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Effects of Food Grade Porcine Pancreatic Lipase on Neutral Volatile Compound Profiles in Cheddar Cheese

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식용 돼지췌장 리파제가 체다치즈의 중성 휘발성 성분 생산에 미치는 영향

곽해수 · 전익준* · 정병수
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Abstract

Effects of commercial food grade porcine pancreatic lipase on the neutral volatile compounds in Cheddar cheese were studied. The enzyme was incorporated into the cheese at two different levels of concentration and ripened at various temperatures. The production of 2-butanone increased at higher amount of lipase and higher temperature, but the production of 2-pentanone was inconsistent trends during ripening periods. The concentration of acetaldehyde was the highest among aldehydes and was increased consistently during ripening periods. In alcohol production ethanol was the most abundant but no further consistent trend was observed after 6 wk. The production of ethyl butyrate was the most abundant ester and related to lipase activities as well as ripening temperatures. Dimethyl sulfide was the only sulfur compound and appeared not to be related to the addition of lipase or ripening temperatures. Statistical analysis suggested that ethyl butyrate was most correlated to aged Cheddar flavor during cheese ripening.

Key words: lipase, Cheddar cheese, flavor, neutral volatile compound

Introduction

Neutral volatile compounds associated with cheese flavor are ketones, aldehydes, alcohols, esters and sulfur compound. These compounds as well as free fatty acids are considered to be important in Cheddar cheese flavor development. Patton *et al.*[1] and Day *et al.*[2] identified odd-numbered methyl ketones (C3-C15) in Cheddar cheese. Odd-numbered methyl ketones are derived from even-numbered fatty acids of milk fat[3], whereas even-numbered methyl ketones (2-butanone) were postulated through the reduction of acetoin into 2,3- butanediol and further transferred into methyl ethyl ketones. However, methyl ketones such as 2-pentanone, and possibly 2-heptanone and 2-nonanone might be a minor direct contributor to the Cheddar flavor.[4] The concentration of 2-pentanone could be used as an indicator of maturity in Cheddar cheese.[5] Aldehydes including formaldehyde, acetaldehyde, propanol, methyl butanal and methional were identified in Cheddar cheese.[6] Lin[7] identified acetaldehyde, propanal and n-hexanal in the enzyme treated Cheddar cheese during ripening periods. He indicated that concentrations of propanal and n-hexanal varied from non-detectable to a trace amount, and it appeared to be less important for the flavor development. On the other hand, he found that the production of acetaldehyde was most abundant of the aldehydes in the cheese, but he also observed inconsistent production of acetaldehyde. Among the alcohols, ethanol and 2-butanol are most common. However, 2-butanol is a reduction product from 2-butanone and is as-

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sociated with off-flavor.[8] Ester such as ethyl butyrate, ethyl hexanoate exist in a relatively small amount in the cheese with normal flavor. However, a fruity flavor could arise with a different starter organism.[9] Sulfur compounds have received attention due to the work of Manning.[10-13] He detected hydrogen sulfide, methanethiol, and dimethyl sulfide in the headspace of Cheddar cheese samples and formulated relationships between the concentrations and the flavor scores of the cheese. However, it was found that although methanethiol showed the highest correlaton coefficient, none of the volatile sulfur compounds was considered to be useful as a reliable indicator for flavor development.[14]

The objectives of this study were to investigate the effects of food grade porcine-pancreatic lipase with different concentrations on the production of neutral volatile compounds in cheese at regular and elevated ripening temperatures, and to study statistical relationships between neutral volatile compounds and flavor development.

Materials and Methods

Enzyme

Food grade pancreatic lipase (Amer. Lab. Inc., Omaha, NB) was selected base on the study by Kwak.[15]

Assay for lipase activity

The pH stat method of Chandan and Shahani[16] was used to determine lipase activities. The detailed procedures were described by Kwak.[15]

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.

