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# Changes of Texture, Microstructure and Free Fatty Acid Contents of Lighvan Cheese during Accelerated Ripening with Lipase

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## ABSTRACT

**In this study, the effect of addition of three different levels of microbial lipase (0, 4, and 8.0 g lipase in 100 kg milk) was investigated on the physiochemical properties, free fatty acids, textural properties, and microstructure of Lighvan cheese during ripening. The addition of lipase did not significantly affect pH, acidity, moisture, and salt content of Lighvan cheese, but increased its free fatty acid content considerably. It also increased hardness and decreased the brittleness of Lighvan cheese in all stages of ripening. The number and the mean diameter of fat globules, which were entrapped in casein network, were affected by all levels of the added lipase. Following the addition of lipase to Lighvan cheese and after 90 days of ripening, individual fat globules or their aggregates totally disappeared and some fingerprints of fat were observed. Disappearance of fat globules along with increase in protein matrix junctions leads to uniform structure of casein consisting voids which are produced from fermentation.**

**Keywords**: Fat globule, Hardness Lipolysis, Traditional cheese.

## INTRODUCTION

Cheese ripening is a slow and very complicated biochemical process, which is costly due to extended storage time. The occurrence of biochemical and physical reactions during aging plays an important role in texture and flavor of ripened cheese (Tunick, 2000). Depending on cheese type, ripening time varies from a few weeks up to three years. Several strategies have been proposed to accelerate the cheese ripening. Since ripening is basically an enzymatic process, increasing the activity of key enzymes could be effective by addition of commercially available enzymes to milk or curd. Several researchers examined the application of this method to accelerate the ripening of different kinds of cheeses such as Cheddar cheese, some Egyptian cheeses, Spanish hard cheese, Tulum cheese, Mihalic cheese, Kashar cheese, and Ultrafiltered-Feta cheese (Fernandez-Garcia *et al.,* 1988; Kheadr-Ehab *et al.,* 2003; Yilmaz *et al.,* 2005; Karami *et al.,* 2009a; Akin *et al*., 2012; Kilcawley *et al*., 2012; Ozcan and Kurdal, 2012). According to these studies, the efficiency of enzyme addition highly depends on cheese type. The focus of most of these studies is on the biochemical properties, free fatty acid composition, and sensory evaluation of cheese during the accelerated ripening and there are a few studies on microstructure and textural properties changes with enzyme addition.

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Lighvan cheese, a cheese variety produced in northwest of Iran, is a traditional starter-free cheese and can be made from a raw ewe's or a mixture of ewe's and goat's milk. It is one of the most popular Iranian traditional cheeses and its unique flavor is appreciated by consumers. In spite of its popularity and ever-growing consumption, there are few studies on its chemical composition and microbial communities (Abdi *et al.,* 2006; Kafili *et al.,* 2009; Aminifar *et al.,* 2010; Aminifar *et al.,* 2012; Rasouli Pirouzian *et al.,* 2012).

Indigenous lipolytic enzymes in milk, rennet and microflora are responsible for the production of free fatty acids as a result of lipolysis. Since Lighvan cheese is produced from raw milk, lipolysis plays an important role in its flavor formation (Aminifar *et al.,* 2012).

Lighvan cheese ripenes in at least three to four months and the shortening of this time without any defect in cheese properties could be very useful. There is no study on the effect of accelerated ripening on Lighvan cheese characteristics. Since accelerating the biochemical reactions such as lipolysis, which occur during Lighvan cheese ripening time, could play an important role in shortening of this time, in the present study, the effect of palatase –a microbial lipase- on microstructure, texture, and free fatty acid content of Lighvan cheese was studied.

## MATERIALS AND METHODS

### Cheese Making and Sampling

Palatase, lipase obtained from *Rhizomucor miehei* with an activity of ≥ 20,000 U $g^{-1}$, was purchased from Sigma (Sigma-Aldrich, Denmark). Experimental Lighvan cheese samples with three different levels of microbial lipase ($L_0$, $L_1$, and $L_2$ with 0.0, 4.0, and 8.0 g lipase in 100 kg milk, respectively) were made at the University of Tehran's dairy pilot in three replicates, according to a method described previously (Kafili *et al.,* 2009) and analyzed at three stages of ripening (0, 30, and 90 days).

