Quantification of Lactones in Ripening Pasteurized Milk Blue Cheese Containing Added Microbial Lipases

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Flavor simulation studies of cheese have shown the importance of lactones. The present study identified and quantified lactones in pasteurized milk blue cheese. It also explored the effect of added lipases on lactone production. Lactones were extracted on a chromatographic column packed with 6 g of aluminum oxide, 35 g of sodium sulfate, and a cheese (20 g)–Celite (25 g) mixture and identified using gas–liquid chromatography. δ-Tetradecalactone and δ-dodecalactone were the major lactones among γ-decalactone, γ-dodecalactone, and δ-decalactone identified in blue cheese. At the beginning of ripening the concentration of δ-decalactone and δ-dodecalactone was low, then increased until 75 days when it decreased. At 75 days the concentration of δ-decalactone and δ-dodecalactone was five times higher in pasteurized milk blue cheese with added microbial lipase than the control pasteurized milk blue cheese. Differences are attributed to the lipolytic effectiveness of microbial lipase incorporated in cheese curds. The application of selected microbial lipase accelerated lactone production and gave superior flavor in blue cheese.

Two lactones, δ-octalactone and δ-decalactone, were tentatively identified in blue cheese by Day and Anderson (1965) and apparently result from five hydroxyalkanoic acids in esterified glyceride form according to the reaction schemes of Boldingh and Taylor (1962) and Patton (1965).

In view of the low threshold values of the δ lactones (Kinsella et al., 1965), it is strongly suggested that these lactones contribute to the flavor of blue cheese. Flavor simulation studies of cheese have shown lactones to be important according to Wong et al. (1973).

Recently, Jolly (1974) showed that the application of microbial lipases with salt to fresh curds of blue cheese accelerated its flavor and the development of free fatty acids, free amino acids, and carbonyls. As a continuation, lactones were studied under similar conditions with regard to their rate of production in pasteurized milk blue cheese and their specific identities and relationship to other classes of compounds. This paper presents the results.

MATERIALS AND METHODS

Cheesemaking. Blue cheese from 500 kg of (a) pasteurized skimmilk (72 °C, 16 sec) and homogenized raw cream, at 500 psig, and (b) pasteurized whole cow's milk was prepared according to Kosikowski (1970). The food grade lipase preparations obtained were individually blended with salt, *Penicillium roqueforti* spores, and microbial enzyme (No. 3500) derived from *Aspergillus* mold (Wallerstein Co., Morton Grove, Ill.). The latter was added at 0.5–1.5 g/7 kg of curd. Periodically, samples were plugged from two wheels of each of the several cheese lots and analyzed in triplicate.

Extraction and Identification of Lactones. The blue cheese was extracted with acetonitrile by a procedure described by Wong et al. (1973) using column chromatography. A 2.5 cm (i.d.) × 50 cm chromatographic tube was packed as follows: 6 g of alumina (heated at 170 °C for 16 hr and then equilibrated with 8% of 5 *N* H_2SO_4), 35 g of anhydrous sodium sulfate, 20 g of cheese, and 25 g of Celite 545. Lactones were eluted with acetonitrile and about 18 ml was collected.

The final eluent from the column was further purified by passing over 0.8 g of treated alumina, concentrated to 0.4 to 0.6 ml from 50 ml of acetonitrile extract, and a 4-μl aliquot was gas chromatographed. Lactones were analyzed by gas–liquid chromatography (Figure 1): helium flow, 28 ml/min; injection port, 250 °C; oven temperature 180 °C; program rate isothermal; 7.5% of 2% phosphorylated ethylene glycol adipate column; sample size 1.9 ml of lactone standard. Authentic lactones obtained from Organic Chemical Corp. were checked for purity. Standard curves for δ-dodecalactone and δ-decalactone were prepared from 0 to 5 μg/μl and the procedure extraction efficiency was calculated to have a correlation coefficient of 0.98.

RESULTS

Cheese samples were analyzed at 15, 30, 45, 60, 75, and 90 days of ripening. The effect of the added food grade microbial lipase on the lactones was determined at various ripening stages.

The GLC separation of various lactones is shown in Figure 1 and the peak area was determined by a triangulation technique. δ-Decalactone, δ-dodecalactone, δ-tetradecalactone, γ-octalactone, γ-decalactone, and γ-dodecalactone were identified by gas chromatography using known standards of these lactones. δ-Dodecalactone and δ-tetradecalactone were the major lactones. The weight percent of various lactones varied over various ripening stages (Table I) but the weight percent of δ-dodecalactone and δ-tetradecalactone as percent of the total remained constant.

The concentration of δ-decalactone and δ-dodecalactone in control blue cheese decreased in the early ripening period followed by an upturn and subsequent lowering in later ripening (Table II). The same lactones in blue cheese containing microbial and animal lipases followed a similar pattern but in blue cheese containing added microbial lipase, the amounts of δ-decalactones were four times higher and of δ-dodecalactone six times higher than in the control cheeses (Table II).

