The effect of using different starter culture combinations on ripening parameters, microbiological and sensory properties of Mihaliç cheese

Elif Özer¹ · Harun Kesenkaş¹

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Abstract The aim of this study was to determine the most appropriate culture combination and scalding temperature to provide standardization in the production of Mihaliç cheese. For this purpose, starter culture combinations consisting of *Propionibacterium freudenreichii*, *Streptococcus thermophilus*, *Lactobacillus helveticus* and *Leuconostoc mesentroides* subsp. *cremoris* were used. Two scalding temperatures (40 °C and 45 °C) were used for the Mihaliç cheese samples with same culture combinations. This study investigated the composition, acidity, proteolysis, lipolysis, microbial counts and sensory properties of Mihaliç cheese during 90 days of ripening. The lipolysis level was higher for cheese scalded at 40 °C. Eye formation, which is a characteristic feature of Mihaliç cheese, was seen in all cheese samples. The viability of the starters generally decreased significantly during ripening, and starter culture usage did not affect the characteristics of the cheeses adversely, which contrarily resulted in better sensory characteristics and increased ripening indexes in comparison to the control cheese. Especially the starter culture combination including *P. freudenreichii*, *S. thermophilus* and *L. helveticus* resulted in more desirable sensory characteristics.

Keywords Mihaliç cheese · Propionic acid · Starter culture · Cheese ripening

Introduction

In Turkey, there are approximately 150 cheese varieties. Kashar, White cheese and Tulum cheese are the most popular ones with economic value. However, other traditional cheeses are getting to become popular (Bulut Solak and Akın 2013). Mihaliç cheese, which has been produced approximately for the last 250 years, is considered to be one of the oldest traditional cheeses in Turkey. It is also known as Maglic, Mahlic or Kelle cheese. It is a semi-hard cheese with a pale cream, yellow or straw yellow colour (Aday and Karagül Yüceer 2014). Mihaliç is a rather salty cheese with roundish holes of 3–4 mm diameter gradually decreasing from the centre to the surface (Özcan and Kurdal 2012). Propionic acid bacteria constitute the main flora of Mihaliç cheese and the cheese has a unique flavour and characteristic 3–4 mm eyes due to propionic acid fermentation. Propionic acid bacteria ferment lactate, which causes release of CO₂, thus creating eye formation. Mihaliç cheese is mostly produced and consumed in the provinces of Bursa and Balıkesir in the Marmara Region of Turkey. Due to the production from raw milk and spontaneous fermentation without starter cultures, the native microflora of raw milk and natural contamination microorganisms are very important for specific properties of this cheese. In case of heat treatment applied to milk, starter cultures have to be used in cheese production for ensuring characteristic features. In cheese production, heat treatment reduces the diversity and level of raw milk microflora, and therefore, the biochemistry of ripening changes. Eventually, the desired flavour cannot be obtained. At this point, pasteurizing cheese milk, as well as using starter cultures, is a good alternative for obtaining quality. The use of commercial starters in cheese production decreases the probability of development of undesired microorganisms due to

✉ Elif Özer
elif.ozer@ege.edu.tr

Harun Kesenkaş
harun.kesenkash@ege.edu.tr

¹ Department of Dairy Technology, Faculty of Agriculture, Ege University, 35100 Izmir, Turkey

the indigenous flora of milk and ensures the production of a standardised product (Garcia et al. 2014). Starter bacteria that are encountered the most often are members of the genera *Lactococcus*, *Lactobacillus*, *Leuconostoc* and *Enterococcus*. Moreover, propionic acid bacteria are the characteristic microflora associated with Swiss-type cheeses. Mihaliç cheese undergoes propionic acid fermentation post-manufacture and the propionic and acetic acids that are produced contribute to the development of characteristic flavours of these cheeses (Beresford et al. 2001). Ripening has a major importance in cheese production. Cheeses have a specific pattern of proteolysis and are mainly characterised and classified using proteolytic data. Ripening index calculated using proteolytic data is used as an indicator of proteolysis in cheese (Hayaloğlu and Karabulut 2013). Cheese ripening is characterised by a series of microbiological and biochemical changes. Proteolysis is the most complex and maybe one of the most important primary biochemical events that occur during ripening of cheeses (McSweeney 2004). Proteolysis involves destabilisation of the casein matrix through the release of peptides and amino acids which have a direct effect on flavour and texture, where free amino acids are also substrates for a series of catabolic reactions that generate many important flavour compounds thiols, esters and others. Most of information available concerning the biochemical modifications that occur during ripening is related to proteolysis (Fox and McSweeney 1996; Grappin and Beuvier 1998). Moreover, lipolysis is one of the major events that contribute to flavour development with proteolysis in cheese. Hydrolysis of lipids is also used as a quality parameter for most types of matured/ripened cheeses. The level of lipolysis depends on the types of cheese (Hayaloğlu and Özer 2011). Extensive lipolysis is inevitable for the cheeses made from raw milk regarding indigenous lipase that is the main lipolytic agent in raw milk. However, lipolytic agents are also originated from rennet paste, starter, adjunct starter, non-starter bacteria and exogenous lipase.

