LWT - Food Science and Technology 169 (2022) 113982

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

# Biocontrol of *Listeria monocytogenes* by *Bacillus coagulans* GBI-30, 6086 in a synbiotic white brined cheese: An *In Vitro* model study

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A R T I C L E   I N F O

*Keywords:*
*Bacillus coagulans* GBI-30, 6086
Inulin
*In vitro*
*Listeria moncytogenes*
White brined cheese

A B S T R A C T

Cheese belongs to the category of foods most frequently contaminated with *Listeria monocytogenes*. Antibiotics, disinfectants, and various preservatives have been conventionally utilized as a microbial control strategy. To address issues such as the emergence of resistance, high cost, and negative effects on health, and the environment, probiotics have been proposed as an environmentally friendly, cost-effective alternative approach to protect against pathogenic microorganisms for better healthcare and food safety. This study assessed the growth and biocontrol of inoculated *L. monocytogenes* in white brined cheese during a 90-day storage at +4 °C. The effect of *Bacillus coagulans* GBI-30, 6086 (BC30) integrated in white brined cheese on *L. monocytogenes* was evaluated using a dynamic system simulating gastrointestinal system conditions. Additionally, the microbiological, physicochemical and sensory characteristics of the cheeses were assessed during ripening. By the termination of ripening, the counts of *L. monocytogenes* were 1.76 and 2.92 $\log_{10}$ CFU/g in groups D (inulin + BC30 + *L. monocytogenes*) and E (*L. monocytogenes*), correspondingly ($P < 0.05$). Group C (inulin + BC30) exhibited higher counts of BC30 compared to group B (BC30) during ripening.

## 1. Introduction

*Listeria monocytogenes* is the responsible pathogen of listeriosis (Eicher et al., 2020). Although listeriosis mortality rate ranges between 20 and 30%, individuals at high risk, including infants, pregnant women, immunosuppressed or seniors, might expect far more severe outcomes (Eicher et al., 2020; Erol & Taşçı, 2021; Gérard et al., 2020; Ziegler et al., 2019). Member states of the European Union (EU) reported 2549 cases of listeriosis in 2018 (Gérard et al., 2020). Worryingly, the number of cases has increased in recent years (EFSA-ECDC, 2019). *L. monocytogenes* constitutes an important concern for the food industry, particularly the dairy sector (Farber & Peterkin, 2000; Lee et al., 2019; Morandi et al., 2020; Melo et al., 2015). Its capability to survive and grow at refrigeration temperatures, in the presence of moderate salt concentrations and neutral pH activity, renders it a hazardous concern for dairy products (Lee et al., 2019; Lim et al., 2020).

The presence of *L. monocytogenes* in RTE products is subject to rigorous criteria. EC No 2073/2005 Regulation on microbiological requirements for foods limits *L. monocytogenes* to <100 CFU/g at the end of the shelf life. Additionally, before placing the food on the market, the pathogen must remain undetected in 25g of RTE foods that support its growth (EC, 2005). Several foods, particularly RTE ones, such as cheese, have already been recognized as possible vectors of *L. monocytogenes* (Ziegler et al., 2019). Within the RTE food category, cheese is a commonly consumed product and has an inherently increased health risk for the consumer. Accordingly, it must conform to Regulation (EC) No 2073/2005 (Gérard et al., 2020). In this framework, because of its recurrent presence in the environment and capacity to grow at low temperatures, *L. monocytogenes* remains a challenging problem and a concern for food safety (Ziegler et al., 2019).

Investigations have studied the utilization of several biocontrol approaches, such as lactic acid bacteria, bacteriocins, bacteriocin-producing cultures, bacteriophages as innovative preservation measures against the development of *L. monocytogenes* in cheeses (Aspri et al., 2017; Falardeau et al., 2021). Cheeses are regarded as a suitable food matrix for probiotic delivery because of their relatively high pH, elevated fat content, and low oxygen concentration, which shield them from the antagonistic conditions faced during gastrointestinal passage

*Abbreviations:* EC, European Commission; EU, European Union; BC30, *Bacillus coagulans* GBI-30, 6086; BHI, Brain Heart Infusion; CFU, Colony Forming Unity; FDA, Food and Drug Administration; FOS, Fructo-oligosaccharides; SGIS, Simulated Gastrointestinal System; RTE, Ready to Eat; WBC, White Brined Cheese.

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https://doi.org/10.1016/j.lwt.2022.113982
Received 18 June 2022; Received in revised form 8 September 2022; Accepted 14 September 2022
Available online 25 September 2022
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(Soares et al., 2019). According to FAO/WHO (2002), probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. Jäger et al. (2018); Keller et al. (2017) have reported various health advantages of probiotic *Bacillus* strains such as enhancements in protein and carbohydrate digestion, meanwhile Nyangale et al., 2015; Nyangale et al., 2014 have stated its effect on senile microbiota modulation and dysbiosis improvement, Hun (2009), Kalman et al. (2009) have described easing of bloating and abdominal ache. Likewise, Honda et al. (2011) has reported the antimicrobial activity of BC30 against pathogens in the alimentary system. FDA (2017) has regarded *B. coagulans* GBI-30, 6086 (BC30) as GRAS (generally recognized as safe*)* under intentional conditions of usage. Synbiotics combine the qualities of probiotics and prebiotics to produce a larger benefit than either probiotics or prebiotics alone (Karetkin et al., 2019; Rugji et al., 2022). Moreover, studies have reported about the effectiveness of probiotics in combination with prebiotics against various pathogens. For instance, co-culturing of *Bifidobacterium* species with various probiotic substrates has been reduced the growth of *Cl. difficile* (Valdes-Varela et al., 2016). Fooks and Gibson (2003) have described the inhibitory effect of synbiotic mixtures on n the human intestinal pathogens *Campylobacter jejuni* and *Escherichia coli.* Akin, the synbiotic mixtures of *Lactobacillus* spp. and *Bifidobacterium* spp. with fructo-oligosaccharide (FOS) were found to inhibit human pathogens such as *E. coli, Shigella* spp., S. Typhimurium and *Cl. difficile* (Piatek et al., 2019).

The combined effect of BC30 and inulin, which were utilized as an add-on culture in the manufacturing of WBC on *L. monocytogenes* was investigated in this study. Additionally, we examined the influence of BC30 and inulin on the microbiological, physical and chemical, textural and sensory profiles of WBC.

## 2. Materials and methods

### 2.1. Materials

Lyophilized *Bacillus coagulans* GBI-30, 6086 was kindly provided by Kerry Inc., Beloit, WI 53511, USA. Commercial starter culture composed of *Lactobacillus casei* and *Lactococcus lactis* subsp. *lactis and* were kindly provided by Chr. Hansen, Turkey. Rennet was kindly provided by Chr. Hansen, Turkey. *Listeria monocytogenes* RSKK 472 (serotype 1/2b) was attained from the culture collection of the Food Hygiene and Technology Department.

Saliva fluid was obtained from *Biochemazone*™, Canada. Fasted gastric fluid, fasted small intestinal fluid and fasted colon dissolution media that simulate human gut fluids were obtained from *Biorelevant,* UK.

### 2.2. Bacterial strains and preparation of inoculum

*B. coagulans* GBI-30, 6086 working inoculum was prepared in obedience to the method described by Abhari et al. (2016). BC30 was inoculated on Nutrient Yeast Extract Salt Medium (NYSM) and incubated at 37 °C for 24 h. Subsequently, a single colony was transferred to NYS Broth and incubated at 37 °C for 24 h. The bacterial solution was centrifuged at 3000×*g* for 20 min washed and re-suspended in 100 mL of sterile saline solution (0.9%). The vegetative forms were then killed by a 15-min heat shock at 80 °C. The spore suspension was serially diluted before being sub cultured on NYS medium. A working solution containing $10^8$ spore/mL was finally kept in the refrigerating temperature until further use.

Stock cultures of *L. monocytogenes* were maintained at −80 °C in Tryptic Soy Broth (TSB) with glycerol (20% v/v) and sub-cultured once again in TSB at 37 °C for 18 h to reach the early stationary phase (~7.5 $log_{10}$ CFU/mL). This culture was then incubated at +5 °C for 20 h for cold adjustment. Strain pools were achieved by merging equal quantities of the cold adjusted stationary phase cultures (Ziegler et al., 2019).

### 2.3. Challenge test

#### 2.3.1. Pilot-scale cheese production and artificial contamination

Pasteurized ewe's milk was utilized to manufacture five batches of white brined cheese in a laboratory scale plant in compliance with the conventional procedure. Raw milk was placed into different stainless-steel vats, pasteurized at 72 °C for 15 s and cooled to 32 °C. Subsequently, starter culture (1 mL/100 mL) and $CaCl_2$ (20 g/100 L) were added. Group A served as the control group (starter culture), group B contained only BC30, group C was inoculated with BC30 and inulin. Group D was inoculated with BC30, inulin and *L. monocytogenes*, meanwhile group E was inoculated with *L. monocytogenes* (Table 1). Inoculated milk was held for 30 min until the pH 6.3–6.4 was attained. Liquid calf rennet (Chr. Hansen, Turkey) diluted (1:10) with sterile $dH_2O$ was added at a proportion of 1g per 16 L milk. After coagulation was reached in 90 min at 32 °C, the curd was fragmented into cubes (2–3 cm) and stirred for 5 min to increase whey separation. After 1 h of draining (without pressing), pressure was applied at ambient temperature (21 °C) for 4 h until whey was completely drained. After pressing, the fresh cheeses were portioned (7 cm × 7 cm x 7 cm) and transferred to polypropylene containers supplied with heat treated brine (15%) and stored at +4 °C. Sampling was carried out at fixed times (D = days), being D1 the starting point of ripening. Precisely, evaluations were carried out at D1, D15, D30, D60 and D90.

