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Original article

Utilisation of *Lactococcus lactis* subsp. *lactis* (PY91K) and *Lactiplantibacillus plantarum* (Y48) to control *Clostridium sporogenes* during Turkish white cheese production and storage

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Summary This study investigated the inhibition of *Clostridium* species, isolated from cheeses with late-blowing defects, by lactic acid bacteria (LAB). Four batches of Turkish white cheeses were produced; control cheese, cheese sample containing *Lactococcus lactis* subsp. *lactis* (PY91K) and *Lactiplantibacillus plantarum* (Y48) as protective cultures (LAB), a cheese sample containing the protective culture and inoculated with *Clostridium sporogenes* spores (CL-LAB) and finally a cheese sample containing only *Cl. sporogenes* spores (CL). White cheese's microbiological and physicochemical properties were also determined throughout the 90-day storage period at 4 °C. Approximately 1 log reduction in *Cl. sporogenes* (ZMP-1-4) spores was observed in the LAB co-inoculated Turkish white cheese samples.

Keywords Biocontrol and *Clostridium sporogenes*, *Lactiplantibacillus plantarum*, *Lactococcus lactis* subsp. *lactis*, Turkish white cheese.

Introduction

White cheese is one of the most important traditional fermented milk products (Öner *et al*., 2006), as it is the most preferred type of cheese produced in Turkey, suitable for every taste. Traditional methodologies produce traditional cheeses at dairy farms using milk from cows, sheep and goats, either separately or in combination. On a small scale of production, raw milk or milk with low heat treatment is used without the addition of starter cultures (Hayaloglu., 2017). Turkish Food Codex Notification (No. 2015/6) on cheese states that Turkish white cheese can be either fresh or matured and is made by coagulating milk with rennet (Turkish Food Codex, 2015). It is brine-ripened for 3 months or longer (Özer *et al*., 2011). Late blowing is one of the most common microbiological problems in cheese production and causes serious economic losses (Silvetti *et al*., 2018). In a study conducted on 50 different cheeses in Turkey, it was reported that 74% of the cheeses contained *Cl. tyrobutyricum*, 50% contained *Cl. butyricum* and 16% contained *Cl. sporogenes* (Şahiner *et al*., 2023). One of the most serious and common defects in hard or semi-hard cheeses is late-blowing failure caused by butyric acid fermentation, which is considered the main cause of late-blowing defects in *Clostridium* spp. cheeses (Garde *et al*., 2016) and can lead to serious safety and financial problems (Lopez-Brea *et al*., 2017). The source of *Clostridium* and its spores in cheese production is mainly raw milk and inadequate hygiene conditions in the production environment (Oliveira *et al*., 2016). Compared to vegetative cells, *Clostridium* spores can withstand far higher temperatures, drying, irradiation and chemicals (Setlow, 2016; Talukdar *et al*., 2017). Under appropriate conditions, the spores turn vegetative and produce gas as a result of butyric acid fermentation, causing cracks and crevices in the cheese matrix and large porous structure, followed by blowing and the emergence of an undesirable odour and aroma (D'Incecco *et al*., 2018). Once they regain access to nutrients, the endospores become metabolically active again and a vegetative cell is formed after germination (Brändle *et al*., 2016; Burtscher *et al*., 2020). Semi-hard and hard cheese varieties have a pH value of 5.2–5.3 and *Cl. butyricum*, *Cl. beijerinckii*, or *Cl. sporogenes* show optimum growth and reproduction at a pH value of 6.5–7.0. These bacteria are known to cause late blowing (Dolenčić Špehar *et al*., 2020). In Turkey, late blowing is observed in ripened kashar and Turkish white cheese. On the other hand, very little information is

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known in the scientific literature about the structural changes that Turkish white cheeses undergo during the ripening process and almost no information is known about late blowing. It is known that potentially pathogenic bacteria are reduced during the ripening period of cheese (Choi *et al.*, 2020), but late-blowing defects may be observed due to the presence of *Clostridium* species.

