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The volatile acids formed from citric and lactic acids by streptococcus citrovorus and streptococcus paracitrovorus

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THE VOLATILE ACIDS FORMED FROM CITRIC AND LACTIC ACIDS BY STREPTOCOCCUS CITROVORUS AND STREPTOCOCCUS PARACITROVORUS

BY

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INTRODUCTION

The studies carried out in various laboratories have indicated that butter cultures are not pure cultures of Streptococcus lactis, as was at one time commonly supposed, but consist of two types of organisms, at least one of which is capable of producing appreciable amounts of volatile acids and possibly diacetyl. In addition to containing these desirable products, good quality butter cultures must be free from materials having an objectionable flavor or odor.

The sources of the volatile acids produced in butter cultures by the organisms associated with S. lactic appear to be (1) citric acid, and (2) lactic acid. The former is naturally present in milk in small quantities, as citrates, and the latter is the result of the breaking down of lactose by bacteria.

The work herein reported deals with (1) the type of volatile acid produced from citric acid; (2) the type of volatile acid produced from lactic acid; and (3) the relationship of the type of volatile acid and the source to the diacetyl produced.

HISTORICAL

The addition of a natural starter to boiled milk to secure a desirable flavor in the resulting butter, cheese or fermented milk was a well established practice among primitive peoples*. Heinemann (12) states that this was described as early as 1776.

According to Henzold (13), a description of the method of using "lange wei" as a starter for edam cheese was published in 1887 in a pamphlet by Boekel. Orla-Jensen (16) has reported that the first commercial butter cultures appeared in 1890.

From time to time various investigators have reported their experimental results and conclusions in regard to cream ripening. They have often emphasized the fact that butter cultures contain organisms other than S.lactis.

Conn (4) studied the effect of various organisms on the flavor of butter, and pointed out that the ripening of cream is a complex matter and that, "While the ripening of cream is undoubtedly dependent upon the presence of bacteria, it is doubtful whether one species can produce what is known as ripened

* The writer has witnessed a comparable practice among the peasants in Turkey, and among the semi-civilized tribes of various sections of Arabia.

cream." He also pointed out that although the acid in butter culture is developed from the sugar, the flavor probably comes from some other source. Weigmann (22), Orla-Jensen (15), and Mc Donnell (14) have done outstanding work on the subject of cream ripening.

Evans, Hastings and Hart (6) noticed that the addition of certain species of streptococci along with Streptococcus lactis, improved the flavor of cheddar cheese made from pasteurized milk. They also emphasized the importance of associative action of these organisms.

As a result of a series of experiments on the ripening of cheddar cheese, Hart, Hastings, Flint and Evans (11) reported that: "Representatives of the coccus groups of organisms isolated from cheddar cheese when grown in milk produced large quantities of the volatile acids, particularly acetic acid. These acids," they assumed, "were produced from citric acid or lactose or protein, as the medium was practically free from fat."

Evans (5) reported that streptococci other than S. lactis are common in ripening cheese of various kinds, and in other foods prepared by fermentation. She called these organisms cheese streptococci and described Streptococcus X and Streptococcus kefir. The addition of them along with S. lactis, improved the flavor in cheese made from pasteurized milk. This investigator observed that S. lactis produced a small quantity of acetic acid in milk cultures.

In 1919 Hammer and Bailey (8) reported that S. lactis from butter cultures produced amounts of volatile acids in agreement with the amounts found by Evans (5) for this particular organism, but much smaller than the amounts formed by good butter cultures. They isolated from butter cultures, an organism which produced no change in litmus milk but which, when grown with S. lactis, gave a volatile acidity essentially the same as that produced by good butter cultures. This organism in pure culture also produced considerable amounts of volatile acidity. The work of Storch (19) and Boekhout and Ott De Vries (2) also shows the presence, in butter cultures, organisms other than S. lactis, that play an important part in the production of desirable flavor and aroma in butter.

In 1920 Hammer (7) isolated two types of streptococci which produced high volatile acidity in milk. He proposed the name Streptococcus citrovorus for the one type and the name of Streptococcus paracitrovorus for the other. He reported that these organisms produced a higher volatile acidity in milk to which a small amount of sterile citric acid had been added, than in milk without this acid. The addition of sterile lactic acid to milk increased the volatile acidity formed by S.citrovorus, but not the volatile acidity produced by S. paracitrovorus. This was attributed to the fact that S. paracitrovorus produces some lactic acid in milk, a portion of which probably was later changed to volatile acid. According to Hammer these experimental results obtained suggested that citric acid, and probably lactic acid, were the sources of volatile acids in butter cultures.

Studies reported by Bosworth and Prucha (3) in 1910, indicated that the growth resulting from the addition of a small amount of buttermilk to sterile milk caused the disappearance of the citric acid content of milk and the production of a high volatile acidity which was made up of acetic acid.

In 1923 Hammer and Sherwood (10) reported that in a highly ripened butter culture the volatile acid is largely acetic, and that ,"the kind of volatile acid present is not the same throughout the ripening period of a butter culture, the acetic acid being less prominent early in the ripening and more prominent later, and the propionic acid accordingly more prominent early and less prominent later."

In 1928 Schmalfuss and Barthmeyer (17) found that, when a culture of Streptococcus acidi lactici Grotenfeldt + Streptococcus cremoris were grown in sterilized milk, one of the products identified was diacetyl, and concluded that it is the result of bacterial metabolism. They further suggested that diacetyl does not arise from the lactic acid formed, nor is it produced by atmospheric oxidation of acetylmethyl carbinol.

In 1929 Van Niel, Kluyver, and Derx (21) reported that the aroma of butter was due to the presence of diacetyl produced by bacteria in the cultures.

Hammer and Farmer reported (9) that, "All of the desirable butter cultures studied contained acetylmethyl carbinol in amounts varying from 0.005 to 0.0138 grams per 200 grams of culture, while unsatisfactory butter cultures gave no acetylmethyl carbinol, or only a trace," and that, "there was no correlation between the acetylmethyl carbinol production of the organisms when 0.4 % citric acid was added to the milk, and their ability to develop satisfactory butter cultures in connection with S. lactis."

METHODS USED

The volatile acid solutions studied were secured by steam distillation (8). Usually 50c.c. of N/1 H_2SO_4 were added to one liter of the fermented material to free any volatile acids that might have been combined with the milk constituents. To secure large quantities of volatile acid solutions, duplicate distillations were usually carried out and the distillate combined. The distillations were continued until sufficient quantities of volatile acids were obtained.

The two methods used in identifying the types of volatile acids present in the distillates were: (1) the determination of the percent barium in the barium salt, and (2) the modified Duclaux method as carried out by Hammer and Sherwood (10).