To make Lighvan cheese, lamb liquid rennet was added to a sheep milk (7.93% fat, 19.7% total solids, pH= 6.58) that contained different levels of lipase ($L_0$, $L_1$, $L_2$) to coagulate at 32°C. The whey was drained from the coagulum by cutting it into walnut-sized pieces and transferring them to rectangular cotton bags. For better whey drainage, after four hours, cotton bags were compressed with a four kilograms mass for 2-4 hours (depending on season). Cheese cubes of 25×25×25 $cm^3$, which were prepared by cutting the mass curd, were placed in a 22% brine stream for six hours. Afterward, curd cubes were transferred to a basin, sodium chloride crystals were strewed on their surfaces, and were stored for three to five days. At this stage, for better whey removal, the cubes were turned upside down nine to fifteen times. The cubes were then kept in 12% salt brine for three months at an average temperature of 10±2°C. During ripening time, physicochemical properties, free fatty acid composition, textural characterization, and microstructure of Lighvan cheese were analyzed.

### Physicochemical Properties

The pH of Lighvan cheese samples was measured by a Knick 766 pH-meter calimatic (Niels, Bohrweg, Utrecht, The Netherlands). The AOAC method (AOAC, 1995) was used to determine total acidity. Standard (4A) FIL-IDF (FIL-IDF, 1982) was used to measure the moisture content. Salt content of cheese samples was determined by a method described previously (Kirk and Sawyer, 1991). ISO method was used to measure fat content of cheese samples (ISO, 2008).

### Analysis of Free Fatty Acid

Extraction of fatty acids was carried out according to a method described previously





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(Garcia-Lopez *et al.,* 1994). According to this method, cheese (10g) was grounded and fat extracted with a mixture of methanol: methylene chloride (1:9) followed by the evaporation of the solvent at 40 ºC under vacuum. Extracted fat was methylated according to a previously described method (Sukhija and Palmquist, 1988). Cheese free fatty acids were analyzed by measurement of their methyl esters with gas chromatography, as used by other researchers (Martin-Hernández *et al*., 1988; Türkoğlu, 2011). Gas chromatograph (Shimadzu GC-17 AAF, V3, 230V series; Shimadzu Corporation, Kyoto, Japan) equipped with flame ionization detector (FID), and fitted with a fused silica capillary column (SP-2380, 100 m×0.25 mm; Supelco Inc., Bellefonte, PA) was used to analyze fatty acid methyl esters. Hellium gas was selected as carrier gas and injector, and detector temperature was 250 °C. The initial column temperature was 40ºC for 1.0 minute and the temperature program rate was 5 ºC $min^{-1}$ and the final temperature was 240ºC. The final temperature was maintained for 10 minutes. Nonanoic acid was used as the internal standard. The standard retention times were determined by a mixture of standard fatty acids and purified known individual fatty acids. Finally, they were identified by comparing the retention times of Lighvan cheese fatty acids with those of fatty acids in the standard samples. All samples were analyzed using three replications.

## Texture Profile Analysis

Uniaxial force-compression was performed with texture analyzer (Testometric M350-10CT, England) fitted with a 0-50 kg texture analyzer to determine fracturability, or brittleness, and hardness of Lighvan cheese at different levels of lipase after 1, 30, and 90 days of ripening. A plunger, 40 mm in diameter, was used to compress the cheese cube samples (20×20×20 $mm^3$) at 30 mm $min^{-1}$ velocity to 70% of their initial height at 12±5ºC. The first break of the compression curve was considered as brittleness (Fredrick *et al.,* 1986) and the force required for compressing the sample to 70% of its initial height determined the hardness (Szczesniak, 1963). All samples were analyzed using three replications.

