Acceleration of Swiss cheese ripening by microbial lipase without affecting its quality characteristics

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Abstract The effect of exogenous microbial lipase enzyme on the ripening of Swiss cheese (0, 200, and 800U lipase in 30 L milk) was investigated for the physico-chemical, textural and sensory properties, along with its microstructure. The aim of this study was to investigate the application of microbial lipase to accelerate the ripening without affecting its original desirable quality characteristics. The effect of the microbial lipase was studied at different time periods (2, 30, 45, and 60 days) of the Swiss cheese ripening stages. Statistical analysis of the results showed that the physico-chemical parameters of cheese slightly increased during the ripening. Also, at all stages of the ripening hardness of Swiss cheese increased while the brittleness decreased. The number and size of the fat globules were also affected by the addition of the lipases. This study also showed that increase in the lipase amount had no significant change in quality and sensory parameters. Therefore, 200U of lipase was found to be sufficient to reduce the ripening time from 90 to 60 days by maintaining its genuine quality. Thus, this study suggested that the addition of microbial lipase may significantly reduce the cost of the cheese production by lowering the ripening period by 1 month and maintaining the quality of the final product.

Keywords Lipase · Cheese ripening · Microstructure · Physico-chemical characteristics · *Bacillus tequilensis* PR13

Introduction

Lipases (triacylglycerol acyl hydrolase, EC 3.1.1.3) are a group of enzymes that hydrolyze the triglycerides to mono and diglycerides, glycerol and free fatty acids (Sharma et al. 2001; Barron et al. 2004; Kumar et al. 2013). Lipases are ubiquitous and are produced by various sources such as plants, animals, and microbes. Among the different sources of the lipases, only the microbial sources have high interest due to their commercial significance. Microbial lipases are cheaper, easier to produce, and their enzyme contents are more predictable and controllable (Kumar et al. 2013).

Microbial lipases are mostly extracellular and have received particular attention concerning their industrial production and broad applications in the processing of fats and oils, detergents and degreasing formulations, food processing, chemical and pharmaceuticals, paper mills, etc. (Houde et al. 2004). However, the most significant industrial applications of lipases have been mainly found in the food, detergent, and pharmaceutical sectors (Houde et al. 2004). In the food industry, carboxylate esters, lipophilic molecules that are released by the action of lipases, have been used as flavoring ingredients. In dairy products, both lipases and esterases are commonly used to produce desired flavors (Moskowitz et al. 1977). Taste and flavor depend on the type of fatty acids chain released during the action of lipase. Short chain fatty acids (C4–C8) impart a cheesy taste and the concentration of long chain fatty acid should be low to avoid the soapy taste (Peng et al. 2014).

The changes in the flavor of mature cheese are because of biochemical actions occurring in the curd during ripening which is accomplished by the interaction of starter bacteria and enzymes from milk, coagulant, and the secondary microbiota (Esteban-Torres et al. 2014). As a result of this, the rubbery or elastic curd is converted into a

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smooth-bodied and fully flavored cheese. Lipase-producing organisms including yeast, lactobacilli, and micrococci contribute to lipolysis during cheese ripening (Aravindan et al. 2007). Free fatty acids (FFAs) are usually released by the actions of lipases (from different sources) during lipolysis. These enzymes contribute directly to cheese flavor, particularly when these enzymes are properly balanced with products of proteolysis and other reactions (Yilmaz et al. 2005). The typical flavor constituents are the methyl ketones, especially 2-heptanone, which are produced by the beta-oxidation of the FFAs (Revah and Lebeault 1989). FFAs also act as precursor molecules for the production of other flavor compounds such as methyl ketones, esters and thioesters (Urbach 1997; McSweeney and Sousa 2000). Short-chain fatty acids themselves contribute directly to the aroma in many ripened cheese varieties (Urbach 1997). In addition to its direct impact on cheese flavor, lipases alone or in combination with the protease increase the lipolysis process (Fernandez-Garcia et al. 1994). However, extensive lipolysis is considered to be undesirable for some cheeses (Fox et al. 1995; Molimard and Spinnler 1996).

