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Lacticaseibacillus paracasei KC39 Immobilized on Prebiotic Wheat Bran to Manufacture Functional Soft White Cheese

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Abstract: In the current study, probiotic *Lacticaseibacillus paracasei* KC39 was immobilized on wheat bran as a carrier. The immobilized synbiotic biocatalyst was freeze-dried and used as an adjunct during the production of functional soft white cheese. Free freeze-dried *Lc. paracasei* cells as an adjunct and a control cheese with a commercial starter were used for comparison. In addition to a fiber content of 1.12%, the functional cheese made using the synbiotic biocatalyst showed higher cell viabilities in the gastric and intestinal phases as well as an enhanced microstructure and favorable sensory characteristics. The presented immobilization method could be applied to the production of soft cheese and other functional food products for the stabilized delivery of both probiotics and dietary fibers.

Keywords: probiotic; *Lacticaseibacillus paracasei* KC39; prebiotic; wheat bran; synbiotic biocatalyst; functional soft white cheese

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1. Introduction

Probiotics are live microorganisms that confer health benefits to the host when administered at adequate levels [1,2]. Commonly used probiotics include *Lactobacillus*, *Bifidobacterium*, *Pediococcus*, *Streptococcus*, *Lactococcus*, and *Enterococcus*. *Lactobacillus* represents a fundamental group among the lactic acid bacteria (LAB) and is generally regarded as safe. LAB are commensals in the human gastrointestinal tract and are frequently used for the fermentation of food products [3].

The biological activity of probiotics, e.g., digestion, the immune modulator production of some bioactive compounds, the detoxification of toxins, and the manipulation of the gut–brain axis' activity, has recently been reported [4]. Factors related to chemical composition and food processing can affect the viable count of probiotics and, therefore, their health utility [5]. In cheese, factors such as pH, the presence of preservatives, microbial competition, the presence of micronutrients, the type of packaging, and the concentration of salt influence probiotic viability [6]. Many health benefits have been associated with the administration of probiotics and/or prebiotics, although the mechanisms of many effects are not fully understood [7]. Novel functional products such as cereal, legumes, and fruit are now being used as carriers for probiotics to enable their survival; these include fermented milk, ice cream, cheese, and other meat products, which help ensure the minimum daily intake of probiotics that provides the host with health benefits and improved immunity [8,9].

The encapsulation and immobilization of probiotic strains is also used to preserve probiotics in food products and in the stomach and the gastrointestinal environments [10,11]. In comparison to free probiotic cells, the presence of prebiotics with probiotic strains can sustain cell viability under simulated gastrointestinal conditions [12].

The use of wheat (*Triticum aestivum*) bran as an immobilization carrier for probiotic strains was previously evaluated in relation to functional dairy production. The immobilized biocatalysts, as ready-to-use dried commercial probiotic starters, had higher cell viabilities during storage, higher survival rates in simulated gastric juice, and they affected the formation of volatile compounds during fermentation, which in turn affected the results of sensory evaluations [12].

The aim of the present study was to immobilize probiotic strain *Lacticaseibacillus paracasei* KC39 (which was previously isolated from Egyptian Karish cheese) on wheat bran as a carrier and use the mixture during the production of functional soft white cheese (CSB). The functional cheese was compared against a control by characterizing the physicochemical, textural, and microstructural properties and stability in simulated gastric intestinal juice. Furthermore, the production of major aroma compounds was analyzed, and a sensory evaluation was conducted.

2. Materials and Methods

2.1. Materials and Microorganisms

Probiotic *Lc. paracasei* KC39 was previously isolated from traditional Egyptian cheese (Karish), genetically identified using a 16S rRNA gene sequencing approach (GenBank accession number MG847589), and then kept at −80 °C until further analysis [13]. *Lactobacillus paracasei* KC39 was activated at 37 °C for 24 h in de Man Rogosa and Sharpe (MRS) broth (Lab M Limited, Lancashire, UK). To produce a soft white cheese, commercial rennet enzyme and yogurt commercial starter culture from OV Dairy Supplies (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) were obtained from the Dairy Pilot Plant, Faculty of Agriculture, Alexandria University. Milk protein concentrate (MPC) (Nzmp Company, Dubai, UAE), reconstituted milk powder (RCM) (Nzmp Company, Dubai, UAE), and butter were obtained from dairy industry suppliers in Alexandria, Egypt. Wheat bran was obtained from Alexandria Flour Mills & Bakeries Co. SAE, Alexandria, Egypt, and Wheat bran was delignified according to the method of Terpou et al. [14].

2.2. Ready-to-Use Freeze-Dried Synbiotic Biocatalyst and Cell Survival Assessment

The MRS medium was selected for *Lc. paracasei* propagation. The immobilization was conducted by mixing 1 g of *L. paracasei* KC39 pellets with 10 g of dry delignified wheat bran in 500 mL of MRS broth and then incubating the mixture at 37 °C for 48–72 h according to the method of Terpou et al. [14]. After decantation, the immobilized cells were washed with sterile Ringer's solution and then freeze-dried in a freeze-drying system (Dura-Dry MP Freeze Drier FTS System, LabX, Midland, ON, Canada). The freeze-dried synbiotic was used to produce soft white cheese (10 g of biocatalyst per 1 kg of cheese). Cell survival analysis was used to evaluate synbiotic functionality of wet culture and freeze-dried culture for free and immobilized cells on wheat bran during 3 months of storage.

