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Changes of free fatty acid contents and sensory properties of white pickled cheese during ripening

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Abstract

In this study, the effects of commercial pregastric lipase enzyme on the accelerated ripening of white pickled cheese were investigated. Sensory evaluation of cheese samples was also performed. Commercial pregastric lipase was added to cheese milk before rennet addition at the level of 2, 4, 6 g/100 l milk and white pickled cheese was made from this milk. The main components (total solids, fat, total nitrogen, salt and titratable acidity), pH and free fatty acids were analysed in cheese samples after 1, 10, 20 and 30 days of ripening. The main components and pH of cheese samples were not significantly affected by the addition of pregastric lipase levels. Generally, when the pregastiric lipase levels increased, volatile free fatty acids (VFFAs) and free fatty acids (FFAs) also significantly increased ($P<0.05$). Generally, sensory scores (appearance, flouvour, body and texture and odour) were significantly higher in both 4 g/100 l treated milk added and after 20 days ripening ($P<0.05$).

Keywords: White pickled cheese; Pregastric lipase; Sensory evaluation; Free fatty acids

1. Introduction

White pickled cheese is probably the most popular, and economically the most important, variety of traditional cheese in Turkey. In almost all parts of Turkey it is produced from raw and heat-treated ewes' milk, or a blend of cows' and ewes' milk and mostly from cows' milk. White pickled cheese represents a type of cheese that owes its character primarily to a strong acidity and a high salt content. A number of volatile compounds contribute to the aroma, which will vary according to the kind of milk used (ewe, cow or a mixture) and the method of manufacture. It is a soft variety, according to the ripening period, which may last from 30 days to 1 year. A typical white pickled cheese made from cows' milk contains high levels of ethanol, propanol, 2-butanol and 2-butanone. Acetic acid is the most abundant volatile acid. But if lipases have been added during preparation of the milk, the cheese also will have high levels of short-chain fatty acids (C_4 to C_8), which will be partly esterified by ethanol. Accelerated cheese ripening has for many years been a subject of interest mainly for two reasons. The first is a need to reduce the storage period and thereby reduce storage cost. The second is a need to obtain more cheese flavour. Many of the fast starter cultures do not result in a rounded, mature cheese flavour; and a bitter taste is even often encountered. This has promoted several researchers (Arnold, Shahni, & Dwivedi, 1975; El-Neshawawy, Abdel Bakey, & Farahat, 1982; Hagrass, El-Ghandour, Hammad, & Hofi, 1983; Obretenov, Dimitroff, & Obretenov, 1978) to search for enzymes, which can speed up the natural processes of cheese ripening.

Lipase, is very often used for production of some varieties of cheeses (especially Italian cheeses) when it is produced from cows' milk instead of ewe or goat milk. This enzyme liberates short and medium chain fatty acids from tryglycerides (Arnold et al., 1975; Kilara, 1985). The use of various pregastric lipase products for Feta cheese has been reported by Ethymiou and Maltick (1964). They showed that the desired mild rancid flavour of Feta cheese was associated with free fatty acids from C_2 through C_{10} whereas objectionable rancid flavour was

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due to high levels of free fatty acids above C_{10}. However, there is a tendency for lipase to be used less, because the younger generation in Turkey seems to prefer Feta cheese with a not-too-pronounced lipolytic taste.

The quality of cheese is determined by its flavour, rheological properties and visual appearance. The relative importance of each of these three quality attributes varies with the cheese variety, and they are interrelated, at least to some extent. The fat fraction of cheese has a major effect on cheese texture and is important for the perception and development of cheese flavour. Poor development of flavour and texture in low-fat cheese is a considerable technological problem that limits the market for such products. In addition to serving as a direct (fatty acids) or indirect source of flavour compounds in cheese, fat serves as a solvent for sapid compounds produced from other constituents. The fat in cheese can undergo degradation via lipolysis (enzymatic) or oxidation. Fatty acids, released upon lipolysis, contribute directly to cheese flavour. However, extensive lipolysis is considered undesirable in most cheese varieties.

Free fatty acids are known to play an important role in flavours of many varieties of ripened cheese. Two major sources are generally considered for free fatty acids in ripened cheese. These are (i) the direct breakdown products of milk fat by lipolysis and (ii) the end-products of carbohydrate and protein metabolism by bacteria. The breakdown of milk fat is carried out by esterases and lipases, which are either endogenous to the milk or of microbial origin (psychotropic and lactic bacteria) (Gripon, Monnet, Lamberet, & Desmazeaud, 1991). The breakdown of milk fat probably occurs in all cheeses but the rate and extent of hydrolysis varies considerably between cheeses varieties (Sahahni, 1975).

White pickled cheese is made from heat-treated milk ripened more slowly than raw milk cheese. It is highly desirable to improve flavour and increase ripening rate of the former. Few references are available on free fatty acid profile of Feta cheese (Efthymiou, 1967; Georgala, Kandarakis, Kaminarides, & Anifantakis, 1999) and changes of individual free fatty acids during cheese ripening (Buruiana & El-Senaity, 1986). The objective of this study was to investigate the free fatty acid profile of white pickled cheese during ripening and to assess the effect of commercial pregastric lipase on white pickled cheese lipolysis made from heat-treated and enzyme-modified cow milks.

