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# Modeling lipolysis in acceleration of ripening of ultrafiltered-feta cheese

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**Abstract**

In this research encapsulated *Aspergillus niger* Lipase was used for accelerated ripening of ultrafiltered-Feta cheese. Four grams of Lipase enzyme, encapsulated lipase, encapsulated lipase accompanied with Arabic Gum was added to 100 kg of ultrafiltered retentate. Fat content, acid degree value and sensory evaluation were measured during 60 days of ripening. Fat content was significant at $p < 0.05$ level in all treatments during ripening . Maximum value of lipolysis was acquired in day 15. Treatments with lipase enzyme created accelerated lipolysis and treatments with encapsulated lipases resulted in gradual lipolysis. Linear regression models were appropriate models to find the relation between development of lipolysis and fat content during ripening($R^2$>80%). Evaluation results of sensory evaluation showed that encapsulated lipase achieved higher score for appearance, odor and texture compared to other treatments.

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## Introduction

Feta cheese is a type of white brine cheese prepared from cow, sheep milk or a mixture of sheep and goat milk and is kept in brine in sliced pieces. It tastes a bit bitter, acidic and salty and has soft, smooth and creamy textures which make it easy to cut. There is no gas bubble inside and its color is white both on top and on sides and lacks hard overlying membrane(Kosikowski and Mistry, 1997).Cheese ripening means applying physical, chemical, biochemical, and microbiological changes in crud resulting in improvement in taste and texture. In scientific point of view, ripened cheese is the product kept at high or low temperature (depending on type) for a long time such that bacteria and enzymes can convert fresh crud into a cheese with special odor and certain appearance. Ripening period varies from zero for fresh cheese to two years for hard ripened cheeses. Biochemical changes of cheese in ripening period is divided into two categories; primary ripening: lipolysis, proteolysis, metabolism of remaining lactose, lactate and citrate, and, secondary ripening: metabolism of free fat acids and free amino acid. Free fat acids and free amino acid are very important compounds for cheese flavor (Kosikowski and Mistry , 1997; McSweeny, 2004).Lipolysis is one of the main biochemical reactions in cheese ripening. Free fat acids accompanied with volatile compounds and products from proteolysis cycle have direct effect on cheese smell and taste(Sousa and McSweeney, 2001). Lipolysis content in different types of cheese varies from low values in Holland cheese to very high values in mold-ripened cheeses (Walstra *et al.,* 2006). The parameters in creation of lipolysis in cheese are: fat-breaking enzymes which naturally exist in milk, milk lipase, rent (pregastric estrase) and microbial flora. The milk lipase involvement in lipolysis depends on heating of milk and pasteurization decreases its activity. In pasteurization process at 72 °C for 15 seconds, lipase activity decreased up to 83%. After heating at 78 °C for 15 seconds, 100% of its activity will be reduced. Lipase activity remarkably decreases and the cheese produced from such concentrated heated milk absolutely will have a very small lipolytic activity (Fox *et al.,* 2000; Castillo *et al.,* 2007). Ripening process is slow, time-consuming and expensive. The common methods that can shorten cheese ripening time are: increasing the temperature level during ripening period, use of microorganisms (El-Soda *et al.,* 1986) and using enzymes (Alais, 1984). Due to this reason, exogenous lipase (EC3.1.1.3), one of the most important enzymes in biological systems, is used for triglyceride hydrolysis to glycerol and fatty acids and acceleration of ripening process. For further stability of enzyme, enzyme immobilization process is carried out using a suitable matrix. This process is effective for reducing financial costs. The best method for lipase enzyme immobilization is using hydrophobic materials and sol-gel technique. Third and fourth structures of protein of trapped enzyme are preserved in sol-gel method, thermal and chemical stability is increased and the effect of inhibitory ingredient is reduced (Guisan, 2006). Due to low lipolysis in Iranian cheese, the purpose of the present research assessment of the ripening acceleration of ultrafiltered Feta cheese by encapsulating *Aspergillus niger* fungus lipase and determination of the optimum model using fat content and acid degree value during ripening process and sensory evaluation. Accordingly, determination of the appropriate model can provide a good solution for product improvement and lowering the costs (reduction of storekeeping expenses).

## Materials and methods

*Materials*

All materials for encapsulation of lipase were provided from Sigma-Aldrich, Fluka ,Acros Organic and Merck. Lipase from *Aspergillus niger* (Lipase Activity ≥ 120000 U/g) was purchased Sigma-Aldrich. Raw cows' milk ,equipment and Ultra filtration were supplied by Pegah dairy company (Shiraz, Iran).The starter cultures include *Lactococcus lactis ssp. Cremoris* and *lactis* (DM-230) and thermophilic *Lactobaciuls delbrueckii ssp. Bulgaricus* and *Streptococcus thermophilus* (Y-502) obtained from Danisco Deutschland GmbH (Alemanha, Germany) and rennet obtained from *Rhizomucor miehei*(≥2200

International Milk Coagulating Unit (IMCU) $g^{-1}$) of DSM Food Specialities (Seclin, France) were used.