2.3. Functional Soft White Cheese Production

Soft white cheese was prepared according to the method proposed by Tamime et al. [15] with some modifications. In standardized reconstituted milk, the total solids, protein, and fat content were 38%, 29%, and 7%, respectively. Milk protein concentrate and reconstituted milk powder were homogenized with water in a laboratory blender (Arion Blender AR–312, Shanghai, China) at 25,000 rpm for 6 min. The mixture was aged at 4 °C overnight to guarantee better powder dispersal before pasteurization. The recombinant mixture was then pasteurized at 78 °C for 60 s and cooled to 35 °C in preparation for inoculation with a starter culture. The mixture was divided into three equal portions (100 g): (1) control cheese with a commercial starter (CS), which was inoculated with the yogurt commercial starter culture Yo-Mix 495 (*S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*) that was previously activated in milk, mixed, and left undisturbed for 2 h; (2) cheese with free *Lc. paracasei* (CFL), which was inoculated with free freeze-dried *Lc. paracasei* cells; and (3) cheese with the synbiotic biocatalyst (CSB), which was inoculated with freeze-dried synbiotic-immobilized

biocatalyst. Stabilizer was added (0.25%); then, 1% salt, 0.02% calcium chloride solution, and butter were added before incubation. The pH decreased during fermentation, and the remaining 0.5% salt was added to the mixture at pH 5.2. Afterwards, the mixture was reheated to 42 °C in a water bath, mixed with the coagulant rennet and 1% citric acid. The samples were immediately poured into containers, incubated at ambient temperature (20 ± 2 °C) overnight, and then stored at 4 °C for 45 days. Cheese samples were analyzed in time intervals: day 1 (fresh) and after 15, 30, and 45 days of storage. Table 1 and Figure 1 show the ingredients used to produce 1 kg of soft white recombined cheese.

Table 1. Ingredients for production of 1 Kg soft white recombined cheese.

Ingredients	Unit	Quantity
Skim Milk Powder	g	145
Milk Protein concentrate	g	145
Butter	g	70
Stabilizer	mL	2.5
Salt	g	15
Calcium chloride	g	2
Rennet (1%)	mL	10
Water added	mL	620

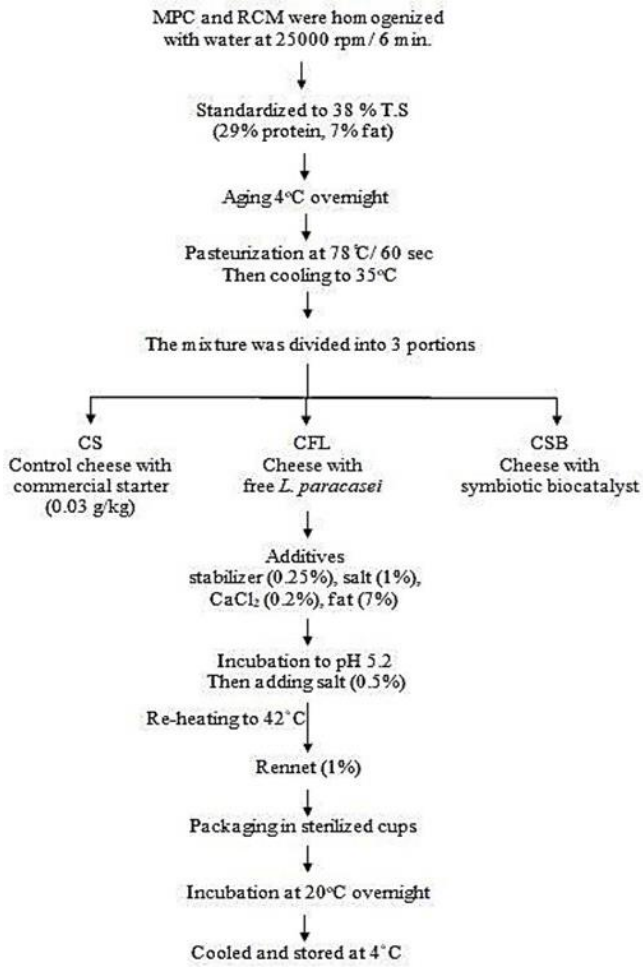

Figure 1. Diagram shows the steps of manufacturing functional soft white cheese. MPC—Milk protein concentrate; RCM—Reconstituted skimmed milk.

2.4. Physicochemical Analysis

A pH meter (ADWA AD1030, Inc., Szeged, Hungary) was used directly by immersing the electrode into cheese samples. The samples were analyzed for their titratable acidity according to AOAC [16]; titratable acidity was expressed as the lactic acid percentage. Moisture, fat, and fiber content were determined according to AOAC [16]. Crude protein was determined using the Kjeldahl procedure [16]. A color meter (Smart Color Pro, Miami, FL, USA) was used to determine the color terms *L* (100 = white; 0 = black), *a* (positive = redness; negative = greenness), and *b* (positive = yellowness; negative = blueness). The physicochemical analysis of functional soft white cheese was performed at zero-time.

2.5. Texture Profile Analyses

Soft cheeses usually contain a moisture level not less than 60% with a fat content not less than 20%. Texture properties are among the key factors in the assessment of rheological characteristics of soft cheeses. Stabilizing systems form solids, increase viscosity, and improve a product's mouthfeel (creamy texture) and flavor release. Texture analysis, therefore, plays a critical role in the development of low-fat foods. According to the method of Bourne [17], texture profile analyses (TPAs) were conducted using a texture analyzer (Texture Pro CT V1.2, AMETEK Brookfield, 11 Commerce Blvd., Middleboro, MA, USA). TPA Machine was equipped with a 50–500 kg load cell. A flat plate probe with a 57 mm diameter was attached to moving crosshead. Cylindrical samples were prepared using a metal borer at 4–6 °C and wrapped with plastic stretch cover. Samples were taken at least 1 cm away from cheese surface. They were left at 25 °C for almost 30 min until they reached the definite temperature (19 ± 1 °C). The central temperature of the control specimen was measured by a thermocouple. The dimensions of cheese specimens were 25 mm both in diameter and height. The operating conditions were crosshead speed 50 mm/min, chart speed 200 mm/min, and 80% of compression ratio from the initial height of the sample in two bites. The texture profile parameters were determined using the TPA curve and data. The TPA parameters were hardness, adhesive forces, adhesiveness, resilience, springiness, and springiness index.

2.6. Scanning Electron Microscopy

Scanning electron micrographs were obtained to examine cell microstructure and immobilization of *Lc. paracasei* on delignified wheat bran and the cross sections of cheese products. These samples were coated with gold for 2 min and then examined using a Scanning Electron Microscope (SEM) (SEM-JEOL JSM6360LA, Otemachi, Chiyoda, Tokyo, Japan) operated at an accelerating voltage of 15 kV.

