The Influence of Certain Bacteria on the Ripening of Cheddar Cheese Made From Pasteurized Milk[1]

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PASTEURIZATION of milk for cheddar cheese making is probably the most important contribution to the cheese industry in recent years. From a public health standpoint the pasteurization of all milk for cheese-making is of great significance since it minimizes the possibility of spreading infectious diseases through cheese. In the pasteurization of milk several changes occur which not only prevent the typical cheese flavor from developing but also materially increase the time necessary for proper ripening. If the increase in time required for the ripening of the cheese is due to the partial destruction of the natural bacterial flora of the milk by pasteurization, the addition of pure cultures of essential bacteria should tend to overcome this difficulty.

Evans, Hastings and Hart (2) concluded that organisms of the *Bacterium casei* group are responsible for the pungent taste that develops late in the ripening of both raw milk and pasteurized milk cheese. When milk cultures of *Bacterium casei* were added to pasteurized milk used for making cheese, however, a pronounced sour flavor developed in the cheese. Davies and associates (1) inoculated milk used for cheddar cheese with cultures of *Lactobacillus casei* or *Lactobacillus plantarum,* which had been isolated from ripened cheese. They found that the Lactobacillus cultures appeared to accelerate the protein degradation in the cheese during the early stages of ripening, but had no effect on the flavor or texture.

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Hucker and Marquardt (4) found that *Streptococcus paracitrovorus,* when added to pasteurized milk in addition to commercial starter, improved the flavor of the cheese; *S. citrovorus* had no effect on the flavor, while certain strains of proteolytic cocci produced a bitter flavor. Hansen, Bendixen and Theophilus (3) found that *S. paracitrovorus* and *S. citrovorus,* when used alone as starters, produced cheese with a bitter flavor and a weak body.

Lane (5) compared the effects of several organisms on the speed of ripening and the flavor of cheddar cheese made from pasteurized milk. Certain strains of *L. casei* produced a mild, buttery flavor in the cheese and hastened proteolysis; *Aerobacter oxytocum* produced an unclean flavor, while *S. liquefaciens* brought about a bitter flavor and abnormal proteolysis. *S. paracitrovorus* produced a typical mellow flavor during the early stages of ripening but had no effect on the hydrolysis of the protein. An unidentified Micrococcus slightly improved the flavor and ripening of the cheese.

The work herein reported is a study of the influence of additional pure cultures of bacteria on the nitrogenous decomposition and the flavor development in cheddar cheese made from pasteurized milk.

METHODS

MANUFACTURE AND SCORING OF CHEESE

For each lot of experimental cheese, 600 pounds of milk were used. The milk was pasteurized at 145° F. for 30 minutes, cooled and divided evenly among four small vats. Two percent of commercial cheese culture was used together with 0.5 percent of a pure culture of L4, a strain of *L. casei* (7). In addition to these cultures, each of three vats was inoculated with a milk culture of a test organism. The cheese manufacturing process employed was that described by Lane and Hammer (6, 7). The cheese were scored for flavor at regular intervals during the ripening by competent judges.

PREPARATION OF TEST CULTURES

The test cultures were cultivated in flasks of sterile milk and were incubated as follows: An unidentified Micrococcus, 70° F. for 7 days; *S. liquefaciens, Alcaligenes viscosus, Achromobacter lipolyticum, Pseudomonas fluorescens, Ps. fragi,* lipolytic acid-forming organism 12 and lipolytic non-acid forming organism 18, 70° F. for 4 days. When inoculated into the milk used for cheesemaking, 0.05 percent of each test culture was used except with the Micrococcus which was added at the rate of 0.5 percent.

CHEMICAL ANALYSIS OF CHEESE

NITROGENOUS DECOMPOSITION IN CHEESE

The methods used for the study of the nitrogenous decomposition in the experimental cheese were those suggested by Lane and Hammer (6).