Cheese ripening is a slow and very complex phenomenon involving a broad range of complicated biochemical process, which is costly due to extended storage time (Beresford et al. 2001). Since ripening is an enzymatic process, increasing the activity of key enzymes could be effective if added exogenously to milk or curd. Glycolysis, proteolysis, and lipolysis are the primary biochemical reactions in the development of flavor in cheese during the ripening. Enzymes of microflora—either exogenous or endogenous contribute to proteolysis and lipolysis during processing and ripening of cheese (Cinbas and Kilic 2006). In the traditional manufacturing method of cheese, apart from starter culture, naturally present microorganisms in milk rely upon fermentation.

The principal pathways for the formation of flavor compounds in cheese are because of the metabolism of lactose and lactate. In the first pathway, depending on variety, microflora, and ripening conditions, lactate may be converted to various compounds that contribute to cheese flavour (El Soda 1993). The second pathway generates fat-derived compounds that are formed by lipolysis and lipid oxidation reactions, such as free fatty acids, esters, lactones, and ketones, with low aroma thresholds (Delgado et al. 2010). The cheese aroma is due to volatile compounds formed during ripening by action of enzyme.

Many studies have been carried out on the addition of lipase to blue cheese ripening (Jolly and Koslowski 1978) and Ras cheese (Omar et al. 1986) however there is no detailed scientific study about lipolysis of Swiss cheese produced from cows' milk and moreover with specific reference to reduction of ripening period. Therefore, in present study we have focussed on reduction of production

cost by reducing the ripening period without affecting its quality characteristics. Swiss cheese produced using the cows' pasteurized milk and its physico-chemical, texture, sensory and microstructure characteristics were determined during a storage time of 0–60 days.

Materials and methods

Materials

Tributyryn agar base, NaOH, NH_4Cl , KH_2PO_4 , Na_2HPO_4 , CaCl_2 , MgSO_4 , MnCl_2 , FeSO_4 (Hi-Media, Mumbai, India), *p*-nitrophenol, silver nitrate and olive oil, *p*-Nitrophenyl Palmitate (pNPP) (Sigma-Aldrich Co. Ltd., Germany) were procured. All other reagents and chemicals used were of analytical grade.

Microorganism

The alkalophilic bacteria were isolated from an oil contaminated soil sample that produced extracellular lipase when grown at 37 °C and pH 9.0. It was identified to be *Bacillus tequilensis* PR13 by 16S DNA sequencing. The isolated organism was maintained on nutrient agar slants at pH 9.0.

Qualitative test for lipase activity

The qualitative lipase produced by microbial strain was determined by using the method described by Kumar et al. (2013) and Samad et al. (1989). The isolated microbial culture was point inoculated on the Tributyrin agar medium (pH 9.0) containing 1% Tributyrin. After incubation for 48 h at 37 °C, a zone of clearance was observed due to hydrolysis of Tributyrin by lipase. Lipolytic activity was also checked on Victoria blue agar plate containing 1% Tween 80 and olive oil with Victoria Blue B as an indicator.

Production of microbial lipase

Minimal salt medium (MSM) was used as a production medium (w/v) as follows containing: 0.4% NH_4Cl , 0.47% KH_2PO_4 , 0.0178% Na_2HPO_4 , 0.001% CaCl_2 , 0.001% MgSO_4 , 0.001% MnCl_2 , 0.0015% FeSO_4 and 2% groundnut oil cake as a sole source of carbon. After autoclaving, the pH of the production medium was adjusted to 9.0 using sterile 10% Na_2CO_3 and was inoculated with 10% of fresh inoculum containing 2×10^8 cells/mL and incubated at 37 °C with constant shaking at 100 rpm for 4 days. After incubation, the cells and media components were removed by centrifugation at 5000 rpm for 10 min; the cell free

supernatant obtained was used as a source of enzyme for further experiments and the enzyme activity was tested using the various assays.

Lipase assay

Titrimetric method

Titrimetric lipase assay was used to determine the activity of enzyme according to the method described by Kumar et al. (2013). Lipase assay was carried out by a titrimetric method using olive oil as a substrate. Olive oil (5% v/v) was emulsified with Gum Arabic (5% w/v) in 50 mM Potassium Phosphate buffer pH 7.0. The suitably diluted enzyme was incubated with the emulsified substrate and the reaction was carried out at 37 °C for 1 h. The reaction was stopped by addition of Acetone: Ethanol mixture solution (1:1). The amount of fatty acid liberated by lipase action was estimated by titrating with 0.05 M NaOH using phenolphthalein as an indicator. One unit of lipase activity was defined as the amount of enzyme required to hydrolyze 1 μmol of fatty acids per minute (1 mL of 0.05 M NaOH is equivalent to 100 μmol of fatty acids liberated per minute). (Hasan et al. 2009).

