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Using Encapsulated Starter Culture to Prepare Semi-Prepared Yogurt Powder: Evaluation of Fermentation Activity and Sensory Characteristics of Set Yogurt During Storage

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ABSTRACT

This study investigated the impact of encapsulating starter cultures with various biopolymers for incorporation into yogurt powder, as well as the effects of two storage temperatures (ambient and refrigerated) on fermentation activity, incubation time, syneresis, and sensory attributes of set- yogurts over time. Encapsulation significantly reduced the solubility of yogurt powder. However, it also led to a statistically significant increase in incubation time, from 3.0 to 4.5 hours, compared to non-encapsulated samples. Incubation time was further influenced by storage temperature and time; those stored in ambiant temperature at the end of storage time revealed extended fermentation times. Among the samples, those encapsulated with soy protein isolate–pectin and gelatin–whey protein concentrate exhibited significantly lower syneresis and higher WHC, likely due to favorable interactions between the encapsulating agents, milk proteins, and water molecules. The microbiological quality of all yogurt powders remained within acceptable range throughout storage, irrespective of the storage conditions or encapsulation material. Sensory evaluation on the first day of production showed no significant differences among samples, all of which received acceptable scores. However, increasing storage time, particularly at ambient temperature, resulted in a significant decline in sensory scores in encapsulated samples. Overall, the findings indicate that encapsulation of starter cultures, in its current form, is not an effective strategy for preserving long-term fermentative activity, and alternative approaches or optimization of encapsulation parameters may be necessary to achieve this goal.

1.Introduction

Yogurt is a fermented dairy product made by inoculating milk with starter cultures, specifically *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*, which are thermophilic and homofermentative strains (1). Despite its high nutritional value, yogurt has a relatively short shelf life. Under ambient conditions (25–30°C), it can be preserved for only about one day, whereas under refrigeration at 7°C, its shelf life extends to approximately five days. This limitation poses a major challenge to the commercialization and export of yogurt. Additionally, maintaining a cold chain at 2–4°C during distribution is crucial, not only to inhibit the growth of yeasts and molds but also to prevent overactivity of the starter cultures and subsequent excessive acidification. These stringent requirements substantially elevate the overall cost of the product (2).

To address the limited shelf life of fresh yogurt, a common and cost-effective strategy involves converting fully fermented yogurt into powder form using various drying techniques. Freeze-drying and spray-drying are the most widely employed methods at both industrial and semi-industrial scales (3). Yogurt powders produced via these methods offer advantages such as reduced transportation and storage costs, along with improved handling and distribution efficiency (2). However, despite these benefits, such powders typically suffer considerable losses in aroma and flavor compared to fresh yogurt. In some cases, the sensory attributes degrade to the extent that the product becomes unappealing to consumers. Furthermore, upon reconstitution with water, these powders frequently fail to reproduce the original texture of fresh yogurt, resulting in diminished consumer acceptance (4). As such, developing yogurt powders that combine extended shelf life with the sensory quality of fresh yogurt represents a promising area of innovation in the field.

An alternative strategy to achieve both extended shelf life and ease of transport, while preserving the aroma, flavor, and texture of fresh yogurt, is to produce yogurt powder from a blend of its original constituents and starter cultures, rather than freeze-drying or spray-drying finished yogurt. In this approach, the resulting powder allows for long-distance storage and transportation without the limitations imposed by cold chain logistics. Upon reconstitution with water, the starter cultures become active, initiating fermentation and producing a yogurt product that closely mimics the sensory attributes of fresh yogurt. Moreover, because fermentation occurs at the point of consumption, post-fermentation pasteurization is unnecessary, allowing the starter cultures to remain viable and active. This preserves the probiotic potential and health benefits associated with live lactic acid bacteria.

A major challenge in developing yogurt powder through this approach lies in preserving the viability of starter cultures throughout storage and transportation up to the point of consumption. This is critical, as these cultures are responsible for generating yogurt's characteristic texture and flavor compounds. Without their metabolic activity, fermentation, and consequently yogurt formation, does not occur (5). A widely adopted strategy to enhance the survival of bacterial cells in various food systems is microencapsulation. This technique involves enclosing bacterial cells within a protective layer of wall materials (6). Entrapment within polymeric matrices helps shield cells from harmful environmental conditions such as thermal degradation, oxidation, and moisture, thereby minimizing cellular damage and maintaining functionality (7).

Although numerous studies have explored the encapsulation of probiotic bacteria for use in dairy products, no research to date

has specifically addressed the encapsulation of yogurt starter cultures. It is important to distinguish between these two approaches, as the encapsulation of starter cultures differs fundamentally from that of probiotics. In probiotic encapsulation, the primary goal is to maintain viable cell counts (typically 10^6–10^7 CFU/g) throughout production, consumption, and transit through the human gastrointestinal tract (8). By contrast, the encapsulation of starter cultures focuses on protecting the cells from environmental stress during storage, while ensuring their rapid release and metabolic activation upon reconstitution with water for fermentation. Given this distinction, selecting suitable wall materials that provide effective protection during storage while enabling rapid dissolution and bacterial release during yogurt preparation remains a significant challenge. Accordingly, the primary objective of this study was to develop a semi-instant yogurt powder incorporating encapsulated starter cultures. The proposed product aims to extend shelf life and preserve fermentation viability through encapsulation with various polymers, while ensuring that the resulting yogurt exhibits acceptable organoleptic properties in comparison to a control sample containing non-encapsulated cultures.