Cheese serum was obtained at regular intervals during the ripening by submitting mixtures of finely divided cheese and sand to relatively high pressures in a hydraulic press. The serum was analyzed for total nitrogen, amino nitrogen and various fractions of protein and protein decomposition products which were soluble or insoluble in trichloracetic acid, ethyl alcohol and phosphotungstic acid. The values for the various forms of nitrogen were expressed as the milliliters of N/10 acid equivalent to the nitrogen of 1 milliliter of cheese serum.

ACID VALUE OF FAT

When the cheese and sand mixtures were submitted to pressure, considerable fat was obtained with the serum. This fat was used to determine the acid values. Twenty grams of fat were boiled with 50 ml. of neutral 95 percent ethyl alcohol and the mixture titrated with N/10 NaOH using phenolphthalein.

RESULTS

EFFECT OF S. LIQUEFACIENS, AN UNIDENTIFIED MICROCOCCUS OR BOTH

Table 1 presents the data on the nitrogenous decomposition and flavor development in one of the duplicate series of cheese in which *S. liquefaciens*, an unidentified Micrococcus or both were used in addition to the regular cultures.

Throughout the ripening little variation was shown in the amounts of total nitrogen in the serums of the four cheese in a series, although slightly more variation occurred at the end of the ripening than at the beginning. The amounts of nitrogen in the fractions soluble in the various reagents increased as the ripening progressed but showed very little variation. Even after 112 days of ripening the variations were small. Increases in the nitrogen content were also shown in the insoluble fractions, with the exception of the fraction insoluble in trichloracetic acid. This fraction increased during the first 28 days and after that gradually decreased so that after 112 days it was only slightly greater than after 3 days. Although the variations in the soluble fractions were small, the data indicate that the serum of the control cheese contained less soluble nitrogen when ethyl alcohol or phosphotungstic acid were used as precipitating agents than the cheese made with the test organisms.

Increases in the amounts of amino nitrogen in the serums were shown by all of the cheese as the ripening progressed, although the increases were generally less in the serums of the control cheese than in those of the cheese made with test organisms.

The data on the effect of the test organisms on the flavor of the cheese indicate that the control cheese regularly scored less than the cheese made with the test cultures. Very little variation was shown in the flavor scores of the cheese made with *S. liquefaciens*, the Micrococcus or both of these organisms.

Bacteriological examinations of the cheese serum at various intervals during the ripening indicated that considerable numbers of living test organisms were present.

EFFECT OF LIPOLYTIC ORGANISM 18, PS. FRAGI, OR LIPOLYTIC ORGANISM 12

The data on the nitrogenous decomposition and flavor development in one of the duplicate series of cheese in which lipolytic organism 18, *Ps. fragi,* or lipolytic organism 12 was used in addition to the regular cultures are given in table 2.

In the very young cheese there was considerable variation in the total nitrogen of the serums. With extended ripening the total nitrogen in the serums increased, and after 112 days it varied from 20.1 to 22.8 ml. of N/10 acid; the value for the control cheese was lowest in the series, and the cheese made with *Ps. fragi* was highest. In general, there was a steady increase in the various nitrogen fractions in the serums of all the cheese as the ripening progressed. Throughout the ripening there were no large variations in the fractions soluble in the various reagents although the fractions of the serum from the control cheese were commonly less than those of the other cheese in the series. The amounts of nitrogen in the fractions insoluble in the reagents regularly increased during the entire ripening except with the fraction insoluble in trichloracetic acid. This fraction did not appear to increase after the 28 days of ripening. Only very small variations were found in the amounts of insoluble nitrogen in the serums of the four cheese at the end of the ripening.

The serums of all of the cheese increased in amino nitrogen content as the ripening progressed. After 112 days the serums of the cheese made with the test organisms, especially that of the cheese made with *Ps. fragi,* were considerably higher in amino nitrogen than the serum of the control cheese.

The results obtained on the effect of the various test organisms on the flavor development in the cheese showed that, in general, the control cheese had a more desirable flavor than the cheese made with the test organisms after continued ripening. The cheese made with *Ps. fragi* or lipolytic organism 12 was criticized as being sour and bitter, respectively.

Bacteriological examinations of the cheese during the ripening indicated that considerable numbers of living test organisms were present.