## Microstructure

Cheese samples were prepared for scanning electron microscopy (SEM) analysis at the beginning of the ripening (after 3 days) and at the end of it (after 90 days) according to the method described previously (Madadlou *et al.,* 2007) with some modifications suggested to prevent damage to fat globules (Karami *et al.,* 2009a). They proposed that, to preserve fat globules, cheese samples should not be washed with water/ethanol solutions and sample preparation must be done at temperatures lower than 10ºC. According to this method, cheese cubes were cut into 5 to 6 $mm^3$ cubes with a sharp razor. The cheese cubes were immersed in 2.5% (w/w) Glutaraldehyde fixative (Merck Chemical Ltd., Darmstadt, Germany) for 3 hours. The prepared cubes were refrigerated until SEM analysis. The cubes were freeze-fractured in liquid nitrogen into approximately 1 mm pieces (Sipahioglu *et al.,* 1999). After drying the cheese pieces to a critical point, they were coated with gold in a sputter-coater (Balzers, Type SCD 005, BalTec Inc., Switzerland) for 6 minutes. The samples were examined with a scanning electron microscope (XL Series, model XL30, Philips, Eindhoven, the Netherlands) operated at 15.0kV. Photomicrographs were recorded at 500 and 1000 magnification levels. The mean diameter of fat globules in three scanning electron micrographs at 1000 magnification levels was measured with microstructure distance measurement software (Manual microstructure distance measurement, Nahamin Pardazane Asia Co., University of Mashhad, Iran), which was





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previously used by Karami *et al*. (2009b) to measure the mean diameter of fat globules in Iranian ultafilterated Feta cheese.

### Statistical Analysis

The analysis of variance (ANOVA) procedure of the SAS system software, Version 6, (1990, SAS Institute Inc., Cory, NJ, USA) was used to evaluate the differences in the physicochemical properties, free fatty acids contents, brittleness, and hardness of Lighvan cheese with different levels of lipase during ripening. All experiments were done in triplicate. Mean values of the physicochemical properties, free fatty acids, brittleness and hardness of Lighvan cheese with different levels of lipase at each stage of ripening were compared with Duncan's test. Evaluation was based on a significance level of $P< 0.05$. Microsoft Excel 2007 was used for drawing the figures.

## RESULTS AND DISCUSSION

### Physicochemical Properties

Changes in pH, acidity, moisture, and salt contents of Lighvan cheese samples at different levels of lipase throughout the ripening are reported in Table 1. Examination of these data revealed that pH, acidity, moisture, and salt content of Lighvan cheese were not affected by addition of lipase. Previously, other researchers showed that the addition of lipase had no significant effect on chemical composition of cheese during ripening (Yilmaz *et al.,* 2005).

The pH of Lighvan cheeses with different levels of lipase did not change significantly in the first 30 days of ripening. This could be related to a balance between lactose fermentation and the production of amino groups by secondary proteolytic activity of bacteria (Fox, 1993). From 30 to 90 days, pH decreased significantly due to the

**Table 1**. Effect of lipase addition and ripening time on physicochemical properties of Lighvan cheese (mean±standard deviation).[a]

| Physiochemical properties | Lipase level | Age (Day) | | |
|---|---|---|---|---|
| | | 1 | 30 | 90 |
| pH | $L_0$ | 5.45±0.32a[1] | 5.74±0.29a[1] | 4.65±0.26b[1] |
| | $L_1$ | 5.42±0.36a[1] | 5.71±0.21a[1] | 4.71±0.27b[1] |
| | $L_2$ | 5.46±0.31a[1] | 5.69±0.21a[1] | 4.59±0.26b[1] |
| Titratable acidity (% w/w) | $L_0$ | 0.36±0.11b[1] | 0.38±0.14b[1] | 0.89±0.03a[1] |
| | $L_1$ | 0.38±0.14b[1] | 0.40±0.08b[1] | 0.85±0.09a[1] |
| | $L_2$ | 0.39±0.10b[1] | 0.43±0.09b[1] | 0.87±0.05a[1] |
| Moisture content (% w/w) | $L_0$ | 68.59±1.21a[1] | 61.37±1.71b[1] | 61.63±1.93b[1] |
| | $L_1$ | 68.84±1.11a[1] | 61.57±1.34b[1] | 61.75±1.12b[1] |
| | $L_2$ | 68.02±1.21a[1] | 61.72±1.91b[1] | 61.95±1.21b[1] |
| Salt (% w/w) | $L_0$ | 5.11±0.77b[1] | 8.08±0.54a[1] | 8.36±0.45a[1] |
| | $L_1$ | 5.81±0.47b[1] | 8.64±0.72a[1] | 8.57±0.41a[1] |
| | $L_2$ | 5.34±0.57 b[1] | 8.85±0.66a[1] | 8.63±0.66a[1] |
| Fat (% w/w) | $L_0$ | 13.11±0.27b[1] | 21.08±0.54a[1] | 21.00±0.44a[1] |
| | $L_1$ | 13.31±0.31b[1] | 21.64±0.72a[1] | 20.57±0.80a[1] |
| | $L_2$ | 13.34±0.21 b[1] | 17.15±0.66a[2] | 17.03±0.66a[2] |