2. Material and methods

2.1. Material

Raw cows' milk was obtained from a farm in Konya (Turkey). Starter culture (R 703 FD-DVS) and calf rennet (Ha-La) were obtained from Peyma-Chr Hansen's Inc. (Istanbul, Turkey). Pregastric lipase, as a powder extracted from frozen epiglottis glands from fresh-slaughtered lambs, was obtained from Peyma-Chr Hansen's Inc. (Istanbul, Turkey). $CaCl_2$ (Merck, Germany) and NaCl (salt) was obtained commercially in Turkey.

2.2. Methods

A simplified flowchart for the production of white pickled cheese is shown in Fig. 1. Experimental cheeses were made in the "Seker Süt" dairy plant. The lots, of 1200 l raw milk, were heat-treated at 83 °C for 5 min, cooled to 32±1 °C and divided into four parts. Varying amounts (0, 2, 4 and 6 g pregastric lipase enzyme 100 l^{-1} milk) of pregastric lipase enzyme (18±4 $LiUg^{-1}$) were mixed with water and added to each part of cheese milk. The pregastric lipase enzyme solution, $CaCl_2$ (20 g 100 l^{-1}) and starter cultures (60 ml 100 l^{-1}) were added to each part of cheese milk. The mixture was left to pre-ripening until the pH 6.3 was reached. The first part was used as a control and coded L_0; to the other parts lipase preparation was added at 2 g 100 l^{-1} milk and coded L_1, 4 g 100 l^{-1} milk and coded L_2 and 6 g 100 l^{-1} milk and coded L_3. Rennet (strength, 1:15,000) was added to coagulate the milk within 90 min. Following coagulation, the coagulum was cut into 1 cm^3 cubes. The curds were transferred into vats lined with cheesecloth to drain whey and pressed for about 3 h at 20±2 °C. Then, curd was cut into 8 cm^3 segments to shape and these shaped curds were put into brine with 16% NaCl (w/v) at about 18 °C for 5 h. At the end of this time, cheeses were packaged in cans, pre-ripened at 18±2 °C for 24 h and ripened at 5 °C in a cold room. Total solids, fat, total nitrogen, salt, pH and titratable acidity (% lactic acids) in cheese amples were determined according to the procedures described by Kirk and Sawyer (1991). Sensory evaluations of cheese samples were obtained for flavour, odour, body and texture and appearance by Turkish Standard-591 for white pickled cheese. In this sensory system, the product is graded on a 100 point scale as follows: 20 points maximum for appearance, 35 points maximum for body and texture, 10 points maximum for odour and 35 points maximum for flavour. Sensory evaluation of cheese samples was performed by five experienced panellists, according to a scoring card (Anonymous, 1995; Bodyfelt, Tobias, & Trout, 1988). Cheeses were periodically analysed at 1, 10, 20 and 30 days during the ripening time. Free fatty acids in cheeses were determined by gas chromatography according to the method of Deeth, Fitz-Gerald, and Snow (1983). A mixture of standard fatty acids to calibrate to the column was supplied from Sigma-Aldrich (Germany). All solvents used for fatty acid extraction were of analytical