### 2.4. Analytical determinations

Prior to cheese production, raw milk was assessed for *L. monocytogenes* presence, in line with EN ISO 11290-1, to verify the absence of former contamination. In the course of the whole ripening, contaminated specimens were subjected to *L. monocytogenes* counts, in accordance with EN ISO 11290-2.

Samples were tested for total aerobic mesophilic counts (TAMC), yeast and moulds (YM), coliforms, *E. coli*, BC30, *L. casei* and *L. lactis*. 10 g of cheese from each group were carried into a sterile bag under aseptic conditions and blended (*IUL Instruments Masticator, Spain*) in 90 mL of sterile peptone water for 2 min. Consecutive decimal dilutions were prepared with peptone water and 1 mL samples of the proper dilutions were poured on the following media: Plate Count Agar (Merck 1.05463, Darmstadt, Germany) for total aerobic mesophilic counts (TAMC), incubated at 30 °C for 48–72 h; M17 Agar (Merck 1.15108, Darmstadt, Germany) for the enumeration of *L. lactis* incubated at 37 °C for 48 h. MRS Agar (Merck 1.10660, Darmstadt, Germany) for *L. casei* incubated at 37 °C for 72 h under anaerobic conditions; Potato Dextrose Agar (Merck 1.10130, Darmstadt, Germany) for yeasts and moulds incubated at 25 °C for 5 days; Violet Red Bile Glucose Agar (Merck 1.01406, Darmstadt, Germany) for coliform counts, overlaid with the same medium and incubated at 37 °C for 18–24 h; Tryptone X-glucuronide agar (Merck 1.16122, Darmstadt, Germany) for *E. coli* counts at 30 °C for 4 h, then at 44 °C for 18 h (Papadopoulou et al., 2018).

The enumeration of BC30 spores was carried after the application of heat shock (80 °C/10 min), using a water bath (*Memmert model WB 14, Germany*), followed by instant cooling in an ice bath. Then, consecutive decimal dilutions were done, appropriate dilutions were poured on Tryptone Glucose Yeast Extract (Condalab, 1190.00, Spain) and incubated at 37 °C for 48 h under anaerobic ambience (Sekhavatizadeh et al.,

**Table 1**
Composition of cheese batches.

| | BC30 | Inulin | *L. monocytogenes* |
|---|---|---|---|
| **A** | – | – | – |
| **B** | + | – | – |
| **C** | + | + | – |
| **D** | + | + | + |
| **E** | – | – | + |

2019).

### 2.5. Microbial survival in simulated gastrointestinal system (SGIS)

A simulated gastrointestinal system was used to establish the survival of BC30 and *L. monocytogenes* in the beginning (D1) and the end of the storage (D90). Four consecutive compartments, each mimicking the saliva, gastric fluid, small and large intestines in a fasting state, were prepared according to the producer's instructions. The transition time, pH and overall temperature of each compartment were selected based on physiological conditions of healthy individuals (Prezzi et al., 2020). pH of saliva, gastric fluid (FaSSGF), small (FaSSIF) and large intestine fluids (FaSSCoF) were 6.5, 2, 7.5 and 7.9, respectively. pH values were adjusted with 1 M NaOH and 1 M HCl solutions. 10 g of cheese from groups B, C, D and E (Table 1) were homogenized with 90 mL of peptone water (*IUL instruments, Barcelona, Spain*). Samples from homogenates (1 mL) were initially placed in the saliva fluid (10 mL) and after the respective incubation was manually transferred in each compartment (5 min in saliva, 2 h in the gastric fluid, 2 h in the small intestine compartment and 2 h in the large intestine compartment) at 37 °C/50 rpm. The samples were diluted at the termination of each step of simulated assimilation, and consequently 1 mL aliquots of the contents, representing each compartment of the gastrointestinal system, were aseptically collected for subsequent serial dilution and viable cell counting.

### 2.6. Proximate analysis

Titratable acidity, total solids, ash, fat, and total protein content were evaluated in accordance with AOAC (1984, pp. 8–34). Titration acidity was calculated by the titration method and the results were expressed as % lactic acid, total solids were determined by drying the samples at 105 °C until constant mass, ash was determined by incineration at 550 °C. Protein content was determined by the Kjeldahl method. Fat content of cheese samples was determined by Gerber method. pH values were measured by digital pH meter (704 pH Meter, Metrohm, Netherlands). Tyrosine content was determined spectrophotometrically as described by Mazzucco et al. (2010). Free fatty acids were extracted from cheese matrix according to methodology described by Rodrigues et al., 2012 with some modifications. Extracted samples were analysed by GC-MS (AGILENT 5975 C AGILENT 7890A GC, U.S.) using a DB WAX column (50 × 0.20 mm x 0.20 μm) operated in MSDCHEM mode. The temperature program started at 80 °C and increased until 240 °C with a flow rate of a sample 1 mL/min and split ratio 20/1.

### 2.7. Sensory evaluation

To assess the descriptive attributes of the cheese samples, quantitative descriptive analysis (QDA) was implemented (Papadopoulou et al., 2018). This method is used in the evaluation of sensory profiling of processed foods, especially dairy products. For the sensory assay, appearance, aroma, taste, texture and overall acceptance were assessed from the panel. Evaluation team consisted of 10 trained members (between 27 and 55 years old) from the Department of Food Hygiene and Technology. All assessments were carried out by the same trained persons, who were all unaware of the sample composition under consideration. Assessment of the groups took place in a well-lighted, odour-free room (20 °C). For all panel sessions, pathogen-free cheeses (A, B and C) approximately 10 g each were placed in plates coded with a letter corresponding to each group. Panellists were allowed to drink water in between evaluations. The evaluation was done using a 9-point hedonic scale. The hedonic scale was oriented from left to right, with increasing intensities (1 = extremely bad; 9 = extremely good).

### 2.8. Statistical analysis

The outcome of BC30, inulin and time on the microbiological, proximate and sensorial profile of the white brined cheeses was determined by analysis of variance (ANOVA), followed by Tukey test. The factors were storage time and treatment: control group (A); probiotic group (B); synbiotic group (C); synbiotic and pathogen group (D); pathogen group (E). All determinations were carried out in triple. The data are evidenced as the mean ± standard deviation. Statistical examination was done using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Significant differences were compared by Mann-Whitney $U$ test on the level of $P < 0.05$. Graphics were done using Microsoft® Exel 2016.

## 3. Results

### 3.1. The survival of BC30 in SGIS

The values ($\log_{10}$ CFU/g) for the spore counts exposed to SGIS in the beginning (D1) and the end of the ripening period (D90) are reported in Fig. 1. All groups showed similar counts of BC30 in the samples exposed to saliva fluid both in the first and last day of ripening. Group C had the highest counts ($P > 0.05$) in the saliva phase on D1 and D90, 6.82 and 7.28 $\log_{10}$ CFU/g respectively. Similar results were observed also in the other compartments of the SGIS for D1 and D90 ($P < 0.05$). Results showed a slight decrease of the BC30 to the different simulated phases of human gastric and intestinal conditions.

### 3.2. The survival of BC30 throughout ripening

The populations of spores of probiotic BC30 studied remained high (~6 $\log_{10}$ CFU/g) at the end of the ripening, being close to the values determined in the beginning of the process (D1) ($P < 0.05$). All groups showed a decrease in the spore counts on D90 ($P < 0.05$). BC30 was inoculated at the concentration of $10^8$ spores/mL, and by the end of the ripening; (D90) remained approximately 6 $\log_{10}$ CFU/g in all groups (Table 2). By the time the ripening was complete, group C had higher spore counts ($P < 0.05$).

### 3.3. The survival of Listeria monocytogenes throughout ripening

*L. monocytogenes* was not detected in the milk used to produce WBC batches. Growth dynamics of *L. monocytogenes* at +4 °C in the two batches are reported in Table 3. Maximum growth rate was measured on D1 in both batches, with 7.47 and 7.51 $\log_{10}$ CFU/g, respectively. In the present study, the use of a 7-log contamination level in the pasteurized milk was necessary to allow the observation of *L. monocytogenes* kinetics during the whole cheese ripening. On the D15, *L. monocytogenes* counts were 5.28 and 5.27 $\log_{10}$ CFU/g in groups D and E, respectively ($P > 0.05$). At the end of ripening (D90) the counts of *L. monocytogenes* were determined 1.76 and 2.92, respectively ($P < 0.05$). Inulin and BC30 containing group (D) revealed lower counts of *L. monocytogenes* during ripening. The difference in *L. monocytogenes* counts from D1 to D90 in this group was found to be statistically significant on D15, D30 and D90 ($P < 0.05$).

### 3.4. The survival of Listeria monocytogenes in SGIS

*L. monocytogenes* counts in SGIS are shown in Table 4. On D1, the counts of *L. monocytogenes* remained at 5.63 $\log_{10}$ CFU/g for both groups at the beginning of the digestion (saliva fluid compartment). Descending to the gastric part of the simulated system, the counts of bacteria were significantly reduced. Inulin and BC30 containing group (D) revealed lower counts of *L. monocytogenes* on the D1 ($P < 0.05$), meanwhile counts were not detected on the D90 for this compartment. No counts were detected in the small and large intestinal fluid compartments both on the D1 and D90.

