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The Relation Between the Volatile and Total Acidity in Starters and in Cultures of *S. Lacticus.*

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During the last few years evidence has been presented which shows that starters are not pure cultures of *S. lacticus.* Hammer and Bailey[1], Storch[2] and Boekhout and Ott de Vries[3] have found that starters contain organisms other than *S. lacticus* that are important from the standpoint of volatile acid and odor and flavor development. More recently Hammer[4] has secured data that indicate the source of the volatile acidity produced by the organisms associated with *S. lacticus* and has also shown that starters do not contain pure *d*-lactic acid as they would be exexpected to if they were pure cultures of *S. lacticus.* The percent of the total acidity represented by volatile acid at various times during the period of rapid acid development is quite different with starters than it is with pure cultures of *S. lacticus,* and this difference offers further proof that starters are not pure cultures; data on this point are presented herewith.

METHODS USED

The methods used were essentially the methods employed by Hammer and Bailey.

The volatile acidity was determined by distilling a 250 gram portion of the milk with steam after the addition of 15 cc. of approximately N/1 H_2SO_4 until 1000 cc. of distillate were secured, after which the distillate was titrated with N/10 NaOH using phenolphthalein as an indicator. The water used in the steam can was allowed to boil for some little time before starting the distillation. Commonly 5 grams of Na_2SO_4 were used in the milk to aid in keeping down the foaming. The results obtained were expressed as the number of cc. of N/10 NaOH required; accordingly the figure means the cc. of N/10 NaOH required for the neutralization of the first 1000 cc. of distillate obtained when a 250 gram portion of the milk was distilled with steam after the addition of 15 cc. of approximately N/1 H_2SO_4. By no means all of the volatile acidity is secured by this method and it is undoubtedly true that the percentage of the total that is obtained is only approximately constant because of the dif-

[1]Hammer, B. W., and Bailey, D. E. The Volatile Acid Production of Starters and Organisms Isolated from Them. Res. Bul. Ia. Agr. Expt. Sta. 55, 1919.

[2]Storch, V. Fortsatte Undersogelser over Fremstillingen af Syrevaekkere. 102de Beretning fra Forsogslaboratoriet, 1919.

[3]Boekhout, F. W. J., and Ott de Vries, J. J., 1919. Aromabildner bei der Rahmsauerung. Centbl. f. Bakt. Abt. 2, 49: 373.

[4]Res. Bul. Ia. Agr. Expt. Sta. 63, 1920.

ferences in the volatile acids present in the various samples; the method, however, gives results that are satisfactory for comparative purposes.

The total acidity of the milk was usually determined by titrating 20 grams with N/10 NaOH, using phenolphthalein as an indicator; in a few instances smaller amounts of milk were used. In all cases the results were calculated as the percent lactic acid.

In determining the percent of the total acid that was represented by volatile acid the results were calculated to the nearest 0.1 percent. These values must be looked upon as comparative instead of absolute because of the errors in the method of estimating the volatile acidity. The total acidity and volatile acidity were frequently determined for the uninoculated milk so that milk which had undergone any appreciable bacterial action would not be used; these values were not deducted from the values secured on the fermented milk because the inaccuracies in the method of estimating the volatile acidities eliminate the possibility of securing anything but comparative results.

RESULTS OBTAINED

The percent of the total acidity in starters that is represented by volatile acidity was determined in a number of instances and a portion of the results secured are given in tables I to V inclusive. The data presented in table I were secured with an excellent starter that had been carried by a creamery for many years and from which *S. citrovorus* had been repeatedly isolated. From these results it is evident that at low acidities the volatile acidity makes up but a small percentage of the total acidity and that this percentage increases as the total acidity increases, reaching in the three trials given from 11.9 to 14.4. The increases are not always regular due undoubtedly to inaccuracies in the method of determining the volatile acidity, but the general relationship outlined is very evident. Table II gives the data secured in three trials with starter from manufacturer A, table III data secured in two trials with starter from manufacturer C, table IV data obtained in one trial with starter from manufacturer D and table V data secured in two trials with a good starter of unknown origin; all of these results show the same general relationship between the total acidity and the percent of this made up of volatile acidity as is shown by table I.

From tables I to V, inclusive, it is evident that as starters ripen there is an increasing percentage of the total acidity made up of volatile acidity. In starters which have practically reached their maximum acid development, the volatile acidity, according to the method of determination used, made up from 10 to 15 percent of the total acidity.

