KOREAN J. FOOD SCI. TECHNOL
Vol. 22, No. 3, 248~254 (1990)

Effects of Food Grade Porcine Pancreatic Lipase on the Production of Short-Chain Fatty Acids and its Contribution

Hae-Soo Kwak, Ike J. Jeon* and Joung-Nam Park
Seoul Dairy CO-OP, Institute of Dairy Food Research
**Department of Animal Sciences and Industry, Kansas State University*

식용 돼지췌장 리파제가 저급지방산 생성과 체다치즈 풍미향상에 미치는 영향

곽해수·전익준*·박정남
서울우유협동조합 기술연구소, *캔사스주립대학교 축산학과

Abstract

Commercial food grade porcine-pancreatic lipase was incorporated into cheese at two different levels of concentration and ripened at 7°, 13° and 21°C. Gas chromatographic analysis showed that the pancreatic lipase-treated cheese produced significantly higher levels of short-chain free fatty acids than controls. At 21°C the high level of pancreatic lipase-treated cheese produced medium flavor cheese at 1 wk and close to sharp flavor cheese at 3 wk without causing distinctive defects. The low level of pancreatic lipase-treated cheese developed a number of good quality cheese. They were roughly equivalent to medium and sharp cheeses when ripened at 7°, 13° and 21°C for 3 to 15 wk. Statistical analyses indicated that there were significant correlations between aged Cheddar flavor and the concentration of C6 as individual short-chain free fatty acids (FFA) or C4 and C6 FFA combinations. Pancreatic lipase may be applicable for the accelerated ripening of Cheddar cheese if appropriate conditions are used.

Key words: porcine-pancreatic lipase, Cheddar cheese, free fatty acid, sensory evaluation

Introduction

The accelerated ripening of Cheddar cheese has been considerably studied due to potential economic benefits. Most studies were done by incorporating various enzymes into milk or cheese curds during cheese manufacturing. It was reported that addition of ruminant-animal and microbial lipases to Cheddar cheese slurries gave pronounced cheese flavor but also gave strong rancidity, whereas microbial acid proteases and decarboxylases produced considerable bitterness[1]. It was also reported by same authors that addition of small controlled amounts of lipase and neutral protease developed cheese flavor rapidly without causing distinctive flavor defects.[2] Another investigation was recently observed that an adding insoluble microbial lipase from *Aspergillus oryzae* in combination with a soluble microbial neutral protease to milk during cheese manufacturing accelerated reasonably good Cheddar flavor development.[3]

The literature indicates that free fatty acids (FFA) might constitute the backbone of Cheedar flavor.[4] Among FFA, short-chain fatty acids (C4 through C10) were considered to be particularly important. Therefore, production of short-chain FFA profiles identical to those in naturally aged cheese was investigated by a number of researchers for the acceleration of Cheddar flavor development.[2,4]

The objectives of this study were to investigate the effects of food grade porcine-pancreatic lipase with different concentrations on the production of short-chain free fatty acids, and flavors in cheese at

Corresponding author: Hae-Soo Kwak, Seoul Dairy CO-OP, Institute of Dairy Food Research, Banwol Industrial Complex, Kyonggi-do, 425-110

regular and elevated ripening temperatures, and to study statistical relationships among flavor development and short-chain fatty acids.

Materials and Methods

Enzyme

Food grade pancreatic lipase (Amer. Lab. Inc., Omaha, NB) was selected based on the study by Kwak.[5]

Assay for lipase activity

The pH stat method of Chandan and Shahani (1963) was used to determine lipase activities. The detailed procedures were described by Kwak.[5]

Cheese sample preparation

Granular cheese was manufactured in the Department of Animal Sciences and Industry dairy plant at Kansas State University. Standard procedures were followed in cheese making using 600 gallon pasteurized milk and Hansen's Red-Set DVS starter culture. Enzyme was added to the granular curds using salt (2.5%) as a vehicle before hooping and pressing. The amount of the lipase added per 11.4 kg of cheese curds were 0.445 g for low-pancreatic lipase and 0.890 g for high-pancreatic lipase-treated cheese. After pressing overnight, cheeses were cut to pound-size blocks, vacuum packaged, and stored at 7 °C for 20 wk, 13 °C for 15 wk, and 21 °C for 10 wk.