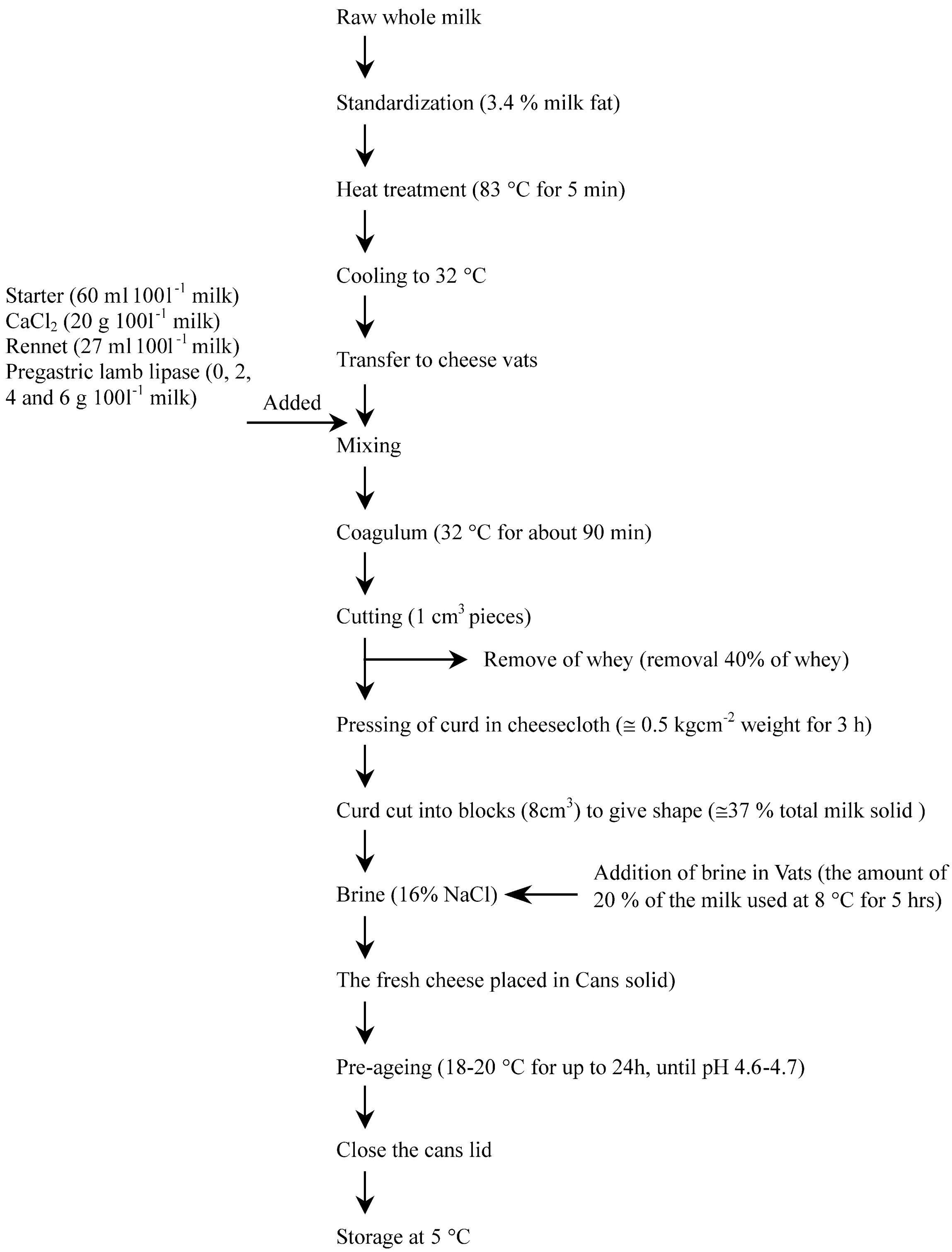

Fig. 1. Simplified flowchart for the production of white pickled cheese.

grade and obtained from Merck. (Germany). A HP5890 Series II Gas Chromatograph (Hewlett Packard, Germany) equipped with an automatic on-column injector and a flame ionisation detector was used with a Nukol capillary column, 15 m×0.53 mm I.D., coated with 0.5 μm film (Sigma-Aldrich, Germany).

The carrier gas (nitrogen) flow rate was 30 ml min^{-1}. During chromatography oven temperature was raised from 110 to 220 °C at a rate of 8 °C min^{-1}. Calibration was based on the internal standard method; heptanoic acid (C_7) was used as the internal standard and was also added to samples before the extraction of free fatty acid from cheese. Free fatty acids were identified by comparison of retention time of the gas chromatographic peaks with those of a commercial standard of free fatty acids. The experiment was replicated three times. For statistical analysis, the effects of pregastric lipase levels (L_0, L_1, L_2 and L_3) and ripening periods (1, 10, 20 and 30 days) were evaluated by Repeated-Measures Analysis of Variance (Zar, 1999). According to the results of Repeated-Measures Analysis of Variance "If necessary", Duncans test was used to determine the groups significantly different from each other. All statistic analysis were done by using STATISTICA (ver. 5.0, 1995) package software. All data are reported as means and standard errors of means.

3. Results and discussion

The mean data of white pickled cheese samples for total solids, fat in total solids, total nitrogen, total nitrogen in total solids, salt, salt in total solids, titratable acidity and pH over the ripening period are presented in Table 1.

Examination of the data revealed that the addition of pregastric lipase had no significant effect on total solids, fat, total nitrogen, salt contents of resultant cheese, titratable acidity or pH in cheese, throughout the ripening time at 5 °C. Salt contents in white pickled cheese increased during the ripening period because of diffusion of salt from the surfaces into the centre of the cheese where samples were taken for these determinations. Total fat contents of samples were from 18.17 to 19.67%. This data represent more than 40% of dry matter of the cheese. The pH in cheese decreased from 4.6 to 4.3 for L_0 and ranged from 4.7 to 4.4 for other (L_1, L_2, L_3 coded) cheese samples. The rate of decrease differed for individual treatments but was not significant. This gradual decrease in pH may be due to lactic acid formation from residual lactose in cheese. It also may result from limited production of alkaline products related to low protein breakdown in white pickled cheese. Titratable acidity also increased for the same reasons. Similarly, several researchers (Hagrass et al., 1983; Nasr, 1983) found that the use of lipase had no significant effect on the gross composition of cheese during the ripening time.

The mean sensory scores for appearance, flavour, body and texture and odour of White Pickled cheese samples are presented in Table 2.