In Turkey, the manufacture of Mihaliç cheese has not reached an industrial level yet. So, a standard product cannot be obtained even in the same plant because of variable manufacturing conditions as well as milk quality. Additionally, manufacturers who cannot afford the cost of production distribute Mihaliç cheeses on the market although the cheese has not fully ripened. Thus, the objective of this study was to obtain a standard and healthy product. For this purpose, the effect of three different starter culture combinations on the composition, proteolytic, lipolytic, microbiologic and sensory characteristics of pasteurized Mihaliç cheeses were investigated. Besides, there is limited information in the literature regarding the effects of different scalding temperatures on the ripening

parameters and microbiological properties of Mihaliç cheese. This study could also determine the effects of different scalding temperatures on the characteristics of Mihaliç cheese. This is necessary to suggest optimal starter culture combination and scalding temperature to produce industrial Mihaliç cheese while preserving the cheese's traditional characteristics.

Materials and methods

Starter cultures

Commercial starter culture containing *Streptococcus thermophilus* (ST-B01) and *Lactobacillus helveticus* (LH-B02) were obtained from Chr. Hansen Holding A/S (Hoersholm, Denmark). *Leuconostoc mesentroides* subsp. *cremoris* NRRL B-3252 was obtained from ARS culture collection (Washington, USA). *Propionibacterium freudenreichii* (Propionici-LI061) was obtained from CSL Centro Sperimentale del Latte, Italy.

Mihaliç cheese production

The traditional method was used for Mihaliç cheese production. Seven different cheeses including a control cheese were produced. Cheese milk was inoculated with three different culture combinations, and two different scalding temperatures were applied. The culture combinations and scalding temperatures that were used for cheese manufacturing are given in Table 1. Raw cow milk was used during the manufacture of the control cheese, whereas the milk was pasteurized for other cheeses, food-grade calcium chloride was added to the milk on a level of 20 g/100 L after cooling. The milk was inoculated with one of the cultures that were tested and then left for approximately 30 min. Liquid rennet was added to cheese milk by an amount sufficient to coagulate the milk within 60 min. Following the coagulation, the coagulum was cut into cubes (approximately 1 cm³). The whey/curd mixture was then scalded by pouring hot water at 70 °C until the mixture's temperature reached 40 °C (for HP40, StHP40, LeuSt40) or 80 °C hot water until the mixture's temperature reached 45 °C (for HP45, StHP45, LeuSt45) within about 30 min. After resting for 15 min, the curds were transferred into a cotton cloth for whey drainage for 2 h. During whey drainage, the cheese cloth was pierced periodically with a needle to accelerate whey-off. After moulding and pressing for 15 h, the Mihaliç cheese blocks were left in an initial brine of 12% for 6 days at 24 ± 1 °C until eye formation occurred, followed by 10 days in 15% brine at 6 °C. Following portioning (~ 400 g) and vacuum packing in polyethylene foils, the cheeses were ripened at

Table 1 Experimental design of Mihaliç cheese production

Cheese	Starter culture						Scalding temperature	
	ST-B01 (1%)	ST-B01 (0.5%)	LH-B02 (1%)	LH-B02 (0.5%)	NRRL B-3252 (0.5%)	Propionici (0.1%)	40 °C	45 °C
HP40			X			X	X	
HP45			X			X		X
StHP40	X			X		X	X	
StHP45	X			X		X		X
LeuSt40		X			X		X	
LeuSt45		X			X			X
Control								X

4 ± 1 °C for 3 months. Each production process was carried out in two replicates, and the cheeses were analysed on the 1st, 15th, 30th, 60th and 90th days of ripening.

Physicochemical analyses

The cheese samples were analysed for total solids (TS), fat and salt according to the method reported by Oysun (2001). Protein (total nitrogen × 6.38) was determined by the micro-Kjeldahl method. The pH values were measured by using a Hanna 210 pH meter. The ripening indexes regarding pH 4.6 soluble nitrogen (pH 4.6-SN) and nitrogen soluble in 12% trichloroacetic acid (TCA-SN) were estimated as percentage of total nitrogen (TN) to monitor the degree of proteolysis based on the method reported by Hayaloğlu et al. (2011). The acid degree value (ADV) was determined according to the method described by Renner (1993).