The percent of the total acidity that is represented by volatile

TABLE I—THE RELATION BETWEEN THE VOLATILE AND TOTAL ACIDITY IN A CREAMERY STARTER
Pasteurized Milk; 21° C. Incubation

Trial 1				Trial 2				Trial 3			
Time elapsed since inoculation (hrs.)	Total acidity calculated as lactic acid (pct.)	Volatile acidity cc. N/10	Pct. total acidity represented by volatile acidity	Time elapsed since inoculation (hrs.)	Total acidity calculated as lactic acid (pct.)	Volatile acidity cc. N/10	Pct. total acidity represented by volatile acidity	Time elapsed since inoculation (hrs.)	Total acidity calculated as lactic acid (pct.)	Volatile acidity cc. N/10	Pct. total acidity represented by volatile acidity
15	.86	18.2	7.6	13	.29	3.1	3.9	15	.34	4.1	4.4
17	.93	29.1	11.2	14	.36	4.0	4.0	16	.38	5.2	4.9
20	.99	39.5	14.4	15	.44	6.0	4.9	17	.45	6.3	5.0
22	1.04	38.8	13.4	16	.61	9.0	5.3	18	.56	7.1	4.5
..	..	...	...	17	.76	13.2	6.3	19	.65	9.0	5.0
..	..	...	...	18	.82	15.8	6.9	20	.77	11.6	5.4
..	..	...	...	35	1.07	35.5	12.0	21	.82	14.7	6.5
..	..	...	...	..	..	...	..	22	.88	17.4	7.1
..	..	...	...	..	..	...	..	47	1.08	35.7	11.9

TABLE II—THE RELATION BETWEEN THE VOLATILE AND TOTAL ACIDITY IN STARTER FROM MANUFACTURER A
Pasteurized Milk; 21° C. Incubation

Trial 1				Trial 2				Trial 3			
15	.70	11.2	5.7	15	.38	4.8	4.5	15	.87	18.1	7.5
16	.78	14.9	6.9	16	.51	6.9	4 8	17	.94	17.2	6.6
17	.84	20.8	8.9	17	.63	8.0	4.5	21	.98	26.2	9.6
18	.87	28.6	11.8	18	.73	11.7	5 8	39	1.06	30.1	10.2
19	.89	30.1	12.2	19	.77	14.4	6.8	42	1.08	30.4	10.2
20	.93	31.4	12.1	20	.80	20.5	9.3	..	..	...	...
21	.94	34.2	13.2	21	.84	22.6	9.7	..	..	...	...
22	.94	35.0	13.5	22	.86	26.4	11.1	..	..	...	...
87	1.09	36.0	11.9	63	1.01	36.2	12.9	..	..	...	...

acidity with pure cultures of *S. lacticus* is shown for four isolations in table VI. The data presented show that in a general way this percentage tends to remain constant altho there are many irregularities, due undoubtedly to inaccuracies in the method of determining the volatile acidity, and that when acid development has practically ceased the percent of the total acid that is volatile is much less than with starters; these results are confirmed in some of the later tables. The data indicate that pure cultures of *S. lacticus* act quite differently than do starters.

Tables VII and VIII present the results secured with mixtures of *S. lacticus* and *S. citrovorus* that had been carried for some time. From these data it is evident that in general the same results were secured with the mixtures as with starters.

In the attempts that were made to prepare starter from pure cultures of organisms, *S. lacticus* was often used alone and for comparison mixtures of the same *S. lacticus* culture with *S. citrovorus*. Such cultures and mixtures were commonly carried thru many generations in pasteurized milk in the same way that commercial cultures are carried. A number of these cultures

TABLE III—THE RELATION BETWEEN THE VOLATILE ACIDITY IN STARTER FROM MANUFACTURER C
Pasteurized Milk; 21° C. Incubation

Trial 1				Trial 2			
Time elapsed after inoculation (hrs.)	Total acidity calculated as lactic acid (pct.)	Volatile acidity	Pct. of total acidity represented by volatile acidity	Time elapsed after inoculation (hrs.)	Total acidity calculated as lactic acid (pct.)	Volatile acidity	Pct. of total acidity represented by volatile acidity
15	.50	6.1	4.4	15	.28	2.1	2.7
16	.63	8.7	5.0	16	.36	2.8	2.8
17	.71	9.4	4.8	17	.41	3.2	2.8
18	.76	12.3	5.9	18	.50	4.9	3.6
19	.79	15.3	7.0	19	.59	5.4	3.3
20	.84	19.2	8.3	20	.68	7.1	3.7
21	.85	23.8	10.1	21	.71	8.3	4.2
22	.89	28.6	11.6	22	.77	10.9	5.1
87	1.13	32.7	10.4	63	.95	33.4	12.6

and mixtures were tried out for volatile acid production and a portion of the results secured are given in table IX.

Each of the tables shows a pronounced difference between the pure culture of *S. lacticus* and the mixture which contained the same *S. lacticus* culture in combination with *S. citrovorus*. The results secured with the pure cultures of *S. lacticus* agree with the results presented in table VI and those secured with the mixtures are essentially the same as those obtained with starters.