There is no interaction between pregastric lipase levels (L_0, L_1, L_2, L_3) and ripening periods (1, 10, 20, 30 days) for all analysed sensory properties ($P > 0.05$). As a result, both pregastric lipase levels and ripening periods were analysed independently of each other. Pregastric

Table 1
Changes of chemical composition in white pickled cheese made from pregastric lipase-treated cows' milk during ripening time (% w/w)

Lipase levels[a]	Ripening period (days)	Total solids (%)	Fat (%)	Fat in total solids (%)	Total nitrogen (%)	Nitrogen in total solids (%)	Salt (%)	Salt in total solids (%)	Titratable acidity (% Lactic acid)	pH
L_0	1	36.0±2.3	18.2±1.7	42.6±1.5	1.9±0.1	5.4±0.3	3.0±0.5	8.3±0.1	1.6±0.1	4.7±0.1
	10	37.2±1.7	18.9±1.3	42.0±1.3	2.6±0.7	6.4±0.7	3.1±0.3	7.4±0.3	1.6±0.5	4.7±0.1
	20	38.4±1.3	19.4±1.8	45.0±1.8	2.1±0.4	5.1±0.5	2.9±0.1	6.9±0.1	1.9±0.6	4.6±0.2
	30	40.9±2.4	19.7±2.2	45.1±1.5	2.3±0.6	5.3±0.6	3.5±0.9	8.2±0.5	1.9±0.6	4.5±0.2
	Mean	38.1±1.1	19.1±0.3	43.7±0.8	2.3±0.2	5.6±0.3	3.1±0.1	7.7±0.3	1.8±0.1	4.6±0.1
L_1	1	36.0±3.8	18.7±1.6	41.3±1.6	2.0±0.3	5.1±0.2	2.9±0.9	7.6±0.3	1.6±0.5	4.7±0.3
	10	37.0±3.1	18.3±1.8	42.6±2.5	2.2±0.3	5.5±0.5	3.0±0.7	7.8±0.8	1.7±0.8	4.7±0.2
	20	39.2±1.6	19.8±1.7	44.7±2.0	2.3±0.5	5.7±0.7	3.2±0.2	8.0±0.4	1.8±0.5	4.6±0.1
	30	41.0±2.9	19.8±2.2	45.1±1.2	2.4±0.3	5.9±0.1	3.3±0.9	8.0±0.1	1.9±0.2	4.5±0.2
	Mean	39.3±1.1	19.2±0.4	43.4±0.9	2.2±0.1	5.6±0.2	3.1±0.1	7.8±0.1	1.8±0.1	4.6±0.1
L_2	1	35.1±2.2	18.7±1.3	43.6±1.1	1.9±0.6	5.2±0.2	3.1±0.6	8.1±0.2	1.6±0.4	4.7±0.1
	10	37.5±2.8	18.5±2.5	44.7±1.6	2.1±0.2	5.4±0.4	3.1±0.5	8.0±0.4	1.7±0.4	4.7±0.1
	20	39.9±1.9	19.2±1.8	43.1±2.2	2.3±0.1	5.9±0.3	3.3±0.4	8.6±0.8	1.9±0.7	4.6±0.3
	30	40.9±3.6	19.1±1.6	44.6±1.4	2.4±0.9	6.3±0.4	3.5±0.7	9.1±0.1	1.9±0.1	4.5±0.2
	Mean	38.4±1.3	18.9±0.2	44.0±0.4	2.2±0.1	5.7±0.3	3.2±0.1	8.4±0.2	1.8±0.1	4.6±0.1
L_3	1	35.8±3.7	18.2±1.7	45.2±1.3	1.9±0.6	5.3±0.5	3.9±0.9	8.6±0.6	1.6±0.2	4.7±0.1
	10	37.8±2.5	18.8±2.2	49.0±2.2	2.1±0.2	5.2±0.9	3.0±0.8	6.8±0.5	1.8±0.3	4.7±0.1
	20	39.4±3.4	19.3±1.3	43.8±1.5	2.3±0.2	5.8±0.2	3.2±0.1	7.9±0.7	2.1±0.4	4.6±0.4
	30	41.4±2.4	19.3±2.5	46.7±1.4	2.4±0.1	5.9±0.4	3.6±0.8	9.0±0.1	2.1±0.2	4.4±0.2
	Mean	38.6±1.2	18.9±0.3	46.2±1.1	2.2±0.1	5.5±0.2	3.4±0.2	8.1±0.5	1.9±0.1	4.6±0.1

[a] L_0: control; L_1: 2 g pregastric lipase/100 l milk; L_2: 4 g pregastric lipase/100 l milk; L_3: 6 g pregastric lipase/100 l milk.

Table 2
Changes of sensory properties in cheese samples during ripening time[a]

