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Enhancing the lipolysis of feta-type cheese made from ultrafiltered cow's milk

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ABSTRACT

This study was conducted to investigate the effects of adding different levels of commercial pregastric lipase enzyme (calf lipase) on the free fatty acid profile of ultrafiltered Feta type cheese during ripening period. With an increase in added lipase level and ripening period, the main components of cheese including total solids, fat, fat in total solids, salt and salt in total solids did not change significantly but water soluble nitrogen increased. Increasing the added pregastric lipase level resulted in a significant decrease in the percentages of $C_{4:0}$-$C_{8:0}$ free fatty acids while that of $C_{12:0}$-$C_{18:0}$ and $C_{18:1}$ free fatty acids increased. According to sensory analysis of cheese samples, the best samples had the combination of 20, 40 and 60 days of ripening with 6 g/100 kg, 4 g/100 kg and 2 g/100 kg lipase in retentate, respectively. The addition of lipase to cheese milk could be recommended for the acceleration of flavor development and ripening of UF-Feta cheese over a short ripening period.

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1. Introduction

Lipolysis is one of the major biochemical changes that occur during cheese ripening (McSweeney & Sousa, 2000). Fatty Acids (FAs) are released usually by the actions of lipases (from different sources) during lipolysis (Deeth, 2006). They contribute directly to cheese flavor, particularly when they are properly balanced with products of proteolysis and other reactions (Collins, McSweeney, & Wilkinson, 2003). However, extensive lipolysis is considered to be undesirable for some cheeses (Molimard & Spinnler, 1996). Short-chain fatty acids contribute directly to aroma in many ripened cheese varieties (Urbach, 1997). In addition to their direct impact on cheese flavor, FAs also act as precursor molecules in a series of catabolic reactions that lead to the production of other flavor compounds such as methyl ketones, esters, and thioesters (McSweeney & Sousa, 2000). Furthermore, analysis of the short- and medium-chain FAs has been suggested as an index for characterizing cheeses over the ripening period (Collins et al., 2003).

The lipolytic agents in cheese include lipolytic enzymes that are naturally present in milk (milk lipase), rennet (pregastric esterases (PGE)) and microflora (Collins et al., 2003). The contribution of milk lipase to cheese lipolysis depends on the heating temperature/period of cheese milk. At UF-Feta cheese production, the used milk is pasteurized twice (first 72 °C for 15 s and again 80 °C for 30 s) and this heating can affect lipase activity. It has been reported that lipase activity was reduced 83% during pasteurization (72 °C for 15 s) and 100% after heating for 15 s at 78 °C (Deeth, 2006; Rao & Renner, 1989). Therefore, it can be assumed that a second thermal treatment of milk during the production of UF cheese (such as heating the pasteurized milk at 50 °C before and during ultrafiltration and re-pasteurization of retentate after homogenization) will cause a further inactivation of lipases; and certainly, heating the UF concentrate should be responsible for the reduced lipolytic activity in the produced cheese (Rao & Renner, 1989). Lipoprotein lipase (LPL) hydrolyzes FA from the *sn-1* and *sn-3* positions in tri-, di- and monoglycerides to give FA, which may have significant contribution in cheese flavor (Collins et al., 2003; Deeth, 2006). Karami, Ehsani, Mousavi, Rezaei, and Safari (2008, 2009a, and 2009b) with direct observation of fat hydrolysis using scanning electron microscopy (SEM) images indicated that, lipolytic enzymes produced by added lactic acid bacteria (LAB) as starter, in the absence of indigenous milk lipase, were the main lipolytic agents. There are several varieties of cheese in Iran such as white pickled, Lighwan (or Liqvan), Jugi (Kuzei) and UF-Feta cheese. UF-Feta cheese has the highest consumption and production per capita in Iran. More than 70 plants produce high quality UF-Feta cheese but some properties such as proteolysis, lipolysis, secondary metabolites and microbial attributes need to be investigated. In this regard, some companies (such as APV, Denmark) recommend the addition

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of lipase to cheese milk (Mistry & Maubois, 1993), but some producers do not add lipase to the UF cheese because they do not have enough information about benefits and conditions of enzyme addition. UF-Feta cheese made from bovine milk is manufactured in modern dairy plants using pasteurized milk with mesophilic starter cultures and commercial rennet. The cheese has a homogeneous structure (without openings), a brittle, crumbly and rather soft consistency, and white color (Karami, Ehsani, Mousavi, Rezaei, & Safari, 2009a, 2009b, 2008). UF-Feta cheese is made from homogenized ultrafiltered milk retentate, at a total solid content of 395 g/kg (for Feta with 40% fat in dry matter) (Mistry & Maubois, 1993). Rennet and starter are added to the retentate and the cheese can be cast directly. The minimum NaCl content is 20 g/kg, but it is typically around 40 g/kg (Karami et al., 2009a, 2009b, 2008) and the pH value is as low as 4.2–4.5. The main characteristics of retentate used for the production of this type of cheese are: minimum total solids of 340 g/kg, 11% protein, 15% fat, 27 °brix, maximum acidity of 42 and pH of 6.20–6.65 (Karami et al., 2009a, 2009b, 2008). Feta made in this way contains most of the whey proteins from the original milk. This may constitute about 15% of total protein of the cheese, while in traditionally-made cheeses, whey proteins account for only 1.0–1.5% of total protein (Mistry & Maubois, 1993). To date, there is not enough information about fatty acid composition of UF-Feta cheese and contribution of lipolysis on its chemical and sensorial characteristics. Reviewing the literature on the FA composition of Feta cheese showed that most of the published results have mainly investigated cheeses made by using the traditional process or without modern technology of ultrafiltration and with the milk of goats or ewes or mixture of their milk (Akin, Aydemir, Kocak, & Yildiz, 2003; Georgala et al., 2005; Katsiari, Voutsinas, Alichanidis, & Roussis, 2000; Mallatou, Pappa, & Massouras, 2003). Frequently, UF-Feta cheese batches are stored 15–20 days at cold storages and then sent to market, because of ripening and texture development. The expiration date of UF-Feta cheese is 60 days after production, therefore, shortening of ripening period with better flavor and texture, have many advantages for both manufacturers and consumers. The studies on FAs contents of UF-Feta cheese are limited and the discussed lipolysis pattern relates to other types of milk or with traditional production conditions, thus the objective of this study was to investigate the effects of added calf lipase on the acceleration of UF-Feta cheese ripening and changes of main cheese components as a result of lipolysis.