Lactic acid bacteria (LAB) can inhibit the growth of spoilage or pathogenic microorganisms in the food environment during the fermentation stage as a result of the antimicrobial compounds they produce (Ibrahim *et al.*, 2021). To pass through the target cell wall, nisin generally makes hydrophobic or electrostatic interactions with anionic components (teichoic acid, teicuronic acid, lipoteichoic acid, acidic polysaccharides or phospholipids) in the cell wall (Kramer *et al.*, 2008). As a result of the change in the cell wall structure, damaged cells cannot produce energy and macromolecules necessary for life and death occurs (Punyauppa-path *et al.*, 2015; Zheng *et al.*, 2022). The use of LAB in the manufacturing of fermented foods and beverages has a long and safe history (Farnworth, 2008). LABs are known to have great potential to control microbial spoilage by using them as a bioprotectant (Chittora *et al.*, 2022). In cheese production, bacteriocins are used as an alternative to non-toxic preservatives and chemical preservatives (Choyam *et al.*, 2019). The choice of bacteriocin-producing starter cultures is known to be more advantageous than conventional preservatives (Dolenčić Špehar *et al.*, 2020). It has been reported that the problem of late blowing caused by *Clostridium* species can be controlled by microfiltration, bactofugation, thermal inactivation, addition of nitrate or lysozyme and LAB commonly applied to milk (Havlíková *et al.*, 2018; Mirkovic *et al.*, 2020; Carvalho *et al.*, 2021). The antagonistic effect of bacteriocin-producing LABs on *Clostridium* species has been the subject of previous scientific studies (González & Zárate, 2015; Ávila *et al.*, 2020). From these perspectives, in this study, *Lc. lactis* subsp. *lactis* (PY91K) and *Lb. plantarum* (Y48) that were previously shown to have an anti-clostridial activity (Demirbaş *et al.*, 2022) were tested as bioprotective cultures to control *Cl. sporogenes* (ZMP-1-4) in a white cheese environment during a 3-month storage period. Both the physicochemical and microbiological characteristics of Turkish white cheese were also determined following the bioprotective culture application. In this study, it was aimed to produce white cheese with lactic acid bacteria whose anti-clostridial, antimicrobial and bacteriocin activities were determined, to examine the physicochemical properties of the cheeses during storage and to determine the logarithmic decrease in the number of *Cl. sporogenes*.

Material and methods

Bacterial strains and culture conditions

Clostridium sporogenes strain ZMP-1-4 was previously isolated from cheese samples with late-blowing defects and used in this study (Demirbaş *et al.*, 2022). The LAB used in this study, *Lc. lactis* subsp. *lactis* (PY91K) and *Lb. plantarum* (Y48), were previously isolated from traditional Turkish white cheese and Turkish yogurt (İspirli *et al.*, 2017; İspirli & Dertli, 2018) and were shown to have anti-clostridial activity (Demirbaş *et al.*, 2022). For the cultivation of microbial species, *Lb. plantarum* (Y48), *Lc. lactis* subsp. *lactis* (PY91K) and *Cl. sporogenes* (ZMP-1-4) were grown in De Man Rogosa Sharpe medium (MRS, Oxoid), LM17 medium (M17, oxoid) and Bryant Burkey Broth at 37 °C under anaerobic conditions, respectively.

Physicochemical features of raw milk

As previously mentioned by (Horwitz & Latimer, 2000), raw cow's milk pH values, ash contents, total solids (%w/w), titratable acidity and fat contents values were measured. Every measurement was applied in duplicate.

Production of Turkish white cheese and application of bio pre-LAB strains

Turkish white cheese production was carried out at Yıldız Technical University, Department of Food Engineering, Dairy Technology Laboratory. Full-fat raw cow's milk for cheese production was provided from Bakır Helke in Istanbul dairy farm and pasteurised at 65 °C for 30 min, then cooled to 37 °C for coagulation. *Lc. lactis* subps. *lactis* and *Lb. plantarum* tested for Turkish white cheese production were inoculated into milk at 37 °C at a level of 10^8 cfu·g^{-1} and *Cl. sporogenes* spores (ZMP-1-4) were inoculated into milk at 35 °C at 10^4 cfu·g^{-1}. The pH value was allowed to rest for 15–20 min to reach a pH of 6.4. By adding a double-strength microbial-derived rennet from Chr. Hansen (Denmark) and 0.02% $CaCl_2$, coagulation started 90 min after. After allowing the coagulum to rest for half an hour, the curd was cut into 1 cm cubes, followed by the pressing of the curd and then roughly 7 × 7 × 7 cm blocks were prepared using a wire knife. The curd was then pressed for 4 h and placed in a 16% NaCl brine solution at 25 °C for approximately 12 h. They each weighed about 100 g. Cheese blocks were individually shrink-wrapped in watertight bags and kept in the fridge (4 °C) until further analysis. The cheese samples were then examined on the 0th, 30th, 60th and 90th days of the ripening

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period. The four production batches of cheeses were coded as follows; C: Control cheese produced only using commercial enzyme, LAB: *Lc. lactis* subsp. *lactis* (PY91K) and *Lb. plantarum* (Y48) inoculated cheese sample; CL-LAB: *Cl. sporogenes* spores (ZMP-1-4) containing in addition to *Lc. lactis* subsp. *lactis* (PY91K) and *Lb. plantarum* (Y48) inoculation, CL: *Cl. sporogenes* spores (ZMP-1-4) containing control cheese. Figure 1 shows the cheese vessels and the White cheese samples produced during different applications.