[a] a, b, c: Means followed by the different letter within the same row are significantly different ($P< 0.05$). [1, 2]: Means followed by the different superscript within the same column for each characterization are significantly different ($P< 0.05$). $L_0$: 0.0 g lipase in 100 kg milk; $L_1$: 4.0 g lipase in 100 kg milk, and $L_2$: 8.0 g lipase in 100 kg milk. For better comparison, Lighvan cheese with no added lipase data were compiled from Aminifar *et al*. (2010).





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fermentative activity of lactic acid bacteria. Titratable acidity of all samples did not change significantly in the first month of the ripening, but increased until the end of the ripening due to lactic acid production by microbial flora of Lighvan cheese as reported previously (Kafili *et al.,* 2009).

The moisture content of Lighvan cheeses with different levels of lipase decreased significantly during the first month of the ripening, this could be related to water movement from cheese blocks to brine due to the osmotic pressure produced by NaCl in brine (Zerfiridis, 2001). After one month of ripening, due to the equilibrium between adsorption of water by amino groups produced from secondary proteolysis (Creamer and Olson, 1982) and desorption of water resulting from osmotic pressure, moisture content did not change significantly until the end of the ripening. Salt content of Lighvan cheeses with different levels of lipase increased significantly in the first month of the ripening due to salt diffusion from brine to cheese texture, and then did not change until the end of the ripening due to equilibrium.

Fat percentage (% w/w) increased during ripening of Lighvan cheese with different levels of lipase due to increase in dry matter within this period. Increase in fat content (% w/w) during cheese ripening in brine due to decrease of moisture was previously reported (Madadlou *et al.,* 2007). On day 1, addition of lipase had no significant effect on fat content (%w/w) of Lighvan cheese, while the samples with added lipase had lower content of fat (%w/w) after 30 and 90 days. This could be related to derivation of short chain fatty acids and their volatility (Collins *et al.,* 2003), which were at higher level in the samples with added lipase due to lipolysis.

## Free Fatty acid Analysis

The changes in free fatty acid (FFA) contents of Lighvan cheeses with different levels of lipase, as determined by gas chromatography during ripening, are given in Table 2.

During ripening, short chain fatty acids increased significantly at different levels of lipase. Most of the short chain fatty acids are esterified on the Sn-3 position – carbon number three of the triglyceride- of the ewe's milk triglycerides (Ha and Lindsay, 1993). Increasing trend of butyric, caproic, and caprylic acids during ripening could be related to the specificity of the milk lipase which is highly selective for fatty acids at this situation (Fox *et al.,* 1993). Besides indigenous lipase, which is the main lipolytic agent in the raw milk cheese (Türkoğlu, 2011), microorganisms with lipolytic activity, such as yeasts and enterococci found in traditional Lighvan cheese (Kafili *et al.,* 2009), and lipoprotein lipase played an important role in its lipolysis. Lavasani and Ehsani (2012) reported butyric acid as one of the most important fatty acid in probiotic Lighvan cheese inoculated with *Bifidobacterium Lactis*. Palatase containing cheeses accumulated higher amounts of short chain fatty acids compared to the control and the effect of enzyme concentration was significant ($P< 0.05$). Our results are in agreement with the results of Ozcan and Kurdal (2012), who reported that lipolysis rate of cheddar cheese was increased by lipase addition.

The FFA ($C_{12:0}$-$C_{18:2}$) increased during ripening period, particularly in the cheese made with palatase added milk. The addition of palatase had a significant effect on the accumulation of medium- and long-chain free fatty acids during cheese ripening, which was compatible with the results reported for Cimi Tulum cheese and Tulum cheese (Kilic *et al.,* 2000; Yilmaz *et al.,* 2005). Our results showed that the level of the FFAs of cheese samples with 8:0 g palatase in 100 kg milk was higher than the other cheese samples at the end of the ripening. Similar results were reported for Tulum cheese made from milk with microbial lipase (Yilmaz *et al.,* 2005).





