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Determination of Some Properties of Traditional Mihalic Cheese Made from Raw and Pasteurized Cow's Milk During Ripening Period

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Abstract: Mihalic cheese, salty, eye formed and semi-hard, is generally consumed after 90-day ripening period. In this study, two types of Mihalic cheese were produced in accordance with traditional techniques. One type of Mihalic cheese was made from pasteurized milk with starter cultures and the other type of Mihalic cheese was made from raw milk as a control cheese group. Changes in the chemical (moisture, non-fat dry matter, fat, protein, salt, titratable acidity and pH) and microbiological (total coliform bacteria [TCB], total aerobic mesophilic bacteria [TAMB], total mesophilic lactic acid bacteria [TMLAB], total thermophilic lactic acid bacteria [TTLAB], total mould-yeast [TMY], total propionic acid bacteria [TPAB] and total psychrophilic bacteria [TPB]) properties of Mihalic cheese made from raw and pasteurized cow's milk were analyzed in different stages of 90-day ripening period. The numbers of TMY and TCB were decreased significantly in 90-day ripening period. TTLAB were the dominant microflora in all cheese samples. The high NaCl content of the cheeses was the main agent and regulated the microbial survival. The results can be recommended that effective pasteurization of raw milk is essential to minimize microbial load in manufacture of Mihalic cheese. Also, standardization of Mihalic cheese is necessary for industrial production.

Key words: Mihalic cheese · Chemical property · Microbiological composition · Ripening · Thermophilic lactic acid bacterium

INTRODUCTION

Cheese is one of the most versatile foods suitable for all age groups which can be consumed in many different meal occasions [1, 2]. Traditionally, cheese has been regarded by consumers as a nutritious food because it is a source of high quality proteins, dietary calcium, fat and other nutrients. The basic composition and structure of cheeses are determined by manufacturing operations, but individual and unique characteristics of each cheese variety develop during ripening period [2, 3].

More than 1000 varieties of cheese are produced around the world [4]. In Turkey, there are approximately 150 varieties of cheese although three of them are the most popular cheeses with economic value. These are Kashar cheese, White cheese and Tulum cheese. However, the other traditional cheeses are getting to become popular. One of them is Mihalic cheese and it is the most consumed cheese in Western Turkey. It is usually consumed after 90-day ripening period.

Mihalic cheese is mostly made from full-fat cow's milk and up to 40% of ewe's milk is sometimes added to cow's milk. In generally, it is made from raw cow's milk because of natural microflora of milk. It is a semi-hard cheese with a pale cream, yellow or straw yellow color. It contains small eyes in the inside. Its shape is rind loaf shape or rectangular-like shape due to handling the curd in pressing cloth. Mihalic cheese is held in brine during ripening period for 90 days. Loaf weight of Mihalic cheese is about 10 kilogram. The flavor of Mihalic cheese tends to become moderate piquant salty and mildly acidic. After ripening period, it has a unique flavor taste, salty and aroma. Manufacture of Mihalic cheese is artisanal or industrial so it depends on whether Mihalic cheese is

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manufactured with raw or pasteurized milk. Besides, Mihalic cheese is often marketed after 4 week storage if it is produced from pasteurized milk.

In Turkey, Mihalic cheese has some problems such as lack of knowledge in manufacturing methods, using raw milk with low quality, insufficient heat treatment, misuse of rennet and/or starter culture, ripening in unsuitable and/or unhygienic conditions [5]. Cheese making has not been reached an industrial level because low-quality milk is sometimes used in cheese making industry [6]. Although Mihalic cheese, consumed high amounts in Western Turkey, has been manufactured for a long time, the techniques used in manufacture of Mihalic cheese have not been developed adequately yet. Moreover, the manufacture of Mihalic cheese has not been reached to standard level industrially. Currently, consumers sometimes are not sure the quality of Mihalic cheese. Standardization of manufacture of Mihalic cheese will be helped to produce Mihalic cheese with high quality. Unfortunately, manufacturers, who can not afford the cost of production, distribute Mihalic cheese on the market even if Mihalic cheese has not been ripened fully. The objective of this study were to investigate properties of chemical and microbiological during different ripening period.