Enumeration of bacteria in Turkish white cheeses

Ten grams of four distinct cheese samples was weighed, combined with a 2% Trisodium citrate-dihydrate solution (1:10 (w/v)); put in a bag mixer and homogenised for 5 min using a Stomacher (VWR Star Blender LB 400, Germany) for microbiological investigations. By using the sterile ringer solution (Merck, 115525, Germany), decimal dilutions were made. Total bacterial count, total anaerobic bacterial spores, *Lactobacilli*, *Lactococci*, yeasts and moulds were all observed during storage in all Turkish white cheese samples. Total mesophilic aerobic bacteria (TMAB) were plated on Plate Count Skim Milk Agar (PCSA, Merck, 155338, Germany) and cultured for 3 days at 30 °C (Hickey *et al*., 2013). Yeasts and moulds were counted, according to ISO 6611: (2004) on Potato Dextrose Agar with 10% tartaric acid (PDA, Merck, 110130, Germany) at 25 °C for 5 days (ISO, 2004). After vegetative forms were inhibited by heat treatment, total anaerobic bacterial spores were determined on RCM agar plates (Merck, 105411, Germany) which were incubated in anaerobiosis for 3 days at 37 °C (Carminati *et al*., 2001). For LAB strains, *Lactobacilli* were determined on MRS agar (ISO, 1998) whereas *Lactococci* were determined on LM17 agar (Merck, 115108, Germany) at 37 °C for 2 days of incubation (Dal Bello *et al*., 2012). Turkish white cheeses were subjected to microbiological investigations on days 0^{th}, 30^{th}, 60^{th} and 90^{th} of storage. Two duplicates of each microbiological count were conducted. After incubation, colonies were counted and the results are given as log cfu·g^{-1}.



Figure 1 Cheese vessels and the White cheese samples produced during different applications.

The physicochemical properties of Turkish white cheeses

The cheeses were analysed for titratable acidity (expressed as lactic acid), pH, dry matter (%), water activity (a_w) and colour measurements. Mettler Toledo (sevencompact S220, Spain) digital pH meter was used to measure the pH (Savello *et al*., 1989). The 10-gram samples of cheese were homogenised in 105 mL of deionised water at 40 °C and filtered. To determine the titratable acidity (%), 25 mL of filtrate was mixed with two drops of phenolphthalein indicator and the samples were titrated with 0.1 N NaOH until the mixture turned pinkish colour (Kurt *et al*., 2007). For dry matter, 5 g of cheese was utilised and samples were dried in an incubator set to 105 °C for dry matter (%) (Kurt *et al*., 2007). The water activity (a_w) of the cheeses was determined by a water activity analyser (Novasina, Labtouch-a_w, Switzerland) during storage (Havlíková *et al*., 2018). CR-400 colorimeter (Konica Minolta Inc., Japan) was used for colour measurements of cheese samples. The results are expressed as L^*, a^* and b^*. The values of L^*, a^* and b^* are represented as darkness/brightness (100-white), red (+a)/green (−a) and yellow (+b)/blue (−b), respectively (Gómez-Torres *et al*., 2014).

Statistical analysis

The replicate mean either the standard deviation plus or minus (±) was used to represent all data. JMP statistical software was used to carry out statistical analysis. An analysis of variance conducted in one way revealed statistically significant differences (ANOVA) ($P \leq 0.05$).

Result and discussion

Physicochemical properties of raw milk used in Turkish white cheese production

The physicochemical properties of the milk used in white cheese production are shown in Table 1. The calculations of the analyses applied to raw cow's milk had a similar composition to cow's milk and were very close to the values given in the Turkish Food Codex and were found to be within acceptable ranges. It was determined that the pH value of raw milk is 6.66, titratable acidity is 0.16 (%LA), dry matter is 11.16%

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Table 1 Some physico-chemical properties of raw milk used in production

Chemical properties	Average ± SD
pH values	6.67 ± 0.01
Titratable acidity values (LA%)	0.16 ± 0.02
Total solid (% w/w)	11.16 ± 0.77
Fat contents (%100)	3.59 ± 0.01
Ash contents	0.73 ± 0.35

Mean ± standard deviation.

and fat content is 3.59% and the results are similar to the study (Altın, 2022).

Microbiological characteristics of Turkish white cheese during storage

The Microbial counts determined in Turkish white cheeses for 90 days are given in Table 2.