Sample preparation, distillation, and headspace gas sampling

Samples of maturing cheese were removed periodically (1, 3, 6, 10, 15, and 20 wk), and redistrilled water (potassium permanganate-treated) was added to the samples to make 30% (w/w) total solids cheese slurries based on their moisture content.[17] The cheese-water mixtures were blended immediately in an Osterizer blender at liquefying speed for 1 min. Fifty m*l* of the blended cheese slurry were transferred into the distillation flask of Kemmer-Hallet type micro-Kjeldahl distillation unit. The slurry was steam-distilled while ice-water was circulated through the condenser by a submersible-type pump. The steam generator (water flask) was heated in a constant manner to collect the first drop of distillate in 2 min after boiling, and 5 m*l* of distillate were collected in 3 min. Two m*l* of each distillate were used for headspace gas sampling as described by Bassette and Ward.[18]

Gas chromatography and quantitation of neutral volatile compounds

A Hewlett-Packard Model 5880A GC equipped with a flame ionization detector was used for all analyses. Headspace gas samples were analyzed on a Supelcowax 10 widebore (30 m × 0.75 mm i.d.) capillary column (Supelco, Inc., Bellefonte, PA). The column was operated with nitrogen carrier gas at a flow rate of 6.0 m*l*/min; hydrogen gas flow rate was 30 m*l*/min; air was 400 m*l*/min. Both temperature for injector and detector was maintained at 230 °C. The column oven was programmed at three temperature levels: initial holding for 5 min at 40 °C, first level heating to 50 °C/min, holding for 0.1 min; second heating to 130 °C at 5 °C/min, holding for 15 min; third heating to 175 °C at 8 °C/min, holding for 20 min. GC

Terminal (Level Four) integrator was used to determine peak areas with a chart speed of 0.5 cm/min. All quantitative analyses were done by relating peak areas of individual neutral volatile compounds to those of standard compounds. The concentrations of volatile compounds were estimated by analyzing cheese samples that contained known concentrations (80 ppb) of standard compounds and those of containing no added standards. The difference between the two treatments was used for the estimation of concentrations of individual volatile compounds. A known concentration (1 ppm) of acetone headspace gas was periodically injected into GC and used as an external standard.

Sample preparation and GC-MS spectrometry for identification

Samples of low pancreatic lipase-treated cheese at 13 °C for 10 wk were distilled as described previously and collected 20 fractions (100 m*l*) in a clean separatory funnel (washed with ether). Distilled ether (20 m*l*) was added to the funnel, and volatile compounds were extracted into ether phase. The ether extract was separated and transferred to an ether-washed Erlenmeyer flask, and 2 g of sodium sulfate were added to eliminate residual water. The extract was treated with deactivated alumina to eliminate free fatty acids that were in extremely high concentrations. The treated ether extract was concentrated under nitrogen stream and made to 0.5 m*l*. One μl of the concentrate was injected into GC-MS.

A Hewlett-Packard Model 5890A GC combined with a mass selective detector (MSD 5970B) was used for identification of volatiles. The compounds were separated on a 5% phenyl methyl silicone crosslinked fused silica capillary column (50 m × 0.2 mm, i.d. × 0.33 μm film thickness). The column was operated with helium carrier gas at a flow rate 1.0 m*l*/min and injector temperature was 230 °C. The column oven was programmed at three temperature levels; initial holding for 1 min at 40 °C, first level heating to 100 °C at 5 °C/min, holding for 0.1 min; second heating to 180 °C at 10 °C/min, holding for 0.1 min; third heating to 250 °C at 15 °C/min for 20 min. The column interface temperature between GC and MSD units was 280 °C. Samples were injected into the GC unit with a splitless mode (0.75 min purge) and 7 min solvent delay was used for MSD. The MSD unit was operated at 2×10^{-5} torr, and compounds were detected with a Scan Mode (mass 40-500) and monitored by a Hewlett-Packard ChemStation (Model 59970). Each compound was identified by utilizing the ChemStation Library Functions. For further identification, standard compounds were injected into GC and retention times were compared.

Data analysis

Statistical correlations were done by utilizing PROC CORR and PROC STEPWISE subcommands of SAS software programs.[19]

Results and Discussion

Neutral Volatile Compounds

A typical chromatogram of neutral volatile compounds in LPL-treated cheese ripened at 13 °C for 6 wk is shown in Fig. 1. Methyl ketones, aldehydes, alcohols, esters, and sulfur compound were major compounds present in various cheese samples.