2- Materials and Methods

All polymers utilized in this study were of food-grade quality, and their types and manufacturers are detailed as follows: iota-carrageenan (Glentham Life Sciences, GC3873, UK); carboxymethyl cellulose (Shandong Yulong Cellulose Technology Co., Ltd., China); soy protein isolate (Shandong Yuxin Bio-Tech Co., Ltd., China); pectin (Cargill Deutschland GmbH, Germany); whey protein concentrate (Lactoprot Deutschland GmbH, Germany); gelatin (Cartino Gelatin Co., Ltd., Thailand); and maltodextrin (Dalian Future International Co., Ltd., China). The yogurt starter culture (YoFlex® Express 1.0) was obtained from Chr. Hansen Company. The formulated yogurt powder, composed of skim milk powder (97.6%), a stabilizer blend (1.5%), and milk protein concentrate (0.9%), was prepared by Gela Dairy Co. (Amol, Iran).

2.1 Formulation of the Wall Polymer Blend

Following an assessment of the behavior of selected polymers regarding water solubility, viscosity, and general behavior at 50 °C and under refrigeration conditions, the final wall-forming polymers were selected based on their optimal concentrations, as outlined in Table 1. Maltodextrin was incorporated into all formulations as a cryoprotectant to protect bacterial cells during the freeze-drying process. The finalized wall polymer blends employed for encapsulating the starter cultures are detailed in Table 1.

Table1. different type and mixing ratios of biopolymers used for encapsulation of bacteria

Treatment code	Polymer percentage for each capsule	Bacteria used in capsules (%)	Encapsulated starter used in 100 g of yogurt powder (g)	Pure bacteria (available in capsules) in 100 g of yogurt powder (g)	Temperature storage
A1	Non-encapsulated (control) Bacteria	-	(0.28 g of pure bacteria without capsules)	0.28	Ambient temperature
R1	Non-encapsulated (control) Bacteria	-	(0.28 g of pure bacteria without capsules)	0.28	Refrigerated temperature

A2	Carboxymethyl Cellulose (0.5%), Iota Carrageenan (5%), Maltodextrin (10%)	2	1.52	0.4	Ambient temperature
R2	Carboxymethyl Cellulose (0.5%), Iota Carrageenan (5%), Maltodextrin (10%)	2	1.52	0.4	Refrigerated temperature
A3	Carboxymethyl Cellulose (1%), Whey Protein Concentrate (5%), Maltodextrin (10%)	2	1.76	0.4	Ambient temperature
R3	Carboxymethyl Cellulose (1%), Whey Protein Concentrate (5%), Maltodextrin (10%)	2	1.76	0.4	Refrigerated temperature
A4	Whey Protein Concentrate (2.5%), Gelatin (3%), Maltodextrin (10%)	1.2	2.80	0.4	Ambient temperature
R4	Whey Protein Concentrate (2.5%), Gelatin (3%), Maltodextrin (10%)	1.2	2.80	0.4	Refrigerated temperature
A5	Isolated Soy Protein (5%), Pectin (1%), Maltodextrin (10%)	1.2	2.88	0.4	Ambient temperature
R5	Isolated Soy Protein (5%), Pectin (1%), Maltodextrin (10%)	1.2	2.88	0.4	Refrigerated temperature

2.2 Encapsulation of Starter Cultures

The starter cultures were encapsulated following the method described by Nguyen et al. (9), with minor modifications. Prior to encapsulation, all polymers were prepared as stock solutions, sterilized via autoclaving, and subsequently mixed in the proportions listed in Table 1. Once cooled to approximately room temperature, commercial yogurt starter culture (1.2%–2%) was added to each formulation and homogenized using an Ultra-Turrax homogenizer for 5 minutes to promote uniform entrapment of bacterial cells within the polymeric matrix. The resulting mixtures were frozen overnight at –20 °C and then subjected to freeze-drying for 48 hours.

2.3 Preparation of Yogurt Powder Containing Encapsulated Starter Cultures

To prepare yogurt powder containing a non-encapsulated starter (control sample), 0.284% of the commercial starter culture, Equivalent to the standard dosage used in commercial products, was added to pre-formulated yogurt powder. The mixture was vacuum-packed in polyethylene pouches. For samples containing encapsulated starter cultures, the same procedure was followed; however, a higher bacterial mass was incorporated to compensate for stress induced during encapsulation and freezing. Each encapsulated treatment contained 0.4% pure starter culture per 100 g of yogurt powder, as detailed in Table 1. For each formulation, two sets of samples were prepared and stored at room temperature and refrigeration conditions, respectively.

2.4 Yogurt Preparation

To prepare yogurt, 13 g of yogurt powder, stored either at ambient or refrigerated conditions, was added to 100 mL of water at 45 °C. The mixture was stirred thoroughly to ensure complete dissolution, avoiding any aggregated or undispersed particles. The samples were then incubated at 45 °C in a laboratory oven until the target pH and titratable acidity of yogurt were achieved. Upon reaching these parameters, the yogurts were refrigerated, and

analytical tests were conducted 24 hours post cold storage (10). Analyses were conducted at biweekly intervals throughout a 6-week storage period.