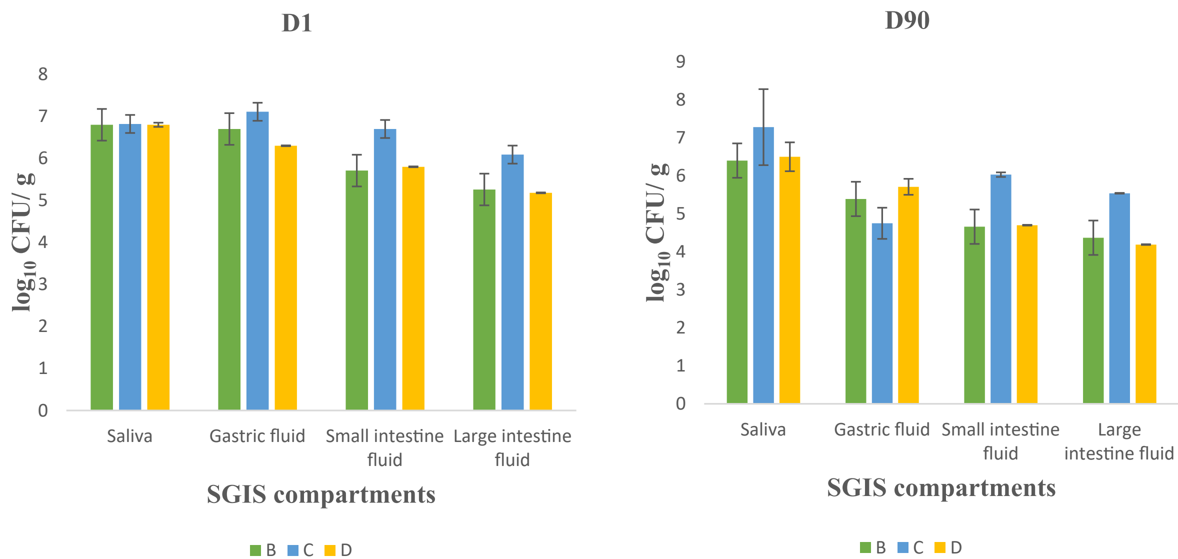


**Fig. 1.** BC30 counts in simulated GIS (M±SD).

**Table 2**
Survival of BC30 throughout ripening ($log_{10}$ CFU/g).

| | D1 | D15 | D30 | D60 | D90 |
|---|---|---|---|---|---|
| **B** | 7.32 ± 0.20$^{Aa}$ | 7.27 ± 0.15$^{Aa}$ | 7.11 ± 0.13$^{Ab}$ | 6.98 ± 0.14$^{Ac}$ | 6.67 ± 0.1$^{Bd}$ |
| **C** | 7.34 ± 0.25$^{Aa}$ | 7.29 ± 0.16$^{Aa}$ | 7.16 ± 0.17$^{Ab}$ | 7.05 ± 0.19$^{Ac}$ | 6.82 ± 0.17$^{Ad}$ |
| **D** | 7.32 ± 0.20$^{Aa}$ | 7.27 ± 0.15$^{Aa}$ | 7.11 ± 0.13$^{Ab}$ | 6.99 ± 0.14$^{Ab}$ | 6.68 ± 0.11$^{Bc}$ |

D-days of ripening.
$^{A-B}$ differences between groups with different superscripts in the same line are important ($P < 0.05$).
$^{a-b}$ differences between days with different superscripts in the same order are important ($P < 0.05$).

**Table 3**
Survival of L. *monocytogenes* throughout ripening ($log_{10}$ CFU/g).

| | D1 | D15 | D30 | D60 | D90 |
|---|---|---|---|---|---|
| **D** | 7.47 ± 0.18$^{Aa}$ | 5.28 ± 0.02$^{Ab}$ | 3.76 ± 0.42$^{Ad}$ | 4.58 ± 0.52$^{Ac}$ | 1.76 ± 0.27$^{Be}$ |
| **E** | 7.51 ± 0.24$^{Aa}$ | 5.27 ± 0.01$^{Ab}$ | 3.98 ± 0.64$^{Ad}$ | 4.61 ± 0.54$^{Ac}$ | 2.92 ± 0.55$^{Ae}$ |

D-days of ripening.
$^{A-B}$ differences between groups with different superscripts in the same line are important ($P < 0.05$).
$^{a-b}$ differences between days with different superscripts in the same order are important ($P < 0.05$).

### 3.5. Microbiological quality

TAMC increased during the ripening period (Table 5). There was a significant increase from baseline to D90 ($P < 0.05$). By the end of the ripening, group C had the highest counts, 9.12 $log_{10}$ CFU/g. The results of counts showed the absence of coliforms and *E. coli* in all the samples, thus ensuring microbiological safety of the product. Changes in YM counts in groups A, B, D and E were found to be statistically significant ($P < 0.05$). In these groups, the lowest counts were detected at the beginning, while an increase was observed on the other days ($P < 0.05$). As a general trend, a gradual decline in *Lactococcus lactis* and *Lactobacillus casei* counts was noted, the degree of which was impacted by all parameters studied. Considering all groups, the highest *L. lactis* counts were found in the group C on D90 (5.86 $log_{10}$ CFU/g). Parallelly, group

**Table 4**
Survival of L. *monocytogenes* in simulated GIS ($log_{10}$ CFU/g).

| | D1 | | D90 | |
|---|---|---|---|---|
| | D | E | D | E |
| **SF** | 5.63 ± 0.05$^{A}$ | 5.63 ± 0.05$^{A}$ | 1.63 ± 0.36$^{B}$ | 2.85 ± 0.57$^{A}$ |
| **GF** | 2.18 ± 0.06$^{B}$ | 2.48 ± 0.05$^{A}$ | ND | ND |
| **SIF** | ND | ND | ND | ND |
| **LIF** | ND | ND | ND | ND |

D1-first day of ripening; D90-last day of ripening.
$^{A-B}$ differences between groups with different superscripts in the same line are important ($P < 0.05$).
ND- Not Detected (<1 $log_{10}$ CFU/g).
SF- Saliva fluid, GF- Gastric fluid, SIF- Small intestinal fluid, LIF- Large intestinal fluid.

C showed the highest counts of *Lactobacillus casei* on D90 ($P < 0.05$).

### 3.6. Proximate composition

The physico-chemical characteristics; pH, titratable acidity, total solids, fat in dry matter, ash and protein in dry matter of the cheese samples are presented in Table 6. During ripening, pH values exhibited variations. Generally, all groups showed higher pH values at the beginning of the evaluation with a tendency to decrease. A continuous increase in titration acidity was observed throughout ripening in all groups. On the last analysis day, titration acidity was determined at the highest level in all groups, while the lowest values were determined at the beginning of ripening ($P < 0.05$). Statistically significant increases and decreases were also observed in ash ratios during ripening in the BC30 and inulin-containing group (C) between the D1 and D30 ($P < 0.05$). The highest ash content of this group was detected on the D30 ($P < 0.05$).

Changes in the fat in the dry matter ratio occurred during ripening (Table 6). All groups showed a continuous decline ($P < 0.05$). The highest fat in dry matter ratios on D1 were found to be 52.78% and 52.81% in groups A and B, respectively. The differences observed between groups on the D15 and D60 were significant ($P < 0.05$). The protein content of milk was found ~21%. Alterations in protein in dry matter content were observed throughout ripening period. None of the groups did not maintain their dominance in the protein ratio in dry matter. On D90 protein in dry matter was found to be lower in all groups compared to D1 ($P < 0.05$). Changes in tyrosine content between groups