*Methods*

*Encapsulation of lipase in sol–gel matrices*

*Aspergillus niger* Lipase (150 mg) was mixed with 390µl buffer of Tird/HCl(0.1M) with the pH 7.5 in a tube ,then 50 mg of Celite, 100 µl of 4% Polyvinyl alcohol aqueous solution, 50µl of 1M Sodium Fluoride aqueous solution, 100µl of Isopropanol was added and mixed with by vertex shaker. For encapsulated lipase to gather with Arabic Gum, 100µl of Arabic Gum was added to mixture.74 µl of 2.5mM Methyltrimethoxysilane (MTMS) and 76 µl of 0.5mM Tetrametoxysilane (TMOS) was added and mixed with by vertex shaker. Gelatinization was observed, then drying process was continued for 24 hours .After gel was washed by 10ml of water, 10-15ml of Isopropanol, 10ml of n-Pentane and distilled water and was scraped by a spatula. Encapsulated lipase dried at room temperature(25 ± 1 °C). At the end Encapsulated lipase and Encapsulated lipase to gather with Arabic Gum was produced(Guisan , 2006).

*Ultrafiltered-Feta cheese making*

The initial milk was clarified. It was bactofugeal to separate the microorganisms and milk fat was standardized to 3.3%. Then milk was pasteurized at 75°C for 15 seconds and cooled at 50 °C. Ultrafiltration process was applied and water, minerals, and lactose were separated by tubular membrane filters to prepare a retentate to 34% (w/w) total solid. The resultant retentate was homogenized at 55°C and pressure of 50bar. Then the produced retentate was pasteurized at 78°C for 1 minute. In order for renneting process to be done, milk temperature was reduced to 37°C. Throughout the process, 1% of lactic starter was added and pH value reached 6.2 and then 30 mg/kg of rennet and encapsulated lipase, encapsulated lipase together with Arabic Gum and lipase enzyme (4g lipase /100Kg retentate) were added. Immediately the containers were filled with the curd and brine (13%) and pass through coagulation (gelation) gel tunnel where the temperature was 37°C (for 30 min) to form curd. Then parchment papers were placed on curds, finally containers were sealed. pH value reached at 4.8. The pre-ripening stage completed at 37 °C in about 20 hours. And the cheese was transferred to cooling storage at 4°C(Bylund ,1995;Hesari et al., 2006 ).

*Fat analysis*

Fat hydrolysis was estimated by measurement of the Acid degree value that ADV was explained as the number of milli equivalents of alkali needed to neutralize the FFA in 100 g of fat (Marshal, 1992).

*Sensory evaluation*

The sensory properties of cheese treatments were evaluated, at 60 days by 40 trained panelists at the Shiraz Pegah Dairy Processing Company .The cheeses were reached to room temperature (25 ± 1 °C) and were cut into small cubes (20×20×20) mm. Deionized water used for palate removing between sensory evaluation of samples .The scores of flavor, body , texture, appearance and odor ,were performed very good (5), good(4), acceptable(3),bad(2) and very bad(1)( Salari and Mortazavi , 2008).

*Statistical analysis*

All the results were explained as the mean of three replicates. The data produced were analyzed by SPSS software (version 20). Analysis of variance (ANOVA) was used to examine the effect of type of lipase (lipase enzyme, encapsulated lipase and encapsulated lipase together with Arabic Gum) and the ripening period (15, 30, 45,and 60 days) on the changes of fat ,acid degree value and sensory evaluation .The least significant difference test used was $P < 0.05$ . Regression analysis was used to describe the relationship between the changes of fat and acid degree value.

**Results and discussion**

*Changes of fat content*

Figure 1 are shown Changes of fat Content during ripening. In all treatments, fat content had a significant difference at $p < 0.05$ during ripening but

there was no significant different between treatments with lipase enzyme and encapsulated enzyme in day 30, 45 and 60. Over the time during cheese ripening, the fat hydrolysis increased in presence of lipase enzyme compared to control treatment and reduced the plasticizer effect of fat. Protein layers slide onto each other and became closer to form a strong gelatin (Dimitreli and Thomareis, 2007). Similar results reported in research works on Changes of fat Content. Cheddar cheese samples having recombinant encapsulated amino peptide had no significant difference at p >0.05 during ripening [16] .Similar opinions reported by Kheadr *et al.* ( 2003 ) and Ong *et al.* ( 2006).