Methyl ketones

Methyl ketones such as acetone, 2-butanone, 2-pentanone, 2-hexanone, and 2-heptanone were present in all samples during cheese ripening. 2-Octanone and 2-nonanone were also present but their presence was inconsistent. As shown in Fig. 2, production of 2-butanone were similar among control, LPL-, and HPL-treated cheese samples ripened at 7 °C for 20 wk. Lipase-treated cheese at 13 °C produced more 2-butanone than control. At 21 °C, similar trends were observed with rapid increase in 2-butanone concentrations. However, the production of 2-butanone in most samples decreased after 6 wk except control. The high temperature appeared to accelerate 2-butanone

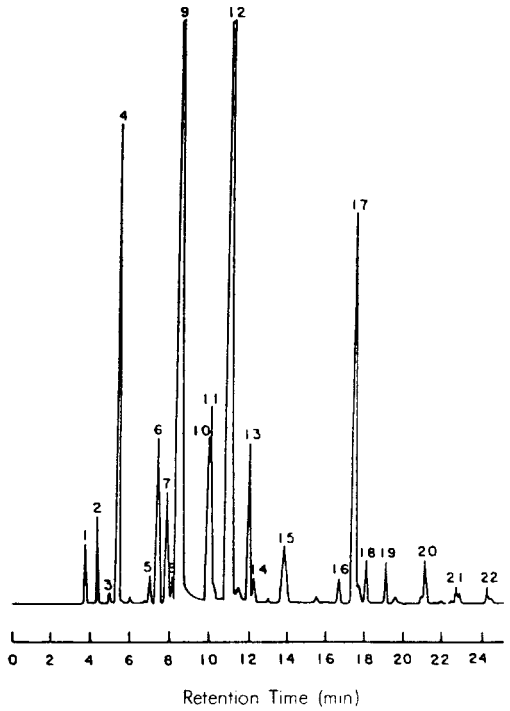

Fig. 1. Typical chromatogram of neutral volatile compounds obtained from low level pancreatic lipase-treated cheese (LPL) at 13°C for 6 wk.
The compounds identified are: 1 = acetaldehyde; 2 = dimethyl sulfide; 3 = propanal; 4 = acetone; 5 = ethyl acetate; 6 = 2-butanone; 7 = unknown; 8 = 2-propanol; 9 = ethanol; 10 = 2-pentanone; 11 = n-pentanal; 12 = unknown; 13 = ethyl butyrate; 14 = n-propanol; 15 = 2-hexanone; 16 = n-hexanol; 17 = 2-heptanone; 18 = 2-hexanol; 19 = ethyl hexanoate; 20 = 2-octanone; 21 = 2-nonanone; 22 = 2-nonanal

production. Similarly, higher amounts of lipase appeared to help accelerate 2-butanone production at 13° and 21°C. Similar results were observed by Lin.[7] There were similar patterns for the production of 2-hexanone and 2-octanone. 2-Butanone has been reported to be a desirable Cheddar flavor component.[8] This results indicate that the production of 2-butanone may be important for relating cheese flavor development during ripening.

The concentration of 2-pentanone in the controls and lipase-treated cheese samples at 7°, 13°, and 21°C is shown in Fig. 3. Inconsistent trends in the production of 2-pentanone were observed during the ripening periods. Although HPL-treated cheese might have produced a higher level of

