Acta Alimentaria, Vol. 46 (4), pp. 492–500 (2017)
DOI: 10.1556/066.2017.46.4.12

THE EFFECT OF USING DIFFERENT STARTER CULTURE COMBINATIONS ON ORGANIC AND FATTY ACID COMPOSITIONS OF MIHALIÇ CHEESE

E. Özer and H. Kesenkaş*

Department of Dairy Technology, Faculty of Agriculture, Ege University, Bornova, TR-35100 Izmir. Turkey

(Received: 1 February 2017; accepted: 20 March 2017)

The objective of this study was to assess the influence of three different starter culture combinations and two scalding temperatures on the organic and fatty acid compositions of pasteurized Mihaliç cheeses. For this purpose, starter culture combinations consisting of *Propionibacterium freudenreichii*, *Streptococcus thermophilus*, *Lactobacillus helveticus*, and *Leuconostoc mesenteroides* subsp. *cremoris* were used. Two scalding temperatures, 40 °C or 45 °C, were used for cheeses with the same culture combination. Samples were evaluated in terms of organic and fatty acid compositions during 90 days of ripening. Eye formation, which is a characteristic feature of Mihaliç cheese, was seen in all cheese samples. Propionic and lactic acids were the most abundant organic acids detected in the cheeses. The most abundant saturated fatty acid was palmitic acid, followed by myristic and stearic acids. Oleic acid content was the highest among total unsaturated fatty acids. The control cheese had lower levels of short-chain fatty acids, which contribute directly to the cheese flavour.

Keywords: Mihaliç cheese, starter culture, organic acids, fatty acids

Mihaliç cheese, which has been produced for approximately 250 years, is considered one of the oldest traditional cheeses in Turkey. Mihaliç is a rather salty cheese with roundish holes and 3- to 4-mm diameter pores gradually decreasing from the centre to the surface (Özcan & Kurdal, 2012). The propionic acid bacteria constitute the main flora of the Mihaliç cheese and provide its characteristic eye formation and unique flavour. Due to the production from raw milk and spontaneous fermentation without starter cultures, the native microflora of raw milk and natural contaminating microorganisms are also very important for specific properties of Mihaliç cheese.

Cheese ripening is characterized by a series of microbiological and biochemical changes affecting the principal components and texture of the cheese (McSweeney, 2004). The flavour of a ripened cheese is connected with numerous chemical compounds, such as organic acids, sulphur compounds, lactones, methyl ketones, alcohols, and phenolic substances (Ong & Shah, 2008). Among them, organic acids are formed as a result of the hydrolysis of fatty acids (FAs), normal bovine metabolism processes, and microbial growth, or appear with the addition of acidulants during cheese making (Akalin et al., 2002; Andiç et al., 2011). Quantitative determination of organic acids is an important tool for studying flavour and nutritional quality and an indicator of microbial activity of ripened cheeses, as the total aroma intensity is correlated with organic acid levels in grated cheeses (Akalin et al., 2002). In addition, FAs may contribute to the savoury taste of cheese, and the latter are considered precursors of aroma compounds (Grappin & Beuvier, 1998). Furthermore, analyses of short-

* To whom correspondence should be addressed.
Phone: +90 232 311 16 39; fax: +90 232 342 57 13; e-mail: harun.kesenkas@ege.edu.tr

and medium-chain FA profiles has been suggested as an index for characterizing cheeses over the ripening period (Georgola et al., 2005).

The production from low-quality raw milk, insufficient heat treatment, ripening under unhygienic conditions, and misuse of starter culture cause quality defects and make it difficult to produce high-quality Mihaliç cheese. Furthermore, limited information exists regarding the effect of different scalding temperatures on the ripening parameters of Mihaliç cheese. Thus, the objective of this study was to assess the influence of three different starter culture combinations and two scalding temperatures on the organic acid content and FA composition of pasteurized Mihaliç cheeses. The results of this study will help to choose the best starter culture combination and scalding temperature to produce industrial Mihaliç cheese while maintaining its traditional characteristics.

1. Materials and methods

1.1. Starter cultures

Thermophilic starter cultures of *Streptococcus thermophilus* (ST-B01) and *Lactobacillus helveticus* (LH-B02) were obtained from Chr. Hansen (Hoersholm, Denmark) in freeze-dried form. *Leuconostoc mesenteroides* subsp. *cremoris* NRRL B-3252 was obtained from Agricultural Research Service (ARS) culture collection (Washington, USA). Propionici (CSL Centro Sperimentale del Latte, Lodi, Italy) containing *Propionibacterium freudenreichii* was used as direct-vat-set culture.