Enumeration of Lactobacilli

It was determined that the initial *Lactobacilli* count in Turkish white cheese samples stored at 4 °C was 8 log cfu·g^{-1} in the LAB and CL-LAB added Turkish white cheese samples whereas in control and CL added Turkish white cheese samples, the initial *Lactobacilli* count was 7 log cfu·g^{-1}. There was no decrease in the number of *Lactobacilli* during storage ($P > 0.05$). However, it was noted that there was no statistically significant variation ($P > 0.05$) between the samples in the LAB and CL-LAB added cheese groups until the 60th day of storage analysis. Within each cheese group, the results were similar ($P < 0.05$). *Lactobacilli* count increased to 9.2 log cfu·g^{-1} at 90 days in the LAB groups. The results were consistent with previous studies on cheddar cheese and Turkish white cheese (Öner *et al*., 2006; Lazárková *et al*., 2021).

Table 2 Changes in microbial count of Turkish white cheese samples during storage (log cfu·g^{-1})

Storage time (days)	Samples	*Lactobacilli*	*Lactococci*	Total aerobic mesophilic bacteria (TAMB)
0.	CONTROL	7.4 ± 0.15^{Cb}	8.5 ± 0.07^{Ab}	8.4 ± 0.09^{Ab}
	LAB	8.6 ± 0.09^{Ba}	9.3 ± 0.05^{Aa}	9.3 ± 0.02^{Aa}
	CL-LAB	8.8 ± 0.06^{Aa}	9.2 ± 0.01^{Aa}	9.0 ± 0.15^{Aa}
	CL	7.6 ± 0.08^{Ab}	7.7 ± 0.01^{Cc}	8.5 ± 0.04^{Ab}
30.	CONTROL	7.7 ± 0.02^{BCb}	8.3 ± 0.00^{Bc}	8.4 ± 0.09^{Ab}
	LAB	8.6 ± 0.03^{Ba}	9.3 ± 0.08^{Aa}	9.3 ± 0.04^{ABa}
	CL-LAB	8.7 ± 0.05^{Aa}	8.9 ± 0.05^{Cb}	8.9 ± 0.31^{Aab}
	CL	7.4 ± 0.02^{Ac}	8.2 ± 0.05^{Ac}	8.6 ± 0.02^{Ab}
60.	CONTROL	8.0 ± 0.04^{Ab}	8.0 ± 0.02^{Cb}	8.3 ± 0.15^{Ab}
	LAB	8.8 ± 0.01^{Ba}	9.3 ± 0.01^{Aa}	9.1 ± 0.05^{BCa}
	CL-LAB	8.8 ± 0.00^{Aa}	9.3 ± 0.02^{Aa}	9.1 ± 0.11^{Aa}
	CL	7.4 ± 0.03^{Ac}	8.1 ± 0.13^{ABb}	8.5 ± 0.00^{Ab}
90.	CONTROL	7.7 ± 0.01^{ABc}	8.3 ± 0.02^{Bb}	8.6 ± 0.05^{Ab}
	LAB	9.2 ± 0.02^{Aa}	9.0 ± 0.07^{Ba}	9.0 ± 0.08^{Ca}
	CL-LAB	8.7 ± 0.00^{Ab}	9.0 ± 0.03^{Ba}	9.2 ± 0.06^{Aa}
	CL	7.4 ± 0.15^{Ad}	7.9 ± 0.03^{BCc}	8.5 ± 0.03^{Ab}

Microorganisms in cheeses during ripening: mean counts ±standard deviation (log cfu·g^{-1}). Control (without starter/protective culture), LAB: Lactic Acid Bacteria *Lc. lactis* subsp. *lactis* (PY91K) and *Lb. plantarum* (Y48), CL-LAB: *Cl. sporogenes* spores (ZMP-1-4) and Lactic Acid Bacteria *Lc. lactis* subsp. *lactis* (PY91K) and *Lb. plantarum* (Y48), CL: With the addition of *Cl. sporogenes* spores (ZMP-1-4). A statistical difference in the same product depending on storage is shown by means marked with various uppercase letters and a statistical difference between the items on the same day is indicated by means denoted with different lowercase letters. ($P < 0.05$).

Enumeration of Lactococci

The initial *Lactococci* count in Turkish white cheese samples supplemented with LAB and CL-LAB was 9 log cfu·g^{-1}. The *Lactococci* counts in the cheese groups that had LAB added did not significantly drop over storage ($P > 0.05$). However, the initial number of *Lactococcal* bacteria in control Turkish white cheese samples was 8 log cfu·g^{-1} ($P < 0.05$). The initial number of *Lactococci* bacteria was 7 log cfu·g^{-1} in Turkish white cheese samples with CL addition ($P < 0.05$). During storage, the results of each cheese groups were similar within their groups. However, higher starter *Lactococci* viability was observed in CL-LAB inoculated cheeses than in control cheeses at day 30 of storage ($P < 0.05$). This viability indicates that the metabolism of *Cl. sporogenes* strains in cheese has a positive effect and creates a favourable environment for *Lactococcal* growth. Some other factors may also affect viability. *Lactococci* shift their metabolism from glycolysis to amino acid catabolism in response to energy depletion and the release of carbohydrates and this additional energy source as the released carbohydrates potentially boosts the survivability of *Lc. lactis* (Stuart *et al*., 1999). The results were consistent with previous studies on Turkish white cheese (Öner *et al*., 2006). Ávila *et al*. (2020), Havlíková *et al*. (2018), Gómez-Torres *et al*. (2014) and González & Zárate (2015), found that *Lactococci* numbers in their studies were maximum 9.3 log cfu·g^{-1} and minimum 8.85 log cfu·g^{-1}. In our study, the maximum 9.3 log cfu·g^{-1} and minimum 9.0 log cfu·g^{-1} value in the LAB-added cheese group is similar to the data in other studies.