2. Materials and methods

2.1. Materials

The starter cultures used in this study, DM-230 and Y-502, were obtained from Danisco Deutschland GmbH (Alemanha, Germany). Rennet [Fromase® 2200 TL granulate (≥2200 International Milk Coagulating Unit (IMCU)/g)] as a microbial coagulant from *Rhizomucor miehei* was provided from DSM Food Specialities (Seclin, France). Pregastric lipase, was used as a powder extracted from frozen epiglottis glands from fresh-slaughtered calves with a strength of 1.2 μkat/g was obtained from Chr-Hansen (Bayswater, Australia). Raw cows' milk, APV full concentrating apparatus (APV, Denmark), and filtration module with spiral wound polymeric ultrafiltration filters were supplied by Hamedan Pegah dairy company (Hamedan, Iran).

2.2. Methods

2.2.1. Cheese making

Experimental cheese samples with different amounts of lipase (L1, L2, L3, L4 with 0, 2, 4 and 6 g lipase/100 kg retentate, respectively) were produced and analyzed during different ripening periods (3, 20, 40 and 60 days) in 3 replicates (three cheeses for each ripening period each analyzed three times) according to the method of Karami et al. (2008; 2009a; 2009b). A schematic of the method used for the production of UF-Feta cheese samples are presented in Fig. 1. After the bactofugation, pasteurization (76 °C × 15 s), ultrafiltration, homogenization, and re-pasteurization (80 °C for 30 s), retentate with volume concentration factor of 5.4 kg milk to 1 kg retentate and about 370 g/kg of total solids, entered the starter tank, where by adding the starter (1 g/1000 kg retentate) and maintaining the temperature at 30 °C, the pH value of milk reached to 6.2. Then, in the filling machine, rennet (2 g/100 kg retentate) and lipase (0, 2, 4 and 6 g/100 kg retentate) were mixed with salt solution (15 g/L) and added to each cheese container. For the better solubility of rennet and more activity of lipase, it must be dissolved in salt solution. Coagulation tunnel, which was set at 37 °C for 30 min, allowed the retentate to convert to a pre-cheese mixture. In the sealing machine, 4.2 g/kg salt was added to the parchment paper on the top of cheese and then it was covered with aluminum foil to seal the container. After these stages, pre-cheese samples stored at 20 °C for about 20 h until their pH value reached to 4.8. This pH value is necessary to proceed fermentation process, the growth of starter cultures, and prevention of the growth of unwanted microorganisms. At the end of production, the samples transferred to a cold-room and maintained at 9 ± 1 °C for 3–60 days, during which physico-chemical analysis and profiling of free fatty acids were performed. Though at this temperature, the ripening reactions such as lipolysis slow down, but to prevent extra bacterial activity, proteolytic, lipolytic, and glycolytic reactions and maintaining cheese for 60 days, this temperature is necessary.

2.2.2. Physicochemical analysis

The cheese samples were analyzed for moisture content by heating to a constant weight using a Sartorius moisture analyzer (Sartorius Ltd., Epsom, UK) at 100 °C for about 30min (Lee, Rankin, Fonseca, & Milani, 2014). Fat content was measured according to British standards institution (1995) method that in this method, cheese fat content is determined using Gerber method. Salt content was determined according to a procedure described by Kirk and Sawyer (1991, pp. 101–105). A Knick 766 calimatic pH-meter (Niels Bohrweg, Utrecht, The Netherlands) was used for measuring the pH of cheese samples. Total nitrogen (TN) was measured using the Kjeldahl method (IDF, 1993) and water soluble nitrogen (WSN) using a method proposed by Alizadeh, Hamedi, and Khosroshahi (2006) as indices of proteolysis. According to this method, 20 g of cheese were homogenized with 100 mL water in a stomacher for 5 min and the suspension was kept at 40 °C for 1 h. After incubation, the insoluble solids were separated by centrifugation at 4 °C for 30 min at 1084 g. The supernatant was filtered through glass wool and the nitrogen content in the filtrate was determined by the Kjeldahl method.

2.2.3. Free fatty acids analysis

The extraction procedures carried out using the method described by De Jong and Badings (1990). According to the method, all FAs ($C_{4:0}$-$C_{18:3}$) were extracted from cheeses using chloroform and methanol at 70:30 ratios, respectively, and methylated in chloroform without concentrating or use of high temperature. In the extraction phase, ethyl ether was used for the interruption of fat globule membrane. All the extraction stages were carried out in cold temperature to avoid the loss of volatile FA, especially butyric acid. FA profiles were determined using gas chromatography according to a method described by Kim et al. (2014) with some

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