Microbiological analyses

For microbiological analyses, 10 g of cheese was added and homogenised in 90 mL of sterile Ringer's solution and subjected to serial dilutions. *Streptococcus thermophilus* counts were determined on ST Agar as described by Dave and Shah (1996). *Lactobacillus helveticus* was enumerated on MRS-Agar (pH 5.4) according to the method used by Valence et al. (2000). *Leuconostoc mesenteroides* subsp. *cremoris* was enumerated on MRS-Agar-containing vancomycin (30 µg/ml) (Güley 2008), and propionibacteria were enumerated on Yeast extract lactate agar (YELA) after anaerobic incubation at 30 °C for 7 days (Darılmaz 2010).

Sensory properties

For sensory properties, ten staff members of the Department of Dairy Technology evaluated the cheese samples by

a scoring test (1–9 points). Panellists assigned scores in partitioned booths equipped with daylight to each cheese sample for colour, regular eye formation, texture, salinity and taste.

Statistical analyses

The effects of ripening and differences between the samples were tested by ANOVA, and significance was indicated by $p < 0.05$, using SPSS 15.0 (SPSS Inc., Chicago, USA). The significant differences were compared by using Duncan's Multiple Range Test.

Result and discussion

Chemical composition

Compositions of experimental cheeses are given in Table 2. Regarding the total solid contents, there were no significant differences between the cheeses ($p > 0.05$). This may be attributed to the similar initial pH values of the cheese samples. This is because a significant decrease in pH level can increase the amount of dehydration from the casein matrix (Özcan and Kurdal 2012).

The differences between the fat contents of cheeses were significant and ranged from 21.50 to 23.25%. The fat levels detected in our cheeses were lower than the Mihaliç cheeses in the previous studies by Bulut Solak and Akın (2013) and Aday and Karagül Yüceer (2014). The differences between the protein contents of the Mihaliç cheese samples were also significant ($p < 0.05$). The difference in the levels of protein may have been caused by different starter cultures, enzyme activities, moisture or titratable acidity (Bulut Solak and Akın 2013). Likewise, Özcan and Kurdal (2012) reported that the protein content of Mihaliç cheese samples changed significantly depending on the addition of enzymes and starter culture.

Table 2 Gross composition of Mihaliç cheeses

	Samples						
	HP40	HP45	StHP40	StHP45	LeuSt40	LeuSt45	Control
Total solid (%)	56.40 ^A	56.71 ^A	57.64 ^A	58.69 ^A	56.87 ^A	58.61 ^A	58.50 ^A
Fat (%)	23.25 ^C	22.75 ^{BC}	21.50 ^A	22.50 ^{BC}	22.25 ^{AB}	23.00 ^{BC}	22.25 ^{AB}
Protein (%)	26.89 ^{AB}	27.16 ^{AB}	27.47 ^{AB}	28.16 ^{CD}	26.57 ^A	27.11 ^{AB}	26.73 ^{AB}
Salt (%)	6.73 ^D	5.61 ^{AB}	6.67 ^D	5.15 ^{AB}	5.85 ^C	5.09 ^A	5.68 ^B

^{A,B,C,D}Means in the same row with different superscripts among cheese samples significantly differ ($p < 0.05$)

Differences between the salt contents of the cheese samples were significant ($p < 0.05$). HP40 had the highest salt content (6.73%). The salt contents of the samples in our study were generally lower than those reported by some previous studies on Mihaliç cheese (Anar and Şen 1991; Öner and Aloğlu 2004). This may be attributed to ripening in vacuum packages instead of ripening in brine. Similarly, Hayaloğlu et al. (2012) reported significantly lower salt contents in vacuum-packaged Mihaliç cheeses than brine-salted samples.

The Mihaliç cheeses scalded at different temperatures (40–45 °C) were generally grouped together in terms of their fat and protein contents except for the StHP cheese batches, while StHP45 had higher levels of fat and protein than StHP40 ($p < 0.05$). However, all cheeses scalded at 40 °C had significantly higher salt content. Similarly, Sheehan et al. (2007) and Yun et al. (1993) reported that the cheeses that were scalded at low temperatures had lower protein but significantly higher salt contents.

Acidity and physicochemical properties

The cheeses' pH values, ripening index expressed by pH 4.6-SN (pH 4.6-SN, %TN) showing the level of primary proteolysis, ripening index expressed by 12% TCA-SN (12% TCA-SN, %TN) showing the depth of proteolysis and ADV values showing lipolysis rate are given in Table 3. Glycolysis of residual lactose and its constituent monosaccharides, which is one of the three primary biochemical events during the ripening process of cheeses has impact on the acidity of the cheeses (Anar and Şen 1991; Öner and Aloğlu 2004). Ripening had a significant effect on pH values except for the control cheese ($p < 0.05$). The lowest pH value was observed for StHP40 initially and for StHP45 at the end of ripening depending on the activity of the starter bacteria present in different culture combinations. A slight increase in pH at the end of ripening except the control may have been due to the formation of alkaline compounds. Similarly, Bulut Solak and Akin (2013) attributed the small increase in pH during ripening to

limited production of alkaline compounds. While the pH values of the cheese samples were initially close to each other, the differences were statistically significant on further days ($p < 0.05$).