Enumeration of total aerobic mesophilic bacteria (TAMB). The count of total aerobic mesophilic bacteria during storage was calculated as 8.4–9.3 log cfu·g^{-1}. Although there was a change in the viable count during storage in the cheese groups, no change was observed in the *Clostridium*-added cheese groups ($P \leq 0.05$). Kahraman-Ilıkkan & Bağdat (2022)

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worked with 15 unpackaged Turkish white kinds of cheese and reported that the number of TAMB in their Turkish white cheeses was a minimum of 4.60 log cfu·g^{-1} and a maximum of 8.67 log cfu·g^{-1}. The numbers of TAMB in the control groups and CL-added cheese groups were similar to the study of (Kahraman-Ilıkkan & Bağdat, 2022). It can be speculated that the higher number of TAMBs in the LAB-added and CL-LAB-added cheese groups than in the other two groups could be due to the addition of the preservative culture *Lc. lactis* subsp. *lactis* (PY91K) and *Lb. plantarum* (Y48).

Enumeration of anaerobic spores

Cheeses produced under controlled conditions were contaminated with *Cl. sporogenes* spores (ZMP-1-4). The number of *Cl. sporogenes* spores (ZMP-1-4) was monitored during storage. Figure 2 shows the microbial change in *Cl. sporogenes* spores (ZMP-1-4) count during storage at 4 °C. *Clostridium* spores were not detected in the control and *Lc. lactis* subsp. *lactis* and *Lb. plantarum* supplemented cheese groups during 90 days of storage at 4 °C. The fact that the spore formation in the cheese samples containing the selected protective cultures decreased from the 30th day onwards supports that the selected cultures have an anti-clostridial effect ($P < 0.05$). *Lc. lactis* subsp. *lactis* (PY91K) and *Lb. plantarum* (Y48) treatment resulted in a 1 log cfu·g^{-1} reduction in *Cl. sporogenes* spores (ZMP-1-4) during 90 days of storage at 4 °C ($P < 0.05$). During storage, there was no discernible shift in the quantity of spores in the cheese groups with CL added ($P < 0.05$). During the storage period, a maximum of 6.40 log cfu·g^{-1} and a minimum of 4.08 log cfu·g^{-1} clostridium spores were detected in the control group, while a maximum of 4.54 log cfu·g^{-1} and minimum of 4.05 log cfu·g^{-1} were detected in the experimental group. In the study conducted by adding *Lb. plantarum* in cheese making, *Cl. sporogenes* spore counts were similar (González & Zárate, 2015).

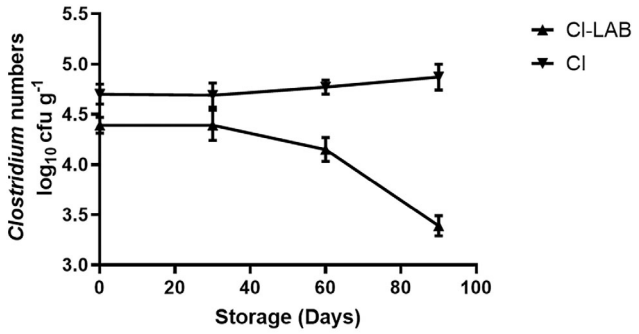


Figure 2 Changes in *Cl. sporogenes* spores (ZMP-1-4) numbers during 4 °C storage of Turkish white cheese.

In our study, a decrease of approximately 1 log was detected in the Lab-added cheese group.

Enumeration of mould and yeast

In the study conducted by Lazárková *et al.* (2021), yeast and mould were not detected in cheeses during 6 months of storage, these results were consistent with the results of our study. According to Mani-López *et al.* (2018), LAB are capable of producing a wide range of antimicrobial substances that can stop the presence of certain yeasts and moulds and examples of these substances are bacteriocins, reuterin, hydrogen peroxide (H_2O_2), organic acids, diacetyl and ethanol.

Physicochemical properties of Turkish white cheese samples

The physicochemical characteristics of the Turkish white cheeses during the ripening period are shown in Table 3.