2.7. Simulated Gastrointestinal Digestion

To simulate the in vitro gastrointestinal digestion of free and immobilized *Lc. paracasei* in cheese, the samples were prepared following the method described by Terpou et al. [14]. The samples were subjected to oral, gastric, and intestinal phases. The oral phase was simulated by adding 9 mL of water and 1 mL of 100 U/mL α-amylase (20,000 U/mL, Creative Enzymes®, New York, NY, USA) diluted in 1 mM $CaCl_2$ (adjusted to pH 6.9 with 1 M $NaHCO_3$) to 1 g of sample. The mixture was vortexed and incubated at 37 °C for 5 min. For the gastric phase, the pH of the mixture was lowered to 2.00 ± 0.05 with HCl (6 M), and 1 mL of pepsin (3000 U/mg, Creative Enzymes®, New York, NY, USA) (10.8 U/mL of 0.1 M HCl) was added. The mixture was incubated for 2 h in a shaking water bath at 37 °C and 70 rpm. After each phase, the mixtures obtained were centrifuged for 12 min at 8000× *g* at 4 °C, yielding the chyme-soluble fraction (CSF) and the pellet fraction (PF). For the intestinal phase, the pH was adjusted to 7.0 ± 0.05 with NaOH (6 M), and 2.5 mL pancreatin (Protease, ≥100 units/mg, Amylase ≥ 50 units/mg, Lipase ≥ 8 units/mg; Creative Enzymes®, New York, NY, USA) (8 U/mL in 0.5 M $NaHCO_3$) was added; then, 2.5 mL bile salt mixture (50 mg/mL in 0.5 M $NaHCO_3$) was added to the mixture. The mixtures of three phases were incubated for 2 h in a shaking water bath

at 37 °C and 70 rpm. After incubation period, the mixtures were subjected to selective counting for *L. paracasei* [18]. This experiment was performed in replicates and the results were expressed as log of mean colony-forming units CFU/g of cheese.

2.8. Microbiological Profile Analysis of Cheese

Representative 10 g portions of cheese samples were analyzed at various intervals (days 1, 15, 30, and 45) throughout the storage period. The samples were blended with 90 mL of sterile saline solution (0.9% *w*/*v*) [19]. Viable counts of total aerobic mesophilic bacteria were counted on plate count agar (Himedia Laboratories, Wagle Industrial Area, Thane West, Maharashtra Pin, India) incubated at 30 °C for 48 h, *Lactobacilli* were counted on MRS agar incubated at 37 °C for 48 h, cocci were counted on M17 agar incubated at 37 °C for 48 h, yeasts and molds were counted on potato dextrose agar incubated at 30 °C for 48–72 h, and coliforms were counted on violet red bile agar incubated at 37 °C for 48 h. Specific counting of *Lc. paracasei* was performed on MRS–vancomycin agar [18]. *Lc. paracasei* clones were white, smooth, shiny disks that were 1–2 mm in diameter [20]. All cell counts were expressed as logs of mean colony-forming units (CFU)/g of cheese. All microbial analyses were performed in 3 replicates and the results were expressed as log of mean colony-forming units CFU/g of cheese [21].

2.9. Solid-Phase Microextraction GC-MS Analysis

After 45 days, three cheese samples (each sample duplicate) were subjected to volatile composition analysis. The samples were placed into a 20 mL vial, which was sealed with a rubber septum, and heated at 60 °C for 5 min. The SPME needle was inserted through the septum and the fiber was exposed to the headspace for 45 min. The absorbed volatile compounds were then analyzed on a Shimadzu GC-17A system, with the Supelco CO WAX-10 column (0.25 µm film thickness; 60 m × 0.32 mm i.d.; set at 70 °C), coupled to a GCMS-QP5050A mass spectrometer. Desorption of volatiles took place in the GC injector port for 5 min at 280 °C in splitless mode. The column temperature program was as follows: 35 °C for 5 min; increase to 50 °C (5 °C/min), hold for 5 min; and increase to 230 °C (5.5 °C/min), hold for 5 min. The total run time was 51.73 min. The carrier gas was He (2 mL/ min). Compounds were identified by comparing their retention times to those of compounds obtained from NIST107, NIST21, and SZTERP libraries produced in previous research [22,23].

2.10. Sensory Evaluation

Six men and four women (average age = 35 years old) were enrolled as participants in a panel test on fresh cheese at the Food Technology Department, Arid Lands Cultivation Research Institute, SRTA-City, Alexandria, Egypt, which was conducted as previously described Polychroniadou et al. [19]. According to Izco and Torre [24], the color, odor, taste, texture, appearance, and overall acceptability were evaluated on a scale from 1 to 9 as follows: 1 = dislike extremely; 2 = dislike greatly; 3 = dislike moderately; 4 = dislike slightly; 5 = neither dislike nor like; 6 = like slightly; 7 = like moderately; 8 = like greatly; 9 = like extremely.

2.11. Statistical Analysis

All assays were performed in triplicate, and the results are expressed as means with standard deviations (mean ± SD). IBM SPSS Statistics 23 software program (IBM SPSS Statistics for Windows, Version 23.0. IBM Corp., Armonk, NY, USA) was applied for statistical analyses [25]. Values were compared using ANOVA with a general linear model followed by Duncan's post hoc test. $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1. Characterization of Freeze-Dried Immobilized Lc. paracasei

3.1.1. Stability of Immobilized *Lc. paracasei* during Storage

Freeze drying is an ideal technique with which to determine the viable count of *Lc. paracasei* in the form of a synbiotic over a three-month period. The results showed that the freeze drying of the free cells decreased cell viability by 19%; however, in a wet culture, the cell viability decreased by 40.7% at the end of the storage period (Table 2). By comparison, the freeze drying of the synbiotic decreased cell viability by 9.7%, whereas the immobilized wet culture decreased *Lc. Paracasei's* viability by 22.8% after three months (Table 2). Wheat bran fibers are immobilized carriers that have previously been used for the delivery of probiotics into the human gut [14,26].

Table 2. Effect of freeze drying on free and immobilized *Lacticaseibacillus paracasei* viability (log cfu/g).