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**Table 2.** Means±standard deviation of the free fatty acid (mg $g^{-1}$extracted fat) found in Lighvan cheeses with different levels of lipase during the ripening.[a]

| Free fatty acid | Age(day) | Lipase level (g 100 $kg^{-1}$ milk) | | |
|---|---|---|---|---|
| | | $L_0$: 0 | $L_1$: 4 | $L_2$: 8 |
| Butyric (C4:0) | 1 | 23.64±1.12c[3] | 27.14±1.10b[3] | 31.19±1.09 a[3] |
| | 30 | 31.04±1.19 c[2] | 35.41±1.11b[2] | 39.74±1.01 a[2] |
| | 90 | 41.05±0.76c[1] | 44.71 ±0.87 b[1] | 48.59±0.96a[1] |
| Caproic (C6:0) | 1 | 14.16±0.25c[3] | 18.43±0.24b[3] | 21.65±0.54a[3] |
| | 30 | 17.67±0.65c[2] | 21.64±0.91b[2] | 24.23±0.89a[2] |
| | 90 | 21.94±0.73c[1] | 23.80±0.39b[1] | 27.3±0.71a[1] |
| Caprylic (C8:0) | 1 | 7.73±0.21c[3] | 9.84±0.11b[3] | 12.92±0.21a[3] |
| | 30 | 11.57±0.21c[2] | 15.97±0.34b[2] | 18.78±0.91a[2] |
| | 90 | 15.34±0.93c[1] | 18.75±0.12b[1] | 22.95±2.21a[1] |
| Capric (C10:0) | 1 | 14.90±0.97 c[3] | 19.8±0.45b[3] | 25.3±0.87 a[3] |
| | 30 | 27.38±0.54c[2] | 32.64±0.96b[2] | 37.85±1.06 a[2] |
| | 90 | 31.61±0.75 c[1] | 35.57±0.91b[1] | 40.63±0.70 a[1] |
| Lauric (C12:0) | 1 | 16.34±0.76c[3] | 19.12±0.54b[3] | 23.5±0.59a[3] |
| | 30 | 24.67±2.21c[2] | 29.29±1.65b[2] | 32.11±1.21a[2] |
| | 90 | 31.75±0.86 c[1] | 36.91±0.49b[1] | 39.12±0.60a[1] |
| Myristic (C14:0) | 1 | 60.01±0.12c[3] | 76.11±0.7b[3] | 81.11±0.98a[3] |
| | 30 | 70.45±0.31c[2] | 84.27±1.54b[2] | 89.30±0.76a[2] |
| | 90 | 80.74±0.97c[1] | 90.68±1.21b[1] | 99.23±1.09a[1] |
| Palmitic (C16:0) | 1 | 200.38±2.98c[3] | 217.13±3.91b[3] | 229.75±1.18 a[3] |
| | 30 | 330.00±2.90 c[2] | 339.71±1.01 b[2] | 347.11±1.56 a[2] |
| | 90 | 349.02±2.11c[1] | 356.05±2.13 b[1] | 364.49±2.23 a[1] |
| Stearic (C18:0) | 1 | 55.11±0.31 c[3] | 59.12±1.20 b[3] | 64.3±1.90 a[3] |
| | 30 | 68.07±1.76 c[2] | 78.36±1.98 b[2] | 82.11±0.32 a[2] |
| | 90 | 76.09±1.07 c[1] | 82.77±1.54 b[1] | 87.91±1.65 a[1] |
| Oleic (C18:1) | 1 | 207.11±1.13c[3] | 211.12±1.11b[3] | 232.2±3.90 a[3] |
| | 30 | 216.06±4.12c[2] | 239.06±3.11b[2] | 266.48±0.90 a[2] |
| | 90 | 246.24±2.82 c[1] | 252.00±5.11 b[1] | 269.00±1.10 a[1] |
| Linoleic (C18:2) | 1 | 20.82±1.14 c[3] | 25.31±1.21 b[3] | 30.12±1.32 a[3] |
| | 30 | 29.55±1.18 c[2] | 38.72±1.53 b[2] | 44.74±2.23 a[2] |
| | 90 | 35.22±1.65 c[1] | 42.56±1.89 b[1] | 49.35±0.98 a[1] |
| Sum C4:0–C18:2 | 1 | 620.2±9.65c[3] | 683.12±15.86b[3] | 752.04±12.3a[3] |
| | 30 | 826.46±14.5c[2] | 921.07±12.5b[2] | 972.22±15.4a[2] |
| | 90 | 929.00±13.3c[1] | 960.80±13.5b[1] | 996.57±14.2a[1] |

[a] Means followed by the different letter within the same row are significantly different P<0.05). [1, 2, 3]: Means followed by the different superscript within the same column for each fatty acid are significantly different (P< 0.05).