**Table 5**
Microbiological determinations throughout ripening ($\log_{10}$ CFU/g).

| Storage | D1 | D15 | D30 | D60 | D90 |
|---|---|---|---|---|---|
| **TAMC** | | | | | |
| A | $8.20 \pm 0.01^{Ce}$ | $8.41 \pm 0.01^{Cd}$ | $8.59 \pm 0.01^{Bc}$ | $8.69 \pm 0.01^{Ab}$ | $8.80 \pm 0.01^{Ba}$ |
| B | $8.28 \pm 0.02^{Bd}$ | $8.41 \pm 0.01^{Cc}$ | $8.60 \pm 0.01^{Bb}$ | $8.70 \pm 0.01^{Aa}$ | $8.70 \pm 0.01^{Ca}$ |
| C | $8.38 \pm 0.02^{Ac}$ | $8.70 \pm 0.02^{Ab}$ | $8.70 \pm 0.02^{Ab}$ | $8.73 \pm 0.06^{Ab}$ | $9.12 \pm 0.01^{Aa}$ |
| D | $8.29 \pm 0.02^{Bd}$ | $8.59 \pm 0.02^{Bc}$ | $8.69 \pm 0.02^{Ab}$ | $8.74 \pm 0.05^{Ab}$ | $8.79 \pm 0.01^{Ba}$ |
| E | $8.20 \pm 0.01^{Cd}$ | $8.41 \pm 0.02^{Cc}$ | $8.58 \pm 0.02^{Bb}$ | $8.70 \pm 0.01^{Aa}$ | $8.70 \pm 0.01^{Ca}$ |
| **YM** | | | | | |
| A | $3.42 \pm 0.01^{Be}$ | $3.65 \pm 0.01^{Ed}$ | $4.18 \pm 0.01^{Cb}$ | $4.02 \pm 0.01^{Dc}$ | $4.48 \pm 0.01^{Ba}$ |
| B | $3.40 \pm 0.01^{Bd}$ | $3.85 \pm 0.01^{Bc}$ | $4.17 \pm 0.01^{Cb}$ | $4.30 \pm 0.01^{Aa}$ | $4.18 \pm 0.01^{Cb}$ |
| C | $3.98 \pm 0.52^{Ab}$ | $4.02 \pm 0.02^{Ab}$ | $4.02 \pm 0.02^{Db}$ | $4.12 \pm 0.01^{Ca}$ | $3.80 \pm 0.01^{Ec}$ |
| D | $3.44 \pm 0.01^{Be}$ | $3.73 \pm 0.01^{Dd}$ | $4.57 \pm 0.01^{Aa}$ | $4.05 \pm 0.01^{Dc}$ | $4.12 \pm 0.01^{Db}$ |
| E | $3.35 \pm 0.01^{Ce}$ | $3.78 \pm 0.01^{Cd}$ | $4.31 \pm 0.01^{Bb}$ | $4.24 \pm 0.01^{Bc}$ | $4.61 \pm 0.01^{Aa}$ |
| ***L. lactis*** | | | | | |
| A | $9.36 \pm 0.04^{Aa}$ | $9.16 \pm 0.04^{Aa}$ | $8.01 \pm 0.13^{Ab}$ | $7.63 \pm 0.06^{Ab}$ | $5.85 \pm 0.29^{Ac}$ |
| B | $9.37 \pm 0.04^{Aa}$ | $9.17 \pm 0.04^{Aa}$ | $8.02 \pm 0.14^{Ab}$ | $7.62 \pm 0.05^{Ac}$ | $5.85 \pm 0.29^{Ad}$ |
| C | $9.37 \pm 0.03^{Aa}$ | $9.21 \pm 0.01^{Aa}$ | $8.06 \pm 0.14^{Ab}$ | $7.68 \pm 0.10^{Ab}$ | $5.86 \pm 0.28^{Ac}$ |
| D | $9.36 \pm 0.04^{Aa}$ | $9.16 \pm 0.04^{Aa}$ | $8.01 \pm 0.14^{Ab}$ | $7.63 \pm 0.07^{Ab}$ | $5.85 \pm 0.29^{Ac}$ |
| E | $9.37 \pm 0.04^{Aa}$ | $9.17 \pm 0.04^{Aa}$ | $8.01 \pm 0.14^{Ab}$ | $7.63 \pm 0.07^{Ab}$ | $5.85 \pm 0.29^{Ac}$ |
| ***L. casei*** | | | | | |
| A | $9.36 \pm 0.04^{Aa}$ | $8.45 \pm 0.06^{Ab}$ | $7.50 \pm 0.01^{Ac}$ | $6.91 \pm 0.01^{Bd}$ | $5.57 \pm 0.01^{Be}$ |
| B | $9.36 \pm 0.04^{Aa}$ | $8.45 \pm 0.06^{Ab}$ | $7.50 \pm 0.01^{Ac}$ | $6.90 \pm 0.01^{Bd}$ | $5.57 \pm 0.01^{Be}$ |
| C | $9.42 \pm 0.01^{Aa}$ | $8.50 \pm 0.10^{Ab}$ | $7.51 \pm 0.01^{Ab}$ | $7.07 \pm 0.06^{Ac}$ | $5.63 \pm 0.04^{Ad}$ |
| D | $9.36 \pm 0.04^{Aa}$ | $8.45 \pm 0.06^{Ab}$ | $7.50 \pm 0.01^{Ac}$ | $6.91 \pm 0.01^{Bd}$ | $5.57 \pm 0.01^{Be}$ |
| E | $9.36 \pm 0.04^{Aa}$ | $8.45 \pm 0.06^{Ab}$ | $7.50 \pm 0.01^{Ac}$ | $6.91 \pm 0.01^{Bd}$ | $5.57 \pm 0.01^{Be}$ |

D-days of ripening.
$^{A-B}$ differences between groups with different superscripts in the same line are important ($P < 0.05$).
$^{a\text{-}b}$ differences between days with different superscripts in the same order are important ($P < 0.05$).

were found to be statistically significant ($P < 0.05$). The proteolytic activity showed appreciable differences between the groups (Fig. 2). Maximum activity was reached on D90 corresponding to group C (260.14 mg tyrosine/100 gr). While the tyrosine content in cheese made from ewe's milk were 97.16 for the three groups at the beginning of the ripening, these values increased by the end of the ripening to 246.34, 245.98 and 260.14 for groups A, B and C respectively ($P > 0.05$).

The content of saturated and unsaturated fatty acids in the WBC throughout ripening is displayed in Table 7. Groups A, B and C exhibited alteration in the fatty acid content. Regardless of the composition all groups showed an increase in the content of decanoic, undecanoic, myristoleic, oleic, linoleic and gamma linoleic acid by the end of the ripening. Eicosanoid acid had the lowest concentration of free fatty acids in the samples, while control group (A) had the highest concentration on D90. Control group also had the highest concentration of caproic and caprylic acids by the end of the ripening. These fatty acids are formed because of triglyceride lipolysis and are responsible for the creation of various smells and flavours in fermented foods.

**Table 6**
Proximate composition throughout ripening.

| | Storage | | | | |
|---|---|---|---|---|---|
| | D1 | D15 | D30 | D60 | D90 |
| **pH** | | | | | |
| A | $5.01 \pm 0.12^{Aa}$ | $5.01 \pm 0.09^{Aa}$ | $4.95 \pm 0.10^{Ab}$ | $4.72 \pm 0.01^{Ac}$ | $4.73 \pm 0.01^{Ac}$ |
| B | $4.90 \pm 0.03^{Ba}$ | $4.91 \pm 0.09^{Ba}$ | $4.79 \pm 0.01^{Bb}$ | $4.71 \pm 0.01^{Ac}$ | $4.73 \pm 0.01^{Ac}$ |
| C | $4.80 \pm 0.03^{Ca}$ | $4.60 \pm 0.64^{Cd}$ | $4.58 \pm 0.04^{Dd}$ | $4.64 \pm 0.01^{Bc}$ | $4.74 \pm 0.01^{Ab}$ |
| D | $4.73 \pm 0.01^{Da}$ | $4.58 \pm 0.01^{Cb}$ | $4.54 \pm 0.01^{Ec}$ | $4.56 \pm 0.01^{Cc}$ | $4.54 \pm 0.01^{Bc}$ |
| E | $4.92 \pm 0.04^{Ba}$ | $4.92 \pm 0.08^{Ba}$ | $4.69 \pm 0.01^{Cb}$ | $4.58 \pm 0.01^{Cc}$ | $4.50 \pm 0.05^{Cd}$ |
| **Acidity (L.A%)** | | | | | |
| A | $1.57 \pm 0.01^{Bd}$ | $1.80 \pm 0.01^{Bc}$ | $2.03 \pm 0.01^{Ab}$ | $2.03 \pm 0.01^{Bb}$ | $2.54 \pm 0.01^{Ba}$ |
| B | $1.57 \pm 0.01^{Be}$ | $1.81 \pm 0.01^{Bd}$ | $1.94 \pm 0.01^{Bc}$ | $2.16 \pm 0.01^{Ab}$ | $2.53 \pm 0.01^{Ba}$ |
| C | $1.65 \pm 0.01^{Ae}$ | $1.85 \pm 0.01^{Ad}$ | $1.90 \pm 0.01^{Cc}$ | $2.04 \pm 0.01^{Bb}$ | $2.57 \pm 0.01^{Aa}$ |
| D | $1.60 \pm 0.01^{Be}$ | $1.79 \pm 0.01^{Bd}$ | $1.90 \pm 0.01^{Cc}$ | $2.16 \pm 0.01^{Ab}$ | $2.50 \pm 0.01^{Ca}$ |
| E | $1.56 \pm 0.01^{Be}$ | $1.81 \pm 0.01^{Bd}$ | $1.84 \pm 0.01^{Dc}$ | $2.03 \pm 0.01^{Bb}$ | $2.49 \pm 0.01^{Ca}$ |
| **Total solids (%)** | | | | | |
| A | $45.18 \pm 0.01^{Ee}$ | $46.73 \pm 0.01^{Cd}$ | $47.28 \pm 0.01^{Dc}$ | $50.75 \pm 0.01^{Ca}$ | $49.38 \pm 0.01^{Db}$ |
| B | $45.86 \pm 0.01^{De}$ | $46.25 \pm 0.01^{Dd}$ | $47.54 \pm 0.01^{Cc}$ | $48.23 \pm 0.01^{Eb}$ | $51.26 \pm 0.01^{Ca}$ |
| C | $52.98 \pm 0.01^{Ad}$ | $52.75 \pm 0.01^{Ad}$ | $54.39 \pm 0.01^{Ac}$ | $55.32 \pm 0.20^{Aa}$ | $54.95 \pm 0.01^{Ab}$ |
| D | $52.40 \pm 0.01^{Bd}$ | $51.83 \pm 0.01^{Be}$ | $52.53 \pm 0.01^{Bc}$ | $54.30 \pm 0.01^{Bb}$ | $54.94 \pm 0.01^{Aa}$ |
| E | $46.10 \pm 0.01^{Ce}$ | $46.18 \pm 0.01^{Ed}$ | $47.19 \pm 0.01^{Ec}$ | $50.38 \pm 0.01^{Db}$ | $51.37 \pm 0.01^{Ba}$ |
| **Fat in dry matter (%)** | | | | | |
| A | $52.78 \pm 1.11^{Aa}$ | $50.08 \pm 0.01^{Bb}$ | $46.79 \pm 0.01^{Ac}$ | $41.84 \pm 0.19^{Bd}$ | $40.74 \pm 0.02^{Ae}$ |
| B | $52.81 \pm 0.01^{Aa}$ | $50.58 \pm 0.01^{Ab}$ | $46.77 \pm 0.20^{Ac}$ | $44.13 \pm 0.31^{Ad}$ | $38.65 \pm 0.23^{Be}$ |
| C | $45.28 \pm 0.01^{Da}$ | $41.92 \pm 0.01^{Eb}$ | $38.86 \pm 0.01^{Dc}$ | $34.72 \pm 0.11^{Ed}$ | $30.73 \pm 0.02^{Ee}$ |
| D | $45.62 \pm 0.05^{Ca}$ | $44.19 \pm 0.05^{Db}$ | $42.32 \pm 0.17^{Cc}$ | $38.98 \pm 0.11^{Dd}$ | $35.87 \pm 0.03^{De}$ |
| E | $51.78 \pm 0.04^{Ba}$ | $49.10 \pm 0.09^{Cb}$ | $46.41 \pm 0.01^{Bc}$ | $41.25 \pm 0.03^{Cd}$ | $38.50 \pm 0.05^{Ce}$ |
| **Ash (%)** | | | | | |
| A | $5.50 \pm 0.01^{Cb}$ | $5.53 \pm 0.01^{Bb}$ | $5.55 \pm 0.01^{Ba}$ | $5.52 \pm 0.01^{Cb}$ | $5.52 \pm 0.01^{Cb}$ |
| B | $5.52 \pm 0.01^{Cb}$ | $5.57 \pm 0.01^{Aa}$ | $5.58 \pm 0.01^{Ba}$ | $5.52 \pm 0.01^{Cb}$ | $5.50 \pm 0.05^{Cb}$ |
| C | $5.89 \pm 0.01^{Bb}$ | $6.03 \pm 0.01^{Aa}$ | $6.05 \pm 0.01^{Aa}$ | $5.60 \pm 0.01^{Bc}$ | $5.89 \pm 0.01^{Bb}$ |
| D | $6.04 \pm 0.01^{Aa}$ | $6.05 \pm 0.01^{Aa}$ | $6.06 \pm 0.01^{Aa}$ | $5.87 \pm 0.01^{Ac}$ | $5.95 \pm 0.01^{Ab}$ |
| E | $5.43 \pm 0.01^{Db}$ | $5.34 \pm 0.01^{Cc}$ | $5.55 \pm 0.01^{Ba}$ | $5.53 \pm 0.01^{Ca}$ | $5.54 \pm 0.01^{Ca}$ |
| **Protein in dry matter (%)** | | | | | |
| A | $46.54 \pm 0.22^{Aa}$ | $36.70 \pm 1.03^{Bb}$ | $34.10 \pm 0.22^{Ac}$ | $27.69 \pm 0.31^{Bd}$ | $26.34 \pm 0.43^{Ae}$ |
| B | $45.41 \pm 0.34^{Ba}$ | $36.55 \pm 0.11^{Cb}$ | $34.13 \pm 0.27^{Ac}$ | $26.62 \pm 0.05^{Cd}$ | $25.01 \pm 0.08^{Be}$ |
| C | $39.35 \pm 0.34^{Da}$ | $31.68 \pm 0.06^{Eb}$ | $29.90 \pm 0.22^{Dc}$ | $25.16 \pm 0.13^{Ed}$ | $23.60 \pm 0.28^{Ee}$ |
| D | $39.62 \pm 0.02^{Ca}$ | $32.83 \pm 0.23^{Db}$ | $30.59 \pm 0.55^{Cc}$ | $26.26 \pm 0.21^{Dd}$ | $23.85 \pm 0.56^{De}$ |
| E | $45.40 \pm 0.22^{Ba}$ | $36.79 \pm 0.27^{Ab}$ | $33.96 \pm 0.93^{Bc}$ | $28.10 \pm 0.33^{Ad}$ | $24.80 \pm 0.22^{Ce}$ |