Chemical compositional analysis

All cheese samples were analyzed for moisture, fat, protein, and salt according to the Methods of Association of Official Analytical Chemists.[6]

Analysis of free fatty acids

One gram samples of maturing cheese were removed periodically at 1, 3, 6, 10, 15, and 20 wk, minced, and extracted with ether and hexane for 2 hr and eluted through a 10 mm i.d. glass column containing neutral alumina according to the method utilized in elsewhere.[5] The column containing alumina with absorbed free fatty acids (FFA) was dried under vacuum and transferred to a stoppered glass tube. One m*l* isopropyl ether containing 6% formic acid was added and mixed with the alumina. The tube was centrifuged at 2,000 rpm for 5 min at room temperature and 1 μl aliquot of the supernatant was injected into gas chromatography (GC). A Hewlett-Packard Model 5880A GC equipped with a flame chromatography (GC). A Hewlett-Packard Model 5880A GC equipped with a flame ionization deterctor and GC Terminal (Level Four) integrator were used for the analysis of individual FFA. The preparation of FFA was achieved using a 91 cm × 2 mm i.d. glass column packed with 10% SP-216-PS on 100/120 Supelcoport (Supelco Inc., Bellefonte, PA). The GC was operated with nitrogen carrier gas at 40 m*l*/min, hydrogen gas 30 m*l*/min, and air at 400 m*l*/min. The column oven was programmed at three temperature levels; initial holdingfor 2 min at 90 °C, first level holding to 100 °C at 15 °C/min, holding for 0.5 min; second heating to 180 °C at 10 °C/min, holding for 15 min; third heating to 195 °C/min, holding for 15 min. Both temperature for injector and detector was 230 °C. All quantitative analyses were done by relating each peak area of individual FFA to the peak area of tridecanoic acid as an internal standard. Each FFA was identified by the retention time of a standard mixture.

Organoleptic flavor analysis

Rectangular cheese samples (1 cm × 5 cm × 1 cm) were prepared periodically (1, 3, 6, 10, 15 and 20 wk) and tempered at room temperature for one hour prior to taste evaluation. A panel of five judges, experienced in judging dairy products, evaluated the cheese samples throughout the study. Aged Cheddar flavor was scored on a 9 point scale (1 = flat or curdy; 9 = extra sharp), and lipolyzed and bitter flavors were scored on a 5 point (1 = none; 5 = extremely). Panelists were encouraged to make additional comments on other flavor defects.

Data analysis

Statistical correlations were done by utilizing PROC CORR and PROC STEPWISE subcom-

mands of SAS software programs[7]

Results and Discussion

Chemical composition

Both experimental and control cheese samples used for analysis were similar in moisture (34.74%), fat (36.13%), protein (25.15%), and salt (1.42%).

Production of short-chain FFA

The production of short-chain FFA (C4, C6, C8, C10) in the control and experimental cheese ripened at 7°, 13°C, and 21°C are shown in Figs. 1, 2, and 3, respectively. Since pancreatic lipase is nonspecific in hydrolyzing milk fat as is natural lipase in cheese[16], the releasing pattern of short-chain FFA from the lipase-treated cheese is expected to be similar to that of the control[5]. At 7°C, capric acid (C10) was at the highest concentration in the lipase-added cheese, while butyric acid (C4), caproic acid (C6), and caprylic acid (C8) were the next in decreasing order (Fig. 1). Similar trends of the releasing pattern of FFA wee shown in Fig. 2 and Fig. 3. The concentrations of short-chain FFA were increased about twice in low pancreatic lipase (LPL)-treated cheese during 20 wk ripening periods, whereas high pancreatic lipase (HPL)-treated cheese showed about three times increase. However, the control cheese showed little increase. HPL-treated cheese produced higher concentrations of FFA than LPL-treated samples in respective fatty acids. At 13°C, the production of FFA was rapid at early stage of HPL-treated cheese up to 6 wk but was slow after 10 wk, whereas LPL-treated cheese was moderate in FFA production (Fig. 2). However, the concentration of FFA ap-

