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Statistical patterns of lipase activities on the release of short-chain fatty acids in Cheddar cheese slurries

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1. Abstract

Twenty-five commercial food grade and alalytical grade lipases were used to study the patterns of release of short-chain free fatty acids (FFA) from milk fat in cheese slurries. Principal Component Analysis showed that there were four distinctive groups by the FFA ratios and five groups by the FFA concentrations. However, Average Linkage Cluster Analysis showed that the patterns of FFA released were dependent upon distance defined between groups of lipases. All the lipases tested with both statistical analysis had distinctive specificities in hydrolyzing short-chain FFA from milk fat. Lipases from ruminant-animal origins produced an extremely high ratio (>40%) of butyric acid and a low ratio (<26%) of capric acid to total short chain FFA. Lipases from porcine -pancreas and some microbial origins showed balanced production in both bytyric and capric acid. However, most lipases from microbial origins released a high ratio of capric acid but similar ratios to other origin enzymes for short-chain free fatty acids. Ruminant-animal origin lipases produced short-chain FFA much higher in concentration than other lipases. Lipases from porcine pancreas as well as microbial origins showed different concentrations of the fatty acids. Ratios of short-chain FFA in each sample were not significantly changed during incubation periods (4 wk), whereas concentrations of the FFA increased considerably.

2. Introduction

The release of fatty acids from milk fat by lipase has been studied considerably. The literature indicates that lipase may be specific or nonspecific with regard to the liberation of fatty acids from milk fat molecules. Milk lipase is reported to be nonspecific ; it released the same ratios of fatty acids that were in milk(Khan et al., 1967 ; Nelson, 1972). On the other hand, calf lipase showed a preferential release of short-chain fatty acids, particularly butyric acid from milk fat. Harper (1957) observed that lipases of ruminant animal origin (calf, kid, and lamb) were specific for the release of butyric acid. He also observed that there was considerable selectivity in the liberation of caproic, caprylic, and capric acids from milk fat. Similar results also were observed in cheese during the ripening process (Arbige et al.. 1986 ; Lin and Jeon, 1987). Pancreatic lipase was reported to be nonspecific (Nelson, 1972). However, Harper (1957) observed that pancreatic lipase liberated predominantly long -chain fatty acids. Microbial lipases derived from different species and strains

differ in specificity for liberating fatty acids from milk fat. Arbige et al. (1986) reported that their experimental Aspergillus oryzae lipase was nonspecifiv. A similar result was also observed with Mucor lopase (Law and wigmore, 1985). Penicillium roqueforti lapase, however, was reported to release higher amounts of butyric acid than other fatty acids (Morris and Jezeski, 1953). Achromobacter lipolyticum and Candida lipolytica lipases were similar in specificity (Wilcox et al., 1955).

The objectives of this work were to investigate the patterns of short-chain fatty acids released from milk fat by various lipases in cheese slurries and to classify activities utilizing sophisticated statistical methods in conjuction with advanced computer software programs.

3. Materials and Methods

Enzymes

Food grade and analytical grade lipases were obtained from various commercial sources (Table 1).

Assay for lipase activity

The pH stat method of Chandan and Shahani (1963) was used to determine lipase activities of each enzyme sample. The assay substrate was a freshly prepared emulsion of 10% (v/v) buterfat in a 10% aqueous solution of gum arabic. To simulate pH conditions similar to a typical young cheese or fresh cheese curds, the substrate was adjusted to pH 5.3 and stored in a refrigerator overnight before being used. The enzyme activities were measured at 32°C for 7 min, maintaining the pH of the substrate at 5.3 by adding 0.02 N NaOH with an automatic titration system (Radiometer Inc., Copenhagen, Denmark). The lipase activities were expressed as units/mg enzyme preparation by computing the total amount of NaOH titrated during the 7 min assay period for each enzyme used.

Preparation of cheese slurries

Cheese slurries were prepared according to the modified method of Sood and Kosikowski (1979). Freshly salted Cheddar cheese curds, which contained 40.2% moisture, 31.3% fat, 23.4% protein, and 2.0% salt according to the methods of the Association of Official Analytical Chemists (AOAC, 1984). were obtained at Kanseas State University Dairy Processing Plant. The curds were mixed in a food processor for 2 min with 4.5% sterilized saline water that contained a premeasured amount of lipase. The lipase added was equivalent to 1.32 units enzyme preparation per 150 g cheese slurry. The mixed slurries were poured into plastic bags (150 g each), vacuum-packaged wiht Multivac (Sepp Haggenmueller KG Wolfertschwenden, W. Germany), and incubated at 32°C for 4 wk. The cheese slurries were analyzed for free fatty acids, pH, and total microbial population (by the standard plate count) at 0, 1, 2, 3, and 4 wk.