S. citrovorus is incapable of growth at 37° C. and in general a starter carried at this temperature produces only a low volatile acidity. In order to determine what the relationship between the total acidity and the volatile acidity would be when milk at 37° C. was inoculated with a good starter the data presented in table X were secured. From these results it is evident that in milk inoculated with a good starter and held at 37° C. the volatile acid production was essentially the same as that of a pure culture of *S. lacticus* at a lower temperature; the check held at 21° C. showed the usual results secured with a starter held at this temperature. The elimination of the acidity of *S. citrovorus* by the use of high temperatures resulted in a

TABLE IV—THE RELATION BETWEEN THE VOLATILE AND TOTAL ACIDITY IN STARTER FROM MANUFACTURER D
Pasteurized Milk; 21° C. Incubation

Time elapsed after inoc. (hrs.)	Total acidity calculated as lactic acid (pct.)	Volatile acidity	Pct. of total acidity represented by volatile acidity
15	.68	7.5	3.9
16	.76	10.0	4.7
17	.81	11.9	5.3
18	.84	15.2	6.5
19	.86	17.1	7.2
20	.88	21.5	8.8
21	.91	26.0	10.2
22	.93	28.3	11.0
39	1.10	34.5	11.3

TABLE V—THE RELATION BETWEEN THE VOLATILE AND TOTAL ACIDITY IN A STARTER OF UNKNOWN ORIGIN
Pasteurized Milk; 21° C. Incubation

Trial 1				Trial 2			
Time elapsed after inoculation (hrs.)	Total acidity calculated as lactic acid (pct.)	Volatile acidity	Pct. of total acidity represented by volatile acidity	Time elapsed after inoculation (hrs.)	Total acidity calculated as lactic acid (pct.)	Volatile acidity	Pct. of total acidity represented by volatile acidity
15	.69	12.1	6.3	17	.52	7.2	5.0
16	.74	16.8	8.1	18	.60	10.1	6.0
17	.78	18.3	8.4	19	.71	11.7	5.9
18	.81	26.6	11.8	20	.76	18.0	8.5
19	.85	27.8	11.8	21	.77	20.4	9.5
20	.88	28.3	11.6	22	.83	21.4	9.3
21	.89	31.4	12.8	24	.85	27.5	11.7
22	.90	35.9	14.4	41	1.06	34.1	11.6
37	.91	37.5	14.8	..	...	...	...

TABLE VI—THE RELATION BETWEEN THE VOLATILE AND TOTAL ACIDITY IN CULTURES OF *S. LACTICUS*
Pasteurized Milk; 21° C. Incubation

Culture 1				Culture 2			
17	.59	4.2	2.6	16	.39	2.9	2.7
19	.65	3.9	2.2	18	.41	2.7	2.4
22	.70	4.2	2.5	21	.46	2.6	2.0
24	,72	4.9	2.5	42	.62	6.0	3.5
65	.81	8.0	3.5	95	.72	7.0	3.5

Culture 3				Culture 4			
18	.27	4.7	6.4	19	.72	7.3	3.6
21	.45	7.0	5.6	21	.77	6.5	3.1
24	.64	6.6	3.8	46	.86	7.2	3.0
40	.78	14.4	6.7	..	..	..	..

TABLE VII—THE RELATION BETWEEN THE VOLATILE AND TOTAL ACIDITY IN TWO MIXTURES OF *S. LACTICUS* AND *S. CITROVORUS*
Pasteurized Milk; 21° C. Incubation

Mixture 1				Mixture 2			
17	.70	16.1	8.3	16	.41	5.2	4.6
19	.77	22.2	10.4	18	.43	4.6	3.8
22	.87	33.1	13.7	21	.49	5.9	4.3
24	.89	35.1	14.3	42	.67	14.1	7.6
65	.94	42.6	16.3	95	.78	37.0	17.0

TABLE VIII—THE RELATION BETWEEN THE VOLATILE AND TOTAL ACIDITY IN A MIXTURE OF *S. LACTICUS* AND *S. CITROVORUS*
Pasteurized Milk; 21° C. Incubation

Trial 1				Trial 2			
16	.74	16.7	8.1	16	.77	19.3	9.1
17	.78	23.3	10.8	17	.81	27.0	12.0
18	.81	25.0	11.0	18	.86	30.9	12.9
19	.86	30.4	12.7	19	.88	33.0	13.5
21	.90	34.7	13.9	40	1.02	34.0	12.0
22	.91	35.1	13.8	..	..	...	...
23	.93	38.2	14.8	..	..	...	...
40	.97	38.3	14.3	..	..	...	...