Spectrophotometric method

The enzymatic activity was determined by the spectrophotometric method, according to the methodology described by Winkler and Stuckmann (1979) using *p*-Nitrophenyl Palmitate (*p*NPP) as the substrate. 10 mL of isopropanol containing 30 mg of *p*NPP was mixed with 90 mL of 0.05 M phosphate buffer, pH 7.0, containing 207 mg sodium deoxycholate and 100 mg of gum Arabic. 2.4 mL amount of this freshly prepared solution was pre-warmed at 37 °C and then mixed with 0.1 mL of the enzyme. After 15 min of incubation at 37 °C, the released *p*-Nitrophenol was measured at 410 nm against an enzyme-free solution. One enzyme unit was defined as 1 μmol of *p*-nitrophenol enzymatically released from the substrate $\text{mL}^{-1} \text{min}^{-1}$ at standard assay conditions.

Experimental plan

The study was conducted to test the effect of exogenous addition of lipase on Swiss cheese ripening. Swiss cheese samples were treated separately in triplicates as follows with different levels of microbial lipase (T_0 , T_1 , and T_2 with 0.0U, 200U, and 800U lipase in 60 litres milk, respectively). These experiments were performed in collaboration with Mr. Benny at ‘La Ferme,’ Auroville, Puducherry. Cheese ripening was carried out using—optimized assay at the commercial plant in the town. In

collaboration with “La Ferme” cheese ripening experiments were carried out at the commercial plant following the cheese production protocol.

Cheese making

For the production of cheese, the raw milk was collected by the commercial manufacturer from the local area. The standard protocol of the ‘La Ferme’ cheese industry was followed (Fig. 1). The raw milk was pasteurized at 62.8 °C for 30 min. For coagulation of milk standard starter cultures and rennet were used. Furthermore microbial lipase was added at different levels in different sets (T_0 : No lipase addition, T_1 : 200U, and T_2 : 800U). After coagulation, the whey was drained from the coagulum by cutting it into small pieces and transferring them to rectangular cotton bags. Afterward, sodium chloride crystals were strewn on the surfaces of curd cubes and mixed uniformly. For efficient whey drainage, cotton bags were compressed after 2 h with a 4 kg mass for 24 h. The cubes were then kept for 3 months at an average temperature of 4–7 °C. During the entire ripening period, test samples (in triplicates) of 50 g were drawn from each set for the comparative study and

Fig. 1 Flowsheet of the experimental plan for the Swiss cheese manufacture

analysis of physico-chemical properties, textual characterization, and microstructure of Swiss cheese.

Physicochemical analysis

Cheese samples from all three sets (each set in triplicates) were analyzed for various parameters at different time periods (2, 30, 45 and 60 days) during the ripening process. The pH of Swiss cheese samples was measured by an EU TECH pH meter and moisture content according to the procedure described by Şengül et al. (2014). Titrability, fat, and ash contents were determined according to method described by AOAC (2000). Volhard method was used to determine salt content of cheese samples (Rajković et al. 2010). For all the analyses, sample in triplicates from each set were used to find out suitable results.

Color analysis

The color was analyzed using Hunter lab colour QUEST II Minolta Chroma Meter CR-400. The equipment was standardized using a white tile and black glass. The cheese sample was filled into the glass sample cup for the analysis. Color of the cheese sample was expressed in terms of L^* , a^* , b^* and ΔE^* parameters indicating lightness/darkness, redness/greenness, yellowness/blueness and total difference of color, respectively. Total difference of color (ΔE^*) was calculated as follows (Sert et al. 2014):

$$\Delta E^* = (\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2$$

where ΔL^* , Δa^* and Δb^* are the differences between the color parameter of the samples and the color parameters of the white standard ($L^* = 96.94$, $a^* = -0.12$, $b^* = -0.27$) used to calibrate the instrument.