MATERIALS and METHODS

Materials: Raw cow's milk was obtained from a farm in Gönen/Balıkesir. Liquid calf rennet (strength 1:16000) (Ha-La) was obtained from Peyma-Chr. Hansen's Inc. (Istanbul, Turkey). Calcium chloride ($CaCl_2$) (Merck, Germany) and sodium chloride (NaCl) (Merck, Germany) were obtained commercially in Turkey. Starter cultures (DVS) were obtained from DSM Food Specialties (The Netherlands).

Starter Culture Preparation: The starter culture coded DX–33A DSL contains *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis biovar. diacetylactis* and *Leuconostoc* ssp. The starter culture coded TX-10A DSL contains *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus helveticus*. The ratio of starter cultures used for cheese manufacture is 1:1. It was inoculated to pasteurized milk (1% w/v) at 35 °C for 30 minutes in manufacture of Mihalic cheese, which is made from pasteurized milk.

Manufacture of Mihalic Cheese: Mihalic cheeses were manufactured in duplicate in Kazanci Dairy Plant. Cow's milk was obtained from a local farm in Gönen. 200 liters of milk was used for each cheese group. Cheese samples coded P1 were manufactured from pasteurized milk. Cheese samples coded P2 were manufactured from raw milk. The raw milk was heat-treated at 65°C for 20 minutes for pasteurization in the manufacturing of cheese coded P1. After pasteurization, it was cooled to 40±1°C and added 0.02% (w/v) $CaCl_2$ (from 40% w/v $CaCl_2$) and held on 15 minutes. 1% (w/v) starter culture was added at 35±1°C. When starter cultures were being added, 0.5% raw milk was added to pasteurized milk as a *Propionibacterium* source in the production of Mihalic cheese coded P1 because *Propionibacterium* species are dominant microorganisms for eye-formation in Mihalic cheese. Then, it was held on 30 minutes. Afterwards, 25 ml liquid rennet which was diluted with 250 ml destile water, was added to the milk at 31±1°C for coagulation. Coagulation of milk took place within 90 minutes. Following coagulation, the coagulum was cut into 1 cm^3 cubes and the curds were held at 30±1°C about 15 minutes until the pieces of coagulum came to the surface of the whey. Then, scalding with hot water (~ 90°C) at 42±2°C about 30 minutes was accelerated whey-off and improved decreasing of pH. The curds were put in pressing cloth and drained for 2 hours. After the curd was cut into medium loaf shape, the loafs were pre-ripened in 15% (w/v) brine for 2 days. Afterwards, the loafs of Mihalic cheese were transferred to 16% (w/v) brine for 2 days and 18% (w/v) brine for 5 days at 20±1°C. Finally, the loafs of Mihalic cheese were transferred to storage room (+4°C) and ripened in 18% (w/v) brine for 3 months. In addition to this, cheese samples coded P2, made from raw milk, were like cheese samples coded P1. But, $CaCl_2$ and starter culture were not added to cheese samples coded P2. The starter cultures were only added to heat-treated cheese milk.

The cheese samples were transferred to the laboratory under cooled conditions (+4°C) during different ripening periods and analyzed immediately for microbiological and chemical analyses. The cheese samples were analyzed in different days of ripening period during 3 months.

Chemical Analyses: The chemical analyses of cheese samples (1^{st} to 90^{th} day) were carried out at different stages of ripening period (on 1^{st}, 3^{rd}, 5^{th}, 7^{th}, 9^{th}, 12^{th}, 30^{th}, 60^{th} and

90th day). Protein [7], fat and salt of cheese samples were determined according to the procedures described by Robert T. Marshall [8]. The total solid contents of the cheese samples were estimated according to Association of Official Analytical Chemists methods [9]. For titratable acidity (SH°) analysis, cheese samples (10 g) were weighed in porcelain mortar. 0.1N NaOH solution was used for titration with phenolphthalein as an indicator. The amount of NaOH used was multiplied by two. Thus, titratable acidity was obtained in Soxhlet-Henkel degree [9]. The pH values of samples were determined at room temperature with a 315i/SET pH-meter and combined sentix 42 electrode (WTW, Weilheim, Germany) [10]. Standard buffer solutions (pH 4.01 and 7.01; WTW, Weilheim, Germany) were used for calibration.