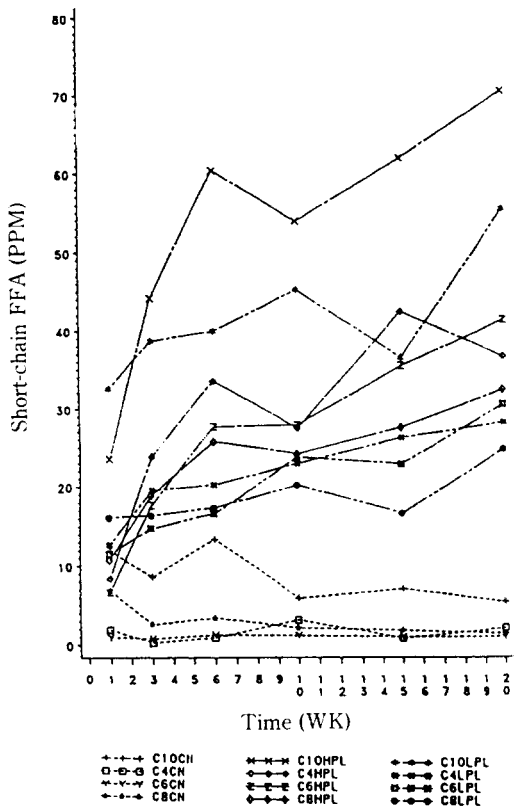

Fig. 1. Production of short-chain free fatty acids in control (CN) and low and high levels of pancreatic lipase (LPL, HPL)-treated cheese at 7°C for 20 wk

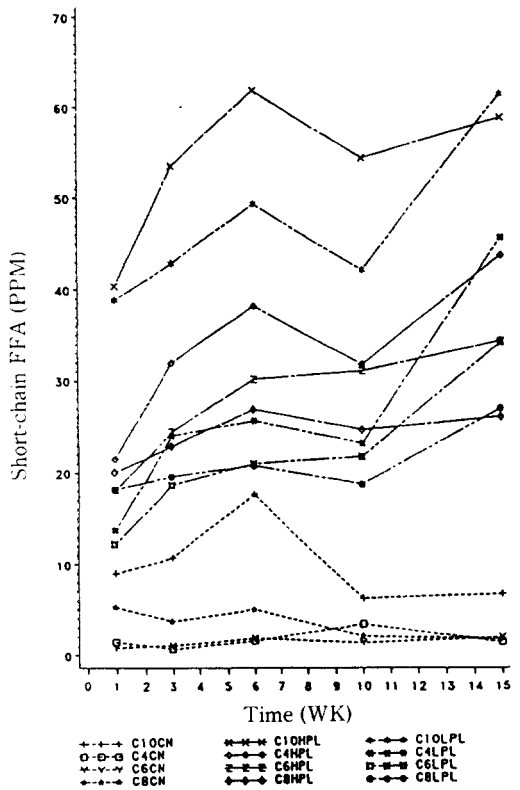

Fig. 2. Production of short-chain free fatty acids in control (CN) and low and high levels of pancreatic lipase (LPL, HPL)-treated cheese at 13°C for 15 wk

peared to be similar at the end of 15 wk between HPL-treated and LPL-treated samples. The production of short-chain FFA both in HLP- and LPL-treated cheeses was accelerated more at 13 °C than at 7 °C. It was also observed similar trends of the acceleration of FFA production in lipase-added cheese in other study[4]. At 21 °C, the production of all short-chain FFA was rapid between 1 and 6 wk but showed no further increases thereafter (Fig. 3). HPL-treated cheese showed higher concentrations of respective FFA than LPL-treated cheese (except C8). The rate of short-chain FFA produced was much higher at 21 °C than at 7° and 13 °C. However, control cheese did not show increase in FFA concentrations at 21 °C. These results indicate that different levels of the pancreatic lipase in experimental cheese had significant effects on the production of short-chain FFA at various repening temperatures.