Texture analysis

Warner–Bratzler Blade was used to shear the cheese samples. The texture was analyzed by using a Texture Analyser (Lloyds Instruments Ltd, Hampshire, England). One cheese sample ($2.5 \times 1 \times 1$ cm) was sheared under the following experimental conditions: Load cell 50 kg, cross head speed 50 mm/min.

Sensory analysis

Sensory analysis was carried out by following the method of Sert et al. (2014). To get unbiased information the sensory evaluation of the 30, 45 and 60 days old cheese samples from all three sets was carried out by ten panelist members using double blind fold method. The members were requested to give their honest opinions about various attributes of cheese like flavour (odour and taste), body and

texture, saltiness, mouth feeling, and acidity and they were requested to give scores between 0 (the worst) and 10 (the best quality).

Microstructure

Cheese samples of 2, 30, 45 and 60 days of ripening were lyophilized to remove the water content and were cut into small pieces for microstructure analysis using Scanning Electron Microscope (SEM) to observe the casein matrices, fat globules, aggregated fat globules and non-fat globules. Samples were viewed with a scanning electron microscope (HITECH S-3400 N) operated at 5.00 kV. Images were recorded at 500 ×, 1000 ×, 2000 × magnification levels.

Results and discussion

Qualitative test and activity of microbial lipase

The *Bacillus tequilensis* PR13 culture was plated onto the tributyrin agar base for the screening of their lipolytic activity. The clear zone of hydrolysis was observed, indicating maximum lipolytic activity. The lipolytic activity of the isolate was also confirmed using Victoria Blue B, methyl red or rhodamine B as indicators (Samad et al. 1989). The enzyme activity of 48 h cell free supernatant was estimated to be 0.8 U/mL using the titrimetric method. In a photometric method, enzyme activity was calculated spectrophotometrically using pNPP assay and found to be 95.4 U/mL.

Physico-chemical analysis of the developed Swiss cheese

Concerning the cheese yield, the addition of microbial enzyme was found more effective as compared to the control (T_0) in Swiss cheese manufacture. Physical properties of the developed Swiss cheese were studied on the basis of its dry matter (% w/w), fat (% w/w), fat in dry matter (%), salt (% w/w), titratable acidity (Lactic acid), and pH. Examination of these data revealed that pH, acidity, moisture, and salt content have been considerably affected by the different treatment due to the addition of lipase enzyme. The results obtained from the three replicates are discussed in detail as follows.

Physico-chemical parameters

The moisture content of Swiss cheese with different levels of lipase decreased during the first month of ripening, which is consistent with the study on Lingshan cheese by Aminifar and Emam-Djomeh (2014). It is possibly due to

the reduced hydration of casein as pH values reaching an isoelectric point and the evaporation of moisture from the cheese through the pores of the casein material (Sert et al. 2014). After 1 month, there was no significant difference observed in all three sets with respect to each other. Creamer and Olson (1982) also reported that after 1 month of ripening, no significant changes in moisture content was observed, this may be due to the equilibrium between adsorption of water by amino groups produced by secondary proteolysis and desorption of water resulting from osmotic pressure. This may be the result of loss of the moisture, which might be associated with the more porous structure of the sample.

The titratable acidity of the samples from all sets increased gradually up to 45 days, and thereafter declined. An increased acidity index up to 90 days has been reported by Yilmaz et al. (2005) on Tulum cheese and Lighvan cheese by Aminifar and Emam-Djomeh (2014). This may be due to the reduced growth of starter culture during the storage period. Maximum acidity observed in T_1 sample, which might be related to the increase in *Lactobacillus* strain when compared with T_0 and T_2 samples.

In this study, there was no significant difference in the pH level with a change in the lipase level along the ripening period which is in accordance with Karami et al. (2009). Despite the slight variation had been observed after 45 days which is believed to be necessary to maintain the good quality of cheese (Karami et al. 2009).

The salt content increased only up to first 30 days of storage with no significant changes found with increasing lipase units. The result of our study are in agreement with the study of Aminifar et al. (2010) on Lighvan cheese. Constancy in the salt content of the cheese after the first month of storage can be allied to the equilibrium attained after 30 days of ripening.

The ash content of cheese samples with the different levels of lipase increased with storage period. The results of the present study are in accordance with those of Sert et al. (2014) on Tulum cheese. However, desired level of mineral was achieved only within 60 days of ripening process for the cheese with 200U of (T_1) which otherwise takes period of 90 days under normal conditions.