In preparing the barium salts of the volatile acids, the usual procedure was as follows: A small portion of the distillate, ordinarily 50 c.c., was titrated with N/10 $Ba(OH)_2$, using phenolphthalein as an indicator, and discarded. From this titration value the remaining distillate was neutralized with the calculated amount of $Ba(OH)_2$. The barium salt was evaporated to dryness in a water bath, re-dissolved in a small amount of distilled water, filtered through a filter paper, and re-crystallized. This re-crystallization was continued until no more $BaCO_3$ precipitation occurred when the salts were re-dissolved in a small amount of distilled water. The $BaCO_3$ free salts were ground to a powder, and either dried to constant weight at 100°C., or heated continuously for about eight hours at the same temperature. In the later part of the investigation the second procedure was used, the accuracy of which was tested as follows: Each of the barium salts was divided into two parts. The one part was heated to constant weight at 100°C., and the other heated continuously for about eight hours at the same temperature. The percentage of barium was then determined on each of the portions. The results obtained are given in Table I. The differences between the averages of the two methods varied between 0.02 and 0.20 percent.

TABLE I.

Comparison of Constant Weight Heating(at 100°C)and Eight Hour Heating (at 100°C)for the Drying of the Barium Salts.

Comparison	Method	% barium in salt			Difference of averages
		A	B	Av.	
1	Heated to constant wgt.	53.07	53.06	53.065	0.20
	Heated for eight hours	52.89	52.84	52.865	
2	Heated to constant wgt.	52.79	52.81	52.80	0.055
	Heated for eight hours	52.88	52.83	52.855	
3	Heated to constant wgt.	53.23	53.18	53.205	0.175
	Heated for eight hours	---	53.38	53.38	
4	Heated to constant wgt.	53.03	53.08	53.055	0.11
	Heated for eight hours	53.2	53.13	53.165	
5	Heated to constant wgt.	53.26	53.29	53.275	0.115
	Heated for eight hours	53.2	53.12	53.16	
6	Heated to constant wgt.	53.65	53.52	53.585	0.095
	Heated for eight hours	53.71	53.65	53.68	
7	Heated to constant wgt.	53.29	53.23	53.26	0.15
	Heated for eight hours	53.31	53.51	53.41	
8	Heated to constant wgt.	53.12	53.25	53.185	0.08
	Heated for eight hours	53.17	53.04	53.105	
9	Heated to constant wgt.	53.32	53.39	53.355	0.02
	Heated for eight hours	53.36	53.39	53.375	
10	Heated to constant wgt.	53.20	53.24	53.22	0.095
	Heated for eight hours	53.11	53.14	53.125	

The percentage of barium was determined as follows: Duplicate samples of about 0.5 gram of the dried salt were weighed out, transferred to a 250 cc. beaker, dissolved in from 60 to 75cc. of hot water, the solution heated to boiling on a low flame, and slightly more than the calculated amount of N/1 H_2SO_4 added slowly to the boiling solution. After digesting this solution (with a watch glass covering the beaker) on hot plate for a reasonable length of time, usually over night, the $BaSO_4$ was filtered off, ignited, weighed to constant weight, and the percent barium in the original sample calculated. Table II gives the barium values obtained by this method on supposedly pure acetic and propionic acids from commercial sources. The barium values secured on each of these acids are slightly below the theoretical values. The percentage variations with the salts of propionic acid are greater than with those of acetic acid. The purity of the acids may have had a bearing on the barium values obtained.

TABLE II.

Percent Barium in Salts Prepared from Commercial Acetic and Propionic Acids.

Barium salts of	Trials	% barium in salt			
		A	B	Av.	Theoretical
Acetic acid	1	53.60	53.59	53.595	53.78
	2	53.66	53.71	53.685	
	3	53.52	53.51	53.515	
	4	53.60	53.56	53.58	
	5	53.65	53.70	53.675	
Propionic acid	1	47.73	47.68	47.705	48.47
	2	47.72	47.83	47.775	
	3	48.03	48.15	48.09	
	4	48.06	48.01	48.035	
	5	48.19	48.14	48.165	
* Propionic and Acetic acids	1	50.76	50.61	50.685	
	2	51.17	51.28	51.225	
	3	50.98	51.02	51.0	
Butyric acid					44.10
Formic acid					60.41

* Approximately 0.5% solution of each acid was prepared and equal volumes of the two mixed together.

The filtrates from the barium determinations were used for the Duclaux method. The volume of the solution in the distilling flask was kept constant at 110 cc., and distilled at the rate of 100cc. in about 45 to 50 minutes. Ten cc. fractions of the distillate were titrated with N/20 NaOH, using phenolphthalein as an indicator, and the percentage of volatile acid in each fraction calculated.

Duclaux values were secured with the method indicated above on commercial acetic and propionic acids so that they could be used as a basis in studying the unknown acids. Table III gives representative titrations and Duclaux values on commercial acetic and propionic acids, while table IV gives a summary of all the Duclaux values.

TABLE III.

Representative Examples of Titration and Duclaux Values
for Commercial Acetic and Propionic Acids

Acetic Acid
cc. of distillate

	10	20	30	40	50	60	70	80	90	100
A+	3.6	3.5	3.3	3.05	2.95	2.6	2.6	2.4	2.3	2.2
B++	3.6	7.1	10.4	13.45	16.4	19.0	21.6	24.0	26.3	28.5
C+++	12.63	24.91	36.49	47.19	57.55	66.67	75.79	84.21	92.28	100

Propionic Acid
cc. of distillate

	10	20	30	40	50	60	70	80	90	100
A+	5.8	5.3	4.65	4.1	3.8	3.35	2.8	2.7	2.55	2.05
B++	5.8	11.1	15.75	19.85	23.65	27.0	29.8	32.5	34.88	36.9
C+++	15.72	30.08	42.68	53.79	64.09	73.17	80.76	88.07	94.44	100

Propionic and Acetic Mixed
cc. of distillate

	10	20	30	40	50	60	70	80	90	100
A+	4.2	4.2	3.7	3.5	3.15	2.85	2.7	2.55	2.25	2.1
B++	4.2	8.4	12.1	15.6	18.75	21.6	24.3	26.85	29.1	31.2
C+++	13.45	26.92	38.8	50.0	60.1	69.25	77.95	86.05	93.25	100

A+ cc. N/20 alk. required for successive cc. fractions.
B++ Sum of values in A for a given amount of distillate.
C+++ Values given in B calculated as percent of the titration values for the 100 cc. of distillate.

TABLE IV.