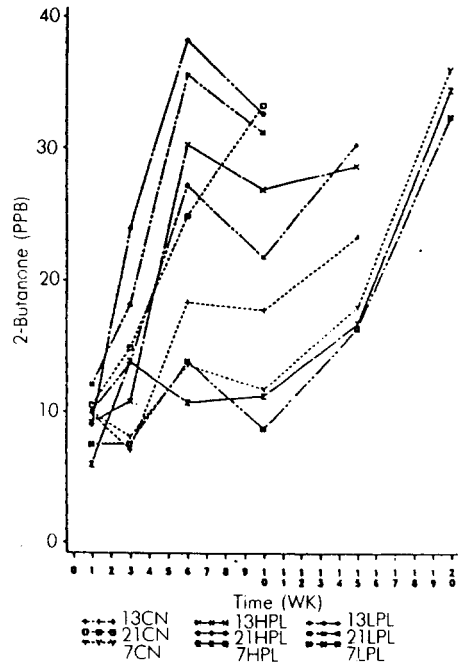

Fig. 2. Production of 2-butanone in control (CN) and low and high levels of pancreatic lipase (LPL, HPL)-treated cheese at 7°C, 13°C, and 21°C for 20 wk

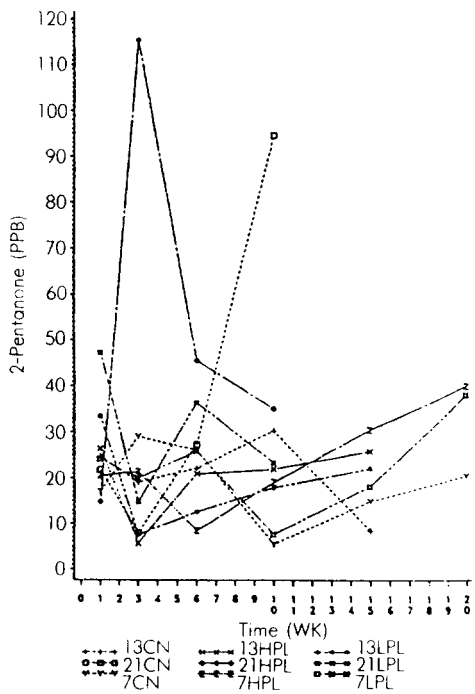

Fig. 3. Production of 2-pentanone in control (CN) and low and high levels of pancreatic lipase (LPL, HPL-)-treated cheese at 7°, 13°C, and 21°C for 20 wk

2-pentanone than LPL-treated or control cheese at the same ripening temperature, the trends were not consistent. The concentrations of 2-pentanone were in the range of 10 to 30 ppb. Similar inconsistent trends were found for acetone, 2-heptanone and 2-nonanone. The concentration of acetone ranged from 15 to 36 ppb for control, 19 to 40 ppb for LPL-treated cheese, and 26 to 46 ppb for HPL-treated at 13 °C throughout the 15 wk period. The concentration of 2-heptanone ranged from 2 to 12 ppb for control, 6 to 22 ppb for LPL-treated, and 7 to 25 ppb for HPL-treated cheese at 13 °C during 15 wk. It was indicated that methyl ketones might contribute to flavor, but they do not directly impart a typical mature Cheddar flavor.[4] However, odd-numbered methyl ketones, 2-pentanone, 2-heptanone, and 2-nonanone may provide a minor contribution to the aged flavor. It was pointed out that the concentration of 2-pentanone could be used as an indicator for the maturity of Cheddar cheese;[5] however, our results could not confirm this due to the inconsistent production of the compound.

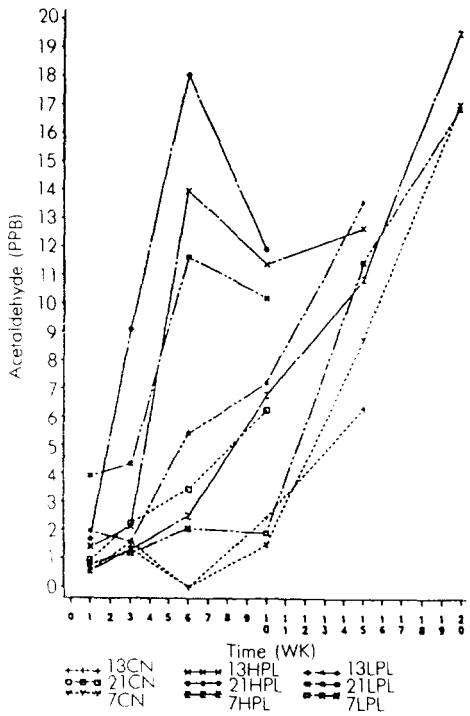

Fig. 4 Production of acetaldehyde in control (CN) and low and high levels of pancreatic lipase-treated cheese at 7°, 13°, and 21 °C for 20 wk