1.2. Mihaliç cheese production

The composition of cow milk used in cheese making trials was as follows: total solids 11.28%, milk fat 3.05%, protein 2.9%, lactose 4.5%, ash 0.7%, titratable acidity 8.8 SH°, and pH 6.58. Seven different cheeses, including a control, were produced according to three starter-culture combinations and two different scalding temperatures. HP40 and HP45 cheeses produced with *L. helveticus* (1%) and *P. freudenreichii* (0.1%) were scalded at 40 °C or 45 °C. StHP40 and StHP45 cheeses produced with *S. thermophilus* (1%), *L. helveticus* (0.5%) and *P. freudenreichii* (0.1%) were scalded at 40 °C or 45 °C. LeuSt40 and LeuSt45 cheeses produced with *Leu. mesenteroides* subsp. *cremoris* (0.5%) and *S. thermophilus* (0.5%) were scalded at 40 °C or 45 °C. Raw cow milk was used during the manufacturing of control cheese, whereas for the production of other samples, the milk was pasteurized at 68 °C for 30 min. After heat treatment, the milk was cooled to 40 °C, food-grade $CaCl_2$ was added at a level of 0.02%; and at 37 °C, it was inoculated with one of the test cultures. The inoculated milk was held for approximately 30 min and coagulated within 60 min at 34 °C using calf rennet (Peyma-Hansen, Istanbul, Turkey). Following the coagulation, the curd was cut into cubes (1 cm^3) and held for approximately 10 min. Then whey/curd mixture was scalded by pouring 70 °C hot water over it and cooled to 40 °C, or it was scalded by pouring 80 °C hot water over it and cooled to 45 °C within approximately 30 min. After resting for 15 min, the curds were transferred to a cotton cloth for whey drainage for 2 h. During whey drainage, the cheesecloth was pierced periodically with a needle to accelerate whey-off. After moulding and pressing for 15 h, Mihaliç cheese blocks were transferred to 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±1 °C. Following portioning (~ 400 g) and vacuum packing in polyethylene foils, the

cheeses were ripened at 4±1 °C for 3 months. Each production was conducted in two replicates, and the cheeses were analysed at the 1st, 15th, 30th, 60th, and 90th day of ripening.

1.3. Determination of organic acids

Seven grams of cheese was taken, 40 ml mobile phase (0.5% $(NH_4)_2PO_4$ – 0.4% acetonitrile) was added and mixed with a homogenizer (Ultra-Turrax, Labortechnik, Wasserburg, Germany) for 1 min. After standing for 1 h in a 40 °C water bath, the slurry was centrifuged at 6000×*g* for 5 min. The upper phase was filtered once through filter paper (Whatman No.1) and then through a 0.45-µm membrane filter (Millex-HV, Millipore, Ireland). A Thermo Dionex UltaMate 3000 Series HPLC system equipped with an UV absorbance detector was used. The chromatographic separation of 20 µl samples was performed on a C18 column (150×4.6 mm, Thermo Fisher Scientific, Waltham, MA, USA) with a flow rate of 1 ml min^{-1}. Individual organic acids were quantified on the basis of the external standard method, using the peak area.

1.4. Determination of FA compositions

FA analysis was conducted using an Agilent Technologies model 6890N gas chromatograph equipped with a FID and an automatic injector. The column was a fused silica capillary "Supelco SP-2380" (60 m×0.25 mm i.d., film thickness 0.2 µm, Bellefonte, PA, USA). The temperature of GC oven was programmed from 100 to 220 °C at the rate of 3 °C min^{-1}. The injector and the detector temperatures were 300 °C. The split ratio was set at 1:20. The identification of the individual FAs of the cheese samples was based on the comparison of the retention times of the unknown FA with those obtained from known FA standards (Supelco TM 37 Component FAME mix, Supelco, Bellefonte, PA, USA).

1.5. Statistical analyses

The effects of ripening and the difference between samples was analysed by one-way analysis of variance (ANOVA) using SPSS 15.0 (SPSS Inc., Chicago, USA), and significant differences were compared using Duncan's multiple range test.

2. Results and discussion

2.1. Organic acids

The changes in the organic acid contents of cheese samples are given in Table 1. The ripening period had a significant effect on the levels of propionic acid in the cheeses except LeuSt45 and control ($P<0.05$). The highest levels of propionic acid were observed in the cheeses manufactured with *P. freudenreichii*, especially on the 15th and 30th days. Sample HP45 had its highest level of propionic acid (27.67 mg g^{-1}) on the 15th day. HP40 (36.48 mg g^{-1}) and StHP40-StHP45 (32.24–36.24 mg g^{-1}) had their highest levels of propionic acid on the 30th day of ripening, whereas the lowest levels were observed for LeuSt40, followed by control cheese during the ripening period.

The fluctuations observed in citric-acid levels during ripening were due to the citrate metabolism. Citrate can act both as a substrate and product in the Krebs or citric acid cycle (Andiç et al., 2011). It is metabolized by many lactic acid bacteria into flavour components,

such as acetate, acetaldehyde, and diacetyl (McSweeney & Sousa, 2000). However, ripening period had a significant effect on the levels of citric acid only for the HP45 and control cheeses ($P<0.05$). Significant differences were observed among the samples during ripening, excluding the 60th day. The highest level of citric acid was determined for StHP45 initially and on the 15th day, with levels of 1.06 and 0.59 mg g^{-1}, respectively, thereafter, it was the highest in control the cheese.

The differences in the acetic acid levels of the Mihaliç cheeses were statistically significant, excluding the 15th day of ripening ($P<0.05$). Acetic acid is formed as a product of several pathways, by the metabolism of lactose by starters, metabolism of citric and lactic acid, and catabolism of amino acids in cheese (McSweeney & Sousa, 2000; Manolaki et al., 2006). Additionally, a higher amount of free amino acids in cheeses with starter as a result of advanced proteolysis might have served as a precursor (Manolaki et al., 2006). Although fluctuations were observed during ripening in the levels of acetic acid, the cheeses had higher acetic acid levels at the end of ripening than the levels observed initially, except for the control cheese. Additionally, the highest level was observed for HP45 (2.49 mg g^{-1}) at the end of ripening.

The production of lactic acid is essential for consistent ripening; also, organic acid is influenced by the age of the cheese. The lactic acid level of the Mihaliç cheeses increased at the end of ripening compared to the initial level, due to the activity of lactic acid bacteria. Lactic acid is a substrate for propionic acid production (Califano & Bevilacqua, 1999). Our results were in good agreement with these findings, because, although the concentration of lactic acid increased for all cheeses, this rise was generally insignificant for cheeses containing propionic acid bacteria as a starter, except HP45.

Significant differences were observed among the samples in the levels of formic acid during the ripening period, except for the 60th day ($P<0.05$). The highest concentration of formic acid was found in StHP40, followed by StHP45 at the end of ripening (2.08–1.02 mg g^{-1}). This might be explained by the presence of *S. thermophilus*, which produces formic acid from lactose (Akalin et al., 2002). Increases in the levels of formic acid were reported in previous studies performed by Lombardi and co-workers (1994) and Califano and Bevilacqua (1999).

2.2. Fatty acid composition

The mean FA compositions of Mihaliç cheese samples were given in Table 2. The range of saturated FAs (SFA) of the samples was determined to be 67.70–72.04%. The lowest SFA level belonged to the control cheese. HP40 had the highest SFA level. Among the SFAs, the most abundant was palmitic acid (C16:0), followed by myristic acid (C14:0) and stearic acid (C18:0). Palmitic acid, unlike other long-chain FAs, can be considered an indication of lipolytic activity. Additionally, rancid flavour can be noticed when a high level of palmitic acid is determined (Urbach, 1997). Hayaloğlu and Karabulut (2013) reported that palmitic acid was the most abundant FA in a ripened Mihaliç cheese sample. Additionally, Öner and Aloğlu (2004) and Dönmez and co-workers (2005) reported that palmitic, oleic (C18:1cis), and myristic (C:14) acids were the most abundant FAs in Mihaliç cheese samples. The total unsaturated FA (TUFA) contents of the samples ranged from 27.98–32.34%. The highest TUFA level belonged to the control cheese, whereas the TUFA level of HP40 was the lowest. Oleic acid (C18:1cis) predominated in the cheese samples, and its content was the highest among the TUFAs. The range of oleic acid in the cheese samples was determined to be 19.96–23.76%.

Table 1. Organic acid concentrations (mg g^{-1}) measured in Mihaliç cheese samples during ripening at 4 °C for 90 days

	Ripening days	Samples						
		HP40	HP45	StHP40	StHP45	LeuSt40	LeuSt45	Control
Propionic acid	1	2.57±0.46^{a}	4.28±0.39^{a}	4.77±1.54^{a}	5.34±0.10^{a}	0.95±0.37^{a}	5.49±0.57	4.74±3.24
	15	27.21±0.37^{cC}	27.67±2.62^{cC}	29.06±2.01^{bC}	32.85±1.10^{cD}	8.37±0.55^{cA}	14.00±0.71^{B}	9.30±1.86^{A}
	30	36.48±1.16^{dC}	24.49±1.63^{cB}	32.24±0.91^{bBC}	36.24±0.97^{cBC}	8.33±0.45^{cA}	8.91±3.62^{A}	11.42±4.25^{A}
	60	9.22±1.63^{bB}	14.10±0.21^{bD}	10.08±0.29^{aBC}	13.81±0.55^{bD}	2.41±0.04^{bA}	13.33±3.71^{CD}	3.64±0.56^{A}
	90	7.87±1.46^{bAB}	14.58±4.43^{bC}	12.77±0.60^{aBC}	5.27±2.05^{aAB}	2.88±0.03^{bA}	16.69±3.97^{C}	6.00±1.55^{AB}
Citric acid	1	0.39±0.02^{AB}	0.19±0.00^{aA}	0.44±0.11^{AB}	1.06±0.15^{C}	0.53±0.07^{B}	0.30±0.07^{AB}	0.64±0.14^{bB}
	15	0.38±0.01^{AB}	0.25±0.09^{abA}	0.51±0.03^{BC}	0.59±0.04^{C}	0.27±0.12^{A}	0.29±0.02^{A}	0.35±0.09^{aAB}
	30	0.25±0.01^{A}	0.36±0.07^{bAB}	0.57±0.05^{BC}	0.49±0.22^{ABC}	0.36±0.09^{AB}	0.46±0.04^{ABC}	0.71±0.10^{bC}
	60	0.34±0.08	0.53±0.02^{c}	0.57±0.07	0.57±0.35	0.35±0.13	0.36±0.19	0.84±0.01^{b}
	90	0.37±0.02^{AB}	0.29±0.08^{abA}	0.51±0.03^{C}	0.54±0.03^{C}	0.26±0.04^{A}	0.43±0.00^{BC}	0.82±0.08^{bD}
Acetic acid	1	1.03±0.07^{aA}	1.40±0.14^{aA}	1.66±0.15^{A}	1.56±0.21^{A}	0.76±0.25^{aA}	1.98±0.47^{bB}	1.10±0.18^{A}
	15	1.32±0.08^{a}	1.48±0.18^{ab}	1.56±0.44	1.88±0.22	1.38±0.13^{c}	1.48±0.05^{a}	1.30±0.65
	30	2.08±0.31^{bC}	2.26±0.00^{cC}	1.91±0.11^{BC}	1.59±0.00^{B}	0.98±0.06^{abA}	1.96±0.09^{aBC}	1.64±0.23^{B}
	60	2.11±0.07^{bC}	1.72±0.02^{bB}	1.71±0.01^{B}	1.59±0.30^{AB}	1.30±0.07^{bcA}	1.83±0.01^{aBC}	1.38±0.01^{A}
	90	1.82±0.11^{bB}	2.49±0.02^{cC}	1.88±0.04^{B}	1.83±0.32^{B}	1.21±0.12^{bcA}	2.12±0.07^{bB}	1.05±0.01^{A}

Table 1. continued

Lactic acid	1	5.67±0.08^{A}	7.15±0.49^{abAB}	8.99±0.32^{BC}	8.35±1.25^{B}	7.11±0.94^{aAB}	11.58±1.02^{bD}	11.10±1.57^{aCD}
	15	6.05±0.47^{A}	6.10±0.16^{aA}	9.77±1.71^{B}	11.27±1.27^{B}	8.8±0.43^{bAB}	8.71±0.78^{aAB}	9.81±1.83^{aB}
	30	6.90±0.82^{A}	8.84±1.19^{cA}	11.76±0.11^{B}	11.71±0.06^{B}	6.46±0.21^{aA}	12.34±0.20^{bB}	15.24±2.21^{bC}
	60	7.55±0.49^{A}	8.21±0.28^{bcA}	10.40±0.29^{B}	10.52±1.99^{B}	7.17±0.24^{aA}	11.90±0.26^{bBC}	12.89±0.28^{abC}
	90	7.02±0.54^{A}	8.27±0.15^{bcA}	10.93±0.37^{B}	11.74±2.31^{BC}	7.33±0.29^{aA}	13.50±0.94^{bC}	15.98±0.08^{bD}
Formic acid	1	0.57±0.03^{aB}	0.69±0.03^{BC}	0.83±0.1^{aC}	0.59±0.07^{B}	0.25±0.10^{A}	0.23±0.02^{A}	0.17±0.06^{A}
	15	0.51±0.03^{aAB}	0.57±0.13^{AB}	0.92±0.28^{abB}	0.89±0.15^{B}	0.34±0.30^{A}	0.26±0.01^{A}	0.22±0.21^{A}
	30	0.71±0.10^{bA}	0.75±0.02^{A}	1.25±0.02^{bB}	0.57±0.45^{A}	0.40±0.05^{A}	0.42±0.04^{A}	0.42±0.05^{A}
	60	0.71±0.00^{b}	0.86±0.18	1.10±0.01^{ab}	0.74±0.48	0.46±0.04	0.74±0.53	0.35±0.06
	90	0.78±0.04^{bC}	0.79±0.01^{C}	2.08±0.08^{cE}	1.02±0.17^{D}	0.45±0.02^{AB}	0.27±0.12^{A}	0.60±0.04^{BC}

(±SD, n=2)

HP40, HP45: Cheeses produced with *L. helveticus* (1%) and *P. freudenreichii* (0.1%), scalded at 40 °C or 45 °C. StHP40, StHP45: Cheeses produced with *S. thermophilus* (1%), *L. helveticus* (0.5%), and *P. freudenreichii* (0.1%), scalded at 40 °C or 45 °C. LeuSt40, LeuSt45: Cheeses produced with *Leu. mesentroides* subsp. *cremoris* (0.5%) and *S. thermophilus* (0.5%), scalded at 40 °C or 45 °C.

$^{a, b, c, d}$: 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).

The mean percentage of short-chain FAs (C4:0–C8:0) and medium-chain FAs (C10:0–C14:0) were 7.40 and 16.73 of total FAs, respectively, whereas the mean percentage of long-chain FAs was 75.87. There were no significant differences between butyric acid (C4:0) level of cheeses, whereas control cheese had significantly lower levels of caproic (C6:0) and caprylic (C8:0) acids. This was an indication of the low lipolytic activity in the control cheese. Although indigenous lipase generally causes significant lipolysis in raw milk cheeses, the effect of the enzyme can be reduced with a scalding procedure. The high degree of lipolysis of other cheese samples was due to the lipolytic activity of the starter microorganisms. Grappin and Beuvier (1998) reported that pasteurized milk cheeses have always lower levels of FAs than raw milk cheeses, excluding Swiss types, in which lipoprotein lipases are partially inactivated during cooking and lipolysis is usually low. Similarly, medium-chain FAs were significantly lower in control cheese, whereas control cheese had significantly higher levels of long-chain FAs, excluding palmitic, myristoleic (C14:1), and linolelaidic (C18:2*t*) acids. Hayaloğlu and Karabulut (2013) reported that long-chain FAs were dominant in many cheeses; however, they do not contribute to the cheese flavour as considerably as short-chain FAs do.

Table 2. Mean fatty acid composition of Mihaliç cheese samples ripened for 90 days (%, n=10[1]).

Fatty acid	Samples						
	HP40	HP45	StHP40	StHP45	LeuSt40	LeuSt45	Control
SFA[2]	72.04	71.74	71.83	71.88	71.90	71.84	67.70
C4:0	4.17^{A}	3.80^{A}	3.94^{A}	3.81^{A}	4.19^{A}	3.98^{A}	3.81^{A}
C6:0	2.20^{B}	2.20^{B}	2.22^{B}	2.16^{B}	2.31^{B}	2.21^{B}	1.93^{A}
C8:0	1.32^{B}	1.28^{B}	1.30^{B}	1.28^{B}	1.29^{B}	1.30^{B}	1.11^{A}
C10:0	3.00^{C}	2.94^{BC}	2.95^{BC}	2.92^{B}	2.99^{BC}	2.93^{B}	2.45^{A}
C11:0	0.29^{B}	0.30^{B}	0.30^{B}	0.29^{B}	0.30^{B}	0.29^{B}	0.25^{A}
C12:0	3.46^{B}	3.43^{B}	3.45^{B}	3.45^{B}	3.45^{B}	3.44^{B}	2.89^{A}
C13:0	0.10^{B}	0.1^{AB}	0.09^{AB}	0.09^{AB}	0.10^{AB}	0.10^{AB}	0.09^{A}
C14:0	12.26^{B}	12.15^{B}	12.13^{B}	12.19^{B}	12.10^{B}	12.12^{B}	11.10^{A}
C15:0	1.08^{A}	1.07^{A}	1.07^{A}	1.07^{A}	1.06^{A}	1.07^{A}	1.11^{B}
C16:0	32.98^{BC}	33.11^{BC}	33.06^{BC}	33.27^{C}	32.88^{B}	33.11^{BC}	29.56^{A}
C17:0	0.57^{A}	0.57^{A}	0.57^{A}	0.57^{A}	0.57^{A}	0.57^{A}	0.61^{B}
C18:0	9.47^{A}	9.62^{A}	9.59^{A}	9.61^{A}	9.51^{A}	9.58^{A}	11.33^{B}
C20:0	0.24^{A}	0.27^{B}	0.24^{AB}	0.24^{AB}	0.26^{AB}	0.24^{A}	0.35^{C}
C21:0	0.90^{A}	0.91^{AB}	0.92^{B}	0.92^{AB}	0.91^{AB}	0.92^{B}	1.13^{C}
TUFA[2]	27.98	28.23	28.22	28.06	28.10	28.15	32.34
MUFA[2]	25.39	25.61	25.61	25.48	25.47	25.57	29.75
C14:1	1.08^{B}	1.07^{B}	1.07^{B}	1.07^{B}	1.07^{B}	1.08^{B}	1.02^{A}
C15:1	0.36^{A}	0.35^{A}	0.35^{A}	0.35^{A}	0.34^{A}	0.35^{A}	0.43^{B}
C16:1	0.51^{A}	0.53^{A}	0.52^{A}	0.52^{A}	0.52^{A}	0.52^{A}	0.58^{B}

Table 2. Continued

Fatty acid	Samples						
	HP40	HP45	StHP40	StHP45	LeuSt40	LeuSt45	Control
C17:1	0.24	0.24	0.22	0.23	0.22	0.24	0.25
C18:1 *trans*	3.13^{A}	3.18^{A}	3.18^{A}	3.10^{A}	3.16^{A}	3.14^{A}	3.57^{B}
C18:1 *cis*	19.96^{A}	20.11^{A}	20.15^{A}	20.09^{A}	20.04^{A}	20.15^{A}	23.76^{B}
C20:1	0.12^{A}	0.13^{A}	0.12^{A}	0.12^{A}	0.12^{A}	0.11^{A}	0.15^{B}
PUFA[2]	2.59	2.62	2.61	2.58	2.63	2.58	2.59
C18:2 *trans*	0.24^{B}	0.23^{B}	0.24^{B}	0.21^{AB}	0.24^{B}	0.21^{AB}	0.19^{A}
C18:2 *cis*	2.30^{A}	2.35^{A}	2.33^{A}	2.33^{A}	2.35^{A}	2.34^{A}	2.36^{A}
C18:3	0.04^{B}	0.04^{AB}	0.04^{AB}	0.04^{AB}	0.03^{A}	0.04^{AB}	0.04^{AB}

[1]: Two trials with five ripening days.
HP40, HP45: Cheeses produced with *L. helveticus* (1%) and *P. freudenreichii* (0.1%), scalded at 40 °C or 45 °C. StHP40, StHP45: Cheeses produced with *S. thermophilus* (1%), *L. helveticus* (0.5%), and *P. freudenreichii* (0.1%), scalded at 40 °C or 45 °C. LeuSt40, LeuSt45: Cheeses produced with *Leu. mesentroides* subsp. *cremoris* (0.5%) and *S. thermophilus* (0.5%), scalded at 40 °C or 45 °C.
[2]: SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; TUFA: total unsaturated fatty acids.
[A, B, C, D]: Means in the same row with different superscripts among cheese samples significantly differ ($P<0.05$).

3. Conclusions

It is important in the commercial production of a traditional product to maintain its traditional characteristics. According to the results obtained in our study, using starter culture combinations after the pasteurizing process was a good alternative to manufacture Mihaliç cheese. For example, eye formation, which is an important quality parameter for Mihaliç cheese, was observed for all cheese samples. Especially the culture combination consisting of *S. thermophilus*, *P. freudenreichii,* and *L. helveticus* can be suggested for supplying the characteristic features of Mihaliç cheeses, possibly due to the interactions between these culture bacteria. Propionic acid was the most abundant organic acid in the cheeses produced by *P. freudenreichii*. Lactic acid, which is an indicator of ripening, increased at the end of ripening according to the initial levels for all samples. Short-chain FAs, which are important for cheese flavour, were in higher concentrations in cheeses manufactured with starter culture combinations than in the control cheese. In addition, the mean percentage of short-chain FAs was higher in the cheeses scalded at 40 °C than the cheeses scalded at 45 °C. For scalding temperatures, 40 °C can be suggested for higher lipolysis and higher consumer acceptability in terms of taste and flavour. It can be concluded that the study provided a new approach to production of Mihaliç cheese. Further studies can be conducted by bacterial species, mainly propionibacteria, which can be isolated from the natural microflora of this traditional cheese and then used as starter culture.

*

This study was supported financially by the Ege University Scientific Research Fund.

References

Akalın, A.S., Gönç, S. & Akbaş, Y. (2002): Variation in organic acids content during ripening of pickled White cheese. *J. Dairy Sci., 85*, 1670–1676.

Andiç, S., Tunçtürk, Y. & Gençcelep, H. (2011): The effect of different packaging methods on the formation of biogenic amines and organic acids in Kashar cheese. *J. Dairy Sci.*, *94*, 1668–1678.

Califano, A.N. & Bevilacqua, A.E. (1999): Freezing low-moisture Mozzarella cheese: Changes in organic acid content. *Food Chem.*, *64*, 193–198.

Dönmez, M., Seçkin, A., Sağdiç, O. & Şimşek, B. (2005): Chemical characteristics, fatty acid compositions, conjugated linoleic acid contents and cholesterol levels of some traditional Turkish cheeses. *Int. J. Food Sci. Nutr.*, *56*, 157–163.

Georgola, A., Moschopoulou, E., Aktypis, A., Massouras, T., Zoidou, E., Kandarakis, I. & Anifantakis, E. (2005): Evaluation of lipolysis during the ripening of traditional Feta cheese. *Food Chem.*, *93*, 73–80.

Grappin, R. & Beuvier, E. (1998): Possible implications of milk pasteurization on the manufacture and sensory quality of ripened cheese. *Int. Dairy J.*, *98*, 751–767.

Hayaloğlu, A.A. & Karabulut, I. (2013): Characterization and comparison of free fatty acid profiles of eleven varieties of Turkish cheeses. *Int. J. Food Prop.*, *16*, 1407–1416.

Lombardi, A.M., Bevilacqua, A.E. & Califano, A.N. (1994): Variation in organic acid content during ripening of Reggianito cheese in air-tight sealed bags. *Food Chem.*, *51*, 221–226.

Manolaki, P., Katsiari, M.C. & Alichanidis, E. (2006): Effect of a commercial adjunct culture on organic acid contents of low-fat Feta-type cheese. *Food Chem.*, *98*, 658–663.

McSweeney, P.L.H. (2004): Biochemistry of cheese ripening. *Int. Dairy J.*, *57*, 127–144.

McSweeney, P.L.H. & Sousa, M.J. (2000): Biochemical pathways for the production of flavour compounds in cheeses during ripening. *Lait*, *80*, 293–324.

Öner, Z. & Aloğlu, H. (2004): Some characteristics of Mihaliç, a traditional Turkish cheese. *Milchwissenschaft, 59*, 628–631.

Ong, N. & Shah, N.P. (2008): Influence of probiotic *Lactobacillus acidophilus* and *L. helveticus* on proteolysis, organic acid profiles and ACE-inhibitory activity of Cheddar cheese ripened at 4, 8 and 12 °C. *J. Food Sci.*, *73*, M111–M120.

Özcan, T. & Kurdal, E. (2012): The effect of using a starter culture, lipase and protease enzymes on ripening of Mihaliç cheese. *Int. J. Dairy Technol.*, *65*, 585–593.

Urbach, G. (1997): The flavour of milk and dairy products: II. Cheese: Contribution of volatile compounds. *Int. J. Dairy Technol.*, *50*, 79–89.