Time (Months)	Wet Culture		Freeze-Dried Culture	
	Free Cell	Immobilized on Wheat Bran	Free Cells	Immobilized on Wheat Bran
0	8.91 ± 0.25^{aA}	8.75 ± 0.30^{aA}	9.06 ± 0.33^{aA}	8.74 ± 0.61^{aA}
1	8.19 ± 0.32^{aB}	8.44 ± 0.14^{aAB}	8.40 ± 0.34^{aB}	8.55 ± 0.29^{aA}
2	7.28 ± 0.19^{bC}	7.73 ± 0.50^{abB}	8.11 ± 0.29^{aB}	8.27 ± 0.45^{aA}
3	5.28 ± 0.24^{cD}	6.75 ± 0.51^{bC}	7.34 ± 0.36^{abC}	7.89 ± 0.49^{aA}

$^{A–D}$ Means in the same column followed by different uppercase letters are significantly different ($p < 0.05$). $^{a–c}$ Means in the same row followed by different lowercase letters are significantly different ($p < 0.05$).

3.1.2. Morphological Analyses

The SEM analysis revealed the morphological structure of the delignified wheat bran (Figure 2A) and immobilized freeze-dried *Lc. paracasei* (Figure 2B,C): many *Lc. paracasei* cells were attached to the wheat bran's surface and cavities. The mechanism of cell immobilization involves weak bonds such as Van der Waals forces [27,28].

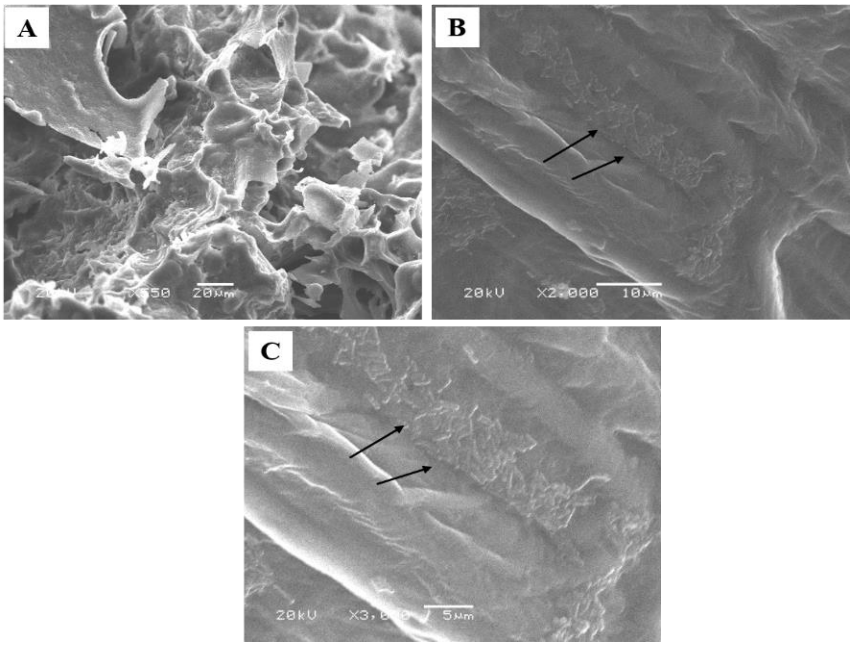

Figure 2. Scanning electron micrographs of immobilized *Lc. paracasei* cells on delignified wheat bran (20 kv). (**A**) Delignified wheat bran (50×); (**B**) immobilized *Lc. paracasei* cells on delignified wheat bran (2000×); (**C**) Immobilized *Lc. paracasei* cells on delignified wheat bran (3000×).

3.2. Physicochemical Characteristics of Functional White Cheese

All the results for the physicochemical analyses of the functional soft white cheese are shown in Table 3. Previous studies [14,29] have indicated that the chemical compositions of functional soft white cheeses are common among those that are manufactured in a similar manner. The total fiber content in the CSB cheese was significantly ($p \leq 0.05$) increased by 55.35% and 48.21% compared with that in the CS and CFL cheeses, respectively. The total protein was also significantly increased in the CSB cheese compared with the CS and CFL cheeses; however, the fat content did not differ among the cheese samples. These results are in agreement with the findings of previous studies in which various adjunct cultures were used in white-brined cheeses [30,31].

The pH and lactic acid were found at levels usually observed in soft white cheeses [32]. In general, soft white cheese production targets high acidification rates using starter cultures that can differ among producers or the areas of the milk's origin [33]. The adjunct synbiotic culture (a dry, ready-to-use culture) was added to enhance the action of the starter culture and, subsequently, reduce the pH during the milk's coagulation. Both immobilized and free *Lc. paracasei* adjuncts significantly affected ($p \leq 0.05$) the studied parameters during storage, and the decrease in pH was significantly ($p \leq 0.05$) higher when the synbiotic adjunct was used.

The color analyses indicated that the CSB was significantly darker (L = 88.84) than the control cheese and the CFL cheese (91.82) with free *Lc. paracasei* (90.42). This was expected due to the bran color (Figure 3) exhibited by the functional soft white cheese product. Additionally, all cheeses were light yellow (b = 4–5), but the cheese fortified with probiotics, either the CFL or CSB, tended to be more yellowish in color than the control. Similar color observations were previously reported for potential probiotic fresh cheeses using two lactobacilli strains [34].