Aldehydes

Among aldehydes, acetaldehyde, propanol, n-pentanal and 2-nonanal were identified in cheese sample (Fig. 1). Propanal and 2-nonanal were present in trace amounts during entire ripening periods. The inconsistent production of n-pentanal was observed after 10 wk in the control and after 6 wk in lipase-treated cheese. On the other hand, the production of acetaldehyde shown in Fig. 4 appears to be the most abundant among aldehydes and consistent in the cheese samples. The concentration of acetaldehyde increased rapidly in the first 6 wk both for HPL- and LPL-treated cheeses at 21 °C as well as for HPL-treated cheese at 13 °C but declined thereafter. This decline may be related to the further reduction of acetaldehyde to ethanol.[20] Acetaldehyde also showed sharply increasing trends in controls ripened at 7° and 13 °C, and LPL-treated cheese at 7 °C after 10 wk ripening. According to our results, the production of acetaldehyde appeared to be dependent upon lipase activity as well as ripening temperatures. It is not clear in the literature whether acetaldehyde contributes to the aged flavor development, but it is known as a precursor of ethanol and has been implicated in the formation of esters.[21]

Alcohols

Alcohols identified in cheese samples were ethanol, n-propanol, 2-propanol, n-hexanol, and 2-hexanol. The production of n-propanol, 2-propanol, n-hexanol, and 2-hexanol were detected in trace amounts during ripening periods (20 wk). These alcohols do not appear to contribute to the flavor development. On the other hand, ethanol was the most abundant alcohol found. The production of ethanol in control and lipase-treated cheese samples at 7°, 13°, and 21 °C is shown in Fig. 5. The initial concentrations of ethanol for all samples were higher than concentrations of other volatile compounds. However, the productions of ethanol appeared to be inconsistent. In most

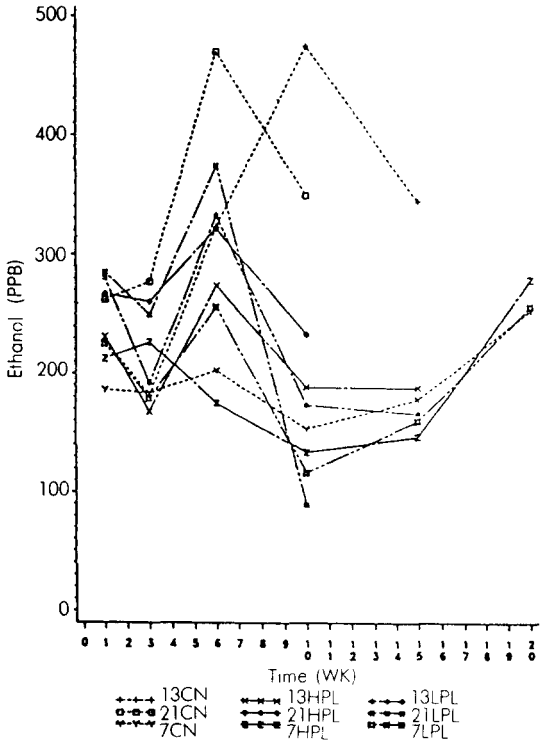

Fig. 5. Production of ethanol in control (CN) and low and high levels of pancreatic lipase-treated cheese at 7°, 13°, and 21°C for 20 wk