Lipase levels[b]	Ripening period (days)	Appearance (20)	Flavour (35)	Body and Texture (35)	Odour (10)
L_0	1	13.5±0.4	28.4±0.6	25.6±0.6A	8.5±0.1A
	10	14.4±0.2	29.2±0.3	26.0±0.1A	8.8±0.1B
	20	15.0±0.2	29.4±0.5	27.6±0.7B	8.7±0.3B
	30	16.0±0.5	29.6±0.6	27.6±0.5B	8.8±0.1B
	Mean	14.7±0.5b	29.2±0.3b	26.7±0.5c	8.8±0.1b
L_1	1	14.8±0.2	29.2±0.8	26.0±0.5A	8.8±0.2A
	10	16.4±0.4	29.2±0.5	26.2±0.6A	9.0±0.4B
	20	16.8±0.6	30.6±0.6	26.6±0.3B	9.6±0.1B
	30	16.2±0.2	31.4±0.6	28.2±0.7B	9.6±0.2B
	Mean	16.1±0.4a	30.1±0.5b	26.8±0.5c	9.3±0.2a
L_2	1	16.2±0.2	31.4±0.8	27.2±0.5A	9.0±0.2A
	10	16.8±0.6	31.4±0.8	28.2±0.7A	9.4±0.4B
	20	17.4±0.6	30.8±0.7	30.8±0.9B	9.8±0.3B
	30	17.8±0.5	31.2±0.9	31.2±0.8B	9.8±0.3B
	Mean	17.1±0.4a	31.2±0.1a	29.4±1.0b	9.5±0.2a
L_3	1	16.5±0.4	31.2±0.5	28.8±0.7A	9.2±0.1A
	10	16.5±0.2	31.6±0.5	30.5±0.6A	9.8±0.2B
	20	17.0±0.5	31.8±0.8	32.5±0.8B	9.8±0.2B
	30	17.0±0.5	32.7±0.7	33.3±0.9B	9.8±0.2B
	Mean	16.8±0.1a	31.8±0.3a	31.3±1.0a	9.7±0.2a

[a] Different letters (a–c) in the same columns were significantly different for lipase levels ($P<0.05$). Different capital letters (A, B) in the same columns were significantly different for ripening periods ($P<0.05$).

[b] L_0: control; L_1: 2 g pregastric lipase/100 l milk; L_2: 4 g pregastric lipase/100 l milk; L_3: 6 g pregastric lipase/100 l milk.

lipase levels and ripening periods, for each of the mean properties scored by sensory test, were compared. According to statistical analysis, results in Table 2 can be summarised as follows: (i) when pregastric lipase levels were increased, sensory scores (appearance, flavour, body and texture and odour) were also significantly increased ($P<0.05$); (ii) different ripening times had no effect on scores of appearance and flavour; (iii) after 20 days ripening, scores for body and texture and odour were significantly higher ($P<0.05$).

The method used to determine the free fatty acids permitted the measurement of fatty acids produced by both lipolytic processes (C_4–$C_{18:1}$) and bacterial fermentation (C_2–C_4). The average free fatty acids of white pickled cheese samples, quantified as acetic, propionic, butyric, valeric, caproic, caprylic, capric, lauric, palmitic, myristic, stearic and oleic acids, throughout the ripening period, are shown in Table 3.

Interaction between pregastric lipase levels (L_0, L_1, L_2, L_3) and ripening periods (1, 10, 20, 30 days) for acetic, propionic, butyric, valeric, caproic, caprylic, capric, myristic, stearic, oleic acids and total free fatty acids (TFFAs) amounts were statistically significant ($P<0.05$). But there are no interactions between pregastric lipase levels (L_0, L_1, L_2, L_3) and ripening periods (1, 10, 20, 30 days) for total volatile free fatty acids (TVFFAs), lauric and palmtic acid amounts ($P>0.05$).

According to statistical analysis, results shown in Table 3 can be summarised as follows: (i) Generally, all free fatty acid amounts were significantly affected by pregastric lipase level addition to cheese milk ($P<0.05$); (ii) generally, all free fatty acid amounts were significantly affected by ripening time ($P<0.05$); (iii) generally, cheese samples with L_2 and L_3 pregastrirc lipase levels showed that all properties analysed were significantly increased (after 20 days ripening time) ($P<0.05$).

In cheese samples, our analyses indicated that, after 30 days of ripening time, white pickled cheese had higher concentrations of oleic, stearic and acetic acids, medium concentrations of capric, caprylic caproic, butyric propionic, valeric and myristic acids and low concentrations of lauric acid; behaviour was similar throughout the ripening period. Therefore, manifold increases were observed for lauric, palmitic, myristic, oleic acids in control samples, for myristic, lauric, palmitic, oleic acids in L_1 coded samples, for lauric, myristic, oleic acids in L_2 coded sample and for palmitic, lauric, myristic, oleic acids in L_3 coded sample and little stearic acid was released during ripening. The data obtained from pregastiric lipase treated samples, compared with control, showed similar increases.

Traditionally, discussion of lipolysis in cheese is based upon actual measured concentrations of free fatty acids (FFAs) at a given ripening time (which are an indication of the extent of lipolysis); however, further useful information on the activity of lipases may be obtained if the actual rate of release of free fatty acids (FFAs) (estimated as the ratio of the amount of fatty acids released in a given time period divided by such time periods) is considered rather than the integrated result thereof. The behaviour was different for the differently made cheese samples; first day releases total volatile free fatty acids (TVFFAs) were 8.5, 14.4, 17.3 and 19.7 mg/100 g cheese/day and total free fatty acids (TFFAs) were 33.7, 42.1, 55.3 and 59.4 mg/100 g cheese/day, for L_0, L_1, L_2 and L_3 coded samples, respectively. The releases of total volatile free fatty acids (TVFFAs) and total free fatty acids (TFFAs) were highest prior to first day of ripening time. The highest rate of hydrolysis for caprylic and capric acids was between 1 and 30 days and they ranged from 0.4 to 1.9 mg/100 g cheese/day for caprylic acid and 0.5 to 1.6 mg/100 g cheese/day for capric acid, while the concentrations of the remaining free fatty acids increased by lower amounts.