Analysis of free fatty acids (FFA)

Cheese slurries (two bags) were extracted with diethyl ether and hexane for 2 hr and eluted through a 10 mm i.d. glass column containing neutral alumina according to the method of Deeth et al. (1983). The column containing alumina with absorbed free fatty acids was dried under vacuum and transferred to a stoppered glass tube. One ml

Table 1. Commercial food grade and analytical lipases incorporated into cheese slurry preparations

		Enzyme		
Sombol	Abbr.	Trade name	Source	Supplier
G	ICD	Italase C 01002	Calf	Dairyland Lab
J	CLM	Lipase # 600	Calf	Miles Lab
D	CKD	Capalase K 01011	Kid	Dairyland Lab
U	KLM	Lipase # 300	Kid	Miles Lab
H	CLD	Capalase L 01013	Lamb	Dairyland Lab
I	LLM	Lipase # 500	Lamb	Miles Lab
F	KLD	Capalase KL 01012	Kid & lamb	Dairyland Lab
C	PPR	Lipase Preparation 7023 C	Porcine Pancreas	Rohm Tech
Q	PLB	Pancreas USP	Porcine Pancreas	Biocon
S	PPSPL	Lipase 30	Porcine pancreas	Sci. Pro. Lab
T	PPALI	Lipase 24	Porcine pancreas	Amer. Lab Inc.
R	RCNT	Control		
M	CVS	Lipase Type XII	C. viscosum	Sigma
O	PFB	Lipase	P. fluorescens	Boeringer
K	CREDC	ENZCO LIpase 1000	C. rogosa	EDC
A	ANR	Lipase Preparation 7051 L	A. niger	Rohn Tech
E	ANNOVO	Palatase 750 L	A. niger	NOVO Lab
V	AOR	Lipase Praparation 2212 E	A. oryzae	Rohm Tech
X	FFRG	Flavor Age FR	A. oryzae	Genencor
Y	FCPG	Flavor Age CP	A. oryzae	Genencor
Z	FCPAG	Flavor AGe CP-A	A. oryzae	Genencor
B	MBR	Lipase Praparation 2212 F	M. bacillus	Rohm Tech
W	MMNOVO	Palatase M 100 L	m. miehei	NOVO Lab
L	PAB	Biolipase	R. arrhizus	Biocon
N	RDCDC	Lipase	R. delema	Chem. Dynamic Corp.
P	WGS	Botanical lipase	Wheat germ	Sigma

isopropyl ether containing 6% formic acid was added and mixed with the alumina. The tube was centrifuged at 2,000 X g for 5 min at room temperature, and a 1 μL aliquot of the supernatant was injected into a gas chromatograph (GC). A Hewlett -Packard Model 5880A GC equipped with a flame ionization detector and GC Terminal (Level Four) integrator were used for the analysis of individual FFA. The separation of FFA was achieved using a 91 cm X 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 flow at 40 mL/min, hydrogen gas at 30 mL/min, and air at 400 mL/min. The column oven was programmed at three temperature levels; initial holding for 2 min at

90°C, first level heating to 110°C at 5°C/min, holding for 2 min ; second heating to 150°C at 8°C/min, holding for 5 min : third heating to 180°C at 5°C/min, holding for 8 min. The temperature for both injector and detector was 230°C. All quantitative analyses were done by relating each peak area of individual free fatty acids (FFA) to the peak area of tridecanoic acid as an internal standard. Each FFA was identified by the retention time of a standard mixture.

Data analysis

All data were treated statistically. For pattern recognition, Principal Component Analysis and Average Linkage Cluster Analysis techniques (Manly, 1986) were appleid utilizing PROC PRINCOMP and PROC CLUSTER subcommands of SAS software programs (SAS, 1985).

4. Results and Discussion

Principal Component Alalysis for FFA

To classify lipase actibities based on their FFA releasing patterns, data on ratios and concentrations of short-chain FFA (C4, C6, C8, C10) obtained form 1 wk of incubation were treated with Principal Component Analysis (PCA). The FFA ratios were calculated by dividing concentrations of each fatty acid by the sum of the four FFA involved, whereas the concentrations were expressed as part per million of each fatty acid. Results of the PCA analysis showed that there were distinctive patterns of liberating FFA from milk fat by the lipases tested (Figs. 1 and 2). As indicated by dotted lines (Fig. 1), there appeared to be four, large, loosely related groups of lipases for the ratios of fatty acids released. However, if more densely clustered neighbors were considered (as indicated by solid lines), eight groups of lipases could be defined. If four of these (K, A, E & X, and Y & Z), however, were considered minor groups or outliers, the lipases could be classified into four major groups. The four major groups are designated as Groups 1-4 in Fig. 1. Group 1 consisted of all lipases of ruminant animal origin lipases (calf, kid, lamb, and mixture of kid and lamb). No lipases of ruminant animal origin tested fell out of the group. This indicates that lipases from domestic ruminant animals will liberate similar ratios of fatty acids from milk fat. Similar ratios of short-chain FFA released from milk fat by ruminant -animal lipases were observed by Farnham et. al. (1956). Group 2 included all porcine -pancreatic lipases and a bacterial lipase (Pseudomonas fluorescens). Group 3 included lipases of mold (Rhizopus arrhizus, Rhizopus delema, Aspergillus oryzae and Mucor miehei) and bacterial (Chromobacterium viscosium) origins. Group 4 lipases were from mold (Mucor bacillus) and plant origin (wheat germ). In contrast to the lipases of ruminant-animal origin or pancreatic lipases which were closely clustered regardless of the source of manufactures, A. oryzae lipases from four different manufactures were scattered into three different groups. The same trend was observed for A. niger lipase. This indicates that lipases from different strains of the same microbial species have different specificities on releasing fatty acids. All the outliers in Figs. 1 and 2 were related to microbial origins.