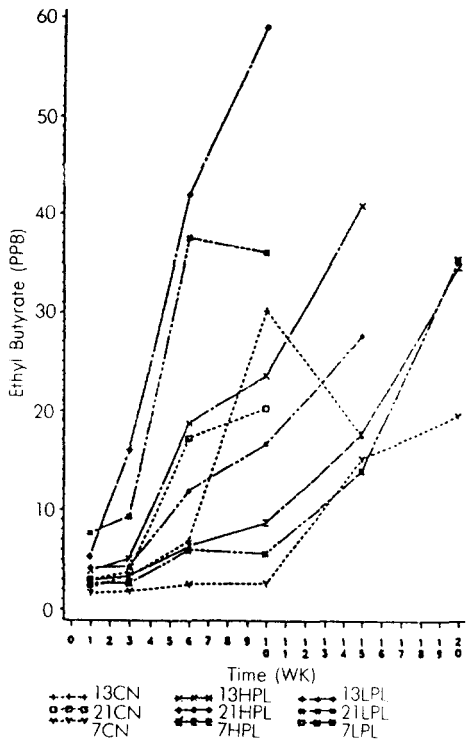

Fig. 6. Production of ethyl butyrate in control (CN) and low and high levels of pancreatic lipase-treated cheese at 7°, 13°, and 21°C for 20 wk

cases, the concentrations of ethanol decreased slightly at 3 wk analysis for all ripening temperatures but considerably increased at 6 wk analysis but no further consistent increases were observed. The controls exhibited the highest concentration of ethanol at 10 and 6 wk for 13° and 21°C, respectively, whereas the rest of the samples did not significantly produce ethanol during the entire ripening periods. Lin[7] observed that ethanol concentration was not high in lipase-treated cheese. It was suggested that ethanol may contribute to the cheese flavor[21]; however, It was reported that high concentrations of ethanol (100 ppm) tended to be associated with high concentrations of esters and fruity defect. Our results showed that the production of ethanol was not increased by lipase additions, but was influenced by ripening temperatures.

Esters

Esters present in the cheese sample were ethyl acetate, ethyl butyrate, and ethyl hexanoate. The trace amount of ethyl hexanoate was present in the control after 6 wk at 13° and 21°C, whereas measurable amount of the ester (1.85 to 1.99 ppb) was produced after 6 wk in LPL- and HPL-treated samples at all ripening temperatures. The production of ethyl acetate was not significantly different at 7°C but significant differences were observed at 13° and 21°C between control and lipase-treated samples. Ethyl butyrate was the most abundant ester. Production of ethyl butyrate in control and lipase-treated cheese samples at 7°, 13°, and 21°C is shown in Fig. 6. The concentrations of ethyl butyrate for LPL- and HPL-treated cheese at 21°C increased rapidly from the early stage of ripening (1 wk), whereas LPL- and HPL-treated cheese at 13°C as well as control at 21°C increased its concentrations moderately after 3 wk. At 7°C, all samples showed little increase up to 10 wk and then showed a steady increasing trend. These results indicate that the production

of ethyl butyrate is related to lipase activities as well as ripening temperatures. It was suggested that the concentration of ethanol has a direct relation to the production of esters.[23] It was reported that a number of esterase-containing bacteria could esterify butyric and caproic acids with ethanol.[24] Since the lipase-treated cheese samples showed ethanol concentrations similar to controls (Fig. 5), production of esters appeared to be less related to the concentrations of ethanol in the cheese.

Sulfur compound

Dimethyl sulfide was the only sulfur compound detected in the headspace analysis of cheese samples. As shown in Fig. 7, the concentration of dimethyl sulfide increased steadily up to 6 or 10 wk but followed by inconsistent changes. Considerable variations observed among control and lipase-added samples. The concentration of dimethyl sulfide appeared not to be related to the addition of lipase or ripening temperatures. It was observed that proteinase-added cheese produced higher levels of dimethyl sulfide than lipase-added or control cheeses.[7] It was reported that methionine was indirectly influenced in the formation of dimethyl sulfide.[25] The presence of dimethyl sulfide appears not to be essential for cheese flavor development because it occurred inconsistently during ripening.[10] However, "sulfide" defect could be related to the high level of dimethyl sulfide in cheese.