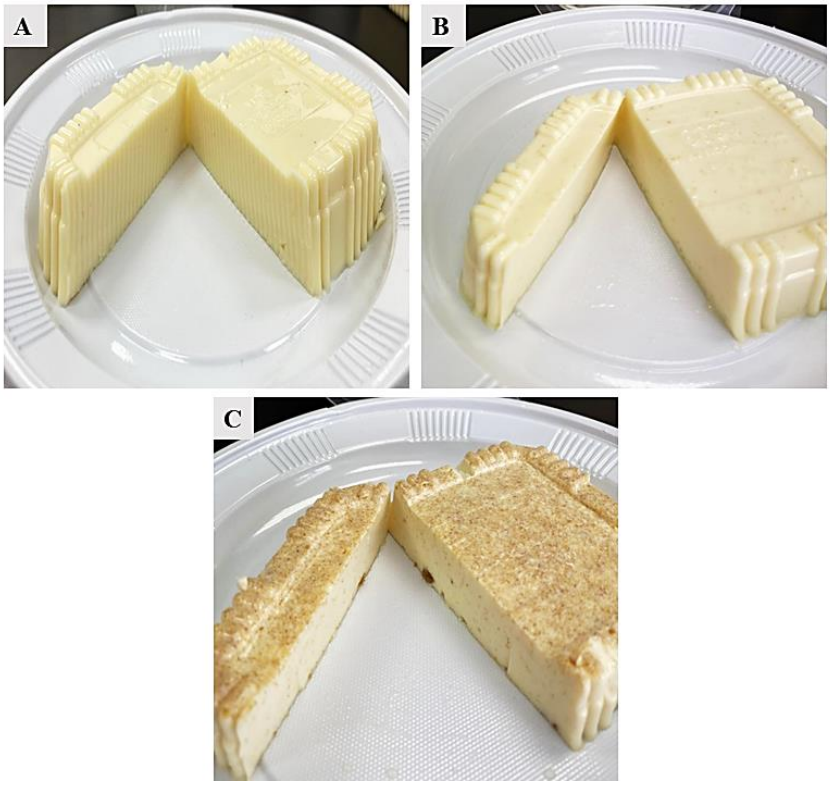

Figure 3. Soft white cheese products. (**A**) Control cheese with commercial starter (CS); (**B**) Cheese with free *Lc. paracasei* (CFL); (**C**) Cheese with symbiotic biocatalyst (CSB).

Table 3. Physicochemical analyses of functional soft white cheese.

Parameters	CS	CFL	CSB
	Chemical composition		
pH	5.21 ± 0.01 [a]	5.13 ± 0.01 [b]	5.08 ± 0.03 [c]
Acidity%	0.54 ± 0.005 [c]	0.58 ± 0.02 [b]	0.64 ± 0.02 [a]
Moisture%	64.15 ± 2.20 [a]	65.36 ± 1.00 [a]	64.30 ± 0.95 [a]
Fiber%	0.50 ± 0.01 [b]	0.58 ± 0.08 [b]	1.12 ± 0.06 [a]
Protein%	8.42 ± 0.05 [b]	8.45 ± 0.05 [b]	8.96 ± 0.20 [a]
Fat%	6.65 ± 0.15 [a]	6.44 ± 0.36 [a]	6.21 ± 0.11 [a]
Fat/DM%	18.55 [a]	18.59 [a]	17.39 [b]
	Color analysis		
*L**	91.82 ± 0.74 [a]	90.42 ± 0.41 [ab]	88.840 ± 0.79 [b]
*a**	−0.24 ± 0.02 [a]	−0.29 ± 0.06 [a]	−0.286 ± 0.05 [a]
*b**	4.00 ± 0.11 [b]	5.14 ± 0.22 [a]	5.096 ± 0.17 [a]

[a–c] Means in the same column followed by different letters are significantly different ($p < 0.05$). CS, Control cheese with commercial starter (Control); CFL, Cheese with free *Lc. paracasei*; CSB, Cheese with symbiotic biocatalyst; DM, Dry matter. *L**, value represents lightness from black (0) to white (100), *a**, value represents color ranging from red (+) to green (−); *b**, value represents yellow (+) to blue (−).

3.3. Texture Profile Analyses

The results of the TPA for the functional soft white cheese are shown in Table 4. The highest hardness values were for the control cheese followed by the CSB and then the CFL cheeses (2648, 2341, and 1761 g, respectively, in cycle 1). The CFL cheese tended to show more springiness, adhesiveness, and adhesive force (7.29 mm, 2.7 mJ, and 191 g, respectively), whereas the CSB cheese with the strains immobilized on bran showed a significant decrease in these parameters (6.66 mm, 0.7 mJ, and 90 g, respectively). The increase in the hardness indicated the increase in the protein content and, consequently, the improvement in the texture and mouth feel. These results are in agreement with those of El-Shibiny et al. [35], who tested cheese supplemented with rice bran.

Table 4. Texture profile analyses of functional soft white cheese.

Texture Parameters	Unit	CS	CFL	CSB
Hardness Cycle 1	g	2648 ± 1.23 [a]	1761 ± 1.17 [c]	2341 ± 1.18 [b]
Adhesive Force	g	156 ± 0.27 [b]	191 ± 0.13 [a]	90 ± 0.32 [c]
Adhesiveness	mJ	1.6 ± 0.02 [b]	2.7 ± 0.05 [a]	0.7 ± 0.03 [c]
Hardness Cycle 2	g	2689 ± 1.16 [a]	1445 ± 1.22 [c]	2075 ± 1.16 [b]
Hardness Work Cycle 2	mJ	66.6 ± 0.01 [a]	38.4 ± 0.02 [c]	53.7 ± 0.07 [b]
Springiness	mm	6.44 ± 0.02 [b]	7.29 ± 0.01 [a]	6.66 ± 0.02 [b]
Springiness Index	-	0.92 ± 0.04 [b]	1.04 ± 0.03 [a]	0.95 ± 0.03 [b]

[a–c] Means in the same row followed by different letters are significantly different ($p < 0.05$). CS, Control cheese with commercial starter (Control); CFL, Cheese with free *Lc. paracasei*; CSB, Cheese with symbiotic biocatalyst.

3.4. Microstructure of Cheese Samples

Scanning electron micrographs of the cross sections of the soft white cheese products are presented in Figure 4. Compared with the control soft white cheese (Figure 4A), the cheese with free *Lc. paracasei* (CFL) (Figure 4B) had a crumbly structure that might have reflected the texture analyses, which indicated its increased adhesiveness and springiness (Table 4). Contrastingly, the CSB (Figure 4C) was structurally superior, with bran particles that caused the least adhesiveness and springiness as well as a mild hardness (Table 4). In addition, the microstructural differences between the cheeses did not negatively affect the panelists' texture scores. Similar observations were reported by Xue et al. [36], who applied oat bran to soft cheese.