D-days of ripening.
$^{A-B}$ differences between groups with different superscripts in the same line are important ($P < 0.05$).
$^{a\text{-}b}$ differences between days with different superscripts in the same order are important ($P < 0.05$).

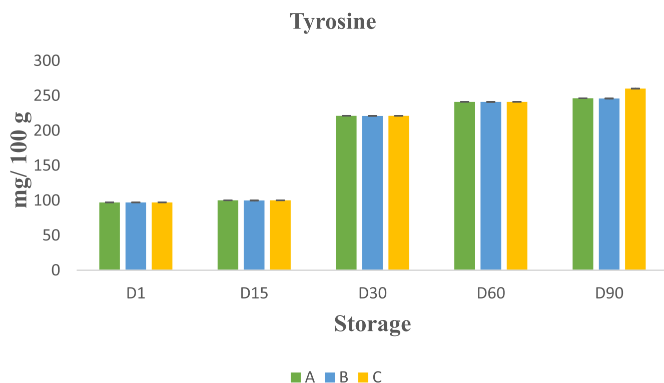


**Fig. 2.** Variations in tyrosine content of cheese samples during ripening (M±SD).

### 3.7. Sensory evaluation

The sensory characteristics of samples in terms of appearance, texture, aroma, taste and general acceptability presented average scores between 7 (liked moderately) and 8 (liked very much), with significant influence ($P < 0.05$) of storage time (Fig. 3).

Regarding appearance, a significant difference in scores ($P < 0.05$) was found between all groups on D1 and D90. Group C was the most liked in terms of appearance on D1, meanwhile group B received the highest scores on D90. In the texture scores, there were increases and decreases throughout ripening that were found to be significant between group A and C on D1, D15, D30 and D60 ($P < 0.05$). In group C, taste scores were found to be 7.15 on D1 and 7.37 on D90 ($P < 0.05$). A constant increase occurred between D1 and D60 ($P < 0.05$).

## 4. Discussion

It is essential to investigate the permanence of probiotics in various foods since the physico-chemical and sensorial characteristics of a probiotic product should be sustained during shelf-life (Rad et al., 2012). Populations of $10^6$–$10^7$ $\log_{10}$ CFU/g in the final product are confirmed as therapeutic extents in probiotic foods (Talwalkar et al., 2004). The significant physical and chemical resistances of spores to gastrointestinal environment are elucidated by the known features of bacterial spores. The spore coatings, the tightness of the spore core and its high content of minerals, and the preservation of the spore DNA by acid-resolvable proteins are all linked to spore resistance to hostile conditions (Nicholson et al., 2000). Almada-Érix et al. (2021), Soares et al. (2019), Keller et al. (2019) have determined results comparable to the present study.

Bacterial spores are exceedingly resistant and can persist in high salt concentrations and long ripening period (Bora et al., 2009). In the current study, even though that BC30 was inoculated at a level of 8 log, evaluation on D1 revealed that the attachment of BC30 was in the range of 7.00 log. By the end of the ripening (D90), BC30 was determined to be ~6.00 $\log_{10}$ CFU/g. Hence, the incorporation of BC30 with claimed probiotic properties in the production of WBC seems feasible. Similarly, Ong et al. (2006) observed a decrease of about 6–7 $\log_{10}$ CFU/g of probiotics in cheese during whey draining. In conventional procedure, the utilization of thermally untreated raw material also could bring safety threats (Soares et al., 2019). BC produces L-lactic acid and coagulin, a bacteriocin with antibacterial action against a variety of pathogens (Ripamonti et al., 2009). According to Koç (2020), BC30 has an inhibiting impact on *Listeria inocua* and biofilm development. Probiotics may assist in food preservation due to their prospective antimicrobial properties, which are settled by the action of various compounds synthesized by these bacteria and delivered in the food matrix (de Vuyst et al., 2004) thus, this effect was also investigated in this study to control