The total fat content of Swiss cheese increased up to 30 days in all the different levels of lipase addition. Madadlou et al. (2007a) and Madadlou et al. (2007b) have previously reported that there was an increase in fat content (% w/w) during cheese ripening due to a decrease in moisture content. Further, reduction in fat content was observed after 30 days in lipase treated samples (T_1 and T_2). The results are quite different from those reported by Aminifar and Emam-Djomeh (2014) on Lighvan cheese, Sert et al. (2014) and Yilmaz et al. (2005) on Tulum cheese. The process of lipolysis might be responsible for

the derivation of short-chain fatty acids and their volatility. Therefore, lipolysis by lipases might play a significant role in the reduction of the total fat content of the Swiss cheese during the ripening period.

Examination of data revealed that the exogenous addition of lipase did not affect pH and salt content of Swiss cheese studied, whereas the moisture, acidity, ash, and fat content of Swiss cheese changed by the addition of lipase. The physical properties of 90 days old sample which is the actual ripening period of Swiss cheese were more similar with the cheese treated with 200U of lipase for 60 days.

The results of the physico-chemical characterization of Swiss cheese samples throughout its ripening period are summarized in Table 1.

Color analysis

Color values of Swiss cheese changed during storage time (60 days) in all the three sets of experiment. Though samples drawn from all three sets (one control and two tests) showed decrease in brightness during storage, bright color intensity produced from T_2 sample was found to be highest. L^* value varied between 73.72 and 87.2 in cheese produced from 800U lipase level (T_2) while it varied between 70.09 and 85.56 in T_1 sample and in T_0 sample it showed variation between 65.63 and 76.14 (Fig. 2a).

On the contrary, the redness value of cheese was found to increase during storage and led to the observation that it was affected only by low levels of 200U lipase enzyme (Fig. 2b). Red color intensity of T_1 sample (200U lipase) was found to be the highest with a^* value ranging between 1.87 and 3.69.

No significant difference was observed between test and control samples in terms of yellow color intensity up to 60 days of ripening (Fig. 2c) that is b^* value of cheese for the control and test samples was not found to be significantly different.

However, increase in yellow color intensity was noted in Swiss cheese with the different levels of lipase. The results of the present study are in agreement with the findings of Rohm and Jaros (1996) and Sert et al. (2014) who reported that there is a decrease in the L^* value and an increase of a^* and b^* values during ripening of Emmental cheese and Tulum cheese respectively.

Texture analysis

Changes in the texture profile (hardness) of Swiss cheese with different levels of lipase enzyme are shown in Fig. 3. The hardness of cheese increased during ripening period in case of all three experimental conditions. Maximum hardness was found in samples treated with lipase than the control samples. This occurrence is common for other

Table 1 Physico-chemical characteristics (Moisture content %, Dry matter %, Titratable acidity %, pH, Ash %, Salt %, Fat %) of Swiss cheese from experimental sets: (T₀: no enzyme; T₁ (200U enzyme); T₂ (800U of enzyme) during ripening period at various time intervals of: days 2, 30, 45, 60 and 90