The patterns of lipase activities according to the concentration of FFA released are shown in Fig. 2. Three large groups were observed as indicated by dotted lines. If two outliers (U & N) near Groups A and B were ignored, the enzymes could be cla-

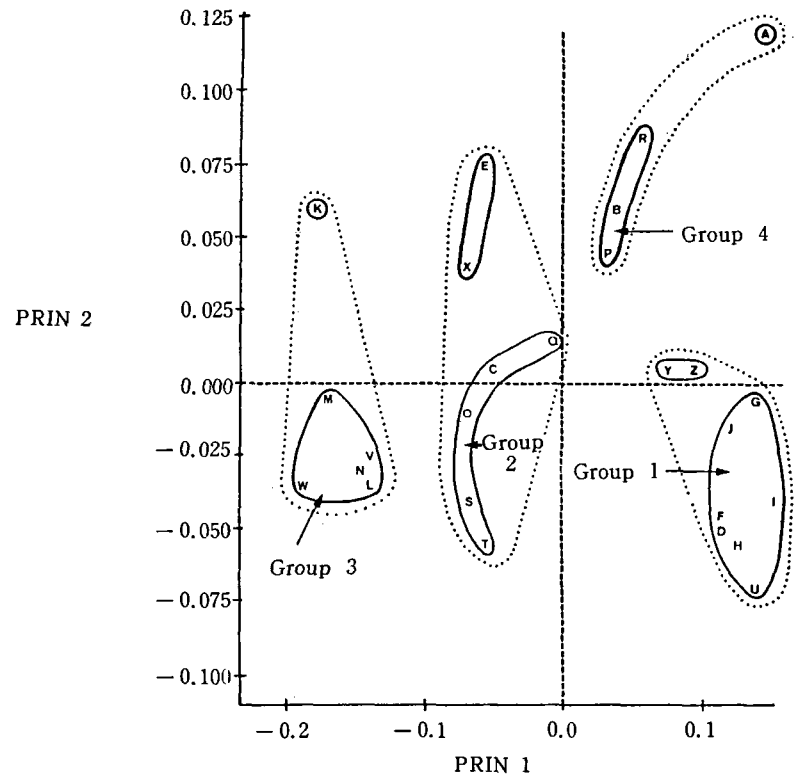

Fig. 1. Grouping of lipases by principal Component Analysis for the ratio of stort-chain fatty acids released at 32°C for 1 wk. Alphabetical letters are symbols for different lipases (see Table 1)

ssified into five major groups indicated by solid lines. Group A included all lapases of ruminant-animal origin, similar to Group 1 delineated by the ratios of FFA in Fig. 1. Group B included lipases of mold (R. arrhizus and M. M. miehei) and bacterial (P. fluorescens) origins. Group C included lipases from one yeast (Candida rogosa), a mold (A. oryzaw), and a porcine pancreas (Rohm Tech.). Group D lipases were from bacterial (C. viscosum), plant (wheat oerm), and porcine pancreas (Biocon and Amer. Lab. Inc.). Group E included lipases from two molds (A. niger, A. oryzae, and M. bacillus), and a porcine pancreas (Sci. Pro. Lab.). Although the total activities of lipase samples were measured and equivalent amounts of enzymes were added to cheese slurries, FFA concentrations during incubation varied considerably. This variability may be related to the difference in the substrate system used for the enzyme activity measurement (milk fat emulsion vs. cheese slurry) as well as difference in the velocity of enzyme activity as a function of incubation time (7 min vs. 1 wk). The difference also may be related to the type of FFA measurements employed (total FFA by pH stat titration vs. individual FFA by GC). Because of this variability, the groupings by FFA concentrations may vary depending upon the amount of lipase actually added to the cheese slurries. On the other hand, it may be difficult to practically obtain equivalent lipase activities. Therefore, classifying lipases by FFA concentrations may be undependable.