The levels of pH 4.6-SN (%TN) increased constantly after 15th day of ripening ($p < 0.05$), while all cheese batches had their highest values at the end of the ripening period due to the increase in the pH 4.6-SN levels for all samples. Similar trends were also observed for Mihaliç cheese in a previous study (Öner and Aloğlu 2004). StHP40 had the highest initial pH 4.6-SN (%TN) value, followed by StHP45 with a level of 16.34 and 16.01%, respectively. At the end of ripening StHP40 had the highest ripening index value followed by StHP45, whereas the control cheese had the lowest values both initially and at the end of ripening ($p < 0.05$). These cheese batches had lower pH values that favour the action of chymosin on α s1-casein, resulting in rapid increases in soluble nitrogen. Lower pH may be more favourable for proteolytic activity of residual chymosin (Hayaloğlu et al. 2012; Sheehan et al. 2007). Besides, this may be related to the synergistic effect of the cultures on proteolytic potential. The degree of ripening in cheeses with the same starter cultures were generally similar although they were scalded at 40 °C or 45 °C except for LeuSt40–LeuSt45 on the 15th day of ripening. This may be attributed to the similar proteolytic activity of residual chymosin in cheese samples.

The levels of 12% TCA-SN increased significantly during ripening ($p < 0.05$), and the highest levels were determined at the end of ripening for all samples, so for the the degree of ripening. In a previous study, the level of total free amino acids in Mihaliç cheeses exhibited a similar trend with soluble nitrogen fractions including 12% TCA-SN (Hayaloğlu et al. 2012). The differences between the 12% TCA-SN (%TN) levels of our cheese samples were significant during ripening ($p < 0.05$). The highest degree of 12% TCA-SN (%TN) was determined in StHP45 initially and at the end of the ripening period, whereas the lowest values were determined for the control cheese at the end of ripening and for LeuSt45 initially. In previous

Table 3 Acidity and physicochemical properties of Mihaliç cheeses during ripening