2.5 Solubility of Yogurt Powder

The solubility of the formulated yogurt powder in water was determined using the procedure described by Zendeboodi et al. (11). 1 g of yogurt powder was added to 10 mL of distilled water and stirred at 600 rpm with a magnetic stirrer at room temperature for 5 minutes. The mixture was then transferred into a Falcon tube and centrifuged at 2000 rpm for 10 minutes. The supernatant was decanted into a glass Petri dish and dried at 105 °C until a constant weight was attained. Solubility was calculated using the following formula:

$$\text{Solubility} = \frac{\textit{initial weight of dry matter}}{\textit{dry matter weight after oven}} \times ١٠٠ \quad \textbf{Eq. 1}$$

2.6 Moisture Content of Yogurt Powder

Moisture content was determined following the procedure outlined by Siyar et al. (12). 5 g of yogurt powder were placed in a glass Petri dish and dried in a laboratory oven at 105 °C for 5 hours. The weight of the dish and sample was recorded post-drying. Moisture percentage was calculated using the following formula:

$$\textbf{Moisture Content} = \frac{\textbf{\textit{initial weight of powder}}}{\textbf{\textit{weight of powder after oven}}} \times \mathbf{100} \quad \textbf{Eq. 2}$$

2.7 Incubation Time (Fermentation activity)

Fermentation power was assessed by recording the incubation time required for yogurt samples to attain a titratable acidity of 72–80 and a pH range of 4.3–4.5. Shorter fermentation time indicated greater metabolic activity of the encapsulated bacterial cultures. pH measurements were taken hourly by inserting the electrode of a calibrated pH meter directly into the yogurt samples (13).

2.8 Titratable Acidity

Titratable acidity was measured by blending 9 g of yogurt with 9 mL of distilled water until a uniform, aggregated-free mixture was obtained. A few drops of 0.5% (w/v) alcoholic phenolphthalein indicator were added, followed by titration with 0.1 N NaOH until the emergence of a stable light pink endpoint. The volume of NaOH consumed was used to calculate acidity (13).

2.9 Syneresis

Syneresis was evaluated based on the method described by Farmani et al. (14) to quantify whey separation under static conditions. Twenty-five grams of stirred yogurt were placed on Whatman filter paper and stored at refrigeration temperature for 2 hours. The volume of liquid collected beneath the paper was weighed, and syneresis percentage was determined using the following formula:

$$\textbf{Syneresis} = \frac{\textbf{\textit{weight of released serum}}}{\textbf{\textit{initial weight of yogurt}}} \times \mathbf{100} \quad \textbf{Eq. 3}$$

2.10 Water Holding Capacity of Yogurt

Water holding capacity was assessed using the protocol of Zamani et al. (15) to evaluate the structural integrity of the yogurt matrix under centrifugation. 10 mL of yogurt were transferred into a 15 mL Falcon tube and centrifuged at 2000 rpm for 20 minutes. The supernatant was discarded, and the retained mass was weighed. The difference in pre- and post-centrifugation weights was expressed as water holding capacity percentage.

$$\text{WHC} = \frac{\textbf{\textit{initial}}\ \textit{weight}\ \textbf{\textit{of yogurt}}}{\textit{weight of}\ \textbf{\textit{yogurt}}\ \textit{after centrifugation}} \times \mathbf{100} \quad \textbf{Eq.}\ 4$$

2.11 Sensory Evaluation

Sensory analysis was conducted following the method developed by Kailasapathy et al. (7) using a five-point hedonic scale. Panelists rated each yogurt sample for taste, aroma, mouthfeel, texture uniformity, and overall acceptability on a scale from 1 (very undesirable) to 5 (very desirable). Samples were coded with random three-digit numbers and presented to a semi-trained panel of ten individuals (six females and four males). Panelists rinsed their mouths

with water between samples to prevent cross-sample interference.

2.12 Microbial Quality

To evaluate microbial quality, tests for total viable count, coliforms, molds, and yeasts were performed immediately post-production and after six weeks of storage. All analyses adhered to the Iranian national standard for dairy microbiology (Standard No. 2406: *Microbiology of Milk and Dairy Products – Specifications and Test Methods*) (16).

2.13 Statistical Analysis

Data were statistically analyzed using a completely randomized design (CRD). All experiments were conducted in triplicate. Mean comparisons were carried out using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test at a significance level of 95%. An independent t-test was applied to compare treatments stored under room and refrigeration conditions. Statistical analyses were performed using SPSS software (version 16.0), and graphical representations were generated using Microsoft Excel.

3. Results and Discussion

As previously mentioned, selecting appropriate polymers for encapsulating starter cultures was critical and guided by the following criteria:

1. The concentration and viscosity of the polymer should be sufficient to entrap the starter cells between the polymer chains. Therefore, the solution concentration should not be too high to prevent dispersion of the starter cells, nor should it be too low such that, due to the low viscosity, the cells settle after mixing with the polymer.

2. The polymer should be easily soluble in water at 45°C and should release the starter rapidly at this temperature to initiate fermentation.

3. The selected polymer should not form a firm gel at refrigeration temperatures, which could result in an unnatural yogurt texture, and should also not leave agglomerates or precipitates at this temperature.

Based on the criteria above, iota-carrageenan, carboxymethyl cellulose, soy protein isolate, pectin, whey protein concentrate, and gelatin were selected as wall materials for the encapsulation of starter cultures.

3.1 Moisture Content and Solubility of Yogurt Powder

Table 2 presents the moisture content and solubility values of various yogurt powder formulations. Results revealed that the control sample (non-encapsulated) exhibited significantly higher solubility than the encapsulated formulations ($P < 0.05$). This reduction in solubility is likely due to the inherent characteristics of the encapsulating polymers. For instance, plant-derived proteins like soy protein isolate generally possess lower solubility than milk proteins, attributed to their hydrophobic groups and complex structural configuration, which hinder complete dissolution in water (17,18).

Furthermore, interactions between milk proteins and polysaccharides, such as iota-carrageenan and pectin, have been shown to decrease the solubility of casein micelles and whey proteins, thereby reducing the overall solubility of milk-based systems (19). Consequently, the lower solubility observed in yogurt powders containing these encapsulating agents can be attributed to these compositional interactions.

In contrast, moisture content did not vary significantly among the encapsulated samples, regardless of the polymer type. However, the highest moisture content was recorded in the control sample and in powders encapsulated with iota-carrageenan and carboxymethyl cellulose. Samples encapsulated with whey protein concentrate and gelatin demonstrated the lowest moisture content, with statistically significant differences between these groups ($P < 0.05$).