TABLE IX—THE RELATION BETWEEN THE VOLATILE AND TOTAL ACIDITY

Pasteurized Milk; 21° C. Incubation

Time elapsed after inoculation (hrs.)	S. lacticus alone			S. lacticus + S. citrovorus		
	Total acidity calculated as lactic acid (pct.)	Volatile acidity	Pct. of total acidity represented by volatile acidity	Total acidity calculated as lactic acid (pct.)	Volatile acidity	Pct. of total acidity represented by volatile acidity
			RUN 1			
21	.34	5.0	5.3	.29	8.2	10.2
24	.60	7.1	4.2	.71	22.7	11.6
29	.74	7.3	3.6	.92	29.1	11.4
41	.87	10.7	4.4	.92	31.8	12.5
46	.88	8.2	3.4	..	...	...
			RUN 2			
18	..	..	..	.72	24.8	12.3
20	.66	7.5	4.1	.75	28.3	13.6
23	.72	6.3	3.2	.83	28.7	12.4
39	.81	8.1	3.6	.89	33.6	13.6
			RUN 3			
22	.76	22.4	10.6	.74	6.7	3.3
25	.77	27.6	12.8	.81	7.3	3.3
27	.85	33.3	14.1	..	..	..
40	.90	33.5	13.3	.86	11.5	4.8
			RUN 4			
18	.44	5.4	4.5	.49	7.0	5.2
21	.57	5.7	3.6	.61	9.6	5.6
23	.68	5.2	2.8	.69	11.7	6.1
41	.83	7.8	3.4	.88	27.2	11.1

starter developing lower volatile acidity in the same way that a pure culture of *S. lacticus* does.

DISCUSSION OF RESULTS

From the data given it is evident that with a starter the percentage of the total acidity represented by volatile acid is low when the total acid is low and gradually increases with an increase in the total acid until it reaches in general from 10 to 15 percent (with the method of determining the volatile acidity used) while with a culture of *S. lacticus* this percentage is more or less constant and never reaches the high value existing in the

TABLE X—THE RELATION BETWEEN THE VOLATILE AND THE TOTAL ACIDITY WHEN A STARTER WAS GROWN AT 37° C. AND AT 21° C.

Pasteurized Milk

Time elapsed after inoculation (hrs.)	37° C.			21° C.		
	Total acidity calculated as lactic acid (pct.)	Volatile acidity	Pct. of total acidity represented by volatile acidity	Total acidity calculated as lactic acid (pct.)	Volatile acidity	Pct. of total acidity represented by volatile acidity
21	.67	6.8	3.7	.74	17.0	8.2
23	.67	5.8	3.1	.79	17.3	7.9
25	.71	9.1	4.6	.80	21.9	9.9
44	.73	8.9	4.4	.86	27.1	11.4

case of a starter. The change, during the ripening period, in the percentage of the total acidity that is made up of volatile acid suggests that a starter is not a pure culture and the practically constant percentage secured with pure cultures of *S. lacticus* seems to prove this conclusively.

The increase (which accompanies the acid development) in the percentage of the total acidity that is made up of volatile acid indicates that the activity of the associated organism is greater late in the ripening period, since this organism is the important one from the standpoint of volatile acid production. It has been impossible to show by trials, in which from 60 to 90 colonies were picked from each plate, that the associated organisms make up a larger percentage of the total flora of a starter late in the ripening period than early, altho the uneven distribution of the associated organisms in a starter makes this method of doubtful value.

Considerable variation occurs in the volatile acid production in different starters. In some cases the percent of the total acidity represented by volatile acidity is about as great at the acidities to which starters are usually ripened (from .7 to .9 percent) as at higher total acidities, while in others it is not. This suggests the possibility of securing a high aroma and flavor without objectionable amounts of total acid. The use of *S. lacticus* cultures, selected because of their low total acid production, in combination with *S. citrovorus* cultures is an interesting possibility along this line, which should overcome the serious, difficultly controlled over-ripening common to certain otherwise excellent starters, and if acid is of any great consequence as a cause of deterioration, the method may result in the production of a desirable flavor and aroma with only a slow deterioration. Preliminary experiments show that it is possible to prepare an unusually mild starter by combining such *S. lacticus* cultures with the associated organisms.

CONCLUSIONS

1. The variation between starters and pure cultures of *S. lacticus* in the percent of the total acidity that is volatile at various times in the ripening period offers another method of proving that starters are not pure cultures of *S. lacticus*.

2. Variations occur in starters in the acidity at which a considerable percent of the total acidity is volatile. This suggests the development of starters having a high aroma and flavor at comparatively low acidities by combining cultures of *S. lacticus*, selected on the basis of a low total acid production, with the associated organisms. Such starters may aid in solving the question of producing a high aroma and flavor in butter without securing butter having poor keeping qualities.