EFFECT OF PS. FLUORESCENS, A. VISCOSUS OR A. LIPOLYTICUM

Table 3 shows the data obtained on the nitrogenous decomposition and flavor development in one of the duplicate series of cheese in which *Ps. fluorescens, A. viscosus* or *A. lipolyticum* was used in addition to the regular cultures.

In the very young cheese there were only slight variations in the amounts of total nitrogen in the serums. With continued ripening the total nitrogen of all of the serums increased and the differences among

TABLE 3. *Effect of Ps. fluorescens, A. viscosus or A. lipolyticum on the nitrogenous decomposition and flavor development in the cheese*

Series 5

Serial number of cheese	Age of cheese (days)	Test organisms used	Moisture (percent)	Ml. of N/10 acid equiv. to nitrogen in 1 ml. of cheese serum						Amino nitrogen (mgs. per ml. serum)	Flavor score of cheese	Remarks on cheese flavor
				Total nitrogen	Nitrogen fractionated into soluble and insoluble fractions with							
					Trichloracetic acid		Ethyl alcohol		Phosphotungstic acid			
					Sol.	Insol.	Sol.	Insol.	Sol.	Insol.		
5-1	3	None	38.6	6.4	4.6	1.8	2.0	4.3	1.5	4.8	1.02		
5-2	3	*Ps. fluorescens*	39.0	5.5	3.7	1.8	1.9	3.5	1.0	4.6	.97		
5-3	3	*A. viscosus*	38.4	5.7	3.4	2.2	2.0	3.7	1.0	4.8	1.07		
5-4	3	*A. lypolyticum*	37.8	5.2	3.6	1.6	1.9	3.2	1.0	4.2	1.07		
5-1	14	None	37.8	10.2	7.8	2.4	2.0	8.2	1.9	8.3	2.18	35.5	Bitter & sour
5-2	14	*Ps. fluorescens*	38.0	10.0	7.0	3.0	2.2	7.8	1.7	8.3	1.85	38.0	Lacks flavor
5-3	14	*A. viscosus*	37.5	10.3	7.6	2.7	2.0	8.3	1.7	8.6	1.74	36.0	Sour
5-4	14	*A. lipolyticum*	37.2	9.7	7.0	2.7	2.0	7.7	1.8	7.9	2.28	37.0	Sour
5-1	28	None	36.8	15.2	11.5	3.8	5.5	9.6	3.4	12.0	2.63	35.5	Bitter
5-2	28	*Ps. fluorescens*	37.2	16.4	12.5	3.9	5.8	10.6	3.5	13.0	3.18	37.5	Lacks flavor
5-3	28	*A. viscosus*	36.8	16.1	12.9	3.2	5.6	10.6	3.2	13.0	2.74	36.5	Sour
5-4	28	*A. lipolyticum*	36.7	16.5	13.9	2.6	4.2	12.4	3.7	12.8	2.78	37.0	Sl. sour
5-1	56	None	36.4	19.6	16.1	3.5	6.5	13.0	4.2	15.3	3.00	36.0	Bitter
5-2	56	*Ps. fluorescens*	36.2	18.0	14.4	3.6	6.0	12.0	3.9	14.0	3.42	37.5	Lacks flavor
5-3	56	*A. viscosus*	36.4	18.0	14.5	3.5	6.6	11.4	3.5	14.5	3.10	37.5	Sl. sour
5-4	56	*A. lipolyticum*	36.0	18.9	15.6	3.4	6.5	12.5	3.8	15.2	3.14	37.0	Sour
5-1	112	None	35.3	24.4	21.8	2.6	11.9	12.4	7.6	16.9	6.01	36.5	Sl. bitter
5-2	112	*Ps. fluorescens*	35.7	22.1	19.5	2.8	8.9	13.5	6.6	15.4	5.28	37.0	Lacks flavor
5-3	112	*A. viscosus*	35.5	23.1	21.2	1.9	10.7	12.6	7.2	16.2	6.37	37.0	Lacks flavor
5-4	112	*A. lipolyticum*	35.4	23.5	20.6	3.1	9.5	14.0	7.1	16.5	6.32	36.0	Sour