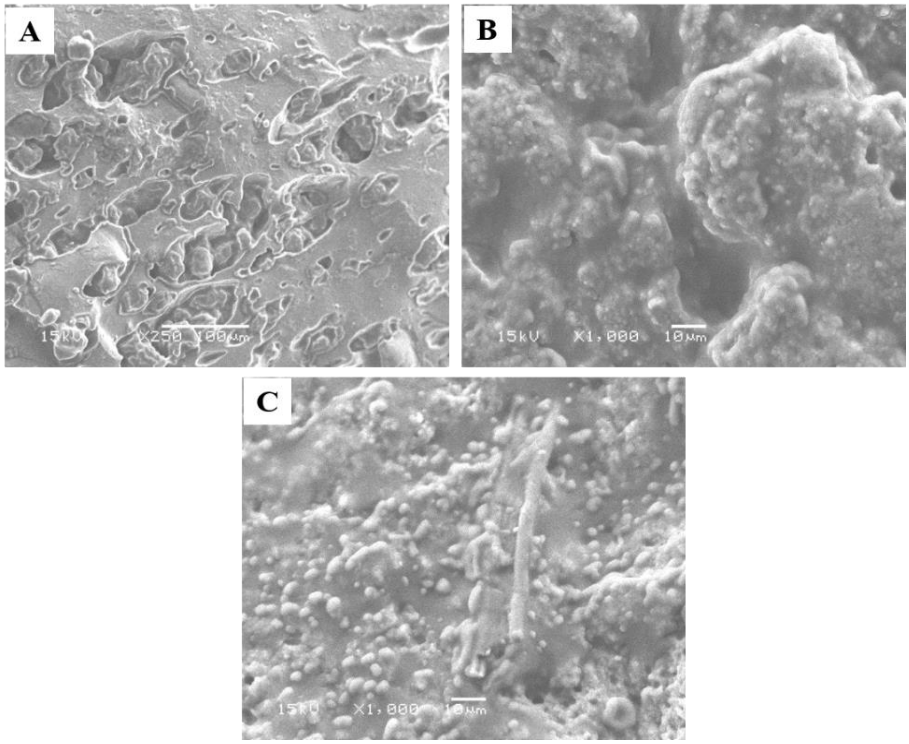

Figure 4. Scanning electron micrographs of cross-sections in soft white cheese products. (**A**) Control cheese with commercial starter (CS); (**B**) Cheese with free *Lc. paracasei* (CFL); (**C**) Cheese with symbiotic biocatalyst (CSB).

3.5. Cell Survival in Simulated Gastric Intestinal Juice

The presence of wheat bran as a prebiotic significantly increased the survival ability of the *Lc. paracasei* cells in the soft white cheeses with the freeze-dried synbiotic biocatalyst (CSB) compared with that of the free freeze-dried *Lc. paracasei* cells (FFDC) in the gastric and intestinal phase ($p < 0.05$; Table 5). A previous study also found that the survival ability of lactobacilli in acidic environments was increased by the presence of metabolizable sugars [37]. Specifically, an incubation in a simulated gastric intestinal phase resulted in a 9.01% decrease in cell viability for the CSB cheese samples with the synbiotic adjunct (from 9.10 ± 0.22 to 8.28 ± 0.28 log cfu/g), whereas the CFL soft white cheeses with free *Lc. paracasei* cells or the FFDCs showed 14.6% (from 9.14 ± 0.23 to 7.80 ± 0.30 log cfu/g) or 22.47% (from 9.39 ± 0.15 to 7.28 ± 0.14 log cfu/g) decreases in cell viability, respectively. Charalampopoulos et al. [26] reported that white-brined cheeses with a high pH could be useful for protecting probiotics from stomach acidity, and the immobilization of these probiotics on wheat bran could supply the probiotics with nutrients and thereby improve their survival ability. Therefore, immobilization on wheat bran represents a promising method for protecting the probiotic *Lactobacillus* sp. during the cheese-manufacturing process, storage, delivery to the gastrointestinal tract, and from antagonistic microorganisms.

Table 5. Simulated gastric and intestinal juice's effect of on survival of freeze-dried *Lc. paracasei* in cheese products (log cfu/g).

Stages	Free Freeze-Dried *Lc. paracasei* Cells (FFDC)	Soft White Cheeses with Free *Lc. paracasei* Cells (CFL)	Soft White Cheeses with Freeze-Dried Symbiotic Biocatalyst (CSB)
Oral phase	9.39 ± 0.15 [a]	9.14 ± 0.23 [a]	9.10 ± 0.22 [a]
Gastric phase	7.87 ± 0.33 [b]	8.48 ± 0.14 [a]	8.55 ± 0.27 [a]
Intestinal phase	7.28 ± 0.14 [b]	7.80 ± 0.30 [ab]	8.28 ± 0.28 [a]

[a,b] Means in the same row followed by different letters are significantly different ($p < 0.05$). CS, Control cheese with commercial starter (Control); CFL, Cheese with free *Lc. Paracasei*; CSB, Cheese with symbiotic biocatalyst.

3.6. Lacticaseibacillus Paracasei Growth Capacity

Lacticaseibacillus paracasei's viability in free and immobilized cases over 45 days of storage is shown in Figure 5. The *Lacticaseibacillus paracasei* immobilized on wheat bran in soft white cheese exhibited high viability throughout the storage period compared with the soft white cheese containing free *Lc. paracasei*. The successful combination of probiotics and other prebiotic constituents has previously been demonstrated in the literature [26]. *Lacticaseibacillus paracasei's* viability decreased in the CFL cheese by 10.8%, whereas the presence of wheat bran in the CSB cheese samples reduced *Lc. Paracasei's* viability by 5.5% (Figure 5). Under these conditions, the minimum live probiotic count of 7 log cfu/g was achieved at consumption [38,39]. These findings agree with those of Terpou et al. [14,40], who reported the suitability of wheat bran during probiotic immobilization and its ability to safely deliver probiotics to the gastrointestinal tract [6,26]. The higher viability of the immobilized *Lc. paracasei* could maximize fermentation rates and enhance the ripening of fermented food products.

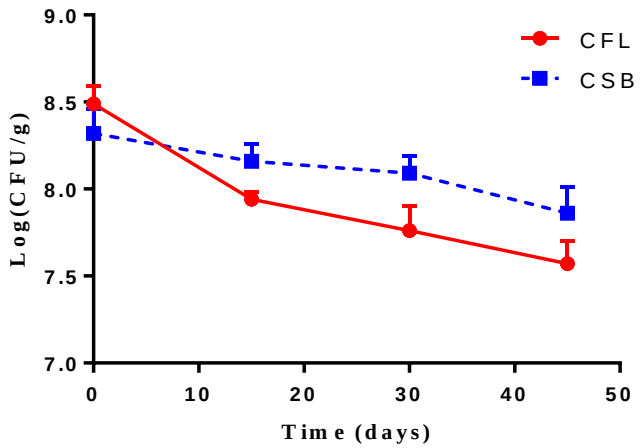

Figure 5. Viability of *Lc. paracasei* (log CFU/g) during 45 days of storage. Cheese with free *Lc. paracasei* (CFL); Cheese with symbiotic biocatalyst (CSB).