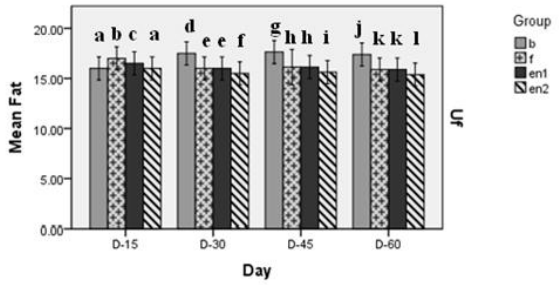


**Fig. 1.** Chenges of fat on Ultrafiltered Feta Cheese treatments. b, Control(no enzyme added); f, Lipase;en1, Encapsulated lipase; en2, Encapsulated lipase together with Gum Arabic ,throughout ripening (Day: 15, 30, 45 and 60).

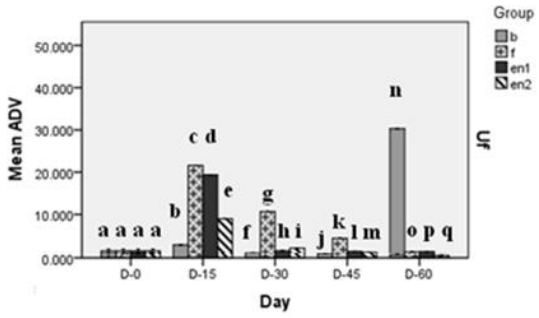


**Fig. 2.** Chenges of acid degree value(meq KOH 100 g fat_1) on Ultrafiltered Feta Cheese treatments. b,Control (no enzyme added); f, Lipase;en1, Encapsulated lipase; en2, Encapsulated lipase together with Gum Arabic, throughout ripening (Day: 15, 30, 45 and 60).

*Lipolysis progress evaluation*

Figure 2 are shown changes of acid degree value in ultrafiltered -Feta cheese during ripening. During cheese ripening, milk fat is affected by the lipase enzymes and its flavor and smell improve by production of free fatty acids. Acid degree value index was used to evaluate lipolysis intensity in the present research. In all the treatments of ultrafiltered -Feta cheese, there was significant difference at p <0.05. During ripening, acid degree value increased from day 1 to day 15 and it decreased from the 15$^{th}$ to 60$^{th}$ day. Maximum acid degree value was respectively acquired in the 15$^{th}$ day for treatments with lipase enzyme (7.8156 ± 7.9724), encapsulated lipase (7.4754 ± 4.9115) and encapsulated lipase together with Arabic Gum (3.2426± 2.7836) in milli equivalent of potassium hydroxide per 100 grams of fat. The obtained acid degree value in the current research were higher than the values reported for Teleme cheese made from cow milk (Mallatoua *et al.,* 2003 ) and Urfa cheese from cow milk during ripening period. Acid degree value and free fatty acids inside the samples containing encapsulated Probiotic was greater than control treatments during ripening period (Zomorodi *et al.,* 2010). Similar results were obtained between encapsulated lipase and encapsulated lipase together with Arabic Gum samples and control sample during the present research. Lipase enzyme caused intensive lipolytic activity but activity of encapsulate lipase changed by adding additives to the gel leaded to difference in enzyme accumulation and its distributed in the gel. Encapsulated lipase without additives have smaller thickness compared to encapsulated lipase with Arabic Gum and they generated gradual and suitable lipolytic activity in cheese but encapsulate lipase with tetramethoxysilane and methyltrimethoxysilane accompanied with Arabic Gum generated gel with small pores that limited substrate transformation and created less lipolytic activity (Soares Cleide *et al.,* 2006). Since lipase is sensitive to heat, they are inactive in cheese prepared from pasteurized milks. Thus, the main reason of lipolysis in such cheeses was exogenous lipase enzyme, encapsulate lipases, encapsulated lipase with Arabic Gum and starter enzymes. Increase of free fatty acids and clod salt concentration probably had a preventive effect on lipase activity, and as a result, a restricted effect on

lipolysis. Irregular changes and reduction of acid degree value result from transformation of fatty acids to compounds like methyl ketones or for provided energy required for fungus (Madkor *et al.,* 1986).

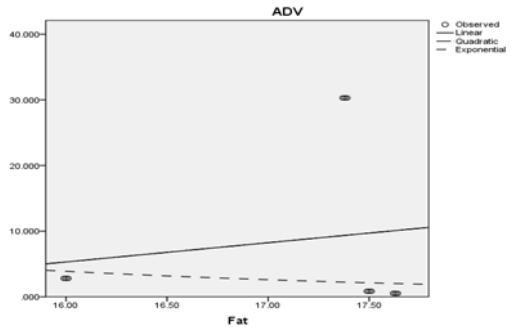


**Fig. 3.** Selection of acceptable model on control treatment of Ultrafiltered Feta Cheese, – linear regression model and --- exponential model.