Duclaux Values of Commercial Acetic and Propionic Acids

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Acid	No. of runs	Average Duclaux Values, cc. of distillate: 10	20	30	40	50	60	70	80	90	100
Acetic acid											
a- max. in	10	13.31	25.18	36.69	47.48	57.91	67.27	75.72	84.53	92.44	100
b- min. in	10	11.94	24.22	34.8	46.3	55.27	65.5	74.7	83.6	91.8	100
c- av. of	10	12.82	24.94	36.54	47.37	57.58	66.96	75.81	84.38	92.42	100
Propionic acid											
a- max. in	10	16.11	30.46	43.15	53.93	64.21	73.35	81.22	88.32	94.54	100
b- min. in	10	14.96	28.0	40.95	51.6	62.05	71.55	79.6	87.0	93.5	100
c- av. of	10	15.68	29.96	42.7	53.91	64.02	72.97	80.82	88.01	94.42	100
Propionic and acetic mixed+											
a- max. in	6	14.33	26.9	38.55	49.7	60.0	70.1	78.2	86.25	93.5	100
b- min. in	6	13.52	26.57	38.26	49.15	59.45	69.0	77.9	85.55	93.25	100
c- av. of	6	13.77	26.62	38.68	49.79	60.01	69.64	78.24	85.27	93.4	100

+ Approximately 0.5% solution of each acid was prepared and equal volumes of the two mixed together.

The unknown volatile acids secured often gave Duclaux values which fell in between those for acetic and propionic acids. When they were much nearer those of acetic acid than with those of propionic acid, the mixture was considered as mainly acetic plus a small amount of propionic; and when the values obtained agreed more closely with those for propionic acid than with those of acetic, the mixture was considered to be propionic plus some acetic acid.

The Duclaux method has been criticized as an unsatisfactory means of determining the kinds of volatile acids present in a mixture. Its use, however, was considered necessary as a rough check on the barium values obtained on the barium salts.

The procedure employed in the examination of cultures for diacetyl consisted of an adaptation by Hammer and Farmer(9) of the widely used Lemoigne (21) method. The first 25 cc. of distillate from steam distillations were treated with 5 cc. of sodium acetate to buffer the solution, then with 5 cc. of hydroxylamine hydrochloride to change the diacetyl, if any is present, to dimethyl glyoxime, and finally with 1 cc. of nickel chloride to change the dimethyl glyoxime to nickel dimethyl glyoxime, which is a very stable red precipitate. Since the amounts of nickel dimethyl glyoxime were so small, weighings were not made but the amounts were graded from a trace to a comparatively large quantity as follows: (1)trace, (2) very slight, (3)slight, (4)very small, (5)small, (6) large amounts.

Chemicals Added

The preparations from which the various additions of chemicals were made had the following concentrations:

Acetic acid	approximately	99.5 %
Beta hydroxy propionic acid	"	92 %
Lactic acid	"	85 %
Phosphoric acid	"	85 %
Propionic acid	"	99.5 %
Sulphuric acid	"	98 %

Citric acid	c.p.	crystals +
Dipotassium phosphate(K_2HPO_4)	"	"
Glycollic acid	"	"
Malic acid	"	"
Succinic acid	"	"
Tartaric acid	"	"

+ Contains one molecule water of crystallization.

EXPERIMENTAL

Fresh milk is a very satisfactory medium for the growth of S. citrovorus and S. paracitrovorus. However, the natural presence of citric acid in milk, which would interfere with the determination of the products formed from lactic acid, suggested the study of other media. Two types were used: (1) beef infusion bouillon with additions of Fleischmann's compressed yeast and di-potassium-hydrogen phosphate, and (2) fermented milk free from citric acid.

The preliminary attempts (carried out in test tubes or small flasks) to grow the organisms in these two media proved encouraging; thereupon further trials were undertaken.

Results Obtained with Phosphate-Yeast-Beef Infusion Bouillon.

The beef-infusion bouillon was prepared in the usual way (1). The clear solution of broth, to which approximately 20 to 25 % Fleischmann's compressed yeast and 0.4 % K_2HPO_4 were added, was divided into portions and put into flasks. After adding amounts of citric or lactic acid or other chemicals to the flasks, they were sterilized in the autoclave under fifteen pounds of pressure for about 30 minutes. The sterile solutions were inoculated with various strains of associated organisms, incubated at 21°C. for some time, filtered through paper to get a fairly clear solution, and steam distilled.

Table V gives the amounts of volatile acids and the barium and Duclaux values obtained on these when various associated organisms were inoculated, while table VI presents similar values secured when culture 5 (S.citrovorus) was inoculated. The data show that the addition of citric acid always increased the volatile acidity; while the addition of lactic, beta hydroxy propionic, tartaric, succinic, malic, or glycollic acids, although they permitted the growth of organisms, as was shown by cultures on beef infusion agar slopes, did not give appreciable increases in volatile acidity. Some lots of bouillon, without added chemicals, inoculated with culture 1 or culture 5; and some with or without added chemicals, not inoculated, gave about the same amounts of volatile acidities as the cultures to which the organic acids other than citric acid had been added.

TABLE V.

Amounts and Types of Volatile Acids Formed in Phosphate-Yeas
with Various Additions by Associated Organ

Batch No.	Designation of run	Chemical added: Kind	Chemical added: Amt. in percent	Associated organisms inoculated	Period incubated at 21°C.	Final acidity calculated as % lactic acid	cc. of culture distilled	cc. of N/10 al for one liter o distillate
L1	1[a]	Lactic acid	0.6	Culture 2	23 da.	0.47	450	16
	1[b]	Citric acid	0.4	Culture 2	26 da.	0.19	400	48
	1[c]	Lactic acid	0.6	Culture 17	37 da.	0.49	550	22
	1[d]	Lactic acid	0.6	Culture 1 + S.lactis	26 da.	0.38	400	7
L2	2[a]	Lactic acid	0.5	Culture 1 +lactis	15 da.	0.66	900	15
	2[b]	Citric acid	0.4	Culture 1 +S.lactis	15 da.	0.60	900	42
L5	7[a]	Tartaric acid	0.6	Culture 1	18 da.	0.88	700	22
	7[b]	Tartaric acid	0.6	Culture 1	18 da.	0.89	700	20
	8[a]	Succinic acid	0.6	Culture 1	17 da.	1.30	650	23
	8[b]	Succinic acid	0.6	Culture 1	17 da.	1.30	650	22
	9[a]	Malic acid	0.6	Culture 1	17 da.	1.13	700	20
	9[b]	Malic acid	0.6	Culture 1	17 da.	1.03	700	20
	10[a]	Glycollic acid	0.6	Culture 1	20 da.	0.23	600	16
	11[a]	None	---	Culture 1	18 da.	0.44	600	26
L4[a]	2[a]	Lactic acid	0.5	Culture 1	15 da.	0.67	600	22
	2[b]	Citric acid	0.4	Culture 1	20 da.	0.67	600	129
	2[c]	All chems. added	--	None	9 da.	1.07	900	14.9
L2	3[a]	Citric acid	0.5	Culture 2	16 da.	0.71	850	84
	3[b]	Lactic	0.6	Culture 2	16 da.	0.68	900	18
	3[c]	Citric acid	0.45	Culture 3	16 da.	0.69	950	56

[+]Value obtained when the entire one liter of distillat instead of calculating from the value for 50 cc.