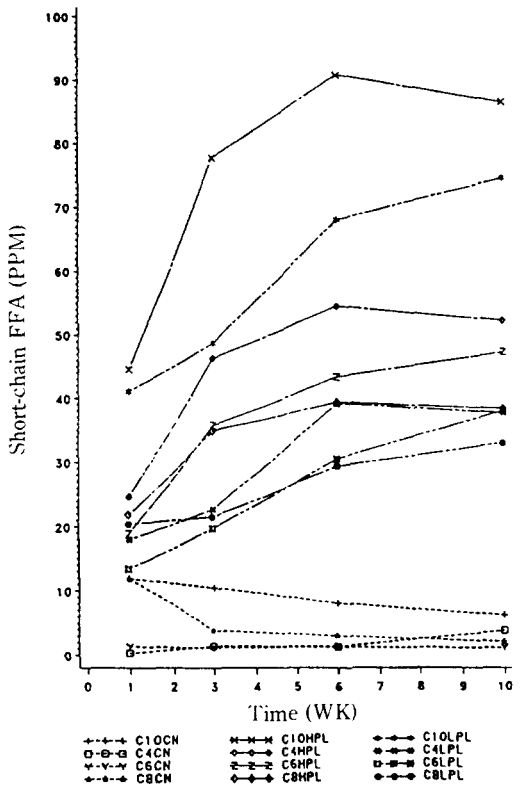

Fig. 3. Production of short-chain free fatty acids in control (CN) and low and high levels of pancreatic lipase (LPL, HPL)- treated cheese at 21 °C for 10 wk

Organoleptic flavor analysis

Sensory scores of aged Cheddar and lipolyzed flavors of control and lipase-treated cheese ripened at 7°, 13°, 21 °C are shown in Fig. 4 and Fig. 5, respectively. In HPL-treated cheese showed good accelerations in aged Cheddar flavor development (Fig. 4). LPL-treated cheese also showed faster Cheddar flavor development than that of the controls. It was reported that the addition of ruminant-animal origin lipases accelerated the Ras cheese flavor development.[8] In aged flavor development, ripening temperatures also played important roles. Sensory scores were consistently higher with the samples ripened at higher temperatures than ripened at low temperatures within the treatment. At 13° and 21 °C, Cheddar flavor development of HPL-treated cheese increased rapidly between 1 and 6

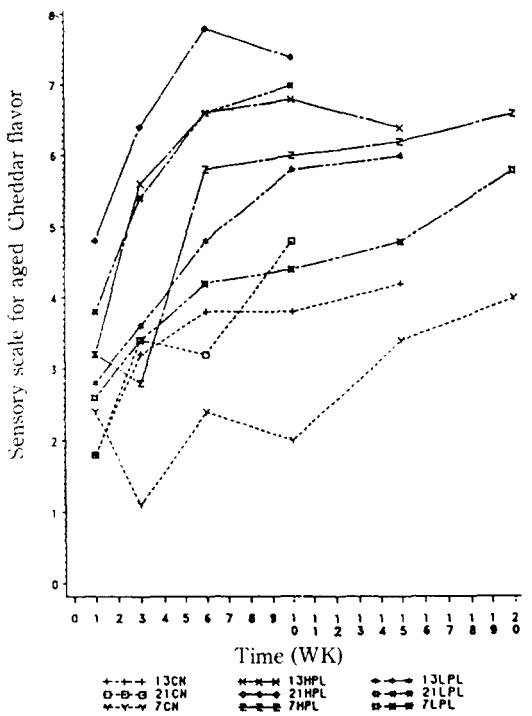

Fig. 4. Cheddar flavor scores in control (CN) and low and high levels of pancreatic lipase-treated cheese ripened at 7°, 13°, and 21 °C. Scale: 1 = flat or curdy, 3 = mild, 5 = medium, 7 = sharp, 9 = extra sharp