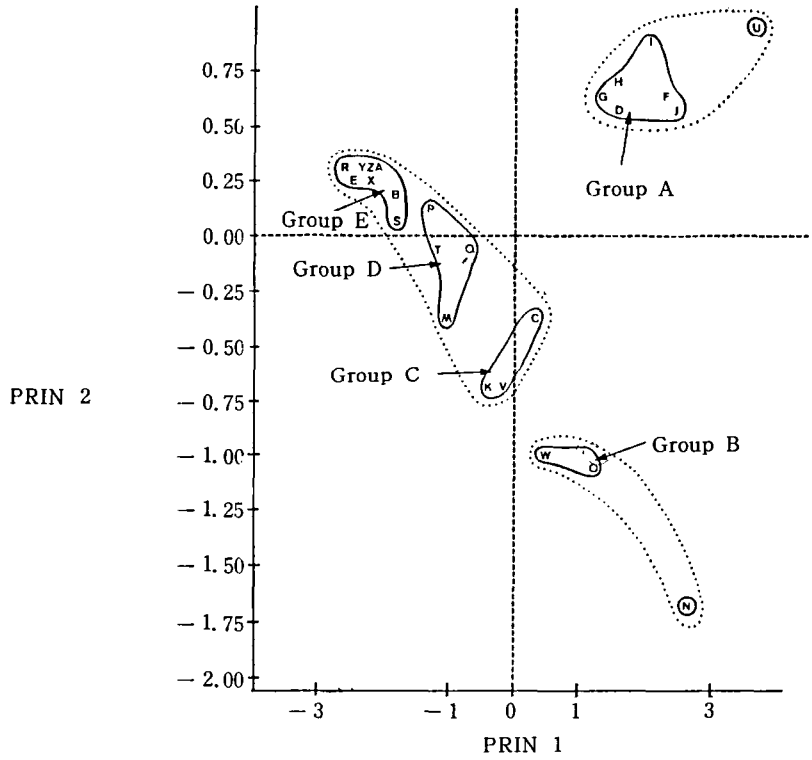

Fig. 2. Grouping of lipases by Principal Component Analysis for the concentration of short-chain fatty acids relased at 32°C for 1 wk. Alphabetical letters are symbols for different lipases (see Table 1)

Eigenvalue of Principal Component for FFA

The eigenvalues for principal component of FFA are summarized in Tables 2 and 3. The first principal component (Prin 1) on the ratio of short-chain FFA accounts for 82.3%, the second for 15.2%, the third for 2.4%, and the fourth for 0.0% (Table 2). Similarly, for concentration, the first principal component accounts for 88.1%, the second for 11.0%, the third for 0.5%, and the fourth for 0.4% (Table 3). This indicates that the first component was the most influential and the second component was next. Contributions of the third and fourth components were insignificant. Therefore, one may consider that the correlation matrices illustrated in Figs. 1 and 2 were mostly contributed by the first and second components (Prin 1 and Prin 2). In addition, the coefficients of eigenvectors (Tables 2 and 3) show interesting statistical results. Prin 1 apperars to be a contrast between variables C4, C6 and variables C8, C10. This means that Prin 1 will be high if C4 and C6 are high but C8 and C10 are low. Howerer, since the effect of the variable C6 is insignificant (a low coefficient), Prin 1 represents a sharp difference in ratios of the C4, C8, and C10 FFA. Prin 2 also shows a contrast between variables C6. C8 and variables C4, C10. suggesting that Prin 2 will be high if C4 and C10 are high but C6 and C8 are low. Howerer, because of a low coefficient for C8, Prin 2 represents difference between the ratios of the C4, C6, and C10 FFA. Similarly, for the concentration of short-chain FFA (Table 3), Prin 1 (88.1%)

Table 2. The eigenvalues and eigenvectors of the correlation matrix for 4 measurements on ratio of short-chain FFA from 26 lipase-added cheese slurries

Component	Eigenvalue*	Eigenvector, coefficient of**			
		C4	C6	C8	C10
1	0.013275	0.797361	0.021434	-0.289932	-0.528862
2	0.002458	0.337910	-0.796171	-0.041912	0.500173
3	0.000391	-0.005714	-0.340078	0.814974	-0.469182
4	0.000000	0.500000	0.500000	0.500000	0.500000
Total	0.016124				

* Eigenvalues are the variances of the principal components.
** Eigenvectrors give the coefficients of the standardized variables.

Table 3. The eigenvalues and eigenvectors of the correlation matrix for 4 measurements on concentration of short-chain FFA from 26 lipase-added cheese slurries

Component	Eigenvalue*	Eigenvector, coefficient of**			
		C4	C6	C8	C10
1	3.52270	0.475378	0.519275	0.491029	0.513088
2	0.44182	0.670235	0.294100	-0.570388	-0.372756
3	0.02126	0.344559	-0.624195	-0.323514	0.622092
4	0.01422	0.453960	-0.504221	0.573486	-0.459214
Total	4.00000				

* Eigenvalues are the variances of the principal components.
** Eigenvectors give the coefficients of the standardized variances.

is related positively to the average values of C4, C6, C8, and C10, whereas Prin 2 shows a contrast between C4, C6 and C8, C10. Therefore, it seems that the variations in Prin 1 are related to the concentration differences of the four variables (C4 to C10). Prin 2 also appears to pepresent all variables since all four variables have relatively large coefficients.

Average Linkage Cluster Analysis of FFA

The data of short-chain FFA obtained from the cheese slurries were also analyzed with Average Linkage Cluster Analysis (ALCA) which showed similar patterns of libera-

ting fatty acids (Fig. 3 and Fig. 4). However, this analysis can be viewed as a matter of distances defined between groups of lipases. For example, there were 25 groups of lipases for zero normalized Root-Mean-Square (RMS) distance, which means that each individual lipase was shown as a group. When the normalized RMS distance was increased to about 0.3, however, lipases were clustered in 10 groups with regard to the FFA ratios (Fig. 3) and 7 groups for the concentrations (Fig. 4). As the RMS distance increased further to 1.2, all of the lipases tested (25 lipases) could be classified into 2 groups. If the results of PCA are compared with these of ALCA, groups of lipases in solid lines and dotted lines (Fig. 1) coincide with those of normalized RMC distances at 0.37 and 0.67 (Fig. 3), respectively. In Fig. 2, groups of enzymes in solid and dotted lines also coincide with those of the enzymes at the distances of 0.3 and 0.4, respectively. The results of both statistical analyses indicated that lipases from different origins and/or manufacturers can be classified into groups by their distinctive patterns of releasing fatty acids from milk fat molecules. In other words, all