TABLE V.

le Acids Formed in Phosphate-Yeast-Beef Infusion Bouillon

ous Additions by Associated Organisms.

	Period incubated at 21°C.	Final acidity calculated as % lactic acid	cc. of culture distilled	cc. of N/10 alk for one liter of distillate	% barium in salt A	B	Av.	Results of Duclaux
)	23 da.	0.47	450	16	48.94		48.94	Acetic +large amt. of propionic acid
)	26 da.	0.19	400	48	53.43	53.38	53.405	Acetic + trace of propionic acid
7	37 da.	0.49	550	22	47.22	-----	47.22	Acetic +large amt. of propionic acid
s	26 da.	0.38	400	7	----	-----	-----	------------
	15 da.	0.66	900	15	48.65	48.36	48.505	Acetic + trace of propionic acid
	15 da.	0.60	900	42	52.26	52.09	52.175	Acetic + large amt of propionic acid
	18 da.	0.88	700	22	47.06	46.91	46.985	Acetic plus large amount of propionic acid
	18 da.	0.89	700	20				
	17 da.	1.30	650	23	50.71	50.63	50.67	Acetic plus large amount of propionic acid
	17 da.	1.30	650	22				
	17 da.	1.13	700	20	50.62	50.46	50.54	Acetic plus small amount of propionic acid
	17 da.	1.03	700	20				
	20 da.	0.23	600	16	50.19	50.28	50.235	Acetic+small amt. of propionic acid
	18 da.	0.44	600	26	51.19	51.20	51.195	Acetic plus trace of propionic acid
	15 da.	0.67	600	22	51.3	51.4	51.35	Acetic + small amt of propionic acid
	20 da.	0.67	600	129	53.21	53.15	53.18	Acetic + small amt of propionic acid
	9 da.	1.07	900	14.9+	---	---	---	----------
	16 da.	0.71	850	84	52.88	52.83	52.855	Acetic + large amt of propionic acid
	16 da.	0.68	900	18	48.89	48.62	48.755	Acetic + large amt of propionic acid
	16 da.	0.69	950	56	52.85	52.81	52.83	Acetic + small amt. of propionic acid

the entire one liter of distillate was titrated
lating from the value for 50 cc.

TABLE Vl.

Amounts and Types of Volatile Acids Formed in Phosphate-Yea

with Various Additions by S.citrovorus (Cu

Batch No.	Designation of run	Chemical added: Kind	Chemical added: Amt. in percent	Period incubated at 21°C.	Final acidity calculated as percent lactic acid	cc. of culture distilled	cc. (N/10 for (liter dist late
L 2	1[a]	Citric acid	0.5	18 days	0.72	850	73
	1[b]	L Lactic acid	0.55	16 "	0.67	650	16
	1[c+]	Lactic acid	0.6	18 "	0.75	650	30
L3	1[a]	Lactic acid	0.5	15 "	0.8	600	25
	1[b]	Lactic acid	0.5	20 "	0.26	600	18
	1[c]	Beta hydroxy propionic acid	0.25	20 "	0.7	600	21
	1[d]	Citric acid	0.4	15 "	0.29	650	67
	1[e]	Citric acid	0.4	15 "	0.2	600	68
	1[f]	None	---	20 "	0.37	600	34
L4[a]	1[a]	Lactic acid	0.3	10 "	0.57	700	7
	1[b]	Citric acid	0.3	10 "	0.5	750	54
	1[c]	Check K_2HPO_4	0.6	10 "	0.18	800	6
	2[c]	Check K_2HPO_4 yeast all chemicals used added	0.6 25.0	9 "	1.07	900	14
L4[b]	1[a]	Check Yeast in 1000 cc. of H_2O without bouillon	25	9 "	0.077	1000	8
	1[a]	Tartaric acid	0.6	18 "	0.9	700	2[illegible]
	1[b]	Tartaric acid	0.6	18 "	0.89	700	2[illegible]
	2[a]	Succinic acid	0.6	17 "	[illegible]	650	[illegible]

TABLE V1.

s Formed in Phosphate-Yeast-Beef Infusion Bouillon

ions by S.citrovorus (Culture 5)

nal acidity lculated percent ctic acid	cc. of culture dis-tilled	cc. of N/10 alk. for one liter of distil-late	% barium in salt A	B	Av.	Results of Duclaux
0.72	850	73	52.85	52.91	52.88	Acetic plus trace of propionic acid
0.67	650	16	49.12	---	49.12	Acetic plus small amt. propionic acid
0.75	650	30	51.39	51.27	51.33	Acetic plus small amt. propionic acid
0.8	600	25	50.46	50.36	50.41	Acetic plus small amt. propionic acid
0.26	600	18	50.51	50.20	50.355	Acetic plus trace of propionic acid
0.7	600	21	50.37	49.99	50.18	Acetic plus small amt. propionic acid
0.29	650	67	52.79	52.81	52.80	Acetic plus small amt. propionic acid
0.2	600	68	53.07	53.06	53.065	Acetic plus trace of propionic acid
0.37	600	34	52.33	52.26	52.295	Acetic plus large amt. propionic acid
0.57	700	7	---	---	---	------------
0.5	750	54	53.20	53.24	53.22	Acetic plus trace of propionic acid
0.18	800	6.2++	---	---	---	-------------
1.07	900	14.9++	---	---	---	-------------
0.077	1000	8.1++	---	---	---	-------------
0.9	700	24	47.87	47.69	47.78	Propionic plus trace of acetic acid
0.89	700	23				
[illegible]	650	23				Acetic plus large

	1[d]	Citric acid	0.4	15 "	0.29	650	67
	1[e]	Citric acid	0.4	15 "	0.2	600	68
	1[f]	None	---	20 "	0.37	600	34
L4[a]	1[a]	Lactic acid	0.3	10 "	0.57	700	7
	1[b]	Citric acid	0.3	10 "	0.5	750	54
	1[c]	Check K_2HPO_4	0.6	10 "	0.18	800	6
	2[c]	Check K_2HPO_4 yeast all chemicals used added	0.6 25.0	9 "	1.07	900	14
L4[b]	1[a]	Check Yeast in 1000 cc. of H_2O without bouillon	25	9 "	0.077	1000	8
L5	1[a]	Tartaric acid	0.6	18 "	0.9	700	24
	1[b]	Tartaric acid	0.6	18 "	0.89	700	23
	2[a]	Succinic acid	0.6	17 "	1.28	650	23
	2[b]	Succinic acid	0.6	17 "	1.2	700	25
	3[a]	Malic acid	0.6	17 "	1.2	650	22
	3[b]	Malic acid	0.6	17 "	1.18	700	23
	4[a]	Glycollic acid	0.6	20 "	0.77	750	14
	5[a]	None	---	18 "	0.42	700	24
	6[a]	Check None	---	18 "	0.44	400	1
	6[b]	Check All the chemicals used added	---	18 "	2.4	400	1

+S. lactis was inoculated with culture 5.