DISCUSSION

The hydroxyalkanoic acids esterified in triglyceride molecules are subject to hydrolyzing action under acidic and mild heat conditions leading to lactone formation. Higher levels of lipolysis in blue cheese ripening ensure splitting of higher amounts of hydroxy acids esterified in triglycerides as compared with other ripened cheeses like cheddar and this indirectly influences the concentration of lactones. The concentration of various lactones in blue cheese was higher than the concentration of corresponding lactones in cheddar cheese reported by Wong et al. (1973), a point attributed to the wider difference in lipolysis occurring in the former. Weight percent data indicated that δ-tetradecalactone and δ-dodecalactone were the major lactones (Table I) as was

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Table I. Lactones at Various Stages of Ripening Pasteurized Milk Blue Cheeses at 5 °C[a]

	Relative wt % at days											
	15		30		45		60		75		90	
Lactones	A	B	A	B	A	B	A	B	A	B	A	B
γ-Deca	t	4.63	0.65	3.24	1.15	6.44	t	1.06	0.87	5.27	1.03	2.24
γ-Dodeca	4.53	4.68	5.80	5.40	6.00	4.14	16.75	1.02	14.81	4.83	9.08	5.98
δ-Deca	9.06	7.93	6.44	6.47	5.54	8.06	14.14	1.87	11.11	1.92	4.65	5.83
δ-Dodeca	15.94	27.17	11.12	21.58	9.87	42.69	32.55	44.78	27.45	17.03	12.46	38.12
δ-Tetradeca	70.47	55.58	76.01	63.31	77.44	38.67	36.55	51.38	45.75	70.96	72.17	47.83

[a] A, pasteurized milk blue cheese without added microbial lipase (control); B, pasteurized milk blue cheese containing added microbial lipase; t, trace.

Table II. Effect of Added Microbial Lipase on δ-Deca- and δ-Dodecalactones in Pasteurized Milk Blue Cheese During Ripening at 5 °C

	μg per 20 g of cheese			
	Control		Lipase 3500	
Days	δ-Deca	δ-Dodeca	δ-Deca	δ-Dodeca
15	278.5	366.5	97.6	171.2
30	114.6	149.8	119.3	204.0
45	106.2	140.2	226.5	265.0
60	87.6	127.2	326.6	518.9
75	108.6	167.2	444.4	925.1
90	83.6	123.4	245.3	679.8

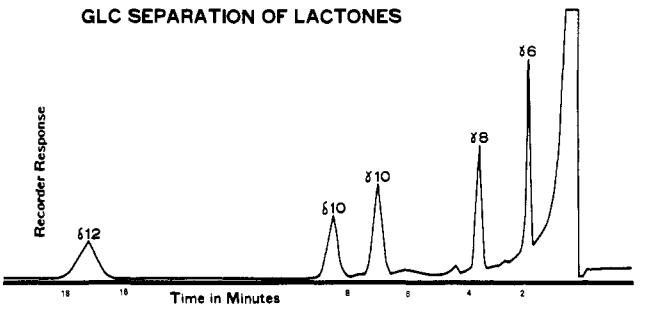

Figure 1. GLC separation of lactones.

the case in cheddar cheese (Wong et al., 1973). The data on relative concentration of δ-dodecalactone and δ-tetradecalactone indicate the latter to be the major lactone formed during ripening of the blue cheese. However, the relative concentration of the two lactones continues to change during the latter part of the ripening. Decreases at the beginning and the end of the ripening are attributed to the hydrolyzing action. However, the highest concentration of δ-decalactone and δ-dodecalactone was observed at the 75th day of ripening and can be attributed to fat hydrolysis by *P. roqueforti*. The chemistry and microbiology of decreased lactone levels are still not well understood. A similar pattern was noticed for cheddar cheese by Wong et al. (1973) where a maximum was observed at about the 210th day which coincided with a maximum accumulation of free fatty acids.

The concentration of δ-decalactones and δ-dodecalactones which have potential for flavor was four times higher in blue cheese containing added microbial lipases than in the control cheese (Table II) and can be attributed to extensive lipolysis of the butterfat by the added food grade microbial lipase. Apparently then the lactone concentration can be increased by accelerated lipolysis of the precursor hydroxy acids. The quantitative data in Table II on lactone concentration should not be confused with Table I as the latter shows only relative weight percentages. Cheese containing microbial lipases also possessed much better blue cheese flavor than the control, as evaluated by four trained taste panelists using American Dairy Science Association score cards. The flavor of cheese containing microbial lipases was expressed as typical strong blue cheese flavor perhaps because lactones blend or modify harsher and sharper flavor notes. The total flavor effect obviously is due to a total effect of free fatty acids, methyl ketones, free amino acids, etc. along with lactones. The data on methyl ketones, free fatty acids, and free amino acids will be published in subsequent publications. The same principle might be applied for improving flavor of other foods, and developing better cheese flavors using microbial enzymes.

LITERATURE CITED

Boldingh, J., Taylor, R. J., *Nature (London)* **194,** 909 (1962).
Day, E. A., Anderson, D. F., *J. Agric. Food Chem.* **13,** 2 (1965).
Jolly, R. C., Ph.D. Thesis, Cornell University, 1974.
Kinsella, J. E., Patton, S., Dimick, P. S., *J. Am. Oil Chem. Soc.* **44,** 202–205 (1965).
Kosikowski, F. V., "Cheese and Fermented Milk Foods", Edwards Bros., Inc., Ann Arbor, Mich., 1970.
Patton, S., "Flavor of Processed Milks", Food Quality Publication No. 77, AAAS, Washington, D.C., 1965, p 165.
Wong, N. P., Ellis, R., LaCroix, D. E., Alford, J. A., *J. Dairy Sci.* **56,** 636 (1973).

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