Total volatile free fatty acid (TVFFA) (C_2–C_{10}) contents of the cheese samples increased throughout the ripening time in all cheese samples, which was in agreement with the results reported by El-Shibiny, Abdel-Baky, Farahat, Mahran, and Hofi (1974) for Domiati cheese, but our total volatile free fatty acid levels were lower than those reported by Georgala et al. (1999). Total free fatty acids and total volatile free fatty acid contents of the cheese samples were increased when pregastric lipase level increased in cheese samples. The

Table 3
Changes of free fatty acids of white pickled cheese made from pregastric lipase-treated cows' milk during ripening time (mg/100 g)[a]

Lipase levels[b]	Ripening period (days)	Acetic acid	Propionic acid	Butyric acid	Valeric acid	Caproic acid	Caprylic acid	Capric acid	TVFFAs[c]	Lauric acid	Palmitic acid	Myristic acid	Stearic acid	Oleic acid	TFFAs[c]
L_0	1	5.9±0.2Aa	0.2±0.0Aa	0.6±0.0Aa	0.6±0.1Aa	0.2±0.1Aa	0.4±0.0Aa	0.6±0.1Aa	8.5±0.1A	0.4±0.1A	0.7±0.1A	0.5±0.0Aa	10.7±0.1Aa	12.9±0.1Aa	33.7±1.9Aa
	10	6.0±0.0Aa	0.4±0.1Aa	0.9±0.1ABa	0.8±0.0Aa	0.4±0.0Aa	0.7±0.0ABa	0.7±0.0ABa	9.8±0.0B	1.0±0.1B	1.0±0.2B	0.7±0.0Aa	11.6±0.2Ba	13.0±0.3Aa	37.0±1.5Aa
	20	8.4±0.2Ba	0.6±0.0Aa	1.4±0.1BCa	1.5±0.1Ba	0.5±0.0Aa	0.8±0.0ABa	1.0±0.4ABa	14.1±0.1C	1.4±0.1C	1.2±0.3B	1.0±0.2Aa	12.2±0.1Ba	26.1±0.2Ba	55.9±2.4Ba
	30	9.9±0.6Ca	0.7±0.0Aa	1.8±0.2Ca	1.9±0.2Ba	1.3±0.0Ba	1.3±0.1Ba	1.4±0.2Ba	18.3±0.2D	1.7±0.1C	1.8±0.4C	1.1±0.2Aa	12.3±0.3Ba	27.6±0.3Ca	63.8±1.4Ca
	Mean	7.6±0.9	0.5±0.1	1.2±0.2	1.2±0.2	0.6±0.2	0.8±0.1	0.9±0.1	12.7±1.6a	1.2±0.2a	1.2±0.2a	0.8±0.1	11.7±0.4	19.9±2.9	47.6±5.2
L_1	1	8.5±0.2Ab	1.1±0.1Ab	1.4±0.0Ab	1.5±0.0Ab	0.7±0.0Aa	0.6±0.0Aab	0.7±0.0Aa	14.4±0.2A	0.5±0.0A	0.9±0.0A	0.6±0.0Aa	11.3±0.1Aa	14.4±0.2Ab	42.1±1.4Ab
	10	10.5±0.1Bb	1.7±0.1Ab	1.8±0.1ABb	1.6±0.1ABb	1.5±0.1Bb	0.9±0.0Aa	1.0±0.1Aa	19.1±0.1B	1.4±0.0B	1.3±0.0B	1.4±0.0Bab	11.8±0.1Aa	20.8±0.1Bb	55.8±0.8Bb
	20	12.3±0.1Cb	2.5±0.2Bb	2.5±0.1Bb	2.3±0.1Bb	4.0±0.2Cb	1.7±0.1Bb	2.0±0.1Bb	27.5±0.1C	2.0±0.0C	1.5±0.0B	2.1±0.0Bb	12.5±0.2Ba	31.4±0.1Cb	76.9±1.1Cb
	30	13.1±0.3Cb	3.8±0.2Cb	3.4±0.1Cb	3.1±0.3Cb	4.3±0.2Cb	2.9±0.2Cb	2.4±0.1Bb	33.0±0.2D	2.1±0.0C	2.2±0.1C	3.4±0.1Cb	12.8±0.2Ca	33.1±0.1Db	86.4±2.1Db
	Mean	11.2±0.7	2.3±0.4	2.3±0.3	2.1±0.3	2.6±0.6	1.5±0.4	1.5±0.3	23.5±3.0b	1.5±0.3b	1.5±0.2a	1.9±0.4	12.1±0.2	24.9±3.1	65.3±7.1
L_2	1	10.2±0.1Ac	1.2±0.0Ab	1.6±0.1Ab	0.8±0.1Aa	1.5±0.1Ab	1.2±0.0Ab	0.8±0.0Aa	17.3±0.1A	0.8±0.0A	1.9±0.1A	1.9±0.1Ab	12.9±0.1Ab	20.6±0.3Ac	55.3±2.4Ac
	10	11.8±0.2Bc	2.1±0.1Bb	2.5±0.0Bbc	1.9±0.1Bb	2.0±0.1Bb	2.0±0.1Ac	1.4±0.1ABb	23.6±0.1B	1.8±0.1B	2.2±0.0B	2.1±0.0Ab	13.0±0.1Bb	27.0±0.2Bc	69.6±1.7Bc
	20	12.7±0.2Cb	2.9±0.0Cc	3.0±0.1Bbc	2.4±0.2Bb	3.8±0.1Cb	2.7±0.1Bc	1.9±0.1Bb	29.4±0.1C	2.4±0.1C	2.8±0.1B	3.4±0.1Bc	13.5±0.1Cb	32.9±0.3Cc	84.5±1.6Cc
	30	13.6±0.4Db	3.2±0.0Cb	3.9±0.2Cb	3.9±0.1Cc	4.5±0.1Db	4.5±0.2Cc	2.1±0.1Bb	35.7±0.1D	2.6±0.0C	3.5±0.1C	4.8±0.1Cc	14.3±0.4Db	35.2±0.4Dc	96.1±2.4Dc
	Mean	12.1±0.5	2.4±0.3	2.8±0.4	2.3±0.4	3.0±0.5	2.6±0.5	1.6±0.2	26.5±2.8c	1.9±0.3c	2.6±0.3b	3.1±0.5	13.4±0.2	28.9±2.3	76.4±6.3
L_3	1	10.6±0.1Ac	1.7±0.1Ab	1.4±0.1Ab	1.7±0.0Ab	2.2±0.1Ab	1.2±0.0Ab	1.0±0.0Aa	19.7±0.1A	1.0±0.1A	1.1±0.0A	2.1±0.0Ab	13.8±0.2Ac	21.7±0.2Ad	59.4±2.4Ad
	10	12.8±0.3Bd	3.6±0.2Bc	2.6±0.2Bc	1.9±0.1Ab	3.3±0.2Bc	1.5±0.2Ac	1.9±0.1Bb	27.6±0.2B	1.9±0.0B	2.6±0.0B	3.1±0.0Bc	16.7±0.1Bc	25.6±0.4Bd	77.4±2.6Bd
	20	13.2±0.3Bb	3.4±0.0Bc	3.7±0.1Cc	2.8±0.2Bb	3.7±0.1Cb	3.2±0.2Bc	3.1±0.1Cc	33.1±0.1C	2.6±0.0C	2.6±0.1B	3.8±0.1Cc	25.6±0.2Cc	36.7±0.3Cd	104.3±1.9Cd
	30	14.5±0.4Cc	4.7±0.1Bc	4.7±0.1Dc	4.7±0.1Cd	4.4±0.2Cb	4.9±0.2Cc	4.2±0.1Db	41.0±0.2D	3.0±0.0C	3.6±0.0C	4.2±0.1Cc	26.4±0.2Dc	38.7±0.2Dd	117.9±2.8Dd
	Mean	12.8±0.6	3.4±0.4	3.1±0.5	3.1±0.5	3.4±0.3	2.7±0.6	2.6±0.5	30.6±3.2d	2.1±0.3c	2.5±0.4b	3.3±0.3	20.6±2.2	30.7±2.9	89.8±9.3