3.7. Microbiological Analysis of Cheese during Maturation and Storage

The microbiological analyses of the free and immobilized *Lc. paracasei* (Figure 6) were monitored during the maturation and storage of the functional soft white cheese for 45 storage days. Fortification with the probiotic strain, either in free or immobilized form, significantly affected the *lactobacilli* counts (8.38 ± 0.29 and 8.66 ± 0.40) for CFL and CSB, respectively ($p < 0.05$), compared with those of the control samples (7.53 ± 0.20). In all the cheese samples, coliforms were not detected in any sample during storage, whereas small counts of yeasts and fungi were found in all the cheese samples from day 15 of storage. The *Lc. paracasei* viable cell counts in the CFL and CFL cheese samples were within the range of 8.4.14 to 7.57 ± 0.13 and 8.32 ± 0.14 to 7.86 ± 0.15 log cfu/g, respectively. Several studies have already found that the presence of an adjunct probiotic during cheese production reduces contamination from *Enterobacteria* and coliforms compared with cheese produced using a single starter culture [41–43]. The presence of immobilized *Lc. paracasei* might have improved lactic acid production and decreased pH, as shown in Table 3. The cocci count did not change significantly ($p \leq 0.05$) among the cheese samples during the 45 days of storage (Figure 6). For free and immobilized probiotics (CFL and CSB), the lactobacilli count (>10^6 log cfu/g) was significantly ($p \leq 0.05$) increased ($p < 0.01$) during the storage period, which provided the cheese samples with probiotic characteristics [38].

The immobilized adjunct significantly affected the lactobacilli in the CSB cheese (8.31 ± 0.16 to 7.90 ± 0.53) compared to the lactobacilli in the CFL cheese containing the free lactobacilli adjunct (8.26 ± 0.55 to 7.70 ± 0.66). In general, lactobacilli largely originate from different starter sources, adjunct cultures, and nonstarter LAB after pasteurization [44].

Ripening and storage may reduce the lactobacilli count due to a decrease in pH and a high salt concentration, which may be due to the production of bacteriocins.

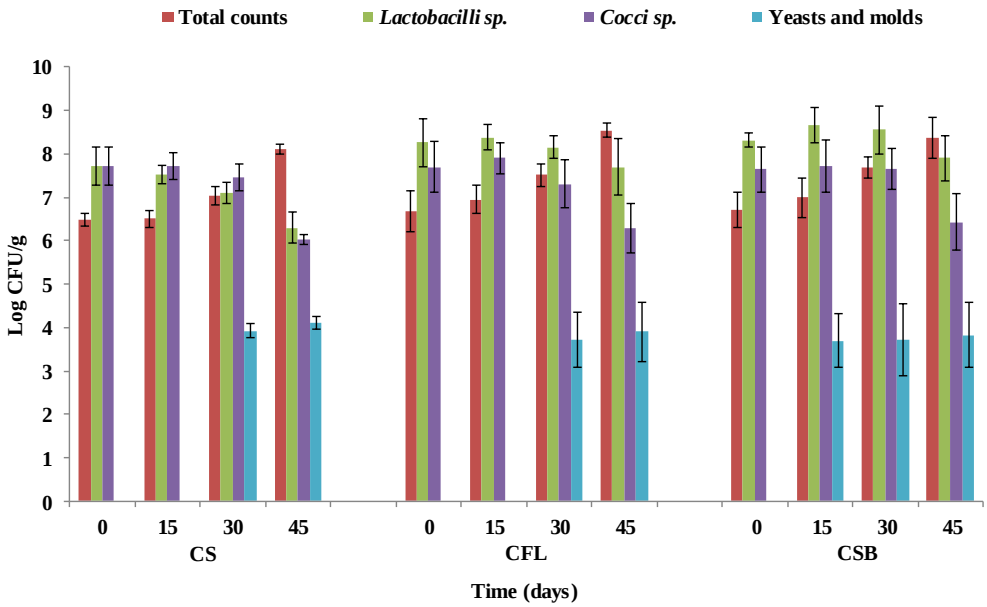

Figure 6. Microbial populations of functional soft white cheese products during cold storage. Control cheese with commercial starter (CS); Cheese with free *Lc. paracasei* (CFL); Cheese with symbiotic biocatalyst (CSB). Coliforms were not detected.

3.8. Volatile Aroma Compounds in Cheese

Cheese manufacturers usually aim to include novel and specific aromatic compounds in their products such as esters, organic acids, alcohols, carbonyl, and flavors [45]. During cheese ripening, aroma and flavor compounds are produced from fat hydrolysis [46]. During cheese production, LAB play a role in fat degradation and the production of aroma compounds. During cheese production, it takes at least one month of maturation to induce the specific aroma of the ripening products. The analyses of the volatile compounds are shown in Table 6 and Figure S1. In total, 24 compounds were detected: 16, 17, and 16 compounds in the CS, CFL, and CSB cheeses, respectively; these compounds can commonly be found in feta-type cheeses and in many other cheese varieties [29,40]. The most important classes of compounds identified were esters, aldehydes, terpenes, aromatic hydrocarbons, alcohols, and organic acids. Esters are known to be the main cause of fruity flavors, and they diminish the rancidity of cheese caused by acids and ketones [47]. In our cheese samples, ethyl butanoate, ethyl octanoate, and ethyl hexanoate were detected, which is in agreement with previous findings [47–49]. Ethyl butanoate is not only found in cheeses but also in wines, black tea, and soybeans, and it is responsible for fruity odors such as that of pineapple [50,51]. Octanoic acid and decanoic acid were the most abundant organic acids in our cheese samples; moreover, these were significantly ($p \leq 0.05$) increased by the presence of free or immobilized *Lc. paracasei*. Such fatty acids are usually found in cheese because of the lipase activity in milk fat [24]. Other fatty acids (with up to 12 carbon atoms) provide the odor characteristics of manufactured cheeses. Based on these results, probiotic immobilization enhances the volatile profile of the cheeses. Terpou et al. [40] reported a similar result, i.e., that a variation in the volatile compounds existed in feta-type cheese due to the presence of a synbiotic. This helps explain the relationship between the microbial presence and the chemical profile, i.e., the microbial interaction with the type of cheese, but an abundance of unknown influencing factors is yet to be examined.