The reduced moisture content in the encapsulated samples may be explained by the formation of protein–protein or protein–polysaccharide complexes, which can limit water uptake and retention. The control sample, devoid of encapsulating agents, exhibited higher moisture content likely due to the presence of lyophilized starter cultures, which have a high affinity for moisture. Freeze-dried materials generally retain a porous microstructure, enhancing their moisture absorption capacity under ambient humidity conditions (20). Although encapsulated bacteria were also subjected to freeze-drying, the presence of encapsulating polymers and their interactions with milk proteins may have modulated water absorption behavior, resulting in comparatively lower moisture levels.

Table2. solubility and moisture content of yogurt powders containing encapsulated bacteria

Treatment	Solubility (%)	Moisture (%)
1	90.6±0.19^{a}	3.93±0.11^{ab}
2	73.7±2.82^{b}	3.97±0.02^{a}
3	72.38±2.88^{b}	3.62±0.05^{bc}
4	71.9±0.17^{b}	3.65±0.54^{c}
5	71.15±0.59^{b}	3.79±0.7^{abc}

Means with same superscripts within a column are not significantly different (p > 0.05)
All values are mean ± standard deviation of three replicates

3.2 Incubation Time (Fermentation Activity)

Figure 1 illustrates the fermentation rates of yogurt powder formulations containing free and encapsulated starter cultures. A key determinant of shelf life is the capacity of these cultures to retain metabolic activity until consumption. On day one, all encapsulated samples reached the target pH and titratable acidity after 4.5 hours of incubation at 46 °C, whereas the control (non-encapsulated) sample reached equivalent acidity within 3 hours.

Throughout the six-week storage period, fermentation rate declined in all samples, with a more pronounced reduction in those stored at ambient temperature compared to refrigerated conditions. This decline is attributed to the negative impact of elevated temperatures on the viability of lactic acid bacteria during storage (21). After six weeks, encapsulated samples stored at room temperature required 6–6.5 hours to reach the target acidity, while refrigerated samples required only 5–5.5 hours (Figure 1A).

The difference in fermentation rates between storage conditions is likely due to temperature-dependent activation of lactic acid bacteria, which typically thrive between 30–45 °C (22). Yogurt powders stored at room temperature (20–30 °C) may undergo partial inactivation, whereas refrigerated samples, kept below this activity threshold, retain higher viability and fermentation activity. These observations align with findings by Patil et al. (2019), who reported superior shelf life and viability of encapsulated lactic acid bacteria stored at 4 °C compared to 37 °C (21).

Figure 2B further highlights that, on day one, all encapsulated samples exhibited slower fermentation compared to the control. This is primarily due to the restricted availability of lactose and other substrates to encapsulated bacteria, as the polymer matrix impedes immediate nutrient contact. Consequently, polymer dissolution in water is necessary for bacterial release and activation, contributing to an extended lag phase. Gutierrez et al. (2024) similarly reported lag phases in hydrogel-encapsulated bacteria that were 1.5–3.5 times longer than those of free cells (23).

Additionally, the encapsulation and freeze-drying processes may compromise cell

integrity and fermentative capability. In the current study, lyophilized starter cultures were vigorously mixed with polymer solutions before freezing, likely exposing cells to shear stress, moisture exposure, and temperature changes. These factors may have contributed to longer incubation times. Over time, all formulations, encapsulated and control, showed reduced fermentation rates, with encapsulated samples taking up to 6.5 hours by week six to achieve target acidity. Coelho-Rocha et al. (2018) similarly found reduced viability of encapsulated lactic acid bacteria compared to free cells post-drying, with CFU counts declining from ~3×10^{14} CFU/g to 1.5×10^{14} CFU/g (24). Passot et al. (2011) also demonstrated that freeze-drying stress negatively affected *Lactobacillus bulgaricus* activity, supporting the results observed in this study (25).