At the end of the ripening, the quantity of long and medium chain free fatty acids in Lighvan cheese was important. Among them, palmitic acid ($C_{16:0}$) was the most abundant, followed by oleic acid ($C_{18:1}$) and myristic acid ($C_{14:0}$). Similar results are reported by Türkoğlu (2011) for Orgu cheese – Turkish raw ewe's cheese-. He showed that palmitic, oleic, myristic, and capric acids were the main free fatty acids in Orgu cheese. In spite of their quantity, they have no important role in the final cheese aroma (Freitas and Malcata, 1998). The quantity of butyric, caproic, and capric acid, which are the most abundant volatile acids in a wide variety of cheeses (Barbieri *et al.,* 1994), significantly increased in Lighvan cheese made from palatase added milk. These FFAs could play an important role in cheese aroma.





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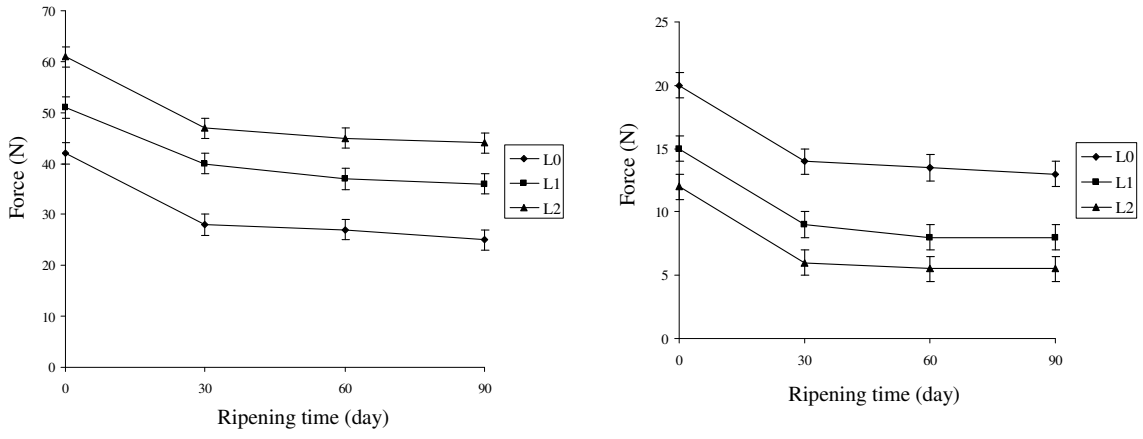
### Texture Profile Analysis

Changes in the texture profile (hardness and brittleness) of Lighvan cheese made from milk with different palatase levels are shown in Figures 1, respectively. Hardness of Lighvan cheese made from milk with different levels of lipase decreased significantly during the first month of the ripening; but it did not change significantly until the end of the ripening. Hardness of cheese was affected by proteolysis (Fredrick *et al.,* 1986) and since the studied Lighvan cheese was made from raw milk, its significant decrease of hardness during the first month of aging was due to the proteolytic activity of microbial diversity of raw milk (Kafili *et al.,* 2009). After one month of ripening, hardness of Lighvan cheese made from milk with different levels of lipase did not change significantly; this could be related to inhibitory effect of salt on proteolysis (Guinee and Fox, 1983). Hardness of Lighvan cheese was affected by the addition of lipase at all stages of ripening. Addition of lipase increased hardness of Lighvan cheese significantly and, when the concentration of lipase increased from 4 to 8 g in 100 kg milk, its hardness also increased significantly. There is an interaction and cross-links between casein matrix and fat phase which can affect textural properties of cheese due to plasticizing effect of fat (Madadlou *et al.,* 2007). Increasing the breakdown of triglycerides and the production of free fatty acids with addition of lipase leads to the disappearance of some fat globules or decreases their diameter. This fact can cause a decrease in the plasticity of cheese samples (Karami *et al.,* 2009a). Decrease in the plasticizing effect of fat results in a compact texture (Karami *et al.,* 2009a), which could be related to the harder texture (Gunasekaran and Ak, 2003)