*Model selection*

The following models were derived in determination of relationship and correlation between fat content during ripening period and lipolysis progress. The treatments with lipase enzyme ,encapsulated lipase and encapsulated lipase together with Arabic Gum followed the linear regression model ($y = a+bX_F$) but the control treatment could not be correlated with linear regression model due to increase in acid degree value on day 60. However, a linear model was achieved after omitting the 60$^{th}$ day data (Figure 3). Increase of ripening time was linearly correlated to fatty acid generation. Change of physical position of milk fat during the ripening period (Carunchia Whetstine *et al.,* 2006), absorption of casein proteins of cheese water onto surface of fat globules during the cheese production lead to restriction of lipolytic activity of lipase enzyme against the fat substrate (Michalski *et al.,* 2001). Secondary metabolism of fatty acids and amino acids affected by starters, non-acid lactic bacteria, mold, yeast, and chemical reactions leading to generation of carbon dioxide, amin, thioester, hydrogen sulphide, keto acid, ammonia, fatty acid esters, phenyl ester, alcohol, aldehyde, lactone, and thiol (Kosikowski and Mistry , 1997; McSweeny, 2004) ,influence decrease and increase of correlation and determination coefficient between fat content and lipolysis progress (Table 1). Analogous results were reported by Salari *et al.* (2008) and Vitro *et al.* (2003).

*Sensory evaluation*

There were significant differences for sensory evaluation such as appearance, smell, flavor, texture, and overall acceptance of ultrafiltered Feta cheese treatments at $p > 0.05$. The highest scores were achieved for: appearance (based on general macroscopic characteristics), flavor (based on chewing and waiting for a couple of seconds and letting out breathe), texture, and overall acceptance for encapsulated lipase treatment of ultrafiltered Feta cheese (Table 2).

**Table 1.** Linear regression fitted of Ultrafiltered Feta Cheese treatments throughout ripening (at 60Days).

| Treatments | $y=a+bX_F$ | $R^2$ |
|---|---|---|
| Control | ADV =24.601-1.36(Fat) | 99.7% |
| Lipase enzyme | ADV = -252.3+16.1(Fat) | 82.5% |
| Encapsulated lipase | ADV = - 496.3 +31.1(Fat) | 85.7% |
| Encapsulated lipase together with Arabic Gum | ADV = - 206.7+13.4(Fat) | 85.9% |

Encapsulated lipase treatment had high score for appearance , odor and texture compared to other treatments, but overall acceptance was similar for encapsulated lipase and lipase enzyme.The sensory evaluation improved as a result of gradual effect of encapsulated lipase, the homogenization process (in ultrafiltered-Feta cheese), and reduction of size of fat globules. Decomposition and conversion of chemical compounds directly affect the sensory evaluation (Fox *et al.,* 2000). With increase of ripening time, lipolysis, lipolysis progress, and generation of short-chained free fatty acids during ripening period, flavored compounds were released and this flavor was more favorable to the consumers.

**Table 2.** Sensory evaluation of Ultrafiltered Feta Cheese treatments throughout ripening (at 60Days ).

| | Sensory attributes | | | | |
|---|---|---|---|---|---|
| Treatments | Appearance | Texture | Odor | Taste | Overall acceptance |
| Control | 3.35 ± 1.8 | 3.95 ± 0.75 | 2.08 ± 1.4 | 2.07 ± 1.2 | 3 ± 0.4 |
| Lipase enzyme | 3.75 ± 0.89 | 3.69 ± 0.91 | 2.42 ± 1.1 | 3.4 ± 0.76 | 3.36 ± 0.72 |
| Encapsulated lipase | 4.1 ± 1 | 4.02 ± 0.75 | 2.57 ± 1.3 | 2.73 ± 1.1 | 3.38 ± 0.73 |
| Encapsulated lipase together with Arabic Gum | 3.3 ± 1.1 | 3.57 ± 1.1 | 2.36 ± 0.9 | 2.45 ± 1 | 2.99 ± 0.67 |

## Conclusions

In analysis of effect of encapsulated lipases on acceleration of lipolysis in ultrafiltered-Feta cheese, it was demonstrated that an extensive lipolysis occurred with high acid degree value in 15$^{th}$ day of 60-day ripening period. Cheese fat changes during ripening, cheese production method, type and amount of starter, and the materials used in immobilization of lipase all affect the lipolysis rate. The linear regression model exhibited the best match for changes of fat content versus acid degree value during ripening of ultrafiltered-Feta cheese. Lower value of correlation coefficient in the treatment having lipase enzyme compared to encapsulated lipases was due to intensified lipolysis that leaded to decomposition and conversion of fatty acids. During the ripening period, release of flavored compounds from milk increased and sensory evaluation improved. With regard to the long time needed for ripening, it seems that use of some methods such as addition of encapsulated lipase enzyme can accelerate this stage and contribute to improvement of the process.

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