival growth that encapsulation

protected probiotics from baking and storage stresses (Bustamante et al., 2017). C demonstrated no beneficial microbial growth counts without probiotics.

3.7. Descriptive sensory evaluation

Sensory evaluation results are presented in Fig. 2, and on day 1, all samples are received high hedonic scores, ranging from 4.5 to 4.8. Scores gradually decreased over time due to staling, moisture loss, and improved hardness. By day 10, C had the minimum score (3.4); however, T9 retained the maximum sensory acceptance (3.8), indicating that higher chia and probiotic levels contributed to promote sensory properties and overall acceptability during shelf life.

Chia containing breads scored higher for sensory acceptance due to more moisture and softer texture. Higher probiotic content was added a slightly acidic, pleasant taste and, positively affecting overall acceptance (Mirzaei et al., 2011), these finding was consistent with the present research. Staling and moisture loss negatively affected sensory scores over time, while chia and probiotics helped maintain favorable sensory features (Alpers et al., 2021).

3.8. SEM analysis procedure

A porous structure observed in C with large cavities and thin cell walls, indicating a weak gluten-free network prone to rapid moisture loss and early staling (Fig. 3).

Cell walls thickened slightly, pores became more diminutive, and initial mucilage layers appeared with small chia additions (T1 to T3), while probiotic capsules dispersed throughout the matrix and were improved in density with higher levels. Moderate chia values (T4 to T6) more effectively reduced pore size, enhanced network

uniformity, and embedded more probiotics, improving moisture retention and texture stability. The highest chia levels (T7 to T9) produced the most compact and uniform structure, with small or closed pores, smooth surfaces and excellent density of probiotic capsules, creating an optimal environment for crumb stability and survival.

The simulated SEM microstructures in previous research demonstrated that chia mucilage fills were voided and strengthened the matrix of gluten-free bakery products, leading to smaller pores and smoother surfaces (Anwar et al., 2024; Hargreaves & Zandonadi, 2018). The present results were agreed with previous findings of researchers, who represented that alginate-based encapsulation allowing probiotics to be integrated in the bakery matrices while retaining structural integrity (Ghasemi et al., 2022; Mirzaei et al., 2011). Higher probiotic levels (e.g., T9) would correspond to capsules that are more visible were in line with past researchers, who highlighted encapsulation role to improve probiotic distribution and survival during baking (Bustamante et al., 2017; Bustamante et al., 2020).

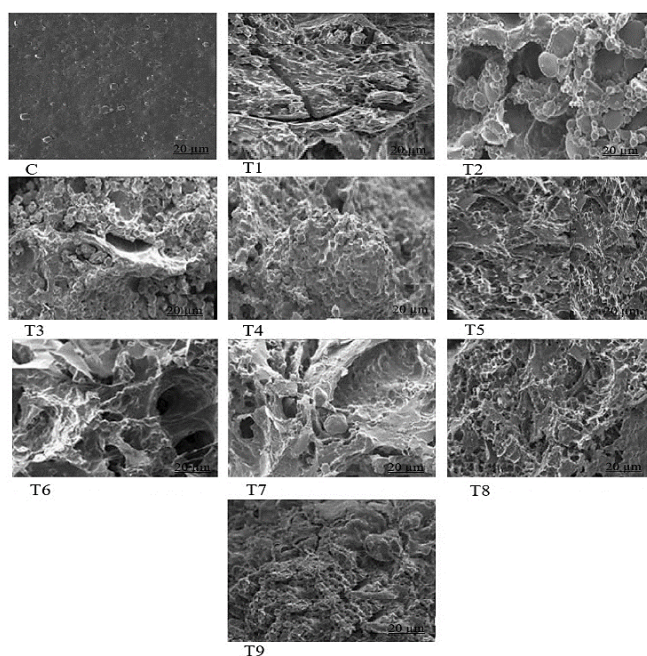


Fig. 3. Scanning electron microscopy images in mini baguette samples.

3.9. DSC result investigation

Logistic fits indicated that ΔH was elevated with shelf life for all samples, but the rate and extent reduced in chia and probiotic-enriched formulations (Fig. 4). C exhibited the largest amplitude and highest ΔH by day 10, whereas T7 to T9 (especially T9: 5 % chia + 3 % probiotic) showed the most minor improvement, indicating delayed amylopectin recrystallization and slower staling. Fit parameters (amplitude A, slope k, and inflection x_0) quantify these differences: chia/probiotic samples had lower A and slightly smaller k or later x_0 , consistent with promoted freshness retention.

Solid lines show logistic (sigmoid) fits to the measured ΔH points (days 1, 2, 5, 7, and 10). The DSC results, displaying reduced further ΔH of chia and probiotic enriched samples, align with past study, who stated lower ΔH in chia fortified wheat bread (Iglesias-Puig & Haros, 2013). Similarly, chia mucilage enhanced crumb stability, indirectly supporting diminished ΔH by maintaining moisture and structural integrity (Romankiewicz et al., 2017). A notable synergy between chia and probiotics was observed, particularly in T9, where the 5% chia and 3% probiotic combination resulted in the smallest ΔH increase. Chia probiotic fibers likely provided a favorable microenvironment for probiotic survival, while further *B. coagulans* enhanced water retention and gel-like properties (Anwar et al., 2024).

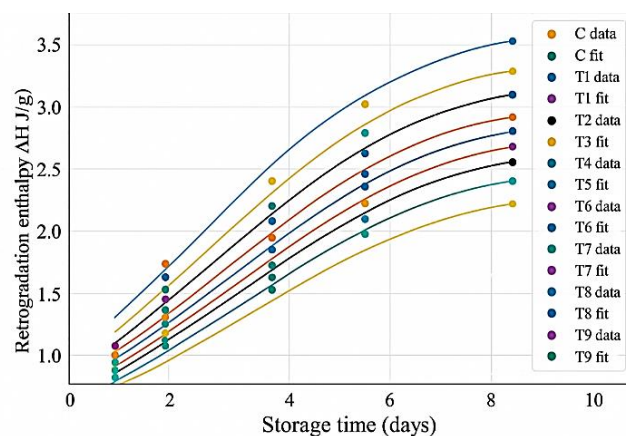


Fig. 4. Retrogradation curves for differential scanning calorimetry (enthalpy ΔH vs. shelf life) in gluten-free mini baguette (C, T1 to T9).

4. Conclusion

The incorporation of chia seeds and encapsulated *B. coagulans* in gluten-free mini baguettes significantly enhanced dough rheology, texture, moisture retention, probiotic viability, and sensory acceptability. The synergistic effects of chia hydrocolloid properties and probiotic metabolic activity effectively address the technological and nutritional limitations for gluten-free bread, offering a functional product with extended shelf life and health benefits. These findings contribute to body growing on synbiotic gluten-free bakery products and highlight the potential for scaling such formulations in commercial applications.

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CRediT authorship contribution statement

Sana Mohafez: Formal analysis, Methodology, Investigation, Drafted manuscript, Resources, Software, Visualization. Marjan Nouri: Conceptualization, Writing-review & editing, Supervision, Conducted statistical, Project administration.

Conflict of interest

The authors declare that there is no conflict of interest.

Data availability

Data will be made available on request from the corresponding author.

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