Microbiological Analyses: The microbiological analyses of cheese samples (1st to 90th day) were carried out at different days of ripening period (on 1st, 3rd, 5th, 7th, 9th, 12th, 30th, 60th and 90th day). Firstly, cheese samples (10g) were weighted in the sterile stomacher bags under aseptic conditions and 10 g cheese samples were diluted with 90 ml of sterile Ringer's solution (Merck). The standard pour plate method was employed to determine the counts of microorganisms. After incubation, plates with 3-300 colonies were counted and the results expressed as a colony forming units (cfu g^{-1}). Decimal dilutions were placed on specific media for viable counts in duplicate. The media used was Plate Count agar (PCA) (Merck, Germany) for counting TAMB (incubated at 32±1°C for 2 days) and TPB (incubated at +4°C for 7 days), Potato Dextrose agar (PDA) (Merck, Germany) of pH 3.5 adjusted with 10% w/v tartaric acid (Merck, Germany) for counting TMY (incubated at 25±1°C for 4 days), Fluorocult Violet Red Bile agar (FVRB) (Merck, Germany) for counting TCB (incubated at 37±°C for 1 day), Rogosa agar (Merck, Germany) for counting TMLAB (incubated at 32±1°C for 2 days), M17 agar (Merck, Germany) for counting TTLAB (incubated at 42±1°C for 2 days) and Sodium Lactate agar (Merck, Germany) for counting TPAB (incubated at 42±1°C for 2 days) [11].

Statistical Analysis: The SPSS computer programme (version 11.5 SPSS Inc. Chicago IL USA) was used for statistical processing. Results were analyzed by multifactor analysis of variance with 95% and 99% significance level using Statgraphics ®Plus 5.1. Multiple comparisons were performed through 95% and 99% significant differences (ANOVA) intervals. Otherwise, stated significant results refer to p=0.01 and p=0.05. All data are reported as means and standard errors of means [12].

RESULTS AND DISCUSSION

Chemical Composition of Mihalic Cheese During Ripening Period: During the ripening period, the mean data of Mihalic cheese samples for moisture, fat, non-fat dry matter, protein, salt, titratable acidity and pH are presented in Table 1. The mean moisture content of Mihalic cheese samples decreased from 1st day to 12th day during pre-ripening period so that the mean total solids content of Mihalic cheese samples increased during first 12 pre-ripening period. The mean moisture of Mihalic cheeses increased from 30th day to 90th day because the protein of Mihalic cheeses decreased between 30th to 90th days. During the ripening period, the decrease in protein level might be due to the decomposition of the water-soluble proteins which migrated into the brine in nitrogen-derivative forms. In this proceeded period, the moisture amount of the cheese samples increased since it might be resulted from the decreases in protein and dry matter [13]. During ripening period, the highest level of protein was observed in cheese samples coded P2. The difference in levels of protein in the two cheese groups might be accounted for the addition of starter culture or variations in enzyme activities, moisture and/or titratable acidity.

Relatively, the fat content remained constant during 90 days of ripening period. Total fat contents of the cheese samples were between 24.00% and 31.50%. A slight upward trend in fat content of cheese samples may be caused by a small amount of fat increases due to the decrease of moisture. The mean percentage of fat value of cheese samples coded P1 was higher than those of cheese samples coded P2. Higher level of acidity in milk may have resulted in better coagulation of milk and less fat loss. It has been suggested that the use of starter culture in cheese-making and high acidity of coagulum may help to retain fat in coagulum. The salt contents of cheese samples increased during 90 days of ripening period. Salt values of Mihalic cheeses were the lowest on the first ripening day and the highest values were on the last ripening day in both cheese groups. The pH of the cheeses did not remain stable relatively during ripening

Table 1: Chemical properties of Mihalic cheeses made from raw and pasteurized cow's milk during ripening periods (X±s.d.)*.