++Values obtained when the entire one liter of distilla
from the value for 50 cc.

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	amt. propionic acid
0.29	650	67	52.79	52.81	52.80	Acetic plus small amt. propionic acid
0.2	600	68	53.07	53.06	53.065	Acetic plus trace of propionic acid
0.37	600	34	52.33	52.26	52.295	Acetic plus large amt. propionic acid
0.57	700	7	---	---	---	------------
0.5	750	54	53.20	53.24	53.22	Acetic plus trace of propionic acid
0.18	800	6.2++	---	---	---	-------------
1.07	900	14.9++	---	---	---	-------------
0.077	1000	8.1++	---	---	---	-------------
0.9	700	24	47.87	47.69	47.78	Propionic plus trace of acetic acid
0.89	700	23				
1.28	650	23	50.43	50.36	50.395	Acetic plus large amount of propionic acid
1.2	700	25				
1.2	650	22	50.94	50.93	50.935	Acetic plus large amount of propionic acid
1.18	700	23				
0.77	750	14	50.18	50.19	50.185	Acetic plus small amt. propionic acid
0.42	700	24	50.09	---	50.09	Acetic plus trace of propionic acid
0.44	400	12	48.66	49.48	49.07	Acetic plus large amount of propionic acid
2.4	400	11				

lture 5.

one liter of distillate was titrated instead of calculating

The barium and Duclaux values of the volatile acids secured with the addition of citric acid, always indicated acetic with large or small amounts or a trace of propionic acid. The barium values on the volatile acids secured with the addition of other organic acids, with and without inoculation, indicated propionic and sometimes small or large amounts of acetic acid. The inoculation of S.lactis along with culture 5, when lactic acid had been added to the bouillon, gave barium values slightly higher than without S.lactis. However, the inoculation of S.lactis along with culture 1 did not produce enough acids for barium value determinations. In two trials when culture 1 or culture 5 was inoculated alone, the volatile acids obtained consisted of a mixture of about equal quantities of propionic and acetic acids. In a different batch of media, without any acids added, however, culture 5 produced volatile acids made-up of acetic plus a large amount of propionic acid.

The barium and Duclaux values did not always agree with each other.

Since associated organisms produced volatile acidities in phosphate-yeast-beef infusion bouillon with no chemicals added, this medium was considered unsatisfactory for further investigation of the problem.

Results Obtained with Fermented Milk.

The preparation of fermented milk, free from citric acid, was based on the fact that the citric acid content of milk (18), soured under ordinary conditions or after inoculation with S.paracitrovorus cultures, completely disappears within two to four days (20). The milk used was that which had been delivered by the College Dairy Farm to the Dairy Industry market milk department. Some lots were allowed to stand at room temperature for at least six days, while other lots were pasteurized and inoculated with S.paracitrovorus or butter cultures, and incubated at 21°C. for at least five days. The milk was then steam distilled, the distillation being continued until the last liter of distillate required only about 3 to 5 cc. of N/10 NaOH for neutralization.

The amount of volatile acids obtained from the impure milk cultures and the barium and Duclaux values on some of them, are given in table VII.

TABLE VII.

Amounts and Types of Volatile Acids Formed in Various

Batch of media	Designation of run	Cultures inoculated	Period incubated at 21°C.	Final acidity calculated as % lactic acid	cc. of culture distilled	cc. of N/10 alk. for one liter of distillate
Raw milk naturally soured at room temperature	1^a	None	5 days	0.88	800	52
	1^b	None	5 "	0.91	800	48
	1^c	3 days of souring + assc.org.culture 15	6 "	0.89	800	44
	1^d	3 days of souring plus assc.org.culture 16	6 "	0.83	800	42
Pasteurized skimmed milk inoculated with pure cultures & butter cultures	2^a	Assc.org. culture 16 for 5 days plus butter culture B 16-1	11 "	0.72	800	53
	2^b	Assc. org. culture 16 plus butter culture b 16-1	11 "	0.96	850	53.
	3^a	Assc. org. culture 15	11	0.55	900	55.5
	3^b	Assc. org. culture 15 for 4 days plus butter culture B 16-1	4 "	0.70	850	57
	3^c	Assc. org. culture 15 plus butter culture B 16 - 1	12 "	0.999	850	59.5
Past. whole milk inoculated with butter culture	4^a	Butter culture No.122	1-2/3"	0.90	900	70
	4^b	" " " "	$4\frac{1}{2}$ "	0.945	950	52
	4^c	" " " "	$6\frac{1}{2}$ "	0.89	950	40
	5^a	" " " "	$\frac{1}{2}$ "	0.756	1000	26
	5^b	" " " "	1 "	0.81	1000	49.5
	5^c	" " " "	1-3/4"	0.89	1000	49
	5^d	" " " "	$2\frac{1}{4}$ "	0.89	1000	50
	5^e	" " " "	2-2/3"	0.88	1000	53
Ster-	[illegible]					

TABLE VII.

le Acids Formed in Various Impure Milk Cultures

Final acidity calculated as % Lactic acid	cc. of culture distilled	cc. of N/10 alk. for one liter of distillate	%barium in salt			Results of Duclaux
			A	B	Av.	
0.88	800	52	51.04	50.84	50.94	Acetic plus small amt.
0.91	800	48	51.13	51.02	51.075	Acetic plus large amt. propionic acid
0.89	800	44	50.45	50.37	50.41	Acetic plus large amt. propionic acid
0.83	800	42	51.28	51.10	51.19	Acetic plus small amt. propionic acid
0.72	800	53	53.01	53.10	53.055	Acetic plus trace of propionic acid
0.96	850	53.				
0.55	900	55.5	53.24	53.18	53.21	Acetic plus small amt. propionic acid
0.70	850	57	52.0	51.99	51.995	Acetic plus trace of propionic acid
0.999	850	59.5	52.82	52.84	52.83	Acetic plus trace of propionic acid
0.90	900	70	51.293	---	51.293	Acetic plus trace of propionic acid
0.945	950	52	52.68	---	52.68	Acetic plus small amt. propionic acid
0.89	950	40	52.34	---	52.34	Acetic plus small amt. propionic acid
0.756	1000	26	51.98	---	51.98	Acetic plus trace of propionic acid
0.81	1000	49.5	51.70	---	51.70	Acetic plus large amt. phopionic acid
0.89	1000	49	52.98	---	52.98	Acetic plus small amt. propionic acid
0.89	1000	50	53.03	---	53.03	Acetic plus large amt. propionic acid
0.88	1000	53	52.96	---	52.96	Acetic pluce trace of propionic acid
						Acetic plus small