Property	Days	T ₀	T ₁	T ₂
Moisture content (%)	2	50.89 ± 2.5	51.75 ± 2.1	48.22 ± 0.2
	30	44.89 ± 2.0	47.07 ± 1.2	46.44 ± 0.1
	45	43.18 ± 1.5	46.78 ± 0.8	46.06 ± 0.1
	60	43.02 ± 1.0	46.48 ± 0.5	46.05 ± 0.2
	90	44.89 ± 1.2	N.D.	N.D.
Dry matter (%)	2	49.11 ± 2.4	48.43 ± 1.0	51.78 ± 0.8
	30	55.10 ± 1.8	52.66 ± 1.0	53.55 ± 0.6
	45	56.82 ± 1.9	53.22 ± 1.0	53.94 ± 0.4
	60	56.98 ± 2.0	53.52 ± 1.2	53.95 ± 0.5
	90	55.11 ± 2.0	N.D.	N.D.
Titratable acidity (%)	2	0.09 ± 0.01	0.115 ± 0.0	0.12 ± 0.01
	30	0.24 ± 0.02	0.29 ± 0.01	0.20 ± 0.01
	45	0.32 ± 0.02	0.39 ± 0.01	0.33 ± 0.02
	60	0.29 ± 0.01	0.30 ± 0.02	0.29 ± 0.02
	90	0.26 ± 0.01	N.D.	N.D.
pH	2	5.80 ± 0.2	5.78 ± 0.02	5.73 ± 0.01
	30	5.75 ± 0.2	5.74 ± 0.03	5.70 ± 0.02
	45	5.76 ± .0.1	5.75 ± 0.01	5.72 ± 0.02
	60	5.28 ± 0.1	5.10 ± 0.03	5.57 ± 0.01
	90	5.25 ± 0.1	N.D.	N.D.
Ash (%) (dry wt. basis)	2	6.39 ± 0.02	7.27 ± 0.03	9.28 ± 0.2
	30	7.91 ± 0.03	4.48 ± 0.02	5.10 ± 0.1
	45	9.81 ± 0.04	11.68 ± 0.05	7.78 ± 0.2
	60	11.49 ± 0.03	10.09 ± 0.01	9.77 ± 0.2
	90	10.38 ± 0.1	N.D.	N.D.
Salt (%)	2	3.01 ± 0.01	2.08 ± 0.0	2.09 ± 0.01
	30	3.00 ± 0.02	3.00 ± 0.01	3.01 ± 0.01
	45	3.00 ± 0.01	3.03 ± 0.02	3.01 ± 0.01
	60	3.01 ± 0.00	3.02 ± 0.01	3.00 ± 0.02
	90	3.01 ± 0.01	N.D.	N.D.
Fat (%) (dry wt. basis)	2	21.19 ± 1.0	32.47 ± 2.0	40.37 ± 2.0
	30	41.42 ± 1.5	41.47 ± 0.5	41.82 ± 0.4
	45	43.37 ± 1.2	33.79 ± 1.0	30.33 ± 1.6
	60	22.74 ± 2.0	30.39 ± 1.0	24.63 ± 1.4
	90	28.52 ± 2.4	N.D.	N.D.

T₀: Control set; with no exogenous addition of microbial lipase, T₁: Experimental set with exogenous addition of 200U of lipase, T₂: Experimental set with exogenous addition of 800U of lipase N.D.: Not determined

cheeses as well indicating that the changes in textural properties of cheese take place as a result of changes in the interaction between casein micelles and fat phase due to plasticizing effect of fat (Madadlou et al. 2007a, b). Decrease in the plasticizing effect of fat results in a compact structure, probably resulting in the harder texture (Karami et al. 2009). Examination of the data obtained clearly showed that the required hardness of Swiss cheese could be achieved by lipase treatment in 60 days instead of

90 days of ripening period by routine procedure that doesn't involve lipase addition.

Sensory analysis

Ten panellist members evaluated the cheese samples drawn from three experimental sets at different time intervals during the ripening period for the various attributes of cheese like: texture, taste and aroma, salinity, odour, acidity, mouth feeling and overall acceptability.

Fig. 2 The changes in color values in Swiss cheeses during storage A.: Lightness, a^* : redness, b^* : yellowness of the cheese samples drawn from three experimental sets (T₀: No exogenous lipase addition; control, T₁: Exogenous addition of 200U of lipase, T₂: Exogenous addition of 800U of lipase) during ripening period at the time intervals of 2, 30, 45, 60 and 90 days and analysed for various colour parameters using Hunter lab colour QUEST II Minolta Chroma Meter CR-400

Fig. 3 Texture analysis of Swiss cheese: T₀: no lipase; T₁: 200U of lipase enzyme; T₂: 800U of lipase enzyme

Differences between the three sets was sensed and judged by the panellist members in the double blind experiment and the scores given by them were used for analysis.

Changes if any in sensory properties of Swiss cheese as judged by the panellist members are presented in Fig. 4.

Texture properties developed during storage were scored maximum 6.9 in T₁ sample. Taste and aroma of fresh Swiss cheeses produced from cow milk scored between 6.4 and 6.8; cheeses from T₁ set were liked more during all the ripening stages. Salinity parameters of cheese were 6.2–7.1. Mouth feeling of cheese was described to be good during ripening period; cheeses produced from T₁ lipase level were liked more in terms of these parameters. During storage, less fatty taste was determined in lipase treated samples. In ripened cheeses, 6.8 average scores were determined in cheese from T₁ set.