Ripening days	Samples						
	HP40	HP45	StHP40	StHP45	LeuSt40	LeuSt45	Control
pH							
1	5.18 ± 0.00 ^{cA}	5.15 ± 0.00 ^{cA}	5.00 ± 0.00 ^{eA}	5.02 ± 0.00 ^{dA}	5.25 ± 0.00 ^{cA}	5.06 ± 0.00 ^{cA}	5.20 ± 0.00 ^{aA}
15	5.08 ± 0.00 ^{bD}	4.81 ± 0.01 ^{aC}	4.70 ± 0.00 ^{cA}	4.74 ± 0.00 ^{cB}	5.21 ± 0.01 ^{bE}	4.75 ± 0.00 ^{aB}	5.20 ± 0.00 ^{aE}
30	5.09 ± 0.01 ^{bE}	4.80 ± 0.00 ^{aD}	4.68 ± 0.00 ^{bB}	4.57 ± 0.02 ^{bA}	5.20 ± 0.00 ^{bF}	4.76 ± 0.01 ^{aC}	5.18 ± 0.00 ^{aF}
60	4.85 ± 0.00 ^{aD}	4.80 ± 0.00 ^{aC}	4.65 ± 0.00 ^{aB}	4.48 ± 0.03 ^{aA}	4.99 ± 0.01 ^{aE}	4.98 ± 0.00 ^{bE}	5.19 ± 0.00 ^{aF}
90	5.20 ± 0.01 ^{dD}	5.00 ± 0.00 ^{bC}	4.81 ± 0.01 ^{dB}	4.76 ± 0.00 ^{cA}	5.22 ± 0.00 ^{bE}	5.19 ± 0.01 ^{dD}	5.19 ± 0.00 ^{aD}
pH 4.6-SN (%/TN)							
1	11.07 ± 0.16 ^{aC}	11.22 ± 0.23 ^{abC}	16.34 ± 0.39 ^{aD}	16.01 ± 0.18 ^{aD}	10.74 ± 0.00 ^{aBC}	10.17 ± 0.43 ^{aB}	10.74 ± 0.00 ^{dA}
15	10.98 ± 0.33 ^{aC}	10.50 ± 0.38 ^{aC}	17.21 ± 0.13 ^{bD}	16.84 ± 0.16 ^{bD}	10.74 ± 0.07 ^{aC}	9.92 ± 0.22 ^{aB}	7.83 ± 0.08 ^{aA}
30	11.73 ± 0.14 ^{bBC}	12.06 ± 0.38 ^{bC}	16.73 ± 0.48 ^{abD}	16.78 ± 0.01 ^{bD}	11.08 ± 0.11 ^{aB}	11.27 ± 0.54 ^{bB}	9.75 ± 0.12 ^{bA}
60	14.53 ± 0.16 ^{cC}	14.77 ± 0.13 ^{cC}	18.94 ± 0.13 ^{cD}	19.07 ± 0.28 ^{cD}	12.09 ± 0.27 ^{bB}	12.76 ± 0.30 ^{cBC}	10.49 ± 0.08 ^{cA}
90	15.21 ± 0.08 ^{dC}	15.27 ± 0.43 ^{dC}	19.78 ± 0.14 ^{dD}	19.72 ± 0.19 ^{dD}	12.86 ± 0.27 ^{cB}	12.89 ± 0.17 ^{cB}	10.88 ± 0.31 ^{dA}
12% TCA-SN (%/TN)							
1	4.25 ± 0.21 ^{aB}	4.34 ± 0.22 ^{abC}	4.76 ± 0.03 ^{aCD}	4.97 ± 0.23 ^{aD}	2.00 ± 0.08 ^{aA}	1.81 ± 0.28 ^{aA}	2.11 ± 0.01 ^{aA}
15	7.06 ± 0.06 ^{bD}	7.00 ± 0.12 ^{bD}	6.48 ± 0.05 ^{bC}	6.29 ± 0.01 ^{bC}	3.98 ± 0.00 ^{bB}	4.04 ± 0.18 ^{bB}	3.72 ± 0.06 ^{bA}
30	7.25 ± 0.02 ^{bCD}	7.50 ± 0.27 ^{bCD}	6.49 ± 0.08 ^{bC}	6.34 ± 0.05 ^{bC}	4.67 ± 0.04 ^{cB}	4.35 ± 0.01 ^{bCB}	3.81 ± 0.21 ^{bA}
60	7.38 ± 0.06 ^{cC}	7.74 ± 0.18 ^{cDD}	7.83 ± 0.21 ^{cD}	7.97 ± 0.19 ^{dD}	4.81 ± 0.06 ^{dB}	4.65 ± 0.02 ^{cDB}	3.95 ± 0.06 ^{bCA}
90	7.81 ± 0.08 ^{dC}	8.11 ± 0.18 ^{dCD}	8.36 ± 0.01 ^{dD}	8.78 ± 0.35 ^{dE}	5.10 ± 0.02 ^{cB}	4.97 ± 0.16 ^{dB}	4.09 ± 0.06 ^{cA}
ADV (mg KOH/g-fat)							
1	2.37 ± 0.17 ^{aE}	1.84 ± 0.24 ^{aD}	1.29 ± 0.03 ^{aBC}	1.15 ± 0.20 ^{aABC}	0.90 ± 0.06 ^{aA}	1.07 ± 0.03 ^{bAB}	1.46 ± 0.00 ^{aC}
15	2.54 ± 0.03 ^{abF}	2.13 ± 0.08 ^{abE}	1.40 ± 0.04 ^{aD}	1.26 ± 0.00 ^{abC}	1.11 ± 0.04 ^{bB}	0.89 ± 0.04 ^{aA}	1.49 ± 0.00 ^{aD}
30	2.77 ± 0.11 ^{bE}	2.19 ± 0.04 ^{bD}	1.59 ± 0.01 ^{bC}	1.36 ± 0.01 ^{abB}	1.10 ± 0.05 ^{bA}	1.06 ± 0.05 ^{bA}	1.51 ± 0.00 ^{abC}
60	3.14 ± 0.04 ^{cF}	2.22 ± 0.05 ^{bE}	1.85 ± 0.11 ^{cD}	1.50 ± 0.05 ^{bcB}	1.19 ± 0.07 ^{bA}	1.16 ± 0.00 ^{cA}	1.64 ± 0.00 ^{bC}
90	3.34 ± 0.04 ^{cE}	2.47 ± 0.11 ^{bD}	2.29 ± 0.06 ^{dD}	1.73 ± 0.12 ^{cB}	1.38 ± 0.07 ^{cA}	1.33 ± 0.02 ^{dA}	2.06 ± 0.12 ^{cC}

^{a,b,c,d}Means in the same column with different superscripts significantly differ ($p < 0.05$)

^{A,B,C,D}Means in the same row with different superscripts among cheese samples significantly differ ($p < 0.05$)

studies a large increase in the amino acid concentration in cheeses that were inoculated with starter bacteria was reported. This is related to the intracellular enzymes released by bacterial lysis (Kawabata et al. 1997; Kiernan et al. 2000).