**Table 7**
Fatty acid composition of cheese samples (%).

| | Storage Period | | | | |
|---|---|---|---|---|---|
| | D1 | D15 | D30 | D60 | D90 |
| Butyric acid methyl ester | | | | | |
| A | 0.63 ± 0.01$^{Cd}$ | 0.97 ± 0.01$^{Cc}$ | 1.01 ± 0.01$^{Cb}$ | 0.96 ± 0.01$^{Bc}$ | 1.57 ± 0.01$^{Aa}$ |
| B | 1.30 ± 0.01$^{Ab}$ | 1.69 ± 0.02$^{Aa}$ | 1.28 ± 0.01$^{Bb}$ | 0.99 ± 0.01$^{Bd}$ | 1.04 ± 1.01$^{Cc}$ |
| C | 0.96 ± 0.01$^{Bd}$ | 1.20 ± 0.01$^{Bc}$ | 2.62 ± 0.01$^{Ab}$ | 2.77 ± 0.01$^{Aa}$ | 1.18 ± 0.01$^{Bc}$ |
| Caproic acid methyl ester | | | | | |
| A | 0.96 ± 0.01$^{Bd}$ | 1.20 ± 0.01$^{Cc}$ | 1.29 ± 0.01$^{Cb}$ | 0.97 ± 0.01$^{Cd}$ | 1.61 ± 0.01$^{Aa}$ |
| B | 1.39 ± 0.01$^{Ab}$ | 1.66 ± 0.01$^{Aa}$ | 1.30 ± 0.01$^{Bc}$ | 1.30 ± 0.01$^{Bc}$ | 1.12 ± 0.01$^{Cd}$ |
| C | 1.35 ± 0.01$^{Ac}$ | 1.40 ± 0.01$^{Bb}$ | 2.78 ± 0.01$^{Aa}$ | 2.80 ± 0.01$^{Aa}$ | 1.42 ± 0.01$^{Bb}$ |
| Heptanoic acid methyl ester | | | | | |
| A | 0.14 ± 0.01$^{Aa}$ | 0.02 ± 0.01$^{Ab}$ | 0.01 ± 0.01$^{Bb}$ | 0.02 ± 0.01$^{Ab}$ | 0.02 ± 0.01$^{Ab}$ |
| B | 0.01 ± 0.01$^{Ba}$ | 0.01 ± 0.02$^{Aa}$ | 0.03 ± 0.01$^{Ba}$ | 0.02 ± 0.01$^{Aa}$ | 0.01 ± 0.01$^{Aa}$ |
| C | 0.01 ± 0.01$^{Bb}$ | 0.01 ± 0.01$^{Ab}$ | 0.07 ± 0.01$^{Aa}$ | 0.01 ± 0.01$^{Ab}$ | 0.01 ± 0.01$^{Ab}$ |
| Caprylic acid methyl ester | | | | | |
| A | 1.00 ± 0.01$^{Cd}$ | 1.30 ± 0.01$^{Cc}$ | 1.37 ± 0.01$^{Bb}$ | 0.96 ± 0.01$^{Cd}$ | 1.70 ± 0.01$^{Aa}$ |
| B | 1.37 ± 0.01$^{Bc}$ | 1.65 ± 0.01$^{Aa}$ | 1.35 ± 0.01$^{Bc}$ | 1.48 ± 0.01$^{Ab}$ | 1.25 ± 0.01$^{Cd}$ |
| C | 1.45 ± 0.01$^{Ac}$ | 1.48 ± 0.01$^{Bc}$ | 2.92 ± 0.01$^{Aa}$ | 1.04 ± 0.01$^{Bd}$ | 1.64 ± 0.01$^{Bb}$ |
| Decanoic acid methyl ester | | | | | |
| A | 0.01 ± 0.01$^{Aa}$ | 0.02 ± 0.01$^{Aa}$ | 0.01 ± 0.01$^{Aa}$ | 0.01 ± 0.01$^{Aa}$ | 0.03 ± 0.01$^{Aa}$ |
| B | 0.01 ± 0.01$^{Aa}$ | 0.01 ± 0.01$^{Aa}$ | 0.01 ± 0.01$^{Aa}$ | 0.02 ± 0.01$^{Aa}$ | 0.02 ± 0.01$^{Aa}$ |
| C | 0.02 ± 0.01$^{Aa}$ | 0.01 ± 0.01$^{Aa}$ | 0.02 ± 0.01$^{Aa}$ | 0.02 ± 0.01$^{Aa}$ | 0.03 ± 0.01$^{Aa}$ |
| Capric methyl ester | | | | | |
| A | 3.22 ± 0.01$^{Cc}$ | 3.91 ± 0.53$^{Cb}$ | 3.95 ± 0.05$^{Cb}$ | 3.07 ± 0.01$^{Cd}$ | 5.26 ± 0.01$^{Ba}$ |
| B | 3.94 ± 0.01$^{Bc}$ | 4.59 ± 0.01$^{Aa}$ | 4.02 ± 0.01$^{Bb}$ | 4.08 ± 0.001$^{Bb}$ | 4.02 ± 0.10$^{Cb}$ |
| C | 4.27 ± 0.01$^{Ae}$ | 4.46 ± 0.01$^{Bd}$ | 8.06 ± 0.01$^{Ab}$ | 9.11 ± 0.01$^{Aa}$ | 5.48 ± 0.01$^{Ac}$ |
| Undecanoic methyl ester | | | | | |
| A | 0.19 ± 0.01$^{Ab}$ | 0.18 ± 0.01$^{Ab}$ | 0.23 ± 0.01$^{Ba}$ | 0.24 ± 0.01$^{Aa}$ | 0.22 ± 0.01$^{Ba}$ |
| B | 0.16 ± 0.03$^{Ab}$ | 0.19 ± 0.01$^{Aa}$ | 0.23 ± 0.01$^{Ba}$ | 0.24 ± 0.01$^{Aa}$ | 0.23 ± 0.01$^{Ba}$ |
| C | 0.14 ± 0.01$^{Bc}$ | 0.20 ± 0.01$^{Ab}$ | 0.29 ± 0.01$^{Aa}$ | 0.25 ± 0.01$^{Aa}$ | 0.26 ± 0.01$^{Aa}$ |
| Undecanoic 10 methyl ester | | | | | |
| A | 0.28 ± 0.01$^{Ba}$ | 0.10 ± 0.01$^{Ab}$ | 0.05 ± 0.01$^{Ac}$ | 0.05 ± 0.01$^{Ac}$ | 0.08 ± 0.01$^{Ab}$ |
| B | 0.65 ± 0.01$^{Aa}$ | 0.03 ± 0.01$^{Bc}$ | 0.05 ± 0.01$^{Ac}$ | 0.04 ± 0.01$^{Ac}$ | 0.09 ± 0.01$^{Ab}$ |
| C | 0.07 ± 0.01$^{Ca}$ | 0.03 ± 0.01$^{Bc}$ | 0.05 ± 0.01$^{Ab}$ | 0.06 ± 0.01$^{Ab}$ | 0.04 ± 0.01$^{Bc}$ |
| Lauric methyl ester | | | | | |
| A | 2.43 ± 0.01$^{Cd}$ | 2.65 ± 0.31$^{Bc}$ | 3.08 ± 0.10$^{Bb}$ | 2.69 ± 0.01$^{Cc}$ | 3.72 ± 0.02$^{Ba}$ |
| B | 3.06 ± 0.18$^{Bc}$ | 3.18 ± 0.01$^{Ab}$ | 3.09 ± 0.10$^{Bc}$ | 3.34 ± 0.01$^{Ba}$ | 2.97 ± 0.01$^{Cc}$ |
| C | 3.23 ± 0.01$^{Ac}$ | 3.20 ± 0.01$^{Ac}$ | 4.99 ± 0.01$^{Aa}$ | 3.85 ± 0.01$^{Ab}$ | 3.82 ± 0.01$^{Ab}$ |
| Undecanoic 10 methyl, methyl ester | | | | | |
| A | 0.28 ± 0.01$^{Ba}$ | 0.10 ± 0.01$^{Ab}$ | 0.05 ± 0.01$^{Ac}$ | 0.05 ± 0.01$^{Ac}$ | 0.08 ± 0.01$^{Ab}$ |
| B | 0.65 ± 0.01$^{Aa}$ | 0.03 ± 0.01$^{Bc}$ | 0.05 ± 0.01$^{Ac}$ | 0.04 ± 0.01$^{Ac}$ | 0.09 ± 0.01$^{Ab}$ |
| C | 0.07 ± 0.01$^{Ca}$ | 0.03 ± 0.01$^{Bc}$ | 0.05 ± 0.01$^{Ab}$ | 0.06 ± 0.01$^{Ab}$ | 0.04 ± 0.01$^{Bc}$ |
| 6nonenoic acid | | | | | |
| A | 0.07 ± 0.01$^{Ab}$ | 0.10 ± 0.01$^{Ab}$ | 0.01 ± 0.01$^{Bc}$ | 0.09 ± 0.01$^{Bb}$ | 0.30 ± 0.01$^{Aa}$ |

(*continued on next page*)