Table 6. GC/MS identification of major aroma-related compounds of cheese samples after ripening for 45 days.

ID	Compound Name	R. Time (min)	Identification Method [1]	Classification	Cheese Samples		
					CS	CFL	CSB
1	Ethyl butanoate	10.0	RT, KI, MS	Esters	+	ND	ND
2	3-methylbutyl acetate	10.4	RT, MS	Esters	+	ND	ND
3	Ethyl hexanoate	11.0	RT, KI, MS	Esters	+	+	ND
4	Hexyl acetate	12.0	RT, KI, MS	Esters	+	+	ND
5	Ethyl dodecanoate	12.4	RT, MS	Esters	+	+	+
6	Hexanal	12.8	RT, MS	Aldehydes	+	+	+
7	Terpene	13.0	RT, MS	Terpenes	+	+	+
8	1,4-p-Menthadiene	13.4	RT, MS	Terpenes	+	ND	ND
9	Ethylbenzene	13.5	RT, MS	Aromatic hydrocarbons	+	ND	ND
10	Isopentyl acetate	13.8	RT, MS	Esters	+	+	+
11	2-Methyl 3-pentanone	13.9	RT, MS	Carbonyl compounds	ND	ND	+
12	2-Pentanol	14.0	RT, MS	Alcohols	ND	+	+
13	1-Penten-3-ol	14.4	RT, MS	Alcohols	+	+	+
14	3-Methyl-1-butanol	14.8	RT, KI, MS	Alcohols	+	ND	ND
15	1-Hexanol	15.0	RT, KI, MS	Alcohols	+	ND	ND
16	1-heptanol	15.3	RT, KI, MS	Alcohols	+	+	+
17	Hexyl butanoate	15.4	RT, MS	Esters	+	+	+
18	Ethyl tetradecanoate	15.5	RT, MS	Esters	+	+	+
19	Ethyl octanoate	15.6	RT, KI, MS	Esters	ND	+	+
20	Ethyl decanoate	15.7	RT, KI, MS	Esters	ND	+	+
21	2-Phenylethyl acetate	16.5	RT, KI, MS	Esters	ND	+	+
22	Hexanoic acid	17.5	RT, MS	organic acid	+	+	+
23	Octanoic acid	18.6	RT, MS	organic acid	ND	+	+
24	decanoic acid	20.0	RT, MS	organic acid	ND	+	+

[1] RT, positive identification by retention times that agree with authentic compounds and by mass spectra of authentic compounds generated in the laboratory; KI, tentative identification by the Kovats retention index; MS, tentative identification by mass spectra obtained from NIST107, NIST21, and SZTERP libraries; ND, Not detected. CS, Control cheese with commercial starter (Control); CFL, Cheese with free *Lc. paracasei*; CSB, Cheese with symbiotic biocatalyst.

3.9. Sensory Evaluation

The sensory assessment of the functional soft white cheese products is shown in Figure 7. The most affected parameter in the sensory evaluation was color, for which the panelists preferred the CS cheese followed by the CFL and CSB cheeses with scores of 8.12, 7.37, and 5.87, respectively. These results are correlated with our color analyses (Table 3), i.e., the yellowish color of the LAB-fortified products. The aroma products detailed in Table 6 had positive effects on enhancing the odor and taste scores of the CSB cheese (7 and 6.62, respectively) to a greater extent than those of the CFL cheese (6.75 and 6.37, respectively). The enhanced microstructure of the CSB cheese (Figure 4) was reflected in the texture scores. The sensory perception of innovative products is crucial because it is a key aspect in maintaining and promoting the flavorful and wholesome image of dairy foods held by consumers. Consequently, sensory measurements are often the final step in experiments or applications including quality or consistency evaluations [52].

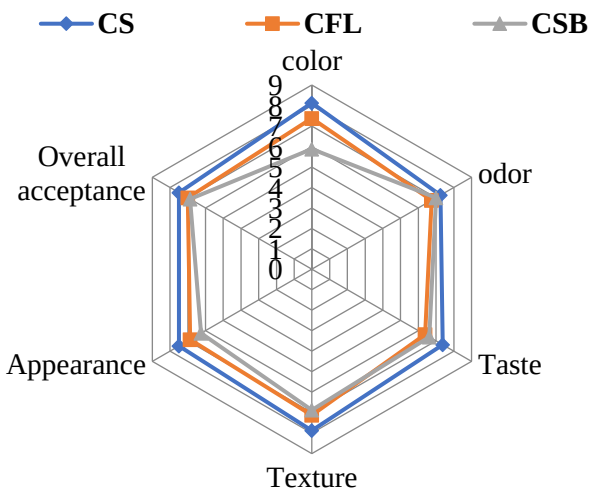

Figure 7. Sensory assessment of functional soft white cheese products. Control cheese with commercial starter (CS); Cheese with free *Lc. paracasei* (CFL); Cheese with symbiotic biocatalyst (CSB).

4. Conclusions

In the current study, a *Lc. paracasei*-immobilized, fiber-fortified functional soft white cheese was prepared using wheat bran, which provided promising processing characteristics to the cheese product. This fortification enhanced the microstructure, texture, aromatic volatile compound content, strain viability, stability, and sensory properties of the functional soft cheese. The presented manufacturing technology could be applied to other soft cheese and functional food products for the stabilized delivery of both probiotics and dietary fibers.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation8100496/s1, Figure S1. Volatile aroma compounds in control soft white cheese (CS) (A), soft white cheeses with free Lc. paracasei cells (CFL) (B) and freeze-dried symbiotic biocatalyst (CSB) (C) after ripening for 45 days.

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