Batch of media	Designation of run	Cultures inoculated	Period incubated at 21°C.	Calculated as % lactic acid	cc. of culture distilled	N/10 alk for one liter of distillate
Raw milk naturally soured at room temperature	1^a	None	5 days	0.88	800	52
	1^b	None	5 "	0.91	800	48
	1^c	3 days of souring + assc.org.culture 15	6 "	0.89	800	44
	1^d	3 days of souring plus assc.org.culture 16	6 "	0.83	800	42
Pasteurized skimmed milk inoculated with pure cultures & butter cultures	2^a	Assc.org. culture 16 for 5 days plus butter culture B 16-1	11 "	0.72	800	53
	2^b	Assc. org. culture 16 plus butter culture b 16-1	11 "	0.96	850	53.
	3^a	Assc. org. culture 15	11	0.55	900	55.5
	3^b	Assc. org. culture 15 for 4 days plus butter culture B 16-1	4 "	0.70	850	57
	3^c	Assc. org. culture 15 plus butter culture B 16 - 1	12 "	0.999	850	59.5
Past. whole milk inoculated with butter culture	4^a	Butter culture No.122	1-2/3"	0.90	900	70
	4^b	" " " "	4½ "	0.945	950	52
	4^c	" " " "	6½ "	0.89	950	40
	5^a	" " " "	½ "	0.756	1000	26
	5^b	" " " "	1 "	0.81	1000	49.5
	5^c	" " " "	1-3/4"	0.89	1000	49
	5^d	" " " "	2¼ "	0.89	1000	50
	5^e	" " " "	2-2/3"	0.88	1000	53
Sterile skim milk inoc. with butter culture	6^a	Butter cult.No.B 16-1	2 "	0.96	900	54
	6^b	" " " "	5 "	1.02	900	58
	6^c	" " " "	7 "	1.02	800	60

calcu-lated as % lactic acid	cc. of culture dis-tilled	N/10 alk. for one liter of distil-late	% barium in salt A	B	Av.	Results of Duclaux
0.88	800	52	51.04	50.84	50.94	Acetic plus small amt.
0.91	800	48	51.13	51.02	51.075	Acetic plus large amt. propionic acid
0.89	800	44	50.45	50.37	50.41	Acetic plus large amt. propionic acid
0.83	800	42	51.28	51.10	51.19	Acetic plus small amt. propionic acid
0.72	800	53	53.01	53.10	53.055	Acetic plus trace of propionic acid
0.96	850	53.				
0.55	900	55.5	53.24	53.18	53.21	Acetic plus small amt. propionic acid
0.70	850	57	52.0	51.99	51.995	Acetic plus trace of propionic acid
0.999	850	59.5	52.82	52.84	52.83	Acetic plus trace of propionic acid
0.90	900	70	51.293	---	51.293	Acetic plus trace of propionic acid
0.945	950	52	52.68	---	52.68	Acetic plus small amt. propionic acid
0.89	950	40	52.34	---	52.34	Acetic plus small amt. propionic acid
0.756	1000	26	51.98	---	51.98	Acetic plus trace of propionic acid
0.81	1000	49.5	51.70	---	51.70	Acetic plus large amt. propionic acid
0.89	1000	49	52.98	---	52.98	Acetic plus small amt. propionic acid
0.89	1000	50	53.03	---	53.03	Acetic plus large amt. propionic acid
0.88	1000	53	52.96	---	52.96	Acetic pluce trace of propionic acid
0.96	900	54	52.36	52.32	52.34	Acetic plus small amt. propionic acid
1.02	900	58	52.11	52.08	52.095	Acetic plus trace of propionic acid
1.02	800	60	52.44	52.52	52.48	Acetic plus trace of propionic acid

The naturally soured raw milk cultures produced high volatile acidities. The volatile acids formed by S.paracitrovorus in pasteurized milk were slightly higher. Butter cultures also yielded high volatile acidities. In general, a longer period of incubation increased the volatile acidities, as is most strikingly shown in runs 5a to 5e. In runs 4a to 4c, the volatile acidities decreased as the incubation period was increased. The barium and Duclaux values of the volatile acids secured from naturally soured milk indicated large amounts of propionic with quantities of acetic acid, while the values of the acids secured from S.paracitrovorus cultures indicated, primarily, acetic with a trace or a small amount of propionic acid. The barium and Duclaux values on the volatile acids secured from butter cultures, in the early stages of ripening indicated acetic plus large amounts of propionic acid; while as the ripening of the cultures advanced, the amount of acetic acid increased and the amount of propionic decreased.

The residue, supposedly free from citric acid and volatile acid, which was left in the flask after the steam distillation, was neutralized with calcium carbonate, and after adding yeast, dipotassium hydrogen phosphate, and citric or lactic acids, it was sterilized in the autoclave. These sterile media were inoculated with associated organisms, incubated for some time at 21°C., and then steam distilled.

The amounts of volatile acids obtained in the cultures, and the barium and Duclaux values on these acids, are given in table VIII. The results recorded are in quite close agreement with those obtained in tables V and VI. The addition of citric acid gave increased amounts of volatile acids, while the addition of lactic acid, although it permitted the growth of organisms, as was shown by cultures on beef infusion agar slopes, did not give appreciable increases. The amounts of N/1 H_2SO_4 added just before the distillation, to the cultures containing lactic acid, had a direct bearing on the volatile acidity obtained in the distillate. The addition of N/1 H_2SO_4 in smaller quantities than the usual amount added, resulted in a corresponding decrease in the volatile acidity.

TABLE VIII.