wk and leveled off thereafter. At 7 °C, HPL-treated cheese showed a samll increase during the same period; however, it showed a steady, good flavor development. LPL-treated cheese increased the Cheddar flavor but at lower rate than HPL-treated. At 7 ° and 13 °C, the control cheeses showed slow but steady increases in the flavor, whereas a moderate increase was observed at 21 °C. It was also observed that elevated ripening temperatures effectively influenced Cheddar flavor development.[9] Our results indicate that both lipase activity and ripening temperature may play an important role in accelerated Cheddar cheese flavor development. Although Cheddar flavor could be accelerated by adding lipase to the cheese, lipolyzed flavor defect was a common problem associated with ripening periods.[1] The levels of flavor defect in various ripening conditions for experimental and control samples are shwon in Fig. 5. Both HPL- and LPL-

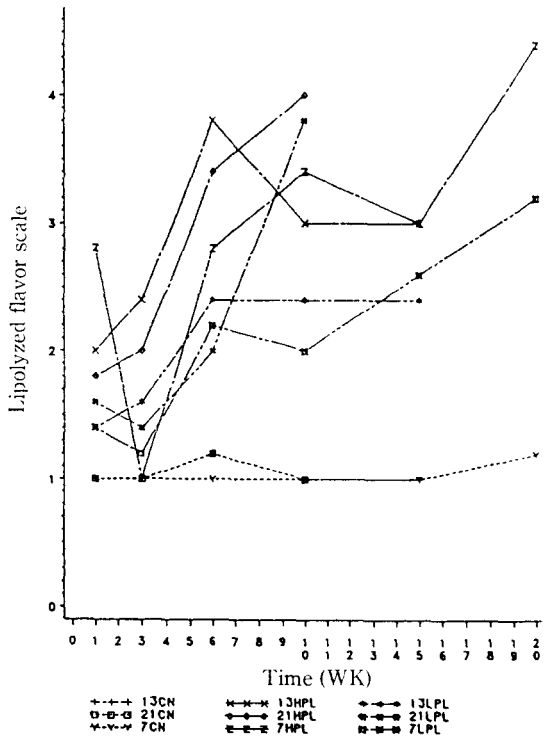

Fig. 5. Lipolyzed flavor scores in control (CN) and low and high levels of pancreatic lipase-treated cheese ripened at 7 °, 13 ° and 21 °C. Scale: 1 = none, 2 = slightly, 3 = moderately, 4 = definite, 5 = extremely

treated cheeses produced only detectable levels of the flavor defect between 1 and 3 wk. The flavor defect in HPL-treated cheese increased rapidly at various temperatures after 3 wk, but only moderate increases were observed in LPL-treated cheese at 6 wk, although it showed a significant lipolyzed flavor defect at 21 °C. The control cheese did not generate the flavor defect as expected. Our results indicate that a combination of a certain lipase activity and ripening temperature could minimize lipolyzed flavor problems in accelerated cheese ripening.

The food grade pancreatic lipase contained a small amount of protease according to the manufacturer, which produced a detectable level of bitterness in some cheese samples (Table 1). LPL-treated cheese developed a slight bitterness at 3 wk and moderate bitterness thereafter at all ripening temperatures. In LPL-treated cheese, bitterness was insignificant at various ripening conditions except at 6 wk for 13 °C and at 10 wk for 21 °C. The results indicate that pancreatic lipase preparations did not significantly influence the bitterness of cheese.