Alteration of pH for different applications

The pH change of Turkish white cheese during storage was between 4.29 and 6.92. The pH values of Turkish white cheese samples were analysed and it was determined that all samples changed during storage ($P < 0.05$). Due to the higher initial pH compared to the control group cheeses, the effect of LAB, CL-LAB and CL-added cheeses and the effect of NSLAB (Non-Starter Lactic Acid Bacteria) on lactose, a rapid decrease was observed in the control cheese groups during storage. The pH differences between the cheese groups caused significant differences in the cheese made in the LAB-added and control cheese groups. The pH values of the control groups were similar to the Turkish white cheese produced without starters in the study (Hayaloglu *et al.*, 2005). Matijašić *et al.* (2007) reported that the high pH values in cheeses were due to the metabolic activity of *Cl. tyrobutyricum*. *Cl. sporogenes* spores (ZMP-1-4) can explain the increase in pH value in cheeses with added spores. Ávila *et al.* (2020) and Gómez-Torres *et al.* (2014) found similar pH values for the cheese made with *Cl. tyrobutyricum* spores in their study. González & Zárate (2015) examined *Lb. plantarum* against *Cl. sporogenes* and obtained similar results with the pH values of spore-added cheeses.

Alteration of TTA for different applications

The %TTA of Turkish white cheeses during storage was between 0.41% and 2.10%. The %TTA values of the LAB-added cheese groups were similar to the results of Hayaloglu *et al.* (2005) in which Turkish white cheese was produced with the addition of *Lc. lactis* ssp. *lactis* NCDO763 and *Lc. lactis* ssp. *cremoris*. It was determined that %TTA values did not

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Table 3 The physicochemical characteristics of Turkish white cheese during 90 days of Ripening

Storage time	Samples	pH	TTA (%)	a_w	Dry matter (%)
0.	CONTROL	6.92 ± 0.00^{a}	0.70 ± 0.02^{c}	0.87 ± 0.00^{a}	58.52 ± 1.16^{Ab}
	LAB	4.96 ± 0.00^{d}	2.10 ± 0.02^{a}	0.87 ± 0.00^{a}	61.82 ± 1.76^{a}
	CL-LAB	6.07 ± 0.01^{c}	0.83 ± 0.00^{b}	0.88 ± 0.01^{a}	55.19 ± 1.14^{b}
	CL	6.54 ± 0.01^{b}	0.59 ± 0.02^{d}	0.86 ± 0.01^{a}	56.97 ± 0.09^{b}
30.	CONTROL	5.51 ± 0.00^{b}	0.81 ± 0.13^{Ab}	0.88 ± 0.00^{a}	59.83 ± 1.20^{a}
	LAB	4.40 ± 0.01^{d}	1.99 ± 0.13^{Ab}	0.88 ± 0.00^{a}	57.15 ± 3.23^{a}
	CL-LAB	5.45 ± 0.02^{c}	1.17 ± 0.38^{a}	0.87 ± 0.01^{a}	53.97 ± 1.18^{a}
	CL	5.60 ± 0.00^{a}	0.41 ± 0.06^{b}	0.88 ± 0.01^{a}	58.00 ± 0.19^{a}
60.	CONTROL	5.56 ± 0.00^{a}	0.77 ± 0.02^{b}	0.91 ± 0.00^{a}	58.57 ± 0.35^{b}
	LAB	4.51 ± 0.00^{b}	2.01 ± 0.04^{a}	0.88 ± 0.00^{b}	55.74 ± 0.45^{c}
	CL-LAB	5.41 ± 0.00^{c}	0.97 ± 0.04^{a}	0.88 ± 0.01^{b}	56.19 ± 0.09^{c}
	CL	5.41 ± 0.01^{c}	0.97 ± 0.00^{a}	0.88 ± 0.01^{b}	60.68 ± 0.39^{a}
90.	CONTROL	5.43 ± 0.01^{a}	0.74 ± 0.02^{c}	0.88 ± 0.00^{a}	60.25 ± 0.57^{a}
	LAB	4.29 ± 0.00^{b}	2.06 ± 0.05^{b}	0.88 ± 0.00^{a}	51.49 ± 0.21^{c}
	CL-LAB	5.19 ± 0.00^{c}	1.37 ± 0.07^{a}	0.88 ± 0.00^{a}	54.88 ± 1.52^{c}
	CL	5.32 ± 0.00^{b}	1.13 ± 0.02^{b}	0.87 ± 0.00^{a}	58.22 ± 1.41^{a}

Mean ± standard deviation. Control (without starter/protective culture), LAB: lactic acid bacteria *Lc. lactis* subsp. *lactis* (PY91K) and *Lb. plantarum* (Y48), CL-LAB: *Cl. sporogenes* spores (ZMP-1-4) and Lactic Acid Bacteria *Lc. lactis* subsp. *lactis* (PY91K) and *Lb. plantarum* (Y48), CL: With the addition of *Cl. sporogenes* spores (ZMP-1-4). A statistical difference in the same product depending on storage is shown by means marked with various uppercase letters and a statistical difference between the items on the same day is indicated by means denoted with different lowercase letters. ($P < 0.05$).