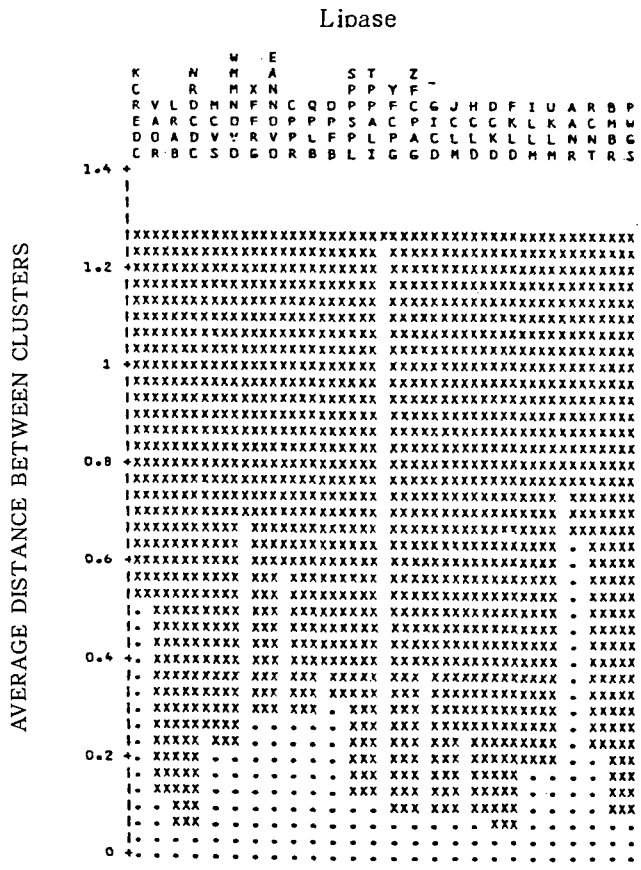

Fig. 3. Clustering of lipases by Average Linkage Cluster Analysis for the ratio of short-chain fatty acids released at 32°C for 1 wk. The first alphabetical letter for lipases represents symbol and the rest of the letters are abbreviation for lipases (see Table 1)

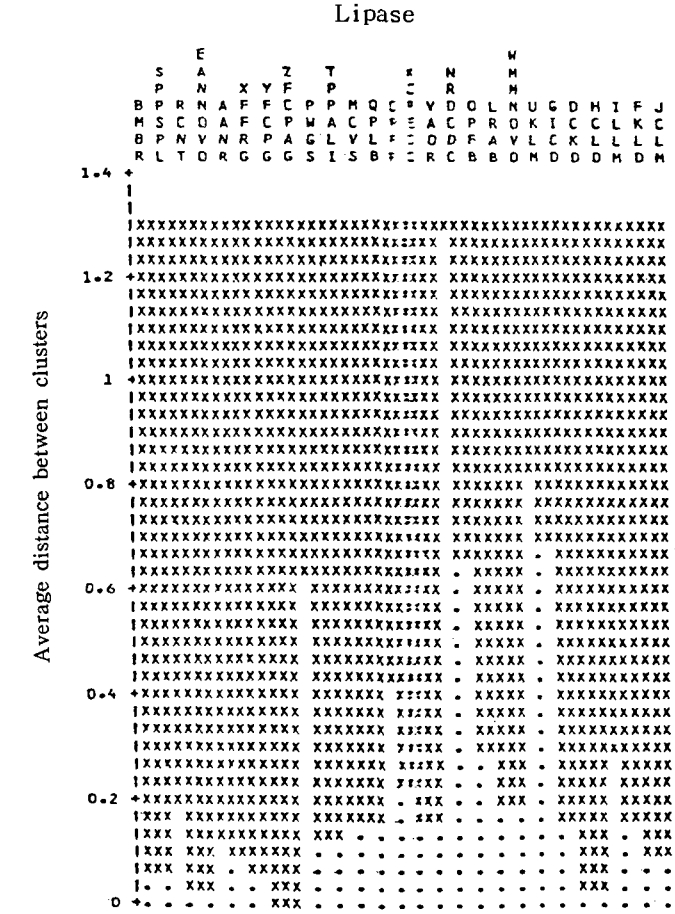

Fig. 4. Clustering of lipases by Average LInkage Cluster Analysis for the concentration of short-chain fatty acids at 32°C for 1 wk. The first alphabetical letter for lipases represents symbol and the rest of the letters are abbreviation for lipases (see Table 1)

of the lipases tested had a considerable preference and selectivity in hydrolyzing short-chain FFA from milk fat.