Brittleness of all Lighvan cheeses –with and without lipase addition– decreased during the first month of the ripening, indicating that all samples became more brittle in the first month of the ripening (Larmond, 1976). This could be related to pore formation during this period (Aminifar *et al.,* 2010), and then became constant until the end of the ripening. In the presence of lipase, brittleness of Lighvan cheese decreased significantly and, therefore, the samples became more brittle, which could be related to the degradation of triglycerides to free fatty acid and the decrease in the diameter of fat globules and lower plasticity of cheese samples. This phenomenon is in agreement with the studies of other researchers indicating that the changes in




(a) (b)

**Figure 2.** Change in (a) hardness of Lighvan cheese (b) brittleness of Lighvan cheese, with different Palatase concentration ($L_0$: 0 g, $L_1$: 4 g and $L_2$: 8 g in 100 kg milk) during ripening ($P<0.05$). For better comparison, Lighvan cheese with no added lipase data were compiled from Aminifar *et al*. (2010).





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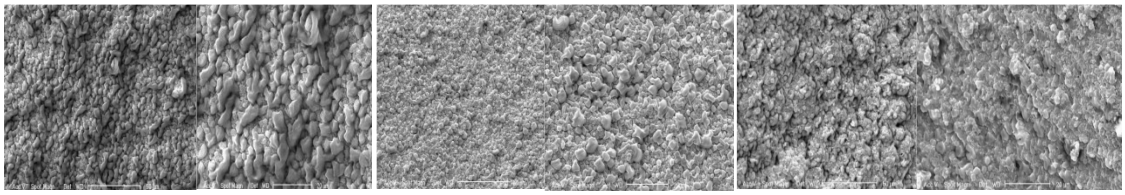
textural properties of cheese takes place as a result of changes in interaction between casein micelles and fat phase due to plasticizing effect of fat (Madadlou *et al.,* 2007).

## Microstructure

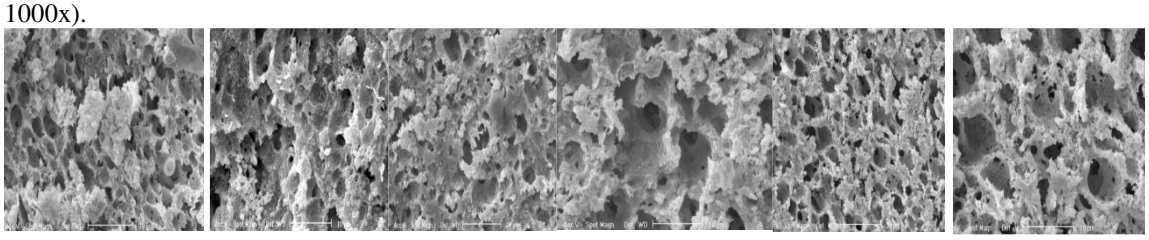
Scanning electron micrographs of Lighvan cheese with different levels of lipase after 3 and 90 days of ripening are shown in Figures 2 and 3, respectively. Figure 2 shows that the number and the mean diameter of fat globules, which were entrapped in casein network, were affected by the levels of the added lipase, after 3 days. Based on scanning electron micrographs, the mean diameter of fat globules was affected by the level of the added lipase. The mean diameter of fat globules, which was 5.20±0.45µm in Lighvan cheese with no added lipase, decreased to 3.71±0.44 µm in Lighvan cheese with 4 g lipase in 100 kg milk. When the added lipase increased to 8 g in 100 kg milk, lipolysis led to the disappearance of some fat globules, consequently, the number of fat globules decreased and also the mean diameter of fat globules decreased significantly to 2.45±0.80 µm. Besides the small fat globules that were formed by lipolysis, there were some large fat globules in Lighvan cheese with added lipase that were formed as a result of partial coalescence and aggregation of small fat globules. Ding and Gunasekaran (1998) showed that, when fat content in dairy product is high, large globules are formed because of partial coalescence and fat aggregation. Interactions between casein network and fat globules play an important role in textural properties of cheese, This could be related to plasticizing effect of fat (Madadlou *et al.,* 2007). Increase in Lighvan cheese hardness with lipase addition could be related to the decrease in plasticizing effect of fat as a result of decrease in the number and size of fat globules.