TABLE 1. *Effect of S. liquefaciens, an unidentified Micrococcus or both on the nitrogenous decomposition and flavor development in the cheese*

Series 1

Serial number of cheese	Age of cheese (days)	Test organisms used	Moisture (per cent)	Ml. of N/10 acid equiv. to nitrogen in 1 ml. of cheese serum						Amino nitrogen (mgs. per ml. serum)	Flavor score of cheese	Remarks on cheese flavor
				Total nitrogen	Nitrogen fractionated into soluble and insoluble fractions with							
					Trichloracetic acid		Ethyl alcohol		Phosphotungstic acid			
					Sol.	Insol.	Sol.	Insol.	Sol.	Insol.		
1-1	3	None	38.9	5.1	3.1	2.0	1.2	3.9	1.0	4.0	.68		
1-2	3	*S. liquefaciens*	38.6	5.0	3.0	1.9	1.1	3.8	1.1	3.9	.64		
1-3	3	Micrococcus	38.7	5.0	3.1	1.9	1.1	3.9	1.1	3.8	.72		
1-4	3	*S. liquefaciens* and Micrococcus	38.9	5.2	3.4	1.7	1.2	4.0	1.2	4.0	.68		
1-1	14	None	37.8	10.9	7.1	3.8	3.8	7.1	2.4	8.4	.90	37.0	Sour
1-2	14	*S. liquefaciens*	37.2	10.7	7.2	3.6	3.2	7.5	2.3	8.5	.86	38.5	Sl. fermented
1-3	14	Micrococcus	37.4	10.8	7.1	3.6	3.1	7.8	1.9	9.0	.94	39.5	Lacks flavor
1-4	14	*S. lique.* and Micro.	37.9	11.0	7.4	3.6	3.1	7.9	2.0	9.0	.93	38.5	Sl. fermented
1-1	28	None	37.2	14.0	10.6	3.3	4.6	9.5	3.4	10.6	1.73	38.0	Sl. sour
1-2	28	*S. liquefaciens*	37.0	14.1	10.7	3.5	4.1	10.1	2.4	11.9	1.73	39.5	Sl sour
1-3	28	Micrococcus	37.3	12.9	10.0	3.7	3.5	9.5	2.4	10.5	1.71	39.0	Sl. sour
1-4	28	*S. lique.* and Micro.	37.5	14.0	10.4	3.6	4.2	9.7	3.6	10.4	2.07	39.0	Sl. sour
1-1	56	None	37.0	16.9	14.6	2.4	5.8	11.1	4.0	13.0	3.90	36.5	Sour
1-2	56	*S. liquefaciens*	37.1	16.4	13.5	3.0	5.9	10.5	4.6	12.0	4.13	37.5	Sl. sour
1-3	56	Micrococcus	37.2	16.6	13.7	2.9	5.8	10.8	3.0	13.6	4.24	38.0	Sl. sour
1-4	56	*S. lique.* and Micro.	37.2	17.8	14.7	3.1	6.2	11.7	3.7	14.0	4.13	37.0	Sl. sour
1-1	112	None	36.4	19.6	17.2	2.5	9.6	9.9	6.8	12.7	5.12	36.5	Sour
1-2	112	*S. liquefaciens*	36.5	19.6	17.0	2.6	7.7	11.0	6.0	13.7	6.53	38.5	Sl. sour
1-3	112	Micrococcus	36.2	20.6	18.0	2.7	8.4	12.1	6.7	13.6	6.21	37.5	Sl. sour
1-4	112	*S. lique.* and Micro.	36.6	20.6	17.9	2.6	8.2	12.5	6.5	14.0	6.26	37.5	Sl. sour

TABLE 2. *Effect of lipolytic organism 18, Ps. fragi or lipolytic organism 12 on the nitrogenous decomposition and flavor development in the cheese*