change in regularity during storage ($P > 0.05$). In this respect %TTA on the first and last days, it was demonstrated that there was not a statistically meaningful difference between the samples ($P > 0.05$). In a study, it was determined that the conversion of lactose to lactic acid following fermentation during the ripening of soft cheeses was fast at first and then slowed down with the decrease in lactose (Topçu, 2004). Changes in titration acidity in storage analyses are predicted to be due to the formation of basic substances caused by proteolysis during storage and changes in the amount of dry matter. In other studies, %TTA values of fresh Turkish white cheeses were found to be between 0.82% and 1.31% (Gürsoy *et al.*, 2001). In mature Turkish white cheeses, these values were found to be between 0.86% and 2.40% (Öner *et al.*, 2006). This information shows that the %TTA values of the Turkish white cheeses produced in our study are similar to the results of other researchers.

Alteration of a_w for different applications

In general, low a_w values are known to inhibit microbial growth (Hickey *et al.*, 2013). Beuchat (1981) reported that most foodstuffs provide effective control for microbial growth when the water activity is below 0.6. In this study, the aw values observed in all four cheese groups (0.86–0.91) are in line with the values for this kind of fermented food published in the literature. However, the differences within each group were insignificant. The water activity values of Turkish white cheese samples were similar to each other ($P > 0.05$).

Dry matter content of Turkish white cheese samples

The % dry matter content of white cheese varied between 51.49% and 61.82%. Although the % dry matter content of the LAB-added white cheese groups decreased during storage, no important change was monitored in the % dry matter content of the other cheese samples during storage. However, the difference between the control, CL and CL-LAB-added samples were determined to be statistically negligible ($P > 0.05$). In the research conducted by Yerlikaya (2014), the highest moisture content of Turkish white cheese was determined to be 56.00%. The % dry matter amounts in our study are similar.

Colour values of Turkish white cheese samples during storage

Colour parameters are one of the important properties in food selection. L^*, a^* and b^* colour parameter values of cheeses are given in Table 4. L^* value gives information about the brightness of the cheeses. The brightness (L^*) values of the cheese samples varied between 81.42 and 89.87. It was determined that the brightness values of the cheeses with the addition of *Cl. sporogenes* (ZMP-1-4) and *Cl. sporogenes* (ZMP-1-4)- *Lc. lactis* subsp. *lactis* (PY91K) and *Lb. plantarum* (Y48) were statistically different ($P < 0.05$) compared to the control cheese groups. It was determined that

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Table 4 Colour features of cheese samples produced

Storage time	Samples	*L**	*a**	*b**
0.	CONTROL	89.25 ± 0.34^{Aa}	-2.20 ± 0.10^{Ab}	10.35 ± 0.39^{Da}
	LAB	89.87 ± 0.49^{Aa}	-2.01 ± 0.16^{Aab}	10.28 ± 0.63^{Ba}
	CL-LAB	87.46 ± 0.13^{Ab}	-1.94 ± 0.18^{Aa}	11.02 ± 0.40^{Ca}
	CL	87.58 ± 0.34^{Ab}	-2.03 ± 0.09^{Aab}	10.83 ± 0.21^{Ba}
30.	CONTROL	83.92 ± 0.84^{Ba}	-2.33 ± 0.09^{ABb}	13.52 ± 0.70^{Bbc}
	LAB	83.10 ± 0.56^{Ba}	-2.21 ± 0.14^{ABab}	13.95 ± 0.44^{Aab}
	CL-LAB	83.03 ± 0.90^{Ba}	-2.03 ± 0.17^{Aa}	14.77 ± 0.52^{Ba}
	CL	81.42 ± 1.55^{Cb}	-2.22 ± 0.15^{Aab}	12.98 ± 0.38^{Ac}
60.	CONTROL	82.10 ± 1.03^{Cb}	-2.58 ± 0.11^{Cbc}	14.71 ± 0.75^{Ab}
	LAB	83.01 ± 0.58^{Bab}	-2.42 ± 0.14^{Bb}	14.86 ± 0.61^{Ab}
	CL-LAB	83.13 ± 1.36^{Bab}	-2.78 ± 0.07^{Cc}	16.08 ± 0.06^{Aa}
	CL	84.17 ± 0.13^{Ba}	-2.21 ± 0.12^{Aa}	13.85 ± 0.30^{Ac}
90.	CONTROL	83.43 ± 0.41^{Ba}	-2.48 ± 0.13^{BCb}	12.47 ± 0.45^{Cc}
	LAB	82.61 ± 0.43^{Bb}	-1.98 ± 0.28^{Aa}	14.06 ± 0.54^{Aab}
	CL-LAB	82.88 ± 0.48^{Bab}	-2.38 ± 0.25^{Bab}	15.09 ± 0.53^{Ba}
	CL	82.65 ± 0.28^{Cb}	-2.18 ± 0.31^{Aab}	13.73 ± 1.12^{Abc}