Production of short-chain fatty acids

The total amount of short-chain FFA released by various lipases is summarized in Fig. 5 and Fig. 6 for the major groups of enzymes as classifies by the statistical analyses. Ratios of short-chain FFA liberated are shown in Fig. 5. Group 1 (ruminant–animal origins) shows an extremely high ratio of butyric acid, but low ratios of caprylic and capric acids. The relative specificity of calf lipase for the preferential release of butyric acid from milk fat seems to be apparent (Harper, 1956; Lin and Jeon, 1987). Harper (1957) also reported that lipases from calf, kid and lamb produced a considerably high ratio of butyric acid but low ratios of short chain FFA in milk fat. Group 2 (porcine pancreas and bacterial origins) showed no extreme ratios in the

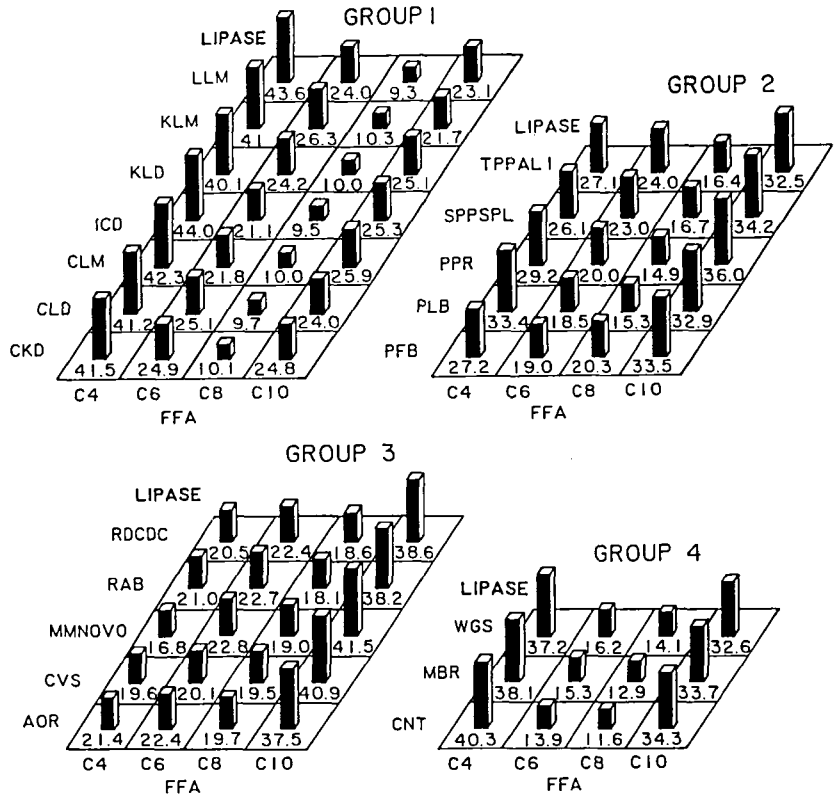

Fig. 5. Major groups of lipases by Principal Component Analysis and Average Linkage Cluster Analysis for the ratio of short-chain fatty accids released at 32°C for 1 wk. For name of lipases, see Table 1 under abbreviations. FFA indicates free fatty acids in carbon numbers of 4 to 10. The numbers in the blocks indicate ratios of the four fatty acids and the heights of the bars are relative to the ratios.

fatty acids liberated. This result was in agreement with Harper (1957) and Nelson (1972), but the ratios of capric acids were low in their studies. Group 3 (mold and bacterial origins) produced lower ratios of butyric acid than other groups but higher ratio of caprylic acid. Moskowitz et al. (1977) reported that M. miehei lipase hydrolyzed at a higher rate in milk than in other substrates, and released high ratios of butyric and caprylic acids but a low ratio of capric acid. Horiuti and Imamura (1977) in a substrate specificity study observed that C. viscosum hydrolyzed more in tricaprylin and less in tricaprin. Group 4 showed high ratios for both butyric and capric acids but low for caproic and caprylic acids.

The concentrations of short-chain FFA in cheese slurries are shown in Fig. 6. Group A (lipase of ruminant-animal origin) produced high concentration of short-chain FFA (more than 350 ppm). Group B (molds and bacteria) produced concentrations over 230 ppm. Group C (molds and a pancreas) was about 170 ppm in concentration. Group D (pancreas, a bacteria and the plant lipase) produced about 110 ppm. Group E showed very low FFA