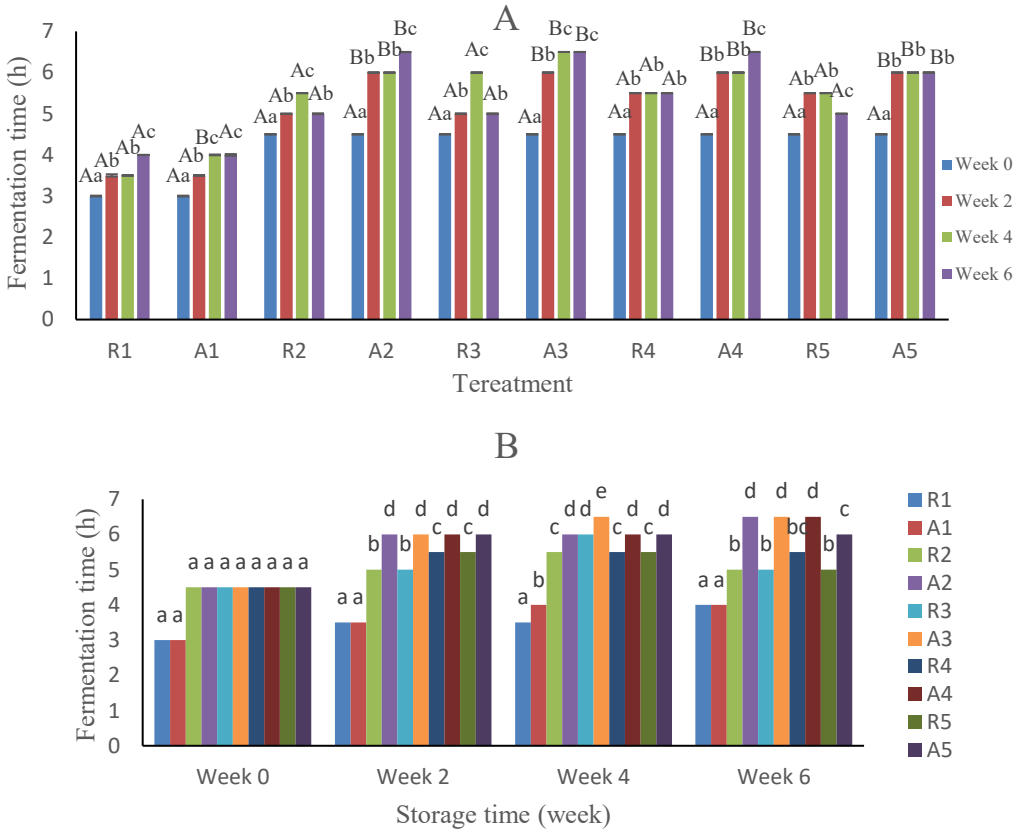

Figure 1-fermentation time of yogurt powders containing different encapsulated bacteria A) comparison of fermentation ability of each formulation during storage period. Lowercase letters show significant differenc between each formulation during storage period and uppercase letters show significant differenc between same samples stored at refrigerated or ambient temperatures., B) comparison of fermentation ability of different formulations in each week.

3.3 Syneresis and Water Holding Capacity of Yogurt Samples

Figures 2A and 2B present the percentage of syneresis and the water holding capacity (WHC) of yogurt samples produced from different yogurt powder formulations over the storage period. Syneresis, defined as whey expulsion from yogurt, is regarded as a negative quality attribute. It reflects the ability of the yogurt matrix to retain structural integrity and entrap whey within its three-dimensional gel network (26). WHC, meanwhile, indicates the matrix's capacity to resist whey expulsion when subjected to physical stress. Both parameters are widely recognized as indicators of gel network strength, with elevated syneresis and reduced WHC suggesting structural weakness (27).

Results demonstrated a general trend of increased syneresis and decreased WHC

with prolonged storage. The lowest syneresis and highest WHC values were observed in week one, with statistically significant differences compared to the corresponding values after six weeks of storage ($P < 0.05$). These findings align with those reported by Supavititpatana et al. (2010), who documented rising syneresis in cow's milk yogurt over time, attributing the increase to weakened protein–protein interactions and subsequent degradation of the gel matrix (27).

The observed increase in syneresis and decrease in WHC toward the end of the storage period may be partially attributed to prolonged incubation times required for fermentation. At this stage, diminished fermentative activity of the starter cultures led to slower acid production, necessitating extended incubation to achieve target acidity. As a result, milk proteins remained at elevated temperatures for longer durations prior to acidification, facilitating extensive structural rearrangements and resulting in a weaker gel matrix, ultimately increasing whey expulsion. Syneresis in yogurt is primarily driven by disruptions in the protein matrix, which compromise its water-binding capacity.

Lee and Lucey (2004) identified two key factors influencing gel network strength: acidification rate and incubation temperature (28). Gel formation is initiated by a pH reduction that dissolves colloidal calcium phosphate from casein micelles, prompting diffusion and structural rearrangement. When acid production is slow, calcium phosphate dissolution occurs earlier and at higher pH levels, yielding atypical micellar aggregates and a weakened gel network. These findings align with the present study's observations linking prolonged incubation to increased syneresis and reduced WHC.

A formulation-wise comparison revealed that Formulations 4 and 5 exhibited the lowest syneresis and highest WHC. Formulation 4 utilized encapsulation with 2.5% whey protein concentrate and 3% gelatin; Formulation 5 employed 5% soy protein isolate and 1% pectin. A likely explanation is that the encapsulating wall materials increased the total solids content, contributing to gel network stability. Increasing milk dry matter, particularly protein, is known to reduce syneresis by enhancing protein–protein interactions and gel matrix strength (29).

Whey protein, widely recognized for improving gel integrity and reducing whey release, likely contributed to the enhanced WHC in Formulation 4. Gelatin, also used in this formulation, serves as an effective stabilizer. Nguyen et al. (2017) demonstrated that gelatin interacts with casein, forming flexible three-dimensional networks that retain both whey and milk proteins (31). Similarly, Motamedzadegan et al. (2015) reported two mechanisms by which gelatin reduces syneresis: increasing dry matter content and reinforcing the casein matrix through interconnective binding (32).

In contrast, Formulations 2 and 3, containing carboxymethyl cellulose (CMC) in combination with iota-carrageenan and whey protein concentrate, respectively, did not exhibit reduced syneresis. Although CMC and carrageenan may enhance viscosity, previous studies indicate they can increase whey separation via depletion-flocculation mechanisms (33,34). Specifically, carrageenan can induce porous gel networks that promote whey release and matrix contraction during gel formation (35,36). Consistent with these findings, Andiç et al. (2013) reported increased syneresis in CMC-stabilized gels, while gelatin yielded firmer textures with less whey loss (33). Nguyen et al. (2017) also observed that gels formed with carrageenan showed greater syneresis than those formed with gelatin (31).

Formulation 3, despite its higher protein content from whey protein concentrate, did not reduce syneresis, likely due to antagonistic interactions with CMC. The negatively charged CMC may have neutralized the positive charges on milk and whey proteins, promoting the formation of

precipitative complexes that disrupted gel strengthening. These outcomes align with observations by Gad and Mohamad (2014), who found similar interactions in CMC-stabilized yogurt drinks (37).