Amounts and Types of Volatile Acids Formed in Fermentec

with Various Additions by Associatec

Designation of run	Chemical added: Kind	Chemical added: Amount in percent	Associated organisms inoculated	Period incubated at 21°C.	Final acidity calculated as % lac. acid	cc. of culture distilled	cc. of N/1 H_2SO_4 added	cc N/ fo li di la
1^a	Lactic acid	0.6	Cult. 2 plus Cult. 16 plus S. lactis	38 da.	---	900	50	
1^b	Lactic acid pepton +F. yeast	00.4 0.2 18.0	Cult. 2 plus Cult. 15 plus S. lactis	21 "	---	1000	50	
1^c	Lactic acid K_2HPO_4 F. yeast	0.4 0.2 5.0	Culture 1	18 "	0.73	800	40	
1^d	Lactic acid pepton F. yeast K_2HPO_4	0.4 0.3 4.4	Culture 3	18 "	0.99	400	45	
1^e	Lactic acid F. yeast	0.4 2.0	Cult. 1 plus S. lactis	17 "	0.84	900	50	
2^a	Citric acid F. yeast	0.4 15.0	Culture 1	23 "	1.07	700	52	
3^a	Lactic acid F. yeast	0.6 15.0	Culture 2	23 "	0.88	800	50	
3^b	Lactic acid F. yeast	0.6 15.0	Cult. 1 plus S. lactis	26 "	0.81	600	50	
3^c	Lactic acid F. yeast	0.6 15.0	Culture 15	26 "	0.47	700	52	
4^a	Lactic acid K_2HPO_4 conc. vitamin B.	0.5 0.5 2.0	Culture 1	15 "	0.71	600	15	
4^b	Lactic acid K_2HPO_4 powdered yeast	0.5 0.5 5.0	Culture 2	14 "	0.64	500	15	
4^c	Lactic acid K_2HPO_4 conc. vitamin B.	0.5 0.5 1.6	Culture 4	15 "	0.75	400	12	
4^d	Lactic acid K_2HPO_4 conc. vitamin B.	0.5 0.5 1.0	Culture 5	15 "	0.82	700	20	
4^e	Lactic acid K_2HPO_4 Powdered yeast	0.5 0.5 5.0	Culture 6	15 "	0.70	650	15	
4^f	Lactic acid K_2HPO_4	0.5 0.5	Culture 7	15 "	0.54	650	15	

TABLE VIII.

e Acids Formed in Fermented Milk (Free from Citric Acid)

ous Additions by Associated Organisms.

iod u- ed t °C.	Final acidity calculated as % lac. acid	cc. of culture distilled	cc. of N/1 H_2SO_4 added	cc. of N/10 alk. for one liter distillate	% barium in salt A	B	Av.	Duclaux values
da.	---	900	50	24	49.01	48.93	49.01	Acetic plus trace of propionic acid
"	---	1000	50	34	49.76	49.57	49.665	Acetic plus small amt.Propionic acid
"	0.73	800	40	26	50.5	50.41	50.455	Acetic plus small amt.propionic acid
"	0.99	400	45	30	49.99	50.01	50.0	Acetic plus small amt.propionic acid
"	0.84	900	50	23	48.03	47.93	47.98	Acetic plus small amt.propionic acid
"	1.07	700	52	83	52.69	52.67	52.68	Acetic plus trace of propionic acid
"	0.88	800	50	22	50.05	49.74	49.895	Acetic plus small amt. propionic acid
"	0.81	600	50	33	48.71	48.58	48.645	Acetic plus small amt.propionic acid
"	0.47	700	52	16	50.78	---	50.78	Acetic plus small amt.propionic acid
"	0.71	600	15	12	47.42	---	47.42	Acetic plus large amt.propionic acid
"	0.64	500	15	14	49.32	---	49.32	Acetic plus large amt.propionic acid
"	0.75	400	12	12	49.38	---	49.38	Acetic plus trace of propionic acid
"	0.82	700	20	13	49.56	49.41	49.485	Acetic plus trace of propionic acid
"	0.70	650	15	14	48.97	48.63	48.80	Acetic plus small amt.propionic acid
"	0.54	650	15	11	48.4	48.05	48.225	Acetic plus small [illegible]

1[c]	Lactic acid K_2HPO_4 F.yeast	0.4 0.2 5.0	Culture 1	18 "	0.73	800	40
1[d]	Lactic acid pepton F.yeast K_2HPO_4	0.4 0.3 4.4	Culture 3	18 "	0.99	400	45
1[e]	Lactic acid F. yeast	0.4 2.0	Cult. 1 plus S. lactis	17 "	0.84	900	50
2[a]	Citric acid F. yeast	0.4 15.0	Culture 1	23 "	1.07	700	52
3[a]	Lactic acid F. yeast	0.6 15.0	Culture 2	23 "	0.88	800	50
3[b]	Lactic acid F. yeast	0.6 15.0	Cult. 1 plus S. lactis	26 "	0.81	600	50
3[c]	Lactic acid F. yeast	0.6 15.0	Culture 15	26 "	0.47	700	52
4[a]	Lactic acid K_2HPO_4 conc. vitamin B.	0.5 0.5 2.0	Culture 1	15 "	0.71	600	15
4[b]	Lactic acid K_2HPO_4 powdered yeast	0.5 0.5 5.0	Culture 2	14 "	0.64	500	15
4[c]	Lactic acid K_2HPO_4 conc. vitamin B.	0.5 0.5 1.6	Culture 4	15 "	0.75	400	12
4[d]	Lactic acid K_2HPO_4 conc. vitamin B.	0.5 0.5 1.0	Culture 5	15 "	0.82	700	20
4[e]	Lactic acid K_2HPO_4 Powdered yeast	0.5 0.5 5.0	Culture 6	15 "	0.70	650	15
4[f]	Lactic acid K_2HPO_4 powdered yeast	0.5 0.5 2.5	Culture 7	15 "	0.54	650	15
4[g]	Lactic acid K_2HPO_4 F.yeast	0.5 0.5 25.0	Culture 8	15 "	0.46	600	50
4[h]	Lactic acid K_2HPO_4 F.yeast	0.5 0.5 25.0	Culture 8	15 "	0.39	550	50
4[i]	Lactic acid K_2HPO_4 F. yeast	0.5 0.5 25.0	Culture 10	15 "	0.38	600	50
4[j]	Lactic acid K_2HPO_4 F. yeast	0.5 0.5 25.0	Culture 11	15 "	0.36	550	45
5[a]	None	----	None	11 "	0.18	550	25
5[b]	F. yeast alone added to 1000cc. dist. H_2O and filtered	25.0	None	9 "	0.077	1000	50

[+] F. stands for Fleischmann's compressed yeast.

[++] Values obtained when the entire one liter of distillate was t

from the value for 50 cc.

"	0.73	800	40	26	50.5	50.41	50.455	Acetic plus small amt.propionic acid
"	0.99	400	45	30	49.99	50.01	50.0	Acetic plus small amt.propionic acid
"	0.84	900	50	23	48.03	47.93	47.98	Acetic plus small amt.propionic acid
"	1.07	700	52	83	52.69	52.67	52.68	Acetic plus trace of propionic acid
"	0.88	800	50	22	50.05	49.74	49.895	Acetic plus small amt. propionic acid
"	0.81	600	50	33	48.71	48.58	48.645	Acetic plus small amt.propionic acid
"	0.47	700	52	16	50.78	---	50.78	Acetic plus small amt.propionic acid
"	0.71	600	15	12	47.42	---	47.42	Acetic plus large amt.propionic acid
"	0.64	500	15	14	49.32	---	49.32	Acetic plus large amt.propionic acid
"	0.75	400	12	12	49.38	---	49.38	Acetic plus trace of propionic acid
"	0.82	700	20	13	49.56	49.41	49.485	Acetic plus trace of propionic acid
"	0.70	650	15	14	48.97	48.63	48.80	Acetic plus small amt.propionic acid
"	0.54	650	15	11	48.4	48.05	48.225	Acetic plus small amt.propionic acid
"	0.46	600	50	28	49.03	48.79	48.91	Acetic plus large amt. propionic acid
"	0.39	550	50	21	49.50	49.62	49.56	Acetic plus small amt.propionic acid
"	0.38	600	50	29	49.18	49.16	49.17	Acetic plus large amt.propionic acid
"	0.36	550	45	26	49.14	49.27	49.205	Acetic plus large amt.propionic acid
"	0.18	550	25	5.1 ++	---	---	---	---------------
"	0.077	1000	50	8.1 ++				

yeast.