**Table 7** (*continued*)

| | Storage Period | | | | |
|---|---|---|---|---|---|
| | D1 | D15 | D30 | D60 | D90 |
| B | $0.10 \pm 0.01^{Ab}$ | $0.09 \pm 0.01^{Ab}$ | $0.10 \pm 0.01^{Ab}$ | $0.10 \pm 0.01^{Bb}$ | $0.18 \pm 0.01^{Ba}$ |
| C | $0.11 \pm 0.01^{Ab}$ | $0.09 \pm 0.01^{Ac}$ | $0.12 \pm 0.01^{Ab}$ | $0.16 \pm 0.01^{Aa}$ | $0.12 \pm 0.01^{Cb}$ |
| Myristoleic acid methyl ester | | | | | |
| A | $1.06 \pm 0.01^{Bb}$ | $1.18 \pm 0.01^{Ca}$ | $0.49 \pm 0.01^{Cc}$ | $0.39 \pm 0.01^{Ad}$ | $0.27 \pm 0.01^{Be}$ |
| B | $0.67 \pm 0.01^{Aa}$ | $0.59 \pm 0.01^{Ab}$ | $0.66 \pm 0.01^{Aa}$ | $0.28 \pm 0.01^{Cd}$ | $0.37 \pm 0.01^{Ac}$ |
| C | $0.69 \pm 0.01^{Aa}$ | $0.53 \pm 0.01^{Bc}$ | $0.59 \pm 0.02^{Bb}$ | $0.34 \pm 0.02^{Bd}$ | $0.25 \pm 0.01^{Be}$ |
| Methyl 12-methyl teradecanoate | | | | | |
| A | $0.74 \pm 0.01^{Bc}$ | $0.53 \pm 0.01^{Bd}$ | $0.76 \pm 0.01^{Bc}$ | $1.02 \pm 0.01^{Cb}$ | $1.08 \pm 0.01^{Ca}$ |
| B | $0.82 \pm 0.01^{Ac}$ | $0.70 \pm 0.01^{Ad}$ | $0.78 \pm 0.01^{Bc}$ | $1.20 \pm 0.01^{Ba}$ | $1.14 \pm 0.01^{Bb}$ |
| C | $0.71 \pm 0.01^{Bd}$ | $0.68 \pm 0.01^{Ad}$ | $0.86 \pm 0.01^{Ac}$ | $1.36 \pm 0.01^{Aa}$ | $1.23 \pm 0.01^{Ab}$ |
| Pentadecanoic methyl ester | | | | | |
| A | $0.10 \pm 0.01^{Bc}$ | $0.11 \pm 0.01^{Ac}$ | $0.11 \pm 0.01^{Ac}$ | $0.24 \pm 0.01^{Ab}$ | $0.27 \pm 0.01^{Aa}$ |
| B | $0.11 \pm 0.01^{Bb}$ | $0.11 \pm 0.01^{Ab}$ | $0.11 \pm 0.01^{Ab}$ | $0.23 \pm 0.01^{Aa}$ | $0.23 \pm 0.01^{Ba}$ |
| C | $0.90 \pm 0.01^{Ab}$ | $0.09 \pm 0.01^{Ab}$ | $0.11 \pm 0.01^{Ac}$ | $0.25 \pm 0.01^{Aa}$ | $0.25 \pm 0.01^{Aa}$ |
| Pentadecanoic acid 14 methyl, methyl ester | | | | | |
| A | $0.25 \pm 0.01^{Aa}$ | $0.22 \pm 0.01^{Ab}$ | $0.15 \pm 0.01^{Ac}$ | $0.13 \pm 0.01^{Ad}$ | $0.11 \pm 0.01^{Ae}$ |
| B | $0.25 \pm 0.01^{Aa}$ | $0.22 \pm 0.01^{Ab}$ | $0.14 \pm 0.01^{Ac}$ | $0.10 \pm 0.01^{Ad}$ | $0.10 \pm 0.01^{Ad}$ |
| C | $0.26 \pm 0.01^{Aa}$ | $0.24 \pm 0.01^{Ab}$ | $0.16 \pm 0.01^{Ac}$ | $0.12 \pm 0.01^{Ad}$ | $0.12 \pm 0.01^{Ad}$ |
| Palmitic acid methyl ester | | | | | |
| A | $32.62 \pm 0.01^{Bc}$ | $34.71 \pm 0.01^{Aa}$ | $34.60 \pm 0.01^{Bb}$ | $31.28 \pm 0.01^{Be}$ | $31.81 \pm 0.01^{Bd}$ |
| B | $33.55 \pm 0.01^{Ac}$ | $34.16 \pm 0.01^{Bb}$ | $34.32 \pm 0.01^{Ca}$ | $31.97 \pm 0.01^{Ae}$ | $32.59 \pm 0.01^{Ad}$ |
| C | $32.01 \pm 0.01^{Cc}$ | $33.34 \pm 0.01^{Cb}$ | $35.92 \pm 0.01^{Aa}$ | $26.02 \pm 0.01^{Ce}$ | $31.23 \pm 0.01^{Cd}$ |
| Heptadecanoic methyl ester | | | | | |
| A | $0.64 \pm 0.01^{Aa}$ | $0.54 \pm 0.01^{Bc}$ | $0.58 \pm 0.01^{Ab}$ | $0.52 \pm 0.01^{Ad}$ | $0.30 \pm 0.01^{Ce}$ |
| B | $0.60 \pm 0.01^{Ba}$ | $0.50 \pm 0.01^{Bb}$ | $0.60 \pm 0.01^{Aa}$ | $0.52 \pm 0.02^{Ab}$ | $0.58 \pm 0.01^{Aa}$ |
| C | $0.66 \pm 0.01^{Ab}$ | $0.79 \pm 0.01^{Aa}$ | $0.43 \pm 0.01^{Bc}$ | $0.36 \pm 0.01^{Be}$ | $0.42 \pm 0.01^{Bd}$ |
| Stearic methyl ester | | | | | |
| A | $28.95 \pm 0.01^{Aa}$ | $25.45 \pm 0.01^{Ab}$ | $21.57 \pm 0.01^{Cc}$ | $19.93 \pm 0.01^{Ad}$ | $16.64 \pm 0.01^{Ce}$ |
| B | $28.70 \pm 0.01^{Ba}$ | $18.70 \pm 0.01^{Cc}$ | $22.60 \pm 0.01^{Bb}$ | $18.05 \pm 0.01^{Cd}$ | $17.90 \pm 0.01^{Ae}$ |
| C | $28.41 \pm 0.01^{Ca}$ | $24.77 \pm 0.01^{Bb}$ | $22.85 \pm 0.01^{Ac}$ | $18.62 \pm 0.01^{Bd}$ | $17.30 \pm 0.01^{Be}$ |
| Palmitoleic acid methyl ester | | | | | |
| A | $0.52 \pm 0.01^{Aa}$ | $0.45 \pm 0.05^{Ab}$ | $0.32 \pm 0.01^{Ad}$ | $0.40 \pm 0.01^{Ac}$ | $0.39 \pm 0.01^{Bc}$ |
| B | $0.51 \pm 0.01^{Aa}$ | $0.43 \pm 0.01^{Ab}$ | $0.32 \pm 0.01^{Ac}$ | $0.42 \pm 0.01^{Ab}$ | $0.49 \pm 0.01^{Aa}$ |
| C | $0.49 \pm 0.02^{Aa}$ | $0.44 \pm 0.01^{Ab}$ | $0.32 \pm 0.01^{Ad}$ | $0.39 \pm 0.01^{Ac}$ | $0.40 \pm 0.01^{Bc}$ |
| Oleic acid methyl ester | | | | | |
| A | $7.29 \pm 0.09^{Cd}$ | $15.7 \pm 0.22^{Cc}$ | $17.26 \pm 0.74^{Ab}$ | $23.89 \pm 0.90^{Aa}$ | $23.92 \pm 0.63^{Aa}$ |
| B | $17.20 \pm 0.08^{Ac}$ | $17.55 \pm 0.01^{Bb}$ | $15.97 \pm 0.5^{Bd}$ | $23.96 \pm 0.90^{Ba}$ | $23.92 \pm 0.63^{Aa}$ |
| C | $15.76 \pm 0.05^{Bd}$ | $26.59 \pm 0.33^{Aa}$ | $10.18 \pm 0.55^{Ce}$ | $21.16 \pm 0.70^{Cc}$ | $22.50 \pm 0.80^{Bb}$ |
| Linoleic acid methyl ester | | | | | |
| A | $2.65 \pm 0.06^{Aa}$ | $2.26 \pm 0.06^{Ad}$ | $2.67 \pm 0.44^{Aa}$ | $2.39 \pm 0.29^{Ac}$ | $2.56 \pm 0.55^{Ab}$ |
| B | $2.21 \pm 0.06^{Bb}$ | $2.10 \pm 0.01^{Bc}$ | $2.36 \pm 0.22^{Ba}$ | $2.01 \pm 0.01^{Cd}$ | $2.22 \pm 0.35^{Bb}$ |
| C | $2.00 \pm 0.01^{Cb}$ | $1.83 \pm 0.01^{Cc}$ | $0.67 \pm 0.33^{Ce}$ | $2.23 \pm 0.55^{Ba}$ | $1.16 \pm 0.11^{Cd}$ |
| Gamma linoleic acid methyl ester | | | | | |
| A | $0.78 \pm 0.01^{Bc}$ | $0.78 \pm 0.01^{Bc}$ | $0.78 \pm 0.17^{Ac}$ | $1.52 \pm 0.46^{Aa}$ | $0.87 \pm 0.01^{Cb}$ |
| B | $0.75 \pm 0.01^{Bc}$ | $0.42 \pm 0.18^{Ce}$ | $0.62 \pm 0.21^{Bd}$ | $0.92 \pm 0.02^{Cb}$ | $2.22 \pm 0.35^{Aa}$ |
| C | $0.85 \pm 0.11^{Ac}$ | $0.97 \pm 0.06^{Ab}$ | $0.63 \pm 0.29^{Bd}$ | $1.11 \pm 0.01^{Bb}$ | $1.37 \pm 0.35^{Ba}$ |
| Eicosanoic acid methyl ester | | | | | |
| A | $0.01 \pm 0.01^{Bc}$ | $0.07 \pm 0.01^{Cb}$ | $0.10 \pm 0.01^{Ab}$ | $0.17 \pm 0.01^{Aa}$ | $0.10 \pm 0.01^{Ab}$ |
| B | $0.04 \pm 0.01^{Bc}$ | $0.62 \pm 0.01^{Ba}$ | $0.07 \pm 0.01^{Ab}$ | $0.10 \pm 0.01^{Bb}$ | $0.07 \pm 0.01^{Ab}$ |
| C | $0.08 \pm 0.01^{Ab}$ | $0.72 \pm 0.01^{Aa}$ | $0.10 \pm 0.06^{Ab}$ | $0.09 \pm 0.01^{Bb}$ | $0.06 \pm 0.01^{Ac}$ |

D-days of ripening.
$^{A-B}$ differences between groups with different superscripts in the same line are important ($P < 0.05$).
$^{a-b}$ differences between days with different superscripts in the same order are important ($P < 0.05$).

the growth of *L. monocytogenes* in white cheese. *L. monocytogenes* counts showed alterations during ripening. Coming to the termination of ripening, the counts of *L. monocytogenes* were 1.76 and 2.92 $\log_{10}$ CFU/g in groups D and E, respectively ($P < 0.05$). Inulin and BC30 containing group (D) exhibited lower counts of *L. monocytogenes* compared to control group (E) during ripening. During the evaluation in simulated gastrointestinal system *L. monocytogenes* counts were detected only in the saliva compartment on D90.

WBC are ripened for extended periods in brine with variable concentrations of NaCl solution (10%–18% NaCl). The degree of salt is selective for the bacteria present in these cheeses (Hayaloglu, 2022). At the beginning of ripening, the TAMC of all samples were determined ~8 log. By the end of the ripening, synbiotic group (C) had the highest counts ($P < 0.05$). BC30 and inulin seemed to reinforce the growth and development of mesophiles. Likewise, Corbo et al. (2001), Yilmaztekin et al. (2004) have stated alike results. *Lactobacillus casei* and *Lactococcus lactis* showed a good resistance to high salt concentration (15%). The decline in the colony counts was faster during the D60 and D90. By the end of the ripening, all groups had similar counts of *Lactobacillus casei* and *Lactococcus lactis* (~6 log). Related results have been outlined for the survival of *B. bifidum* together with *L. acidophilus* in WBC (Yilmaztekin et al., 2004).