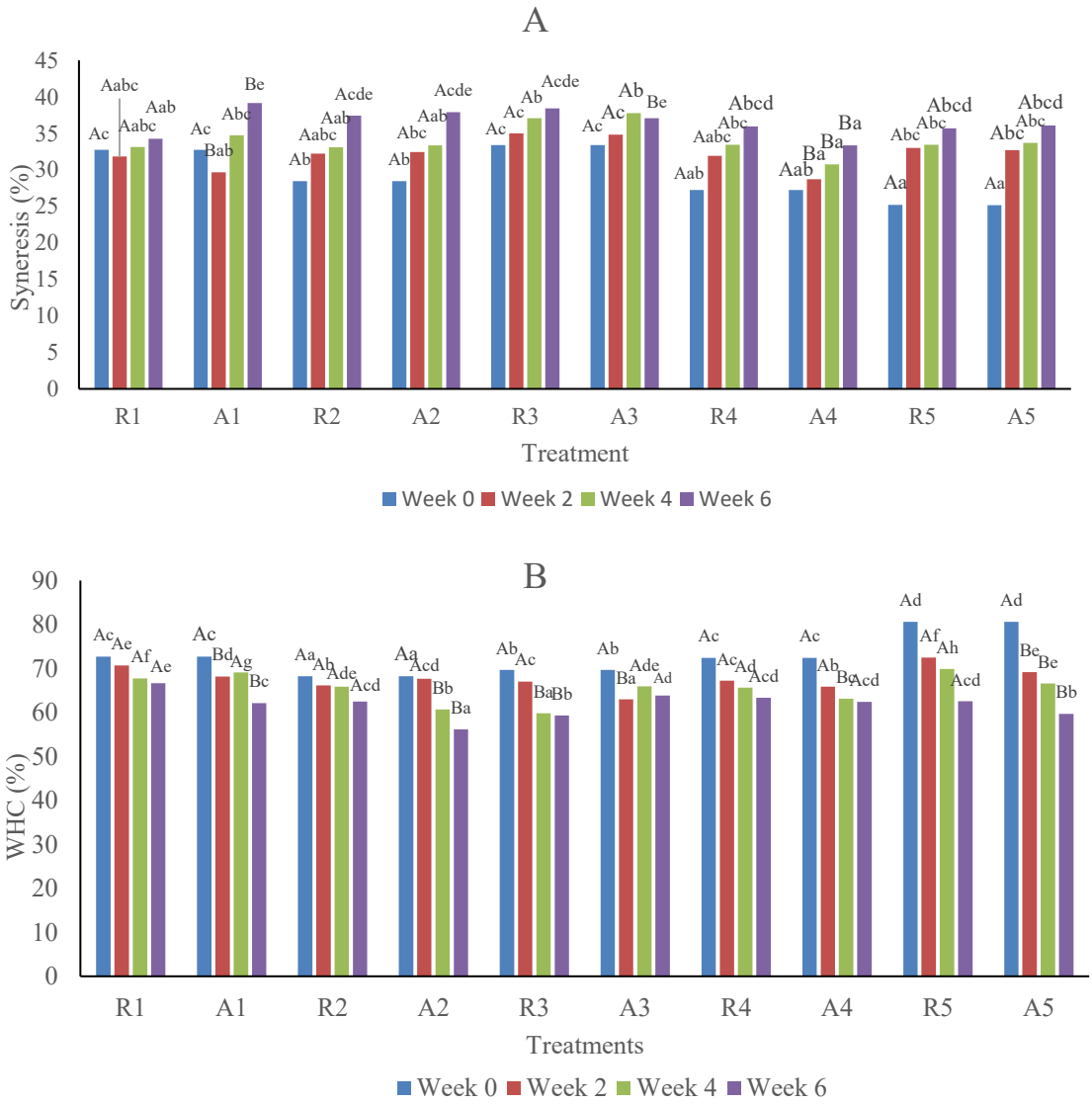

Figure 2 A) syneresis, and B) water holding capacity of yogurt powders containing different encapsulated bacteria. Lowercase letters show significant differenc between each formulation during storage period and uppercase letters show significant differenc between same samples stored at refrigerated or ambient temperatures

3.4 Microbiological Quality

Table 9 summarizes the microbial contamination levels of yogurt powder samples at the first day and after six weeks of storage. Mold, yeast, and coliform counts in all formulations remained below 10 CFU on both 1st day and sixth week, well within acceptable regulatory limits. These results suggest that the encapsulation technique and yogurt powder production were conducted under hygienic conditions.

A slight increase in total viable count (TVC) was observed across all samples after six weeks, potentially due to the favorable conditions that supported the recovery and proliferation of sublethally injured bacteria over time. Despite this increase, all formulations maintained microbial levels within the standard range, reinforcing the effectiveness of the encapsulation and storage approach.

Increases in bacterial populations during storage are well-documented. For example,

Akbari et al. (2023) reported that even when high ozone concentrations were used to reduce microbial load in fresh skim milk, TVC still increased after 15 days of storage relative to initial measurements (38). Similarly, Jalili and Seifzadeh (2021) noted elevated bacterial counts in low-fat dairy butter formulated with gelatin and pectin after five weeks of storage (39), mirroring the trends observed in this study.

The microbial contamination levels of the powder samples at the beginning and end of the storage period are shown in Table 9. The mold and yeast counts as well as coliform counts of all samples on day zero and after six weeks of storage were less than 10 CFU, which falls within the standard range. However, the total viable count slightly increased in all samples after six weeks, likely due to favorable conditions over time that allowed for the proliferation of previously injured bacteria. Nevertheless, the microbial contamination levels remained acceptable in all samples, indicating that the encapsulation and yogurt powder production processes were carried out under hygienic conditions.