Series 3

Serial number of cheese	Age of cheese (days)	Test organisms used	Moisture (percent)	Ml. of N/10 acid equiv. to nitrogen in 1 ml. of cheese serum						Amino nitrogen (mgs. per ml. serum)	Flavor score of cheese	Remarks on cheese flavor
				Total nitrogen	Nitrogen fractionated into soluble and insoluble fractions with							
					Trichloracetic acid		Ethyl alcohol		Phosphotungstic acid			
					Sol.	Insol.	Sol.	Insol.	Sol.	Insol.		
3-1	3	None	39.8	4.5	2.8	1.7	1.5	3.1	1.0	3.6	.82		
3-2	3	Lipolytic 18	39.2	5.1	3.3	1.8	1.9	3.2	1.0	4.1	.89		
3-3	3	*Ps. fragi*	39.3	4.8	3.3	1.7	1.6	3.4	0.9	3.4	1.26		
3-4	3	Lipolytic 12	39.3	6.6	4.6	2.0	1.6	3.9	1.1	5.5	1.21		
3-1	14	None	39.4	9.3	6.0	3.2	3.5	5.7	2.0	7.2	1.90	39.0	
3-2	14	Lipolytic 18	38.3	10.0	7.6	2.5	3.9	6.0	1.9	8.0	2.34	39.5	
3-3	14	*Ps. fragi*	38.2	9.8	7.3	2.5	3.6	6.2	1.5	8.3	1.90	37.0	Sour
3-4	14	Lipolytic 12	38.8	11.9	9.2	2.7	4.1	7.8	1.5	10.4	2.60	36.0	Bitter
3-1	28	None	39.0	13.1	10.3	2.8	3.7	9.4	2.0	11.1	2.21	38.5	
3-2	28	Lipolytic 18	38.0	13.6	9.8	3.8	3.8	9.8	2.2	11.4	2.85	38.0	
3-3	28	*Ps. fragi*	38.2	13.7	10.1	3.7	4.3	9.4	1.6	13.0	2.40	37.5	Sl. sour
3-4	28	Lipolytic 12	38.2	13.6	10.5	3.0	3.5	10.1	1.9	11.8	2.25	36.5	Sl. bitter
3-1	56	None	37.8	16.0	13.5	2.5	4.3	11.7	2.5	13.5	3.79	38.0	
3-2	56	Lipolytic 18	37.6	16.0	13.0	3.0	4.5	11.5	2.5	13.7	3.68	38.0	
3-3	56	*Ps. fragi*	37.7	16.7	13.9	2.8	4.8	11.9	2.0	14.7	3.57	37.0	Sl. sour
3-4	56	Lipolytic 12	37.9	16.5	14.2	2.3	4.2	12.3	2.3	14.2	3.84	37.0	Bitter
3-1	112	None	36.8	20.1	17.0	3.0	8.0	12.0	7.1	13.0	5.38	38.0	
3-2	112	Lipolytic 18	36.7	22.3	19.0	3.2	9.8	12.5	8.2	14.0	7.01	37.0	
3-3	112	*Ps. fragi*	36.7	22.8	19.7	2.9	9.7	12.8	6.2	16.5	8.25	36.0	Sour
3-4	112	Lipolytic 12	36.4	21.7	19.5	2.1	8.6	13.2	8.0	13.6	6.97	35.5	Bitter

them became slightly greater. After 112 days the total nitrogen varied from 22.1 in the serum of the cheese made with *Ps. fluorescens* to 24.4 in the serum of the control cheese. Although there was a steady increase in the various nitrogenous fractions in the serums of all the cheese during the curing, the variations among the fractions were relatively small. With the fraction insoluble in trichloracetic acid, however, no increase was apparent after 14 days; and after 56 days there was an actual decrease.

The serums of all the cheese showed about the same increases in amino nitrogen during the curing. No variations that could be attributed to the test organisms were apparent.

The data showing the effect of the test organisms on the flavor of the cheese showed that, in general, the cheese made with the test organisms scored higher than the control cheese.

Bacteriological examination of the cheese from time to time during the ripening indicated that relatively large numbers of living test organisms were present.