The ripening period significantly affected the ADV values of all cheese samples ($p < 0.05$). While the ADV of the HP and StHP cheese batches showed a continuous increase, the ADV of LeuSt samples showed fluctuations during ripening. Additionally, all cheese samples had their highest ADV values at the end of the ripening period. HP40–HP45 had significantly higher ADV levels at any stage of ripening followed by StHP40–StHP45 due to the culture combination used for their manufacturing, especially propionic acid bacteria. Propionic acid bacteria, especially *P. freudenreichii*, had 10–100 times higher lipolytic activity than lactic acid bacteria (Chamba and Irlinger 2004). Among the cheeses manufactured with the same culture but at different scalding temperatures, the samples scalded at 40 °C had higher ADV values than the batches scalded at 45 °C in their ripening periods when they were placed in different groups according to Duncan's test. This may be due to the higher level of the curd-scalding process which inhibits the enzymes that come from the milk and the microorganisms. Therefore, lipolysis developed fairly slowly in the cheeses that were scalded at 45 °C.

Microbial composition

Changes in the populations of *S. thermophilus*, *L. helveticus*, *Leu. mesentroides* subsp. *cremoris* and *P. freudenreichii* during ripening are shown in Fig. 1. The viability values of all starters in the cheese samples were found significantly different from each other throughout the ripening period ($p < 0.05$). The counts of *S. thermophilus* decreased significantly as the ripening period progressed ($p < 0.05$). A significant decrease was reported for *S. thermophilus* in previous studies where it was used in addition to *L. helveticus* (Ong and Shah 2008) and those with adjunct *Lactobacillus* strains (Kocaoğlu-Vurma et al. 2008). Among the starters, the highest decline was noticed in the growth rate of *S. thermophilus* (8.64–7.89 log cfu/g). This decrease may be due to the unfavourable conditions in cheeses such as anaerobic atmosphere because of vacuum packaging, low water activity, low pH, low redox potential and high salt content (Ong and Shah 2008).

Viable counts of *L. helveticus* decreased constantly for HP45 and StHP40, while fluctuations were observed in the counts of *L. helveticus* for HP40 and StHP45. Additionally, all had their lowest counts at the end of ripening ($p < 0.05$). Porcellato et al. (2013) reported that, microbial counts of *Lactobacillus* and *Lactococcus* in cheese made

with added propionibacteria showed a large decrease because of high contents of propionic acid that have been correlated with the inhibition of other microorganisms.

A significant decrease was observed for *L. mesentroides* subsp. *cremoris* counts. It was reported in a previous study salt concentration did not seem to affect the growth of *Leuconostoc* strains up to a certain level. On the contrary, low pH seemed to exert a greater effect. Moreover, Liu et al. (1997) reported that low water activity in cheese reduce the activity of *L. mesentroides* subsp. *cremoris*.

Viable cell counts of *P. freudenreichii* showed a significant decrease during ripening. However, among the starters, the lowest decrease was noticed in *P. freudenreichii* counts. Bulut Solak and Akin (2013) reported a significant decrease in the counts of propionic acid bacteria during ripening for Mihaliç cheese due to the decline in water activity and the amount of nutrition elements, as well as acidity in the cheese. Moreover, Sheehan et al. (2008), reported that increased cheese pH was significantly correlated with increased counts of propionic acid bacteria. Porcellato et al. (2013) observed that viable cell counts of propionic acid bacteria for different cheese samples were between 7.0 and 9.4 log cfu/g.

Regarding the scalding temperatures, StHP45 had significantly higher cell counts of *S. thermophilus* than StHP40. HP45 had higher cell counts of *L. helveticus* than HP40, StHP45 had higher cell counts of *P. freudenreichii* than StHP40, and the cell counts of *P. freudenreichii* for the HP batches were initially the same. So, it may be thought that scalding at 45 °C did not show an inhibition effect on starter bacteria. The viable cell counts of *Leu. mesentroides* subsp. *cremoris* were higher for LeuSt45 than LeuSt40 throughout ripening, and similar results were found for the same cheese batches regarding the *S. thermophilus* counts. Sheehan et al. (2007) showed that increasing scalding temperature from 47 to 53 °C had no significant effect on the mean viable cell counts of *L. helveticus*, but it significantly reduced the growth rate of *S. thermophilus*.

Sensory evaluation

The sensory evaluation scores of cheeses in terms of colour, regular eye formation, texture, salinity and taste during 90 days of ripening are given in Table 4. Higher scores were obtained at the end of the ripening period than the scores obtained initially for all attributes of the Mihaliç cheeses except for LeuSt40 regarding to salinity. The ripening period had a statistically significant effect ($p < 0.05$) on colour scores except for HP40–HP45. Among the samples, the highest colour score was in HP45 at the beginning, both in HP45 and LeuSt40 at the end of ripening, whereas the control sample received the lowest

Fig. 1 Changes in **a** *S. thermophilus* counts (log cfu/g); **b** *L. helveticus* counts (log cfu/g); **c** *L. mesenteroides* subsp. *cremoris* counts (log cfu/g); **d** *P. freudenreichii* counts (log cfu/g) during ripening (n = 2)

scores on these days. The differences between the colour scores of the samples were significant on the 1st and 60th days of ripening ($p < 0.05$).