Content of ash, total solids, acidity, pH, fat and protein in dry matter of cheeses during ripening are shown in Table 6. Integration of inulin in the cheese composition showed to affect the pH, total acidity, total solids and ash content. While the total solids content increased by the end of ripening, pH and total acidity decreased. Sojica et al. (2011), Mendoza et al. (2001), Huang et al. (2011), Alaei et al. (2018) have reported akin findings.

The tyrosine content of the cheese samples during ripening is related to the peptidase activity of the various microorganisms utilized in cheese production; thus, it is reasonable to conclude that the greater the inoculation of probiotics, the higher the proteolysis in cheese (Yilmaztekin et al., 2004). BC30 has also been found to exhibit proteolytic, amylytic, and lipolytic activities, and hence has the capability to help in nutrient assimilation (Prihanto et al., 2013; Reyes-Mendez et al., 2015). In addition, spores of BC30 investigated by Keller et al. (2017), Keller et al. (2019), have exhibited to release intracellular peptidase in a dynamic, computer-administered *in vitro* model of the gastrointestinal system consequently contributed to increased protein digestion.

Bacterial lipolytic esterases, often known as lipases, are important lipolytic agents in cheese (Erkaya et al., 2015). Oleic acid concentration was the highest among the long-chain unsaturated fatty acids in all samples followed by gamma linolenic and linoleic acids. Gamma linoleic

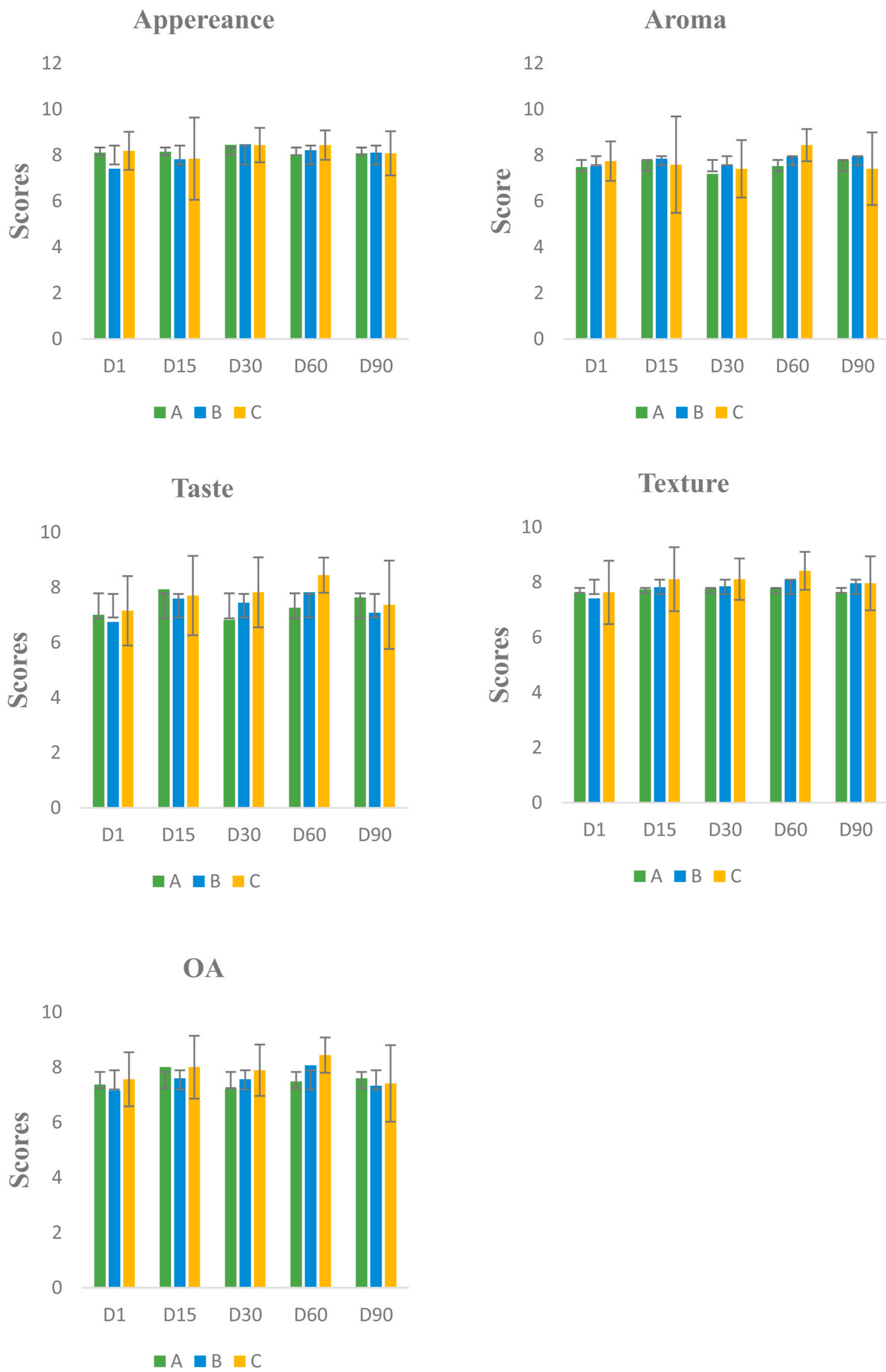


**Fig. 3.** Sensorial determinations throughout ripening (M±SD).

acid content was found 0.87 (A), 2.22 (B) and 1.37% (C) by the end of the storage. The presence of linoleic acid in dairy products is determined by several factors such as livestock diet, locus, recurrent diversity, food supply for animals in pasture, forage type used, the initial quantity of linolenic acid in raw milk, temperature and starter type used (Serafeimidou et al., 2012). Butyric acid is another major free fatty acid identified in the free fatty acid profile. In the current investigation, group B had the top percentage of butyric acid on D1, meanwhile at the ending of the ripening control group (A) had the highest, followed by group C and B (Table 7). It is produced by the lipolytic action of a lactic acid bacteria, while linoleic acid is produced by the hydrolysis of milk fat (Ogawa et al., 2005; Sekhavatizadeh et al., 2019).

Sensory assessment is crucial for investigating the impact of BC30 and inulin in WBC. The cheese samples supplemented with BC30 and inulin were satisfactory in terms of overall acceptability (Fig. 3), with scores ranging from 7.15 to 8.07 on a 9-point scale, indicating a product with commercial promise in the dairy sector. There was no difference in acceptability between the beginning and end of the ripening showing that the panellists would purchase the cheese nevertheless of time. Ehsannia and Sanjabi (2016a), Ehsannia and Sanjabi (2016b) revealed comparable results for the sensory assessment of *Bacillus coagulans* inoculated processed cheese. The sensory evaluation revealed that the BC30-containing group outperformed the control group in texture, taste, and aroma in apricots impregnated with BC30 (Ayrıç-Danşman et al., 2022). In contrast to the control groups, Sekhavatizadeh et al. (2019) discovered that taste scores in kashk cheese enhanced with *B. coagulans* spores were slightly lower. In another study, BC30 was used as an adjuvant culture in the production of yogurt. As storage time passed, the appearance, texture, and taste of yogurt samples deteriorated. However, as compared to the control group, the sensory quality of BC30-enhanced yogurt samples did not decline (Cao et al., 2022).

## 5. Conclusion

Storage temperature, pH, ambient salinity, and chlorine stress may all have a role in inducing the viable but non-culturable (VBNC) condition in *L. monocytogenes*. The present investigation allowed us to understand and authenticate a dynamic growth-death model for the evaluation of *L. monocytogenes* viability in WBC supplemented with probiotic BC30 and inulin via a challenge test, as well as obtain applicable information about this product and its safety levels. There were no substantial changes in the physico-chemical parameters of the WBC, confirming the product's stability, which is critical from an industrial standpoint. The product was shown to be safe for human ingestion and to be a good carrier of BC30 and inulin. BC30 demonstrated a survival range of 6.67–6.82 $\log_{10}$ CFU/g on D90 in WBC thus indicating the potentiality of the carrier matrix. Although the use of BC30 and inulin in WBC had a mild effect on *L. monocytogenes*, this study demonstrates that their co-usage is a distinctive and propitious substitute for the dairy sector, since it expands the choice of different age groups that care about health and welfare.

## Authorship contributions

During the study's preparation, all authors contributed equally.

## Author declaration

[Instructions: Please check all applicable boxes and provide additional information as requested.]

## 1. Conflict of interest

No conflict of interest exists.

## 2. Funding

Funding was received for this work.

## 3. Intellectual property

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

## 4. Research ethics

We further confirm that any aspect of the work covered in this manuscript that has involved human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

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All listed authors meet the ICMJE criteria.
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## CRediT authorship contribution statement

**Jerina Rugji:** carried out processing, experimental work, interpretation of results and manuscript writing and editing. **Ahmet Hulusi Dinçoğlu:** was in charge of the conceptualization, Methodology, development, Supervision, and reviewing and editing of the manuscript.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Acknowledgements

This work was financially supported by Burdur Mehmet Akif Ersoy University Scientific Research Projects Coordinator (0677-DR-20). The

authors gratefully acknowledge Kerry Inc., Beloit, WI 53511,USA for their support. We would like to thank Catherine Tuttle Korona for her contribution in the reviewing of the manuscript.

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