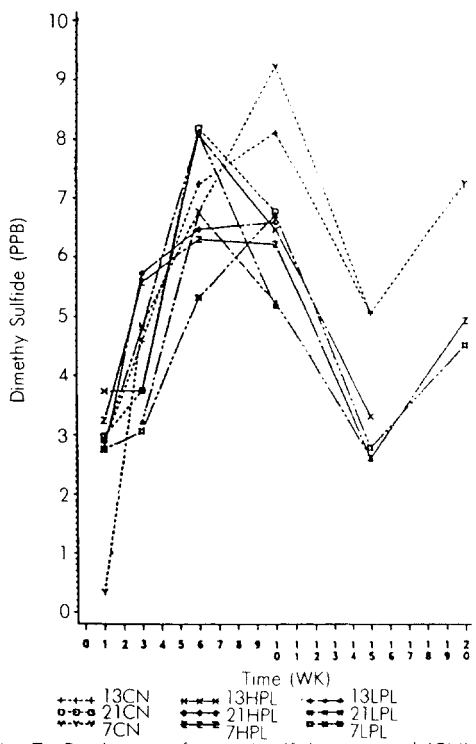

Fig. 7. Production of methyl sulfide in control (CN) and low and high levels of pancreatic lipase-treated cheese at 7°, 13°, and 21°C for 20 wk

Correlation of sensory flavors and neutral volatile compounds

Correlations of sensory flavors and neutral volatile compounds for control and lipase-treated cheeses are shown in Table 1. To aged Cheddar flavor, ethyl butyrate was correlated most (r = 0.762) during cheese ripening. Other esters (ethyl acetate and ethyl hexanoate) also appear to have good correlations with Cheddar flavor. In lipase-treated cheese, esters (especially ethyl butyrate) was reported to be a good indicator for maturity of the cheese[7], although it has been related to the fruity defect.[23] In our experimental cheese, fruity

Table 1. Correlation coefficients (r) of neutral volatile compounds and Cheddar or lipolyzed flavor scores for control and porcine pancreatic lipase treated-cheese ripened at 7, 13, and 21°C for 20, 15, and 10 wk, respectively

Volatile compounds	Sensory characteristics[26]	
	Cheddar flavor	Lipolyzed flavor
Acetaldehyde	0.758	0.670
Dimethylsulfide	0.087[a]	-0.001[a]
Acetone	0.486	0.337
Ethyl acetate	0.630	0.423
2-Butanone	0.691	0.479
Ethanol	-0.058[a]	-0.239[a]
2-Pentanone	0.261[a]	0.647[a]
Ethyl butyrate	0.762	0.632
n-Hexanal	0.133[a]	0.086[a]
2-Heptanone	0.489	0.305
Ethyl hexanoate	0.652	0.587
2-Octanone	-0.056[a]	-0.017[a]
2-Nonanone	0.118[a]	0.087[a]

a) Not significant at $p<0.05$.

defect was rarely observed. It may be due to the low concentration of esters (5.16 to 37.51 ppb of ethyl butyrate in good matured Cheddar cheeses). Acetaldehyde as well as 2-butanone also showed good correlations with Cheddar flavor, although acetaldehyde might not contribute Cheddar flavor development.[21] The concentration of 2-butanone reported to vary in matured cheese[4,23], although Lin[7] observed a significant correlation between 2-butanone and ripening time.

요 약

식용 돼지췌장 리파제가 체다치즈에서 중성 휘발성 성분의 생산에 미치는 영향에 대하여 고찰하였다. 치즈 제조시 이 효소를 두 가지 함량으로 혼합하여 여러 온도에서 숙성시켰다. 2-butanone의 생산은 효소의 양과 숙성온도를 높였을 때 증가했으나, 2-pentanone의 생산은 숙성기간 중에 일관성이 없었다. Acetaldehyde는 aldehyde 중에서 가장 많이 생성되었고 일관성있게 증가하였다. Alcohol 생산에서는 ethanol이 가장 높았지만 숙성 6주 후에는 일관성있는 증가가 없었다. Ethylbutyrate는 ester 중에서 가장 많이 생성되었고 리파제의 활성도와 숙성온도에 연관이 있었다. Dimethyl sulfide는 유일한 유황 성분이었지만 리파제의 첨가와 숙성온도에 영향이 없는 것으로 나타났다. 통계분석결과 치즈 숙성 중에 ethyl butyrate와 체다풍미가 유의성있는 상호관계가 있었다.

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