Overall quality of experimental cheese

Based on organoleptic flavor analysis of pancreatic lipase-treated cheese (Figs. 4, 5, and Table 1), acceptable quality cheeses were selected and summarized in Table 2. HPL-treated cheese, which ripened 1 wk at 21 °C generated medium flavor and ripened 3 wk generated flavors between medium and sharp with having only a slight level of lipolyzed and bitter flavors. In LPL-treated cheese, a number of good quality cheeses were observed. Those ripened at 7 °C for 10 wk and 21 °C for 3 wk produced medium cheese; those ripened at 13 °C for 10 wk gave flavors between medium and sharp; and those ripened at 13 °C for 15 wk and 21 °C for 6 wk produced sharp cheese with little lipolyzed and bitter flavor problems.

Correlation of sensory flavors and chemical components

As shwon in Table 3, significant correlations ($p < 0.0001$) existed among short-chain FFA of con-

Table 1. Effect of added food grade pancreatic lipase in granular Cheddar cheese on bitterness in relation to ripening time and temperature

Time (wk)	Temperature (°C)	Bitterness[a] Control	LPL[b]	HPL[c]
1	7	1.0	1.0	1.0
3	6	1.0	1.0	1.8
6	7	1.2	1.6	2.8
10	7	1.0	1.8	2.8
15	7	1.2	1.8	3.0
20	7	1.2	2.4	3.0
1	13	1.0	1.0	1.0
3	13	1.2	1.0	1.2
6	13	1.0	2.8	3.4
10	13	1.2	2.0	3.0
15	13	1.2	1.8	2.4
1	21	1.0	1.2	2.2
3	21	1.4	1.8	2.2
6	21	1.6	2.4	3.0
10	21	1.0	2.8	3.2

[a]Scale: 1 = none, 2 = slightly, 3 = moderately, 4 = definite, 5 = extremely

[b]Low concentration of pancreatic lipase: 0.66 units

[c]High concentration of pancreatic lipase: 1.32 units

Table 3. Correlation coefficients (r) of short-chain free fatty acids and Chedar flavor scores or lipolyzed flavor scores for control and porcine pancreatic lipase-treated cheese ripended at 7, 13, and 21 °C for 20, 15 and 10 wk, respectively[a]

Free fatty acids	Sensory characteristics: Cheddar flavor	Lipolyzed flavor
C4	0.854	0.807
C6	0.877	0.873
C8	0.797	0.810
C10	0.835	0.819
Low 1 (C4 + C6)	0.870	0.843
Low 2 (C4 + C8)	0.840	0.817
Low 3 (C4 + C10)	0.848	0.820
Low 4 (C6 + C8)	0.848	0.853
Low 5 (C6 + C10)	0.855	0.843
Low 6 (C8 + C10)	0.824	0.818
Med 1 (C4 + C6 + C8)	0.856	0.840
Med 2 (C4 + C6 + C10)	0.859	0.836
Med 3 (C4 + C8 + C10)	0.840	0.820
Med 4 (C6 + C8 + C10)	0.844	0.838
Total (C4 + C6 + C8 + C10)	0.850	0.834

[a]All are significant at 0.0001

Table 2. Effect of added porcine-pancreatic lipase on sensory flavor characteristics of granular Cheddar cheese ripened at various conditions

Amount of lipase (units)[a]	Time (wk)	Temperature (°C)	Flavor characteristics: Cheddar[b]	Lipolyzed[c]	Bitter[c]
0.66	10	7	4.4	2.0	1.8
0.66	10	13	5.8	2.4	2.0
0.66	15	13	6.8	2.4	1.8
0.66	3	21	5.4	1.4	1.8
0.66	6	21	6.6	2.0	2.4
1.32	1	21	4.8	1.8	2.2
1.32	3	21	6.4	2.0	2.2

[a]$\text{Units/mg} = \frac{\text{ml NaOH/min} \times \text{M NaOH}}{\text{mg enzyme used}} \times 100$

The amountsof pancreatic lipase were added into each 150g of cheese curd.