During storage, acidity in cheese produced from lipase treated sample (T₂) was graded to be less sensible. This result also showed that an appropriate concentration of lipase is required to obtain the desirable flavour but would not cause undesirable rancid off-flavors as excessive lipolysis is considered undesirable in many varieties such as Dutch-type cheeses, Cheddar cheese, and Emmental and cheeses containing even a moderate level of free fatty acids (FFAs) may contribute to sour flavor (Sert et al. 2014).

Fig. 4 The changes of sensory properties in Swiss cheese during ripening; the scores are calculated as an average of the individual scores given by ten panellist members in double blind experiment. T₀: no lipase; T₁: 200U of lipase enzyme; T₂: 800U of lipase enzyme

In fresh cheeses, overall acceptability was found to be highest for cheese from T₁ set (with 200U of lipase). Predilection of panellist members for cheese samples from T₁ set increased with increase in the ripening period and the cheese sample drawn on 60 days of ripening from T₁ set were given maximum score and unanimously graded as the most desirable amongst all three sets of double blind experiment.

Fig. 5 Ultrastructure of Swiss cheese: SEM images of cheese samples at $\times 1000$ on Day 2, 30, 45 and 60 from three experimental sets: (T₀: no enzyme; T₁ (200U enzyme); T₂ (800U of enzyme) respectively

Microstructure

Microstructure obtained by SEM images of Swiss cheese samples from three sets of experiment (control and two tests) at 2, 30, 45, and 60 days of ripening periods are shown in Fig. 5 that shows the porous structure of casein network and the fat globules entrapped inside the matrix. The number and the diameter of fat globules are affected by addition of lipase which was seen even just after 2 days.

On day 30 as shown in Fig. 5 with the addition of enzyme, the number of fat globules and their distribution areas reduced. This could be due to hydrolysis by the added lipase that led to the disappearance of some fat globules. Consequently, the number of fat globules was decreased but irregularity in fat globules shape got increased. Besides the small globules, large fat globules were also formed as a result of coalescence and aggregation of small fat globules. These kinds of changes have been noticed in case of Lighvan cheese ripening by Aminifar and Emam-Djomeh (2014). The interaction between casein network and fat globules are important in determining textural properties of cheese that give plasticizing effect of fat (Madadlou et al. 2007a). The increased hardness of Swiss cheese after lipase addition could be consequence of the plasticizing effect of fat.

After 45 days of ripening (Fig. 5), no apparent changes in individual fat or aggregated fat globules were observed. However, on day 60 some small fat globules were observed in Swiss cheese with no added enzyme which can be

attributed to hydrolytic activity of starter culture during ripening. Studies reported by Collins et al. (2003), showed that levels of added starter culture to the samples consequently produced esterases from lactic acid bacteria (LAB).

The present study showed that the activity of the lipase enzyme was found to be active only up to 45 days and thereafter, no further change was found in cheese samples. Aminifar et al. (2010) reported that during cheese aging, porosity in the cheese also increases significantly.

It appears that cheese porosity had maximum change during the first month of aging and this change could be related to a diverse microbial population during this period. Changes in cheese porosity could also affect the salt transport profile. It is well-known fact that the salt transport profile can influence ripening behaviour since salt content affects the microbial and biochemical processes (Aminifar et al. 2010). The protein and fat components are responsible for the resulting texture and functionality of cheeses.

In resume, the Swiss cheese with same desirable properties was produced only in 60 days by exogenous addition of 200U of lipase enzyme in 60 litres of pasteurized milk. Therefore this led to lowering the total production cost reducing the ripening period by 1 month.

Conclusion

The results of the present study showed that an appropriate level (200U) of microbial lipase reduced the Swiss cheese ripening period from 3 to 2 months. This was achieved with no compromise on the desirable physico-chemical properties of the final product. Thus, an addition of lipase would result in decreased production cost by reducing ripening period without affecting quality characteristics the Swiss cheese therefore the enzyme could be used for the cost-effective production of the Swiss cheese.

Further, analysis with free fatty acid and volatile fatty acids can prove the role of the enzyme in flavor enhancement.

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