[a] Different letters (a–d) in the same columns were significantly different from each other for lipase levels ($P<0.05$). Different capital letters (A–D) in the same columns were significantly different from each other for ripening periods ($P<0.05$).

[b] L_0: control; L_1: 2 g pregastric lipase/100 l milk; L_2: 4 g pregastric lipase/100 l milk; L_3: 6 g pregastric lipase/100 l milk.

[c] TVFFAs = total volatile free fatty acids; TFFAs = total free fatty acids.

levels of total free fatty acid and total volatile free fatty acids in 30-day-old cheeses made from milk with added pregastric lipase (6 g/100 l) were higher than those from other cheese samples. Similar results were observed by El-Neshawy et al. (1982) in Domiati cheese, but our total volatile free fatty acid levels were lower than those reported by Efthymiou (1967). Obretenov et al. (1978) reported that the total volatile fatty acid content of white brined cheese was 123 mg/100 g, of which 89% was acetic acid. Harwood, Lloy, and Stark (1981) carried out a comprehensive investigation of the fat and free fatty acid contents of Feta cheeses of different origin, using gas chromatography and mass spectrometry. Their results show that acetic acid was the principal acid present and the level of butyric, caproic and caprylic acids were low. The total volatile free fatty acids (TVFFAs) (C_2–C_8) was from 45 to 81 mg/100 g sample. Lower results were observed in our results. Lipases are active at the pH of ripening cheese. These lipases may represent intracellular lipases of lactic acid bacteria liberated by bacterial autolysis and pregastric lipase. Part of the butyric acid and all of the free caproic, caprylic and capric acids shown to be present in 30-day-old cheese, are the result of the action of pregastric lipases on the milk fat of the cheese. The differences in amounts of these fatty acids in cheese samples, made different enzyme levels, are possibly due to large reductions in numbers of bacterial species in milk capable of liberating intracellular lipases by autolysis. The differences in the free fatty acid concentrations found by various authors may be related, either to the techniques used for the distillation and extraction and type of cheese, or the formation of acetic acid, due to lactate fermentation. Valeric acid and some butyric acid, acetic acid and propionic acid, are produced by oxidative deamination of leucine-isoleucine, valine, glycine, alanine, serine and threonine, respectively (Hemme, Bouillane, Metro, & Desmazeaud, 1982).