An increase in bacterial count over time is an expected outcome and has been reported by many researchers. For instance, Akbari et al. (2023) injected ozone at various concentrations into fresh skim milk to reduce its microbial load and observed that even at high ozone doses, the total viable count increased after 15 days of storage compared to day one (38). Similarly, Jalili and Seifzadeh (2021), who produced low-fat dairy butter using gelatin and pectin, also reported increased bacterial counts after five weeks of storage (39), which is consistent with our findings.

Table 3. microbial analysis of yogurt powders at first and end days of storage

	Week 0			**Week 6**		
Treatment	Mold and Yeast	Total count	Coliform	Mold and Yeast	Total count	Coliform
R1	10>	10>	10>	10>	10>	10>
A1	10>	$0*10^2$-$4*10^1$	10>	10>	$0*10^2$-$0*10^1$	10>
R2	10>	$1*10^2$	10>	10>	$20*10^1$	10>
A2	10>	$1*10^2$	10>	10>	TNTC$*10^3$	10>
R3	10	$2*10^2$-$10*10^1$	10>	10>	$15*10^1$	10>
A3	10	$2*10^2$-$10*10^1$	10>	10>	TNTC$*10^3$	10>
R4	10	$12*10^2$-$1*10^1$	10>	10>	$15*10^2$-$20*10^1$	10>
A4	10	$12*10^2$-$1*10^1$	10>	10>	TNTC$*10^3$	10>
R5	10>	$4*10^2$-$5*10^1$	10>	10>	$4*10^2$-$5*10^1$	10>
A5	10>	$4*10^2$-$5*10^1$	10>	10>	$12*10^2$-$16*10^1$	10>

***A: Ambient temperature**
***R: Refrigerator temperature**

3.5 Sensory Evaluation

The results of sensory evaluation are presented in Table 4. In the first week, all samples received acceptable scores across evaluated attributes, with no statistically significant differences observed. This suggests that bacterial encapsulation using various polymers did not adversely impact sensory qualities, and all treatments displayed comparable characteristics to the control. These findings are in agreement with Kailasapathy (2006), who reported no notable sensory distinctions between yogurts containing free and encapsulated probiotic cultures (7).

Over the six-week storage period, however, scores for all sensory attributes, excluding aroma, declined across all samples. This reduction is attributed to decreased bacterial fermentation activity and prolonged incubation times, which weakened gel formation and negatively influenced mouthfeel, texture, and flavor. These sensory trends correlate with earlier observations concerning fermentation rates, syneresis, and water holding capacity

(WHC). Specifically, extended storage resulted in slower fermentation, longer incubation times, reduced gel strength, and increased syneresis, all contributing to diminished sensory quality.

Although the polymers used as wall materials mildly affected gel integrity and syneresis among treatments, these differences were imperceptible to panelists, likely due to the low polymer concentrations in yogurt powder, which remained below typical sensory detection thresholds. A comparable decline in sensory attributes during storage was reported by Karimi and Manafi (2021) in low-fat yogurts formulated with soy protein isolate, where texture, flavor, and overall acceptability diminished due to increased syneresis, reduced gel firmness, and lipid oxidation (40). Similar findings were observed by Azarashkan et al. (2022) (41).

Conversely, Nateghi (2020) reported improvements in non-oral textural qualities and overall acceptability upon incorporating Zedo gum, although mouthfeel and flavor did not significantly differ from the control (42). This variation may stem from the higher hydrocolloid concentrations used in Nateghi's study. While the hydrocolloids applied as wall materials in the present research possess functional properties that enhance viscosity, water retention, and textural stability, their concentrations were likely too low to elicit perceptible sensory changes.

Table 4. sensorial properties of yogurts prepared from yogurt powders containing encapsulated bacteria

	week 6					week 4					week 2					week 0			
Treatment	General acceptance	Uniformity	Mouthfeel	Odor	Flavor	General acceptance	Uniformity	Mouthfeel	Odor	Flavor	General acceptance	Uniformity	Mouthfeel	Odor	Flavor	General acceptance	Uniformity	Mouthfeel	Odor	Flavor
R1	3.2±0.4	2.8±0.4	3±0	4.6±0.5	3.6±0.5	3.6±0.9	3.8±0.4	3.6±1.1	3.8±0.8	3.6±1.1	4.6±0.5	4.2±0.4	4.8±0.4	5±0	4.2±0.8	3.6±1.3	4.2±0.8	4±1.4	3.6±1.1	3.6±1.3
A1	1.8±0.4	2.4±0.9	1.8±0.8	3.6±0.9	1.8±0.4	2.6±1.1	3.2±1.6	2.6±1.8	2.6±1.5	2±1	3.8±0.4	4.2±0.4	4.2±0.4	4.6±0.5	3.2±1.5	3.6±1.3	4.2±0.8	4±1.4	3.6±1.1	3.6±1.3
R2	3.8±0.8	4.6±0.5	4±0.7	4±0.7	3±0.7	3.2±1.1	3±1	3.4±1.8	4.4±0.9	3.4±1.8	4.2±0.4	3.4±0.5	4.2±0.8	4.4±0.9	3.8±0.8	3.2±1.1	3.8±1.6	3.4±1.7	4±1.4	3.2±1.1
A2	3±0.7	2.6±0.9	3.4±1.1	4±0.7	3.6±1.1	4±0.7	4.4±0.9	4±1	3.8±1.6	4.2±0.8	3.4±0.9	3.6±0.5	3.8±1.3	4.2±1.1	2.8±0.8	3.2±1.1	3.8±1.6	3.4±1.7	4±1.4	3.2±1.1
R3	3.8±0.8	4.4±0.5	3.6±0.9	4±0.5	3.6±0.9	3±1.2	4±1	3.2±1.1	4.2±0.8	3.2±1.1	4.2±0.4	4.4±0.5	4.4±0.5	4.4±0.5	3.8±0.4	3±1	3.6±1.5	3.8±1.3	3.8±1.3	3.4±0.55
A3	3.4±1.1	2.8±1.3	4.4±0.5	4.8±0.4	3.6±1.1	4.4±0.5	4.8±0.4	3.8±0.4	4.4±0.9	4.2±0.4	4.6±0.5	5±0	4.8±0.4	4.2±0.8	4.8±0.4	3±1	3.6±1.5	3.8±1.3	3.8±1.3	3.4±0.5
R4	3.8±0.4	4.4±0.5	3.8±0.8	4.6±0.5	3.4±0.5	3.4±1.1	3.2±1.6	3.8±0.8	2.6±0.9	3±0.7	4.2±0.8	4.6±0.5	4.2±0.8	4.6±0.9	4±0.7	3.4±0.9	3.4±1.5	4±1	3.2±1.5	3.8±1.1
A4	3.8±0.4	4.6±0.5	4.2±0.8	4.6±0.5	3.8±0.4	3.2±0.4	3.6±1.1	3.6±1.1	4.2±0.8	3.2±0.4	2.8±0.4	2.4±0.9	2.8±0.4	3±0.7	3±0	3.4±0.9	3.4±1.5	4±1	3.2±1.5	3.8±1.1
R5	3.8±0.4	4±0	3.6±0.9	4.6±0.5	3.6±0.9	4±0.7	3.6±1.1	3.8±0.8	4.2±0.8	3.8±0.8	4±0	4.6±0.5	5±0	4.6±0.9	4±0	3.4±0.9	3.6±1.5	4±0.7	3.8±0.8	3.4±0.9
A5	3.4±0.5	4.6±0.5	3.8±0.4	3.6±0.9	3±0	3.6±0.5	3.4±1.1	3±1.6	3.8±1.1	3.4±0.9	4.2±0.4	4.6±0.9	4.6±0.5	4.2±0.8	4.4±0.5	3.4±0.9	3.6±1.5	4±0.7	3.8±0.8	3.4±0.9