Mean ± standard deviation. Control (without starter/protective culture), LAB: lactic acid bacteria *Lc. lactis* subsp. *lactis* (PY91K) and *Lb. plantarum* (Y48), CL-LAB: *Cl. sporogenes* spores (ZMP-1-4) and Lactic Acid Bacteria *Lc. lactis* subsp. *lactis* (PY91K) and *Lb. plantarum* (Y48), CL: With the addition of *Cl. sporogenes* spores (ZMP-1-4). A statistical difference in the same product depending on storage is shown by means marked with various uppercase letters and a statistical difference between the items on the same day is indicated by means denoted with different lowercase letters. ($P < 0.05$).

Cl. sporogenes (ZMP-1-4) addition decreased the brightness values of the cheese samples compared to the control and there was a statistically significant decline ($P < 0.05$). The yellowness (a^*) value indicates the redness value of the cheeses and varies between −1.94 and −2.78. Due to the increase in dry matter content during storage, the L^* and a^* parameters of the cheeses (except LAB added cheese) decreased numerically and b^* values increased numerically during cheese storage ($P < 0.05$). The redness (b^*) value indicates the yellowness value of the cheese samples and these values varied between 10.28 and 16.08. The b^* parameter for all cheeses displayed positive values in a yellow direction, whereas the a^* readings indicated negative values in a greenish direction. It was concluded that the increase in a^* value with storage in the LAB-added Turkish white cheese sample increased the greenish colour. The addition of *Cl. sporogenes* spores (ZMP-1-4) had an important effect on the yellowness values of the cheeses ($P < 0.05$). At the end of the 90-day storage period, the L^*, a^* and b^* values of the cheese samples differed and a statistically significant difference was found ($P < 0.05$). Additionally, a statistically significant difference was discovered ($P < 0.05$) between the samples in terms of L^*, a^* and b^* values on the first day of storage and at the end of storage. The colour characteristics were similar to those of Ávila *et al.* (2008), Gómez-Torres *et al.* (2014) and Garde *et al.* (2016). Regarding the b^* parameter, colour saturation and chroma (C), there was no discernible difference ($P > 0.05$) between the cheeses contaminated with *Cl. sporogenes* spores (ZMP-1-4) and the control groups. Since pores were formed by butyric acid fermentation in the *Clostridium* spores added cheese group, remarkable measurements were taken in colour measurements compared to other cheese groups.

Conclusion

In Turkey, white cheese is a highly popular choice among consumers, constituting around 60% of domestic cheese production. The spoilage of Turkish white cheese is a major concern. The highlight a concerning level of microbial contamination in white cheese produced in small dairy plants in Turkey, surpassing the acceptable limits set by the Turkish Food Codex. While there are many studies on various yeast species and pathogens causing spoilage in different types of white cheese, studies on *Clostridium* species in white cheese are limited.

In this study, an anti-clostridial challenge test was applied during Turkish white cheese production where *Lc. lactis* subsp. *lactis* (PY91K) and *Lb. plantarum* (Y48) as preservative cultures were inoculated at 10^8 cfu·g^{-1} level and *Cl. sporogenes* spores (ZMP-1-4) were added at 10^4 cfu·g^{-1} level. *Lc. lactis* subsp. *lactis* (PY91K) and *Lb. plantarum* (Y48) were found to be

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effective in the inhibition of *Cl. sporogenes* during the production and storage of Turkish white cheese. Additionally, the use of these cultures improved the physicochemical characteristics of Turkish white cheese suggesting their starter potential together with anti-clostridial role.

In this research *Clostridium* species contamination highlights the importance of monitoring and addressing spoilage issues in Turkish white cheese production. Constant monitoring and adherence to microbiological standards are crucial to ensure the safety and quality of white cheese available to consumers in Turkey, emphasising the importance of stringent quality control measures in dairy production processes. The results presented here have led to the first conclusions on the inhibition of *Clostridium* species in white cheese production and are thus expected to form the basis for further research.

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Author contributions

Fatma Nur Demirbaş: Conceptualization; investigation; methodology; validation; visualization; writing – original draft. **Muhammet Arıcı:** Investigation; funding acquisition; writing – review and editing; supervision. **Enes Dertli:** Conceptualization; writing – review and editing.

Ethical guidelines

Ethics approval was not required for this research.

Peer review

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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