ter of distillate was titrated instead of calculating

The barium and Duclaux values on the volatile acids secured when citric acid was added to the fermented milk, indicated acetic acid with a trace of propionic acid. The barium values on the volatile acids secured when lactic acid was added indicated, primarily, propionic acid with a small amount, a trace, or no acetic acid. The Duclaux values did not always check with the percentages of barium obtained. Although enough checks were unavailable because of contamination, nevertheless the data showed in table VIII suggested that lactic acid was not a probable source of volatile acids, and that either the yeasts used or the compounds present in the fermented milk, may have been sources of volatile acids.

Results Obtained with Fresh Milk.

Since S. paracitrovorus is capable of producing a small amount of lactic acid from lactose in milk, while S. citrovorus is not (7), the volatile acids produced in sterile freshly drawn milk by the latter type are presumably formed from the citric acid normally present. It is difficult, however, to draw a sharp line of demarcation between the two types of organisms and yet, judging from their cultural characteristics in milk, it is possible to make a reasonably definite distinction between them.

Freshly drawn milk was secured from the College Dairy Farm, brought to the laboratory under careful conditions, and sterilized immediately. The time elapsing between the milking and the beginning of the sterilization process never exceeded thirty minutes. The chemicals to be used were sterilized, as aqueous solutions, in small test tubes, then added to the inoculated milk cultures. The cultures were then incubated for some time at 21°C. and then steam distilled.

The amounts of volatile acids produced in various cultures, and the barium and Duclaux values obtained on these acids, are given in table IX.

TABLE IX

The Amounts and Types of Volatile Acids Formed in
and
with Citric or Lactic Acid Added by Associated

Designation of run	Chemical added: Kind	Chemical added: Amount in per cent	Associated organisms inoculated	Period incubated at 21° C.	Final acidity calculated as lactic acid	cc. of culture distilled	cc. of N/10 alk. for one liter of distillate	
1a+	None	----	Culture 1	12 da.	0.39	900	42	5
1b+	None	----	" "	14 "	0.35	900	76	[illegible]
2a	Citric acid	0.3	" "	13 "	0.45	900	86	[illegible]
2b	Citric acid	0.3	" "	18 "	0.35	900	99	[illegible]
3a	Lactic acid	0.4	" "	12 "	0.46	900	54	[illegible]
3b	Lactic acid	0.4	" "	5-1/3"	0.58	900	62	[illegible]
4a	None	----	Culture 2	12 "	0.41	900	56	[illegible]
4b	None	----	" "	14 "	0.42	900	63	[illegible]
5a	Citric acid	0.3	" "	13 "	0.657	900	137	[illegible]
5b	Citric acid	0.3	" "	18 "	0.71	900	156	[illegible]
6a	Lactic acid	0.4	" "	12 "	0.594	900	66	[illegible]
6b	Lactic acid	0.4	" "	5-1/3"	0.67	900	67	[illegible]
7a	Lactic acid	0.4	Culture 3	6 "	0.75	900	78	[illegible]
7b	Citric acid	0.3	" "	18 "	0.69	950	177	[illegible]
7c	None	---	Culture 1 plus Culture 3	27 "	0.315	950	69	5

+a and b are different batches of milk which were run at differe

TABLE IX

Types of Volatile Acids Formed in Fresh Milk Alone
and
r Lactic Acid Added by Associated Organisms.

Period incubated at 21° C.	Final acidity calculated as lactic acid	cc. of culture distilled	cc. of N/10alk. for one liter of distillate	Per cent barium in salt			Results of Duclaux.
				A	B	Av.	
12 da.	0.39	900	42	51.9	---	51.9	Acetic plus small amt.of propionic
14 "	0.35	900	76	52.83	53.05	52.94	Acetic plus large amt.propionic
13 "	0.45	900	86	52.97	52.95	52.96	Acetic plus small amt.of propionic
18 "	0.35	900	99	52.41	52.49	52.45	Acetic plus small amt. of propionic
12 "	0.46	900	54	53.08	53.17	53.125	Acetic plus trace of propionic
5-1/3"	0.58	900	62	52.64	52.56	52.6	Acetic plus slight amt.of propionic
12 "	0.41	900	56	53.18	53.1	53.14	Acetic plus trace of propionic
14 "	0.42	900	63	52.89	52.74	52.815	Acetic plus slight amt.of propionic
13 "	0.657	900	137	53.36	53.35	53.355	Acetic plus trace of propionic
18 "	0.71	900	156	53.16	53.11	53.135	Acetic pluc trace of propionic
12 "	0.594	900	66	53.55	---	53.55	Acetic plus trace of propionic
1/3"	0.67	900	67	53.76	53.72	53.74	Acetic plus trace of propionic
6 "	0.75	900	78	52.89	52.75	52.82	Acetic plus slight amt. of propionic
18 "	0.69	950	177	52.47	52.82	52.645	Acetic plus slight amt. of propionic
7 "	0.315	950	69	53.04	52.97	53.005	Acetic plus trace of propionic

of milk which were run at different times.

The addition of sterile citric acid to milk inoculated with associated organisms, always greatly increased the amounts of volatile acids. The addition of sterile lactic acid to S. paracitrovorus (culture 2,3,) cultures did not appreciably increase the volatile acid production, while with S. citrovorus (culture 1) it gave a marked increase in the amount of volatile acid produced. The barium and Duclaux values on the volatile acids obtained from cultures with and without the addition of citric or lactic acid, in all cases indicated acetic plus a trace or a small amount of propionic acid. The types of volatile acids secured from the addition of sterile lactic acid to the milk did not show any appreciable difference from those obtained when sterile citric acid was added. The source of milk seemed to have a bearing upon the barium values secured, since the same organism (culture 1 or culture 2) inoculated into different batches of milk without added chemicals, produced types of volatile acids which differed slightly in their proportional make-up.

To secure further evidence as to the types of acids formed from citric and lactic acids, a series of experiments were carried out on sterilized fresh milk, using a number of strains of associated organisms. Cultures of associated organisms were obtained from the research laboratory of