As shown in Figures 3-a and -b, 90 days ripening, there were some small fat globules in Lighvan cheese with no added lipase. Lipolysis can take place during ripening by indigenous milk lipoprotein (Jensen and Pitas, 1976) and lipolytic enzymes of psychrotrophic bacteria in raw milk (Law, 1979). Since



(a) (b) (c) (d) (e) (f)

**Figure 2**. Scanning electron micrographs of Lighvan cheese with different levels of lipase after 3 days of ripening (a: no lipase added at 500x; b: no lipase added at 1000x; c: 4 g lipase in 100 kg milk at 500x; d: 4 g lipase in 100 kg milk at 1000x; e: 8 g lipase in 100 kg milk at 500x, f: 8 g lipase in 100 kg milk at 1000x).



(a) (b) (c) (d) (e) (f)

**Figure 3**. Scanning electron micrographs of Lighvan cheese with different levels of lipase after 90 days of ripening (a: no lipase added at 1000x; b: no lipase added at 2000x; c: 4 g lipase in 100 kg milk at 1000x; d: 4 g lipase in 100 kg milk at 2000x; e: 8 g lipase in 100 kg milk at 1000x, f: 8 g lipase in 100 kg milk at 2000x).





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Lighvan cheese is produced from raw milk, lipolytic enzymes of diverse microbial flora play an important role in degradation of fat globules. Figure 3 panels (c), (d), (e) and (f), show that when lipase was added to Lighvan cheese, after 90 days of ripening, individual fat globules or their aggregates totally disappeared and some fingerprints of fat were observed. Apparently, fat was in the form of free fat within voids inside the casein matrix (Lopez *et al.,* 2007). The disappearance of fat and increase in matrix junction protein led to uniform structure of casein consisting of voids produced from fermentation. Carbon dioxide and other atmospheric gasses were entrapped in the large holes in casein matrix formed by fermentative activity of raw milk microbial flora during ripening (Fox *et al.,* 1993).

## CONCLUSIONS

The results of this research showed that free fatty acid content, texture, and microstructure of Lighvan cheese were affected by the addition of lipase during the accelerated ripening. The main changes resulting from the addition of microbial lipase were:

Accumulation of higher amounts of short, medium, and long chain fatty acids in Palatase containing cheeses compared to the control cheese and the effect of enzyme concentration is significant.

Increase in hardness and decrease in brittleness of Lighvan cheese with lipase addition, which could be related to the degradation of triglycerides to free fatty acid, decrease in the diameter of fat globules, and also lower plasticity of cheese samples.

The number and the mean diameter of fat globules which were entrapped in casein network were affected by levels of the added lipase.

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## تغییر بافت، ریزساختار و اسیدهای چرب آزاد پنیر لیقوان در طول رسیدن تسریع شده با لیپاز

**م. امینی فر و ز. امام جمعه**

## چکیده

در این تحقیق، اثر اضافه کردن سه غلظت متفاوت از لیپاز میکروبی (۰، ۴ و ۸ گرم در ۱۰۰ کیلوگرم شیر) بر ویژگیهای فیزیکوشیمیایی، مقدار اسیدهای چرب آزاد، بافت و ریزساختار پنیرلیقوان در طول رسیدن مطالعه شده است. اضافه کردن لیپاز pH ، اسیدیته، درصد رطوبت و نمک پنیر لیقوان را تحت تاثیر قرار نمی دهد ولی سبب افزایش معنی دار اسیدهای چرب آزاد می شود، همچنین اضافه کردن آنزیم سبب افزایش سختی و کاهش بریتلنس پنیر در تمام مراحل رسیدن می شود. تعداد و قطر متوسط گلبولهای چربی که در شبکه کازئینی به دام افتاده اند، تحت تاثیر لیپاز اضافه شده می باشند. زمانی که لیپاز به پنیر لیقوان اضافه می شود، در پایان ۹۰ روز رسیدن، گلبولهای چربی و تجمعات آنها ناپدید شده و فقط آثار آنها مشاهده می شود. ناپدید شدن گلبولهای چربی همراه با افزایش اتصالات کازئینی، سبب ایجاد ساختار یکنواخت از کازئین به همراه حفرات ناشی از تخمیر می شود.





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