Characteristic eye formation produced by the action of propionibacteria has a critical importance because of consumer acceptability and visual appearance in Mihaliç cheeses (Özcan and Kurdal 2012). *Leuconostoc* creates an opening in cheese resulting from CO_2 production, and when cell concentrations are between 5×10^6 and 5×10^7 cells/mL, very high numbers of cells in cheese trials provide an excess of opening (Hemme and Foucaud-Scheunemann 2004). HP45 had the highest regular eye formation score initially, while LeuSt40 had the highest score at the end of ripening. The control cheese had the lowest scores in these periods. These scores were generally consistent with the texture scores. It was reported in previous studies that an elastic, pliable texture is crucial in the formation of regular eyes (Lucey et al. 2003). A recent study found a similar trend related to texture attributes (Özcan and Kurdal 2012). Alvarado Gamboa et al. (2013) also reported that the positive changes in terms of the texture attributes of cheeses manufactured by lactic culture

may be due to the extent of protein–protein interactions during the ripening process.

The salinity score of Mihaliç cheese was the highest in StHP40 initially, whereas it was the highest in LeuSt45 at the end of ripening. Generally, the chemically determined salt ratio is consistent with the salinity scores determined as a sensory attribute of Mihaliç cheeses. Among our cheeses, the ripening period had a significant effect in the scores of salinity only for LeuSt45. This may be attributed to vacuum packaging instead of packaging in brine due to the fact that more salt uptake occurs in cheeses stored in brine (Hayaloğlu et al. 2012). The highest taste scores were found in StHP45 initially. The panellists observed a nut-like, sweet flavour, due to either free fatty acids, peptides, amino acids or interactions between them, by the activity of the propionibacteria in StHP45 and StHP40. It was reported in previous studies that cheeses undergoing propionic acid fermentation have a characteristic nutty flavour (Beuvier et al. 1997; Thierry et al. 2004). It may be stated that the cheeses manufactured with the combination including *S. thermophilus*, *L. helveticus* and *P. freudenreichii* gained the highest acceptability from the panellists.