*A: Ambient temperature
*R: Refrigerator temperature

4-Conclusion

The findings of this study demonstrated that all yogurt powder formulations exhibited comparable moisture content (3.62–3.97%). However, the solubility of samples containing free starter culture (90.6%) was significantly higher than that of encapsulated formulations (71–73%), likely due to the reduced solubility of encapsulating polymers. On the 1st day of storage, encapsulated samples required longer incubation times (4.5 hours) than the control (3 hours), indicating that encapsulation had no beneficial impact on the immediate viability and fermentative activity of starter cultures. Fermentation rates declined progressively over the six-week storage period in all samples, accompanied by increased incubation time. Encapsulated formulations also exhibited elevated syneresis and reduced water holding capacity (WHC), although those prepared with soy protein isolate–pectin (25.19% syneresis) and gelatin–whey protein concentrate (27.25% syneresis) showed improved gel integrity and WHC, likely due to the matrix-enhancing properties of these biopolymers. All powder samples remained within acceptable microbial count thresholds throughout storage. Sensory evaluations revealed no significant differences in taste, texture, or flavor between encapsulated and non-encapsulated samples at the first day of storage. Nonetheless, sensory scores declined over time, particularly under ambient conditions, correlating with

weakened gel structure and reduced fermentation activity. In summary, while bacterial encapsulation may enhance some physical properties of the yogurt matrix, it did not prove effective for preserving long-term fermentative activity. Further studies are warranted to explore alternative strategies that better support bacterial viability during extended storage.

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چکیده

در این مطالعه، اثر انکپسوله کردن استارتر با پلیمرهای مختلف جهت افزودن به پودر ماست و همچنین اثر دو دمای نگهداری محیط و یخچال بر قدرت تخمیر، زمان گرمخانه‌گذاری، آب‌اندازی و خصوصیات حسی ماست قالبی در طی زمان مورد بررسی قرار گرفت. نتایج نشان داد که انکپسوله کردن باکتری، سبب کاهش معنی‌دار حلالیت پودر ماست شده و در مقابل زمان گرمخانه‌گذاری آنها نسبت به نمونه های بدون کپسول به صورت معنی‌داری از ۳ ساعت به ٤/٥ ساعت افزایش یافت. این پارامتر همچنین تحت تاثیر دما و دوره نگهداری قرار گرفت و دمای بالاتر و زمان ماندگاری بیشتر هردو سبب افزایش زمان گرمخانه گذاری شد. در بین تمامی نمونه‌ها، دو نمونه انکپسوله شده در ایزوله پروتئین سویا-پکتین و ژلاتین-کنسانتره پروتئین آب پنیر به دلیل برهمکنش این ترکیبات با پروتئین‌های شیر و مولکول‌های آب، آب‌اندازی کمتری داشتند. کیفیت میکروبی تمامی نمونه‌های پودر ماست تحت تاثیر دوره نگهداری، دمای نگهداری یا نوع باکتری های انکپسوله قرار نگرفتند و در تمامی دوره در محدوده مجاز بودند. آنالیز حسی در روز اول تولید اختلاف معنی‌داری بین نمونه‌ها نشان نداد و تمامی آنها امتیاز قابل قبولی داشتند. با این حال، افزایش زمان نگهداری خصوصا در نمونه‌های انکپسوله و نگهداری شده در دمای محیط، سبب کاهش معنی دار تمامی خصوصیات حسی شد. به طور کلی می‌توان نتیجه‌گیری کرد که انکپسوله کردن باکتری جهت حفظ فعالیت تخمیری آنها در طولانی مدت راهکار مناسبی نبوده و برای دستیابی به این هدف باید روش‌ها و متغییرهای دیگری را مورد آزمون قرار داد.