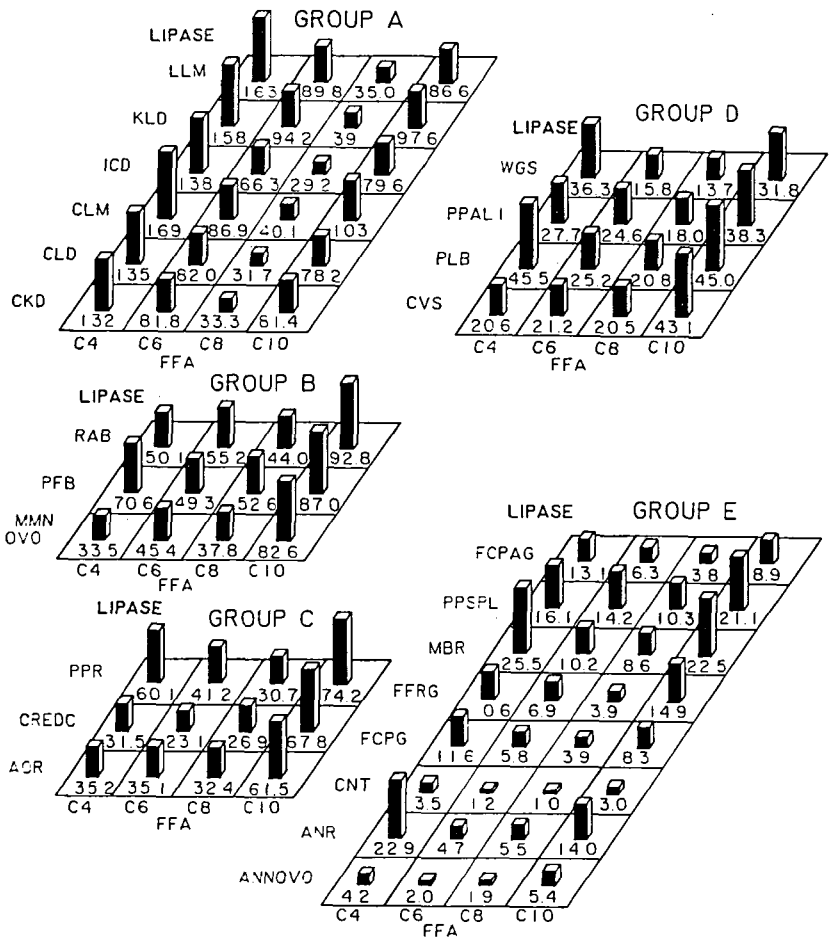

Fig. 6. Major groups of lipases by Principal Component Analysis and Average Linkage Cluster Analysis for the concentration of short-chain fatty acids released at 32°C for 1.wk. For name of lipases, see Table 1 under abbreviations. FFA indicates free fatty acids in carbon numbers of 4 to 10. The numbers and heights of the bars in the blocks indicate concentrations (ppm) of the fatty acids.

production. The concentration differences in groups might have been due to different optimum conditions for enzymes such as substrate, temperature, pH, or ionic strength.

Effect of Time on production of short-chain FFA

Data of short-chain FFA in Table 4 were from a 4 wk incubation of cheese slurries treated with lipases from the four major groups indicated by ratios (Fig. 1). Ratios of each sample were comparatively constant throughout whole periods of ripening. However, some ratios at the initial time show that they were not stable. Lipase from M. bacillus (B in Table 4) had a higher standard deviation than others. Data for production of short-chain FFA (TAble 5) were from a 4 wk ripening of the slurries using lipases from the

Table 4. Effect of time on ratio of short-chain fatty acids from lipase-treated cheese slurries

Symbol*	Ripening periods (wk) 0	1	2	3	4	Mean	S.D.**
				C4 (%)			
F	43.77	40.73	39.76	39.93	36.40	40.12	2.63
Q	30.61	33.37	30.19	38.09	40.22	34.50	3.40
N	17.91	20.46	23.46	20.58	21.67	20.82	2.02
B	29.74	38.13	28.56	35.51	35.39	33.47	4.54
				C6 (%)			
F	27.25	24.19	23.61	23.85	24.61	24.70	1.47
Q	19.54	18.46	21.44	20.79	20.10	20.07	1.06
N	21.69	22.35	23.01	23.85	21.28	22.44	1.03
B	10.60	15.30	16.55	18.75	19.27	16.09	3.47
				C8 (%)			
F	8.69	10.01	10.51	11.47	11.92	10.52	1.27
Q	15.61	15.25	16.08	13.12	14.85	14.98	1.95
N	20.26	18.62	17.56	18.41	17.65	18.50	1.09
B	19.42	12.91	13.74	13.09	13.85	14.60	2.77
				C10 (%)			
F	20.29	25.07	26.12	24.78	27.07	24.67	2.64
Q	34.24	32.93	35.35	28.00	24.82	31.07	4.48
N	40.14	38.57	35.97	36.74	39.39	38.16	1.76
B	40.23	33.66	41.15	32.65	31.49	35.84	4.57

All analyses were in duplicates.

* Symbols are from Table 1 for individual lipase preparations and the enzymes are selected to represent each of the 4 major groups in fig. 1.

** Standard deviation

five major groups indicated in Fig. 2. The production of each short-chain FFA was considerably increased by longer ripening time. However, for some groups (Group 1, 2, and 4), concentration of the fatty acids showed no change or decreased at 4 wk. Even though the sample from Group 5 increased in concentration of fatty acids during the incubation periods, the initial amounts of FFA released were smaller than those with other groups. During the course of 4 wk analysis, however, the effect of extraneous microbial activities could have been added to the relase of FFA in cheese slurries although their effect seemed least likely to alter the pattern of FFA released in enzymetreated slurries. There was no significant difference in pH and total microbial population for the initial and one week analysis. After 1 wk, however, the microbial population showed a considerable decrease in some enzyme-added samples as the FFA concentrations increased. The fatty acids released might have had an antimicrobial effect in cheese slurries (Oulare, 1986).