[b]1 = flat or curdy, 3 = mild, 5 = medium, 7 = sharp, 9 = extra sharp

[c]1 = none, 2 = slightly, 3 = moderately, 4 = definite, 5 = extremely

trol and pancreatic lipase-treated cheeses for aged Cheddar flavor. The concentration of C6 was correlated most (r = 0.877) among individual short-chain FFA, whereas the concentration of C8 was least correlated (r = 0.797). Low 1 (C4 + C6) showed the best correlation (r = 0.870) among combinations of FFA. It was reported that the combinations of C4 and C6 might be an indicator for flavor development.[4] Similarly, it was suggested that the combination of C4, C6 and C8 was responsible for the flavor development during lipase-treated cheese ripening.[2] To the lipolyzed off-flavor, the concentration of C6 was correlated most (r = 0.873) as individual short chain FFA, whereas C4 was least

(r = 0.807) correlated. Low 4 (C6 + C8) showed slightly higher correlation (r = 0.807) than other combinations. Lipase of ruminant animal orgins produced high concentrations of C4, and the lipase-added cheese developed highly rancid flavor.[4] However, pancreactic lipase did not produce high amounts of C4 as compared to ruminant animal lipases. It appears, therefore, that C4 is the least important lipolyzed flavor defect for pancreatic lipases. It appears, therefore, that C4 is the least important lipolyzed flavor defect for pancreatic lipase-treated cheese.

요 약

식용 돼지췌장 리파제를 두 가지 함량으로 치즈제조시 혼합하여 7°, 13°, 21℃에서 숙성시켰다. GC 분석결과 효소처리한 치즈에서는 control에서 보다 훨씬 많은 양의 저급지방산이 생성되었다. 돼지췌장 리파제를 많이 넣어 21℃에서 숙성시킨 치즈의 경우 1주 후에 medium flavor 치즈, 3주 후에 거의 sharp flavor 치즈가 특별한 결함없이 생산되었다. 또한 이효소를 조금 넣은 치즈에서는 양호한 질의 치즈가 많았는데, 7°, 13°, 21℃에서 3주부터 15주의 숙성기간 중 medium과 sharp flavor 치즈가 생산되었다. 통계분석에 의하면 체다풍미와 caproic acid의 함량에서, 또 체다풍미와 butyric acid, caproic acid 혼합함량 상호관계에서 유의성이 있었다. 적합한 조건을 사용한다면 돼지췌장 리파제는 체다치즈의 숙성을 촉진시키는데 사용 가능한 것으로 사료된다.

References

1. Kosikowski, F.V. and Iwasaki, I.: Changes in Cheddar cheese by commercial enzyme preparation. *J. Dairy Sci.*, **58**, 963 (1975).
2. Arbige, M.V., Freund, P.R., Silver, S.C. and Zelko, J.T.: Novel lipase for Cheddar cheese flavor development. *Food Technol.*, **40**(4), 91 (1986).
3. Patton, S.: Volatile acids and the aroma of Cheddar cheese. *J. Dairy Sci.*, **46**, 856 (1963).
4. Lin, J.C.C. and Jeon, I.J.: Effects of commercial food grade enzymes on fatty acid profiles in granular cheese. *J. Food Sci.*, **52**, 78 (1987).
5. Kwak, H.S. Jeon, I.J. and Perng, S.K.: Statistical patterns of lipase activities on the release of short chain fatty acids in Cheddar cheese slurries. *J. Food Sci.*, **54**, 1559 (1989).
6. SAS, SAS User's Guide: Statistics, SAS. Institute Inc., Box 8000, Cary, NC. 27511-800 (1985).
7. AOAC: *Official Method of Analysis*, 14th ed. Association of Official Analytical Chemists Washington, D.C. (1984).
8. Nelson, J.H.: Enzymatically produced flavors for fatty systems. *J. Amer. Oil Chem. Soc.*, **49**, 559 (1972).
9. Aston, J.W., Giles, J.E. and Dulley, J.R.: Effect of elevated ripening temperatures on proteolysis and flavour development in Cheddar cheese. *J. Dairy Res.*, **52**, 565 (1986).

(1989년 12월 21일 접수)