ACID VALUES ON FAT FROM CHEESE MADE WITH VARIOUS CULTURES

Acid values on the fat from the experimental cheese were obtained to determine changes in the values at various periods during the ripening. It is not unlikely that fat hydrolysis, in a relatively small degree, may benefit the flavor of cheddar cheese. The data showing the acid values of the fat from the cheese at different periods of curing are given in table 4; the values are expressed as the milliliters of N/10 NaOH required to neutralize 20 grams of fat.

TABLE 4. *Acid values on fat from cheese made with different cultures*

Series and number of cheese	Test culture used[1]	Ml. of N/10 NaOH required to neutralize 20 grams of fat after:				
		3 days	14 days	28 days	56 days	112 days
1-1	Control cheese				4.1	5.2
1-2	*S. liquefaciens*				4.2	5.3
1-3	Micrococcus				4.0	4.9
1-4	*S. liquefaciens* and Micrococcus				4.2	5.3
3-1	Control cheese	2.9	3.3	3.5	4.0	4.2
3-2	Lipolytic 18	3.0	3.5	4.1	4.5	4.7
3-3	*Ps. fragi*	3.0	3.8	4.8	4.9	4.9
3-4	Lipolytic 12	2.8	3.4	4.6	4.5	5.7
5-1	Control cheese	2.9	3.4	4.3	4.4	4.3
5-2	*Ps. fluorescens*	2.8	3.3	4.5	5.2	6.7
5-3	*A. viscosus*	2.8	3.3	4.1	4.4	6.7
5-4	*A. lipolyticum*	2.9	3.5	4.1	4.5	5.4

[1] Butter culture (122) and *L. casei* (L4) were used in all cheese.

A regular increase in the acid values on the fat from all the cheese was apparent as the ripening progressed. With the control cheese and the cheese made with organisms which were not lipolytic, the values were relatively low, whereas comparatively high values were obtained when lipolytic organisms were used in making the cheese. The data show, however, that none of the organisms employed hydrolyzed the cheese fat to any great extent. No rancid flavors were detected in any of the cheese even after 112 days of ripening.

SUMMARY AND CONCLUSIONS

The work reported involved a study of the effect of certain bacteria on the ripening of cheddar cheese made from pasteurized milk. All of the experimental cheese were compared with control cheese made by adding a pure culture of *L. casei* (L4) and a butter culture (122) to the pasteurized milk, because, according to the work of Lane and Hammer (7), the addition of certain strains of *L. casei* to pasteurized milk used for making cheddar cheese appeared to have a desirable effect on the nitrogenous decomposition, the flavor development and the uniformity of the resulting cheese. These cultures were also used in the milk inoculated with the various test organisms.

1. Within the limits of the study, as imposed by the numbers of cheese made and the scope of the chemical analysis, the inoculation of small amounts of milk cultures of the test organisms into pasteurized milk appeared to have the following effects on the cheese:

a. *S. liquefaciens* improved the flavor of the cheese but did not materially influence the nitrogenous decomposition.
b. An unidentified Micrococcus improved the flavor of the cheese but did not significantly influence the nitrogenous decomposition.
c. When both *S. liquefaciens* and the unidentified Micrococcus were added to the milk, the flavor of the cheese was improved and there was a small increase in the total nitrogen in the cheese serum.
d. Lipolytic organism 18 did not influence the flavor development of the cheese but increased the total nitrogen in the cheese serum.
e. *Ps. fragi* decreased the flavor score of the cheese but did not materially influence the nitrogenous decomposition.
f. Lipolytic acid-forming organism 12 decreased the flavor score of the cheese but had little effect on the nitrogenous decomposition.
g. *Ps. fluorescens* did not significantly affect the flavor score or the nitrogenous decomposition in the cheese.
h. *A. viscosus* did not appreciably affect the flavor score or the nitrogenous decomposition of the cheese.
i. *A. lipolyticum* had little effect on the flavor score of the cheese and the nitrogenous decomposition.

2. The acid values obtained on the fat from all of the cheese increased with continued ripening; relatively large increases occurred when *Ps. fluorescens*, *A. viscosus* and *A. lipolyticum* were employed as test organisms.

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