Since the releasing patterns of lipases were related to the ratios of short-chain FFA as well as their concentrations, it would be significant to find and/or use a

Table 5. Effect of time on concentration of short-chain fatty acids from lipase -treated cheese slurries

Symbol*	Ripenining Periods (wk)				
	0	1	2	3	4
	C4 (ppm)				
F	88.8	158.9	179.9	285.6	211.3
L	13.2	51.1	58.9	74.8	67.7
V	14.1	35.2	63.0	96.4	166.1
T	3.5	27.7	44.0	64.3	68.4
X	0.9	10.6	20.1	23.3	35.9
	C6 (ppm)				
F	55.3	94.2	106.9	170.6	142.8
L	12.3	55.2	64.7	86.1	73.6
V	12.9	35.1	47.9	78.9	145.4
T	6.1	24.6	29.9	46.5	43.7
X	1.9	6.9	7.6	12.1	20.4
	C8 (ppm)				
F	17.6	39.0	47.6	82.1	69.2
L	11.8	44.0	50.3	57.3	54.5
V	12.5	32.4	40.7	64.0	107.1
T	6.9	16.8	21.3	34.8	34.6
X	3.5	3.9	4.2	7.4	9.6
	C10 (ppm)				
F	41.2	97.6	118.2	170.1	157.1
L	24.9	92.8	115.4	124.9	127.6
V	25.8	61.5	86.8	123.2	198.4
T	13.8	33.2	51.0	83.0	84.1
X	11.6	15.0	16.4	20.0	18.3

All analyses were in duplicates.

* Symbols are from Table 1 for individual lipase preparations and the enzymes are selected to represent each of the 4 major groups in Fig. 1.

lipase that has a specific activity similar to that in natural fermentation of dairy products. This finding also may be significant in understanding the behavior of lipases and may be useful for desirable flavor development in accelerated cheese ripening.

5. Conclusions

Lipase could be classified into four of five major groups according to patterns of fatty acid release. Similar ratios or concentrations of FFA were observed within a group of lipase. However, the pattern of liberating FFA differed from one enzyme source to another. Principal Component Analysis and Cluster Linkage Analysis appeared to be applicable for the classification of lipases based on their specificities.

6. References

1. AOAC. 1984. "Offical Method of Analysis", 14th ed. Association of Official Analytical Chemists, Washington, D.C.
2. Arbige, M.V., Freund, P.R., Silver, S. C., and Zelko, J.T. 1986. Novel lipase for cheddar cheese flavor development. Food Technol. 40(4):91.
3. Chandan. R.C.. and Shahani. K.M. 1963. purification and characterization of milk lipases. I. Purification. J. Dairy Sci. 46:275.
4. Deeth, H.C., Fitz-Gerald, C.H. and Snow, A.J. 1983. A gas chromatographic method for the quantitative determination of free fatty acids in milk and milk products. New Zealand. J. Dairy Sci. and Technol. 18:13.
5. Farnham, M.G., Nelson, J.H., and Kemp, A.R. 1956. A study of the lypolysis induced in various milk by pregastric esterase obtained obtained from suckling mammals. J. Dairy SCi. 39:928.
6. Harper, W. J. 1956. Apparent selective liberation of butyric acid from milk fat by the action of various lipase system. J. Dairy Sci. 38:1391.
7. Harper, W.J. 1957. Lipase systems used in the manufacture of Italial cheese. II. Selective hydrolysis. J. Dairy Sci. 40:556.
8. Horiuti, Y., and Imamura, S. 1977. Purification of lipase from Chromobacterium viscosum by chromatography on palmotoyl cellulose. J. Biochem. 81:1639.
9. Khan, I.M., Bill, C.W., Chandan, R.C., and Shahani, K.M. 1967. Production and properties of the extracellular lipase of Achromobacter lipolyticum. Biochim. Biophys. Acta. 132:68
10. Law, B.A.. and Wigmore. A.S. 1985. Effect of commercial lipolytic enzymes on flavour development in Cheddar cheese. J. Soc. Dairy Technol. 38(3):86.
11. Lin, J.C.C., and Jeon, I.J. 1987. Effects of commercial food grade enzymes on free fatty acid profiles in granula cheese. J. Food Sci. 52:78.
12. Manly, B.F.J. 1986. "Multivariate Statistical Methods. A Primer." London and New York. Chapman Hall.
13. Morris, H.A., and Jezeski, J.J. 1953. The action of microorganisms on fats. II. Some characteristics of the lipase system of Penicillium roqueforti. J. Dairy Sci. 36:1253.
14. Moskowitz, G.J., Shen, T., West, I.R. Cassaigne, R., and Feldman, L.I. 1977. Properties of the esterases produced by Mucor miehei to develop flavor in dairy products. J. Dairy Sci. 60:1260.
15. Nelson, J.H. 1972. Enzymatically produced flavors for fatty systems. J. Amer. Oil Chem. Soc. 49:559.
16. Oulare, K. 1986. Antimicrobial effect of fatty acids in laboratory media and in meats. Ph.D. dissertation, Kansas State University, Manhattan, Ks.
17. SAS. 1985. SAS User's Guide:Statistics. SAS Institute Inc., Box 8000, Cary, NC. 27511-8000.
18. Sood, V.K., and Kosikowski, F.V. 1979. Repening changes and flavor development in microbial enzyme treated cheddar cheese slurries. J.Food SCi. 44:1690.
19. Wilcox, J.C., Nelson, W.O., and Wood, W. A. 1955. The selective release of volatile acids from butterfat by microbial lipases. J. Dairy Sci. 38:775.