In conclusion, these results indicate that the use of pregastric lipase in white pickled cheese milk, at different concentration levels, enhanced the development of free fatty acids and changed the pattern of volatile free fatty acid composition of cheese samples. Information about specificities of pregastric lipase, for individual volatile free fatty acids (FFAs), obtained in this study, should provide important guidance for the development of improved flavours in white pickled cheese. Cheese samples, with added pregastric lipase, showed the characteristic flavour of white pickled cheese and were more acceptable than the control cheese sample within the 30 day ripening time. Higher amounts of butyric, caproic and, caprylic acid in cheese seem to contribute to the development of piquant taste. The high level of pregastric lipase addition to cheese milk could be recommended for accelerating the development of flavour in cheese in a short ripening time.

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References

Anonymous. (1995). *Beyaz Peynir Standardı. TS-591*. Ankara: Türk Standartları Enstitüsü.

Arnold, R. G., Shahni, K. M., & Dwivedi, B. K. (1975). Application of lipolytic enzymes to flavour development in dairy products. *Journal of Dairy Science*, *58*, 1127–1243.

Bodyfelt, F. W., Tobias, J., & Trout, G. M. (1988). *The sensory evaluation of dairy products*. London: Van Nostrand Reinhold.

Buruiana, L. M., & El-Senaity, M. H. (1986). Volatile free fatty acid in Teleme cheese during ripening. *Egyptian Journal of Dairy Science*, *14*, 201–205.

Deeth, H. C., Fitz-Gerald, C. H., & Snow, A. J. (1983). Gas chromatographic method for the quantitative determination of free fatty acids in milk and milk products. *New Zealand Journal of Dairy Science & Technology*, *18*, 13–20.

Efthymiou, C. (1967). Major free fatty acid of Feta cheese. *Journal of Dairy Science*, *50*, 20–24.

Efthymiou, C. C., & Maltick, A. (1964). Development of domestic Feta cheese. *Journal of Dairy Science*, *47*, 593–598.

El-Neshawawy, A. A., Abdel Baky, A. A., & Farahat, S. M. (1982). Enhancement of flavour of flavour development in Domiati cheese by animal lipase preparations. *Dairy Industries International*, *47*, 29–31.

El-Shibiny, S., Abdel-Baky, A. A., Farahat, S. M., Mahran, G. A., & Hofi, A. A. (1974). Development of free fatty acids in soft cheese during pickling. *Milchwissenschaft*, *29*, 666–669.

Georgala, A. K., Kandarakis, I. G., Kaminarides, S. E., & Anifantakis, E. M. (1999). Volatile free fatty acid content of feta and white-brined cheeses. *The Australian Journal of Dairy Technology*, *45*, 5–8.

Gripon, J. C., Monnet, V., Lamberet, G., & Desmazeaud, M. J. (1991). Microbial enzymes in cheese ripening. In P. F. Fox (Ed.), *Food enzymology*, *vol. 1* (pp. 131–148). London: Elsevier Applied Science.

Hagrass, A. E. A., El-Ghandour, M. H., Hammad, Y. A., & Hofi, A. A. (1983). Production of Ras cheese from recombined milk. III. Effect of some ripening agents. *Egyptian Journal of Dairy Science*, *11*, 271–279.

Harwood, J. F., Lloy, G. T., & Stark, W. (1981). Some flavour components of Feta cheese. *The Australian Journal of Dairy Technology*, *36*, 34–37.

Hemme, D., Bouillane, C., Metro, F., & Desmazeaud, M. J. (1982). Microbial catabolism of amino acids during cheese ripening. *Sciences. des Aliments*, *2*, 113 123.

Kilara, A. (1985). Enzyme-modified lipid food ingredients. *Process Biochemistry*, *April*, 35–45.

Kirk, R. S., & Sawyer, R. (1991). *Pearson's composition and analysis of foods* (9th ed.). London: Longman Science and Technical.

Nasr, M. (1983). Accerleration of Romi cheese ripening by addition of fungal esterase lipase powder. *Egyptian Journal of Dairy Science*, *11*, 309–315.

Obretenov, T., Dimitroff, D., & Obretenova, M. (1978). Application of microbial enzyme preperations in cheese production. 5. Study of volatile fatty acids of brine-ripened cheese manufactured with Mesenterin. *Milchwissenschaf*, *33*, 545–548.

Sahahni, K. M. (1975). Lipases and esterases. In G. Reed (Ed.), *Enzymes in food processing* (2nd ed.) (pp. 181). New York: Academic Press.

STATISTICA (ver. 5.0). (1995). Stat Soft, Inc. 2325 East 13th Street, Tulsa, OK 74104, USA.

Zar, J. H. (1999). *Biostatistical analysis* (4th ed.). New Jersey: Prentice Hall.