Group	Ripening periods (day)	Moisture (%)	Fat (%)	Non-fat dry matter (%)	Salt (%)	Protein (%)	Titratable acidity (SH°)	pH
	1st	48.700±0.00	29.500±3.00	21.800±3.00	1.515±0.42	21.150±0.19	99.000±5.01	4.875±0.02
	3rd	47.200±0.80	31.250±4.76	21.550±3.96	4.445±0.34	20.570±0.78	112.500±1.50	4.690±0.01
	5th	44.850±1.86	30.500±3.51	24.650±1.65	5.540±0.62	21.585±1.39	107.500±8.52	4.655±0.02
	7th	43.200±2.21	30.150±3.66	26.650±1.45	7.630±0.38	22.005±0.97	114.500±5.93	4.685±0.02
P1	9th	42.200±0.50	31.250±3.26	26.550±2.76	8.180±0.00	21.645±0.21	111.750±7.77	4.760±0.01
	12th	40.900±0.50	31.500±3.51	27.600±3.00	8.890±0.01	22.180±0.47	99.000±0.00	4.720±0.08
	30th	44.700±1.00	29.750±3.26	25.550±2.26	9.965±0.09	19.840±0.49	90.000±1.00	4.855±0.01
	60th	45.500±1.50	29.500±2.51	25.000±4.01	10.070±0.05	19.465±0.63	79.000±16.05	4.890±0.10
	90th	46.000±1.00	31.500±1.50	22.500±0.50	10.360±0.00	18.900±0.81	75.000±5.01	4.935±0.11
	1st	46.700±1.70	28.550±1.55	24.750±0.15	1.924±0.06	23.290±0.65	104.000±2.01	4.775±0.01
	3rd	43.850±0.15	29.650±0.65	26.500±0.80	2.477±0.32	22.945±1.36	106.000±1.00	4.760±0.04
	5th	41.520±0.18	30.000±1.00	28.480±1.18	5.493±0.23	23.830±0.88	100.000±8.02	4.820±0.00
	7th	40.900±0.50	29.650±0.85	29.450±1.35	6.685±0.59	23.530±0.74	108.500±1.50	4.745±0.06
P2	9th	40.500±0.50	30.250±0.25	29.250±0.75	7.635±0.89	24.190±1.29	106.000±6.02	4.830±0.01
	12th	39.000±0.00	30.500±0.00	30.500±0.00	7.920±0.67	23.780±2.03	102.000±13.0	4.830±0.05
	30th	42.350±1.65	29.500±0.50	28.150±2.16	10.100±0.96	21.935±0.79	90.500±5.52	4.930±0.03
	60th	45.500±2.51	24.750±1.76	29.750±4.26	11.560±1.51	20.125±2.23	68.500±652	4.935±0.05
	90th	45.500±2.21	24.000±1.00	30.800±3.20	11.570±1.50	18.880±177	71.250±8.77	5.010±0.20

*Chemical properties of the cheese samples were not significant at all levels ($p > 0.05$)

Table 2: Microbiological properties of Mihalic cheeses made from raw and pasteurized cow's milk during ripening period (X±s.d.) *(cfu g^{-1}).