Table 4 Sensory evaluation of Mihaliç cheeses during ripening

Ripening days	Samples						
	HP40	HP45	StHP40	StHP45	LeuSt40	LeuSt45	Control
Colour							
1	6.17 ^{aAB}	7.38 ^{aC}	6.61 ^{aBC}	6.48 ^{aBC}	6.42 ^{aABC}	6.53 ^{BC}	5.43 ^{aA}
15	6.78 ^{aA}	7.31 ^{aA}	7.14 ^{abA}	7.53 ^{bcA}	6.87 ^{abA}	6.96 ^{abA}	7.27 ^{bA}
30	7.43 ^{aA}	7.57 ^{aA}	7.79 ^{cA}	7.29 ^{bA}	7.21 ^{bcA}	7.64 ^{bcA}	7.21 ^{bA}
60	7.40 ^{aB}	7.90 ^{aC}	8.40 ^{dC}	8.10 ^{cC}	7.00 ^{abAB}	8.10 ^{cC}	6.80 ^{bA}
90	7.41 ^{aA}	7.94 ^{aA}	7.56 ^{bcA}	7.63 ^{bcA}	7.94 ^{cA}	7.50 ^{bcA}	6.94 ^{bA}
Regular eye formation							
1	6.09 ^{aB}	6.59 ^{aB}	5.33 ^{aA}	6.14 ^{aB}	6.11 ^{aB}	6.06 ^{aB}	4.42 ^{aA}
15	6.36 ^{aA}	7.67 ^{aB}	6.73 ^{bA}	7.32 ^{cB}	6.74 ^{aA}	7.46 ^{bB}	6.25 ^{bA}
30	7.14 ^{aA}	7.14 ^{aA}	7.29 ^{bA}	6.86 ^{bcA}	6.79 ^{aA}	7.21 ^{bA}	6.50 ^{bA}
60	7.50 ^{aC}	7.40 ^{aC}	7.20 ^{bBC}	6.50 ^{abAB}	7.50 ^{aC}	7.40 ^{bC}	6.20 ^{bA}
90	7.31 ^{aA}	7.19 ^{aA}	7.31 ^{bA}	7.13 ^{bcA}	7.72 ^{aA}	7.03 ^{bA}	5.44 ^{bA}
Texture							
1	6.68 ^{abA}	7.14 ^{aA}	7.05 ^{aA}	6.82 ^{aA}	6.11 ^{aA}	6.31 ^{aA}	5.63 ^{aA}
15	6.96 ^{abA}	7.32 ^{aA}	7.07 ^{aA}	7.46 ^{aA}	7.08 ^{bcA}	6.92 ^{abA}	6.79 ^{aA}
30	6.29 ^{aA}	7.50 ^{aB}	7.71 ^{abB}	7.50 ^{aB}	6.57 ^{aA}	7.43 ^{bcB}	6.79 ^{aA}
60	7.50 ^{bA}	7.70 ^{aA}	7.50 ^{abA}	7.50 ^{aA}	7.40 ^{cA}	7.70 ^{cA}	6.80 ^{aA}
90	7.44 ^{bB}	7.88 ^{aB}	8.00 ^{bB}	7.75 ^{aB}	7.78 ^{cB}	7.63 ^{cB}	6.75 ^{aA}
Salinity							
1	5.05 ^{aA}	6.36 ^{aA}	6.89 ^{aA}	6.30 ^{aA}	6.72 ^{aA}	5.69 ^{aA}	5.64 ^{aA}
15	6.08 ^{aA}	6.13 ^{aA}	7.35 ^{aBC}	7.77 ^{aC}	6.54 ^{aAB}	7.19 ^{bBC}	6.47 ^{aAB}
30	5.64 ^{aA}	7.29 ^{aC}	7.57 ^{aC}	7.39 ^{aC}	5.71 ^{aA}	7.57 ^{bC}	6.29 ^{aB}
60	6.10 ^{aA}	6.50 ^{aAB}	6.70 ^{aABC}	7.20 ^{aBC}	6.10 ^{aA}	7.50 ^{bC}	6.60 ^{aABC}
90	6.44 ^{aAB}	6.69 ^{aABC}	7.69 ^{aBC}	7.75 ^{aBC}	6.72 ^{aABC}	8.00 ^{bC}	5.75 ^{aA}
Taste							
1	5.54 ^{aA}	6.33 ^{aA}	6.50 ^{aA}	6.56 ^{aA}	5.73 ^{aA}	5.79 ^{aA}	4.75 ^{aA}
15	6.36 ^{abAB}	7.04 ^{aBC}	6.65 ^{aAB}	7.63 ^{aC}	6.43 ^{aAB}	7.27 ^{bBC}	5.99 ^{cA}
30	6.36 ^{abA}	7.64 ^{aB}	7.75 ^{aB}	7.71 ^{aB}	6.14 ^{aA}	7.54 ^{bB}	5.75 ^{cA}
60	6.85 ^{bBC}	7.30 ^{aBC}	7.50 ^{aC}	7.30 ^{aBC}	6.60 ^{aB}	7.40 ^{bBC}	5.20 ^{bAB}
90	7.09 ^{bB}	7.25 ^{aBC}	8.09 ^{aC}	7.82 ^{aBC}	7.28 ^{aBC}	7.84 ^{bBC}	5.59 ^{bcA}

^{a,b,c,d}Means in the same column with different superscripts significantly differ ($p < 0.05$)

^{A,B,C,D}Means in the same row with different superscripts among cheese samples significantly differ ($p < 0.05$)

All cheese samples with starter cultures were preferred more than the control cheese was. It was reported in a previous study that eye formation in Mihaliç cheese occurred from the addition of starter culture which also produced the desired colour, appearance, taste and aroma (Özcan and Kurdal 2012).

Conclusion

The use of different starter culture combinations in the manufacture of Mihaliç cheeses influenced some quality parameters of the cheeses. According to the results, using starter cultures in the manufacture of Mihaliç cheese

provides contributions in terms of physical, chemical and sensory characteristics. The Mihaliç cheeses with starter culture combination had generally higher ripening indexes than control at any stage of ripening period. Proteolysis and lipolysis increased for all cheese samples throughout ripening. At the end of ripening all the cheeses had the highest ripening indexes and ADV values. The cheeses with *P. freudenreichii* had higher lipolysis levels than other cheeses owing to lipolytic characteristic of this starter bacteria. The starter cultures showed significant decrease during ripening although small fluctuations were observed. In terms of scalding temperature, 45 °C may be more favourable than 40 °C with regard to proteolysis, whereas 40 °C may be recommended for higher levels of lipolysis.

In terms of sensory properties all cheese samples with starter cultures were generally preferred more than the control cheese was. According to the results, accelerated ripening with higher sensorial acceptability may be obtained by the starter culture addition to the pasteurized milk, especially by the starter culture combination including *S. thermophilus*, *L. helveticus* and *P. freudenreichii*. It may be concluded that this study provided a new approach for the production of Mihaliç cheese.

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