Group	Ripening days	TCB	TAMB	TMLAB	TTLAB	TMY	TPAB	TPB
	1st	$2.30\times107\pm4.0\times10^{6}$	$3.80\times108\pm2.00\times10^{7}$	$9.30\times10^{6}\pm3.71\times10^{6}$	$1.03\times109\pm3.75\times10^{8}$	$2.30x104\pm1003^{CD}$	$1.25\times108\pm5.01\times10^{6}$	$3.80\times108\pm2.00\times10^{7}$
	3rd	$2.70\times107\pm2.0\times105$	$5.20\times108\pm2.10\times10^{8}$	$6.40\times107\pm4.61\times10^{7}$	$7.15\times108\pm1.50\times10^{7}$	$2.20x104\pm2006^{CDE}$	$4.75\times108\pm4.66\times10^{8}$	$5.20\times108\pm2.10\times10^{8}$
	5th	$1.66\times107\pm9.4\times10^{6}$	$3.40\times108\pm1.50\times10^{8}$	$1.55\times108\pm4.51\times10^{7}$	$4.50\times109\pm1.60\times10^{9}$	2.70x104±13040C	$3.90\times108\pm9.00\times10^{7}$	$3.40\times108\pm1.50\times10^{8}$
P1	7th	$5.35\times106\pm3.2\times10^{6}$	$3.55\times108\pm1.50\times10^{7}$	$1.10\times108\pm1.0\times10^{7}$	$1.20\times1010\pm1.00\times10^{9}$	$5.90x104\pm1003D^{EF}$	$7.65\times108\pm3.51\times10^{8}$	$3.55\times108\pm1.50\times10^{7}$
	9th	$7.40\times105\pm5.0\times10^{3}$	$3.25\times108\pm1.04\times10^{8}$	$3.65\times108\pm5.01\times10^{6}$	1.20×1010±0.00	$1.40x104\pm2006^{CDEF}$	$3.00\times108\pm6.01\times10^{7}$	$3.25\times108\pm1.04\times10^{8}$
	12th	$2.85\times105\pm1.5\times10^{3}$	$3.65\times108\pm1.75\times10^{8}$	$3.40\times108\pm2.0\times10^{7}$	$1.16\times1010\pm7.44\times10^{9}$	$5.10x10300\pm401^{EF}$	2.35×108±5.01×106	$3.65\times108\pm1.75\times10^{8}$
	30th	$1.81x104\pm9.9\times10^{3}$	$1.80\times108\pm2.00\times10^{7}$	$1.45\times108\pm2.5\times10^{7}$	$9.55\times108\pm5.01\times10^{6}$	1.13x103±70F	$7.60\times108\pm6.41\times10^{7}$	$1.80\times108\pm2.00\times10^{7}$
	60th	$3.45x103\pm1.9\times10^{3}$	$2.75\times108\pm4.51\times10^{7}$	2.30×108±0.00	$4.30\times109\pm5.01\times10^{8}$	3.49x103±3220F	$2.90\times108\pm6.01\times10^{7}$	$2.75\times108\pm4.51\times10^{7}$
	90th	$3.92x103\pm3.8\times10^{3}$	$9.60\times108\pm5.41\times10^{8}$	$4.65\times107\pm1.75\times10^{7}$	$1.18\times109\pm9.21\times10^{8}$	7.90x102±170F	$6.65\times108\pm3.36\times10^{8}$	$9.60\times108\pm5.41\times10^{8}$
	1st	$2.14\times107\pm1.9\times10^{7}$	$3.70\times109\pm1.90\times10^{9}$	$1.27\times108\pm8.29\times10^{7}$	$1.95\times109\pm5.51\times10^{8}$	$3.90\times105\pm150448^{A}$	$7.00\times108\pm6.02\times10^{8}$	$3.70\times109\pm1.90\times10^{9}$
	3rd	$1.65\times108\pm1.6\times10^{8}$	$4.90\times109\pm3.51\times10^{9}$	$3.25\times108\pm1.65\times10^{8}$	$1.80\times109\pm7.02\times10^{8}$	8.35x104±6699B	$5.78\times108\pm5.23\times10^{8}$	$4.90\times109\pm3.51\times10^{9}$
	5th	$4.30\times107\pm4.3\times10^{7}$	$1.65\times109\pm1.5\times10^{8}$	$3.50\times108\pm1.2\times10^{8}$	$9.30\times108\pm2.70\times10^{8}$	$2.2x104\pm10030^{CDE}$	2.70×109±2.31×109	$1.65\times109\pm1.50\times10^{8}$
P2	7th	$2.55\times108\pm2.24\times10^{8}$	$6.45\times109\pm3.51\times10^{8}$	$4.95\times108\pm7.5\times10^{7}$	$5.85\times109\pm4.16\times10^{9}$	$2.35x104\pm9528^{CD}$	$4.95\times109\pm4.56\times10^{9}$	$6.45\times109\pm3.51\times10^{8}$
	9th	$5.50\times106\pm5.5\times10^{6}$	$4.95\times109\pm2.75\times10^{9}$	$4.80\times108\pm1.25\times10^{8}$	$7.95\times109\pm6.07\times10^{9}$	$3.50x104\pm1906^{F}$	2.37×109±2.03×109	$4.95\times109\pm2.75\times10^{9}$
	12th	$1.75x107\pm1.7\times10^{7}$	$6.10\times109\pm3.31\times10^{9}$	$8.10\times108\pm2.9\times10^{8}$	$6.55\times109\pm4.46\times10^{9}$	$8.65x103\pm7372D^{EF}$	$2.52\times109\pm2.18\times10^{9}$	$6.10\times109\pm3.31\times10^{9}$
	30th	8.58×104±8.4×104	$5.33\times109\pm4.42\times10^{8}$	$1.75\times108\pm3.61\times10^{8}$	$5.00\times108\pm1.80\times10^{8}$	$1.30x103\pm797^{F}$	$4.35\times108\pm2.85\times10^{8}$	$5.33\times109\pm4.42\times10^{8}$
	60th	5.50x104±5.5×104	$2.20\times109\pm6.00\times10^{7}$	$2.65\times108\pm2.5\times10^{7}$	$1.70\times109\pm4.01\times10^{7}$	$1.75x103\pm1755^{F}$	$7.65\times108\pm4.36\times10^{8}$	$2.20\times109\pm6.02\times10^{8}$
	90th	4.50x102±4.21x102	$1.70\times109\pm5.01\times10^{8}$	$8.60\times107\pm8.43\times10^{7}$	$9.21\times108\pm8.79\times10^{8}$	$3.81x104\pm3861^{F}$	$2.50\times108\pm2.00\times10^{7}$	$1.70\times109\pm5.01\times10^{8}$

*Total mould-yeast ($p \leq 0.01$) of the cheese samples were significant at all levels. The other results of cheese samples were not significant statistically ($p>0.05$).

periods. This small increase of pH may be due to lactic acid formation from residual lactose in cheeses (Table 1). It also may be resulted from limited production of alkaline products which are related to low protein breakdown in cheese samples. Relatively, titratable acidity also did not remain stable during ripening period as indicated in Table 1. The difference in titratable acidity might be mainly responsible for the variations in salt values of cheese samples. When the levels of titratable acidity of the cheese samples were compared each others, the titratable acidity of cheese samples coded P1 was higher than cheese samples coded P2. The higher titratable acidity of cheese samples coded P1 was the cause of the lower salt of this cheese group. Some researchers showed that there was a reverse interaction between acidity of cheeses and salt immigration [14]. It is well-known that cheese samples with high acidity retain less water and absorb less salt [15].

Microbiological Composition of Mihalic Cheeses During Ripening Period: Cheese ripening is a complex microbiological and enzymatic process characterized by the production of compounds that lend the cheese certain aroma and texture characteristics [16]. During the ripening period, the secondary flora promotes a series of complex biochemical reactions vital for the proper development of both flavor and texture of the cheese. Many physical parameters such as water content, NaCl concentration and pH influence microorganism growth in cheese during ripening [17]. The results shown in Table 2 for cheese samples indicated that in generally microbial counts of cheeses were high. The high level of TCB was an indication of unhygienic conditions in the small dairy where the cheese was produced and this also showed that it was not complied with the hygienic conditions in all stages ranging from the provision of raw material and process to storage conservation and transportation. During the ripening period, the most important reason for the decline in TCB was the increase in brine concentration and acidity in cheese mass and cold conditions during ripening period. On the other hand, TCB probably decreased due to unfavorable conditions created by the increasing population of LAB and the lower pH. Just as it was in pasteurized cheese samples, TCB did not disappear completely at the end of 90-day ripening period in cheese samples coded P2. The 1^{st} and 90^{th} day counts of the TAMB were determined as 3.8×10^{8} cfu g^{-1} and 9.6×10^{8} cfu g^{-1} in cheese samples coded P1 and 3.7×10^{9} cfu g^{-1} and 1.7×10^{9} cfu g^{-1} in cheese samples coded P2 respectively. Lactic acid bacteria were the major components of the cheese microflora in cheese samples. TMLAB content of cheese samples coded P1 increased at the end of the ripening period while it decreased in the other cheese samples at the end of the ripening period compared with first ripening day. This might suggest that metabolic matters released during ripening period and other matters from biochemical reactions promoted the growth of mesophilic microflora. As shown in other food ecosystems, metabolic interactions among microorganisms may increase the number of a certain species [18]. The fact that bacteria content in cheese samples coded P2 was low at the end of the ripening period was due to the decrease in water activity in cheese mass and to the increase in titratable acidity and salt content.

According to results, TTLAB in cheese samples coded P1 increased at the end of the ripening period whereas it declined in the other cheese group. The reason for this decrease could be the existence of inhibitory metabolic products and scarcity of nutritional factors. It was observed that during ripening period, TTLAB in the cheese samples were higher than the TMLAB. The dominant microflora in Mihalic cheese samples was TTLAB. The reason for the difference in the number of the two types of bacteria might be mainly the existence of inhibitors, starter cultures and stimulators in these cheese samples. Salt in cheese would not have had any significant effects on bacteria growth but the combined effect of pH and salt might have had some influences. One of these influences may have been greater on microbial decline in cheese samples with a higher NaCl concentration. Growth rates of TMY of cheese samples coded P2 were higher than the other type of cheese. This was due to the use of pasteurized milk in samples coded P1; therefore TMY diminished at the end of the ripening period, but they did not totally disappear in both cheese samples due to the environmental temperature and a suitable pH value for mould and yeast growth. TPAB fermented lactate which causes the release of CO_2 thus creating eye forms characteristic of Mihalic cheese. NaCl and diffused oxygen content varies greatly between the centre and the surface of the cheese in brine. These factors form a suitable setting in the centre of the cheese for the growth of propionic acid bacteria. The number of *Propionobacterium* varies according to the depth of the cheese mass [19]. The reason for the reduction in TPAB during the ripening period might have been the decline in water activity and the amount of nutrition elements and the increase in NaCl concentration and acidity in cheese mass. On the contrary, the cell concentrations of TPB decreased slightly during cheese ripening period. The major reason for the reduction in the count of TPB during the ripening period might have been the decrease in water activity and nutrition elements and the increase in NaCl content in cheese mass. Pasteurization process was observed not to have made a significant effect on TPB in cheese samples coded P1. Because micrococcus the kind of microorganism that constitutes 50% of the psychrophilic bacteria is resistant to pasteurization process. The warmer environment during first 12 days pre-ripening period may have contributed to some extent to a faster growth of psychrophilic bacteria.

CONCLUSION

The dominant microflora in Mihalic cheese samples was TTLAB. It seemed possible that high NaCl content of the cheeses was the main agent and regulated the microbial survival. The cheese manufactured without adding starter culture had significantly a high level of mesophilic aerobic microflora. The high NaCl content of the brine in which Mihalic cheese was ripened slowed down both enzyme activity and microorganism growth thereby, extending the ripening period. Some of the fat content in the Mihalic cheeses migrates to the brine due to the extended ripening period which adversely affects the flavor and texture of the cheese. Changing the brine less frequently might reduce fat loss in Mihalic cheese. Too much NaCl content may lead to a decrease in customer demand for this kind of cheese. Taking such factors into consideration necessary changes should be made in the NaCl content of the brine and ripening process. The microbial flora of Mihalic cheese should be determined precisely for industrial manufacture of standard cheese of this type. Microflora obtained from traditional cheese should be analyzed in order to achieve a desirable texture and flavor in hard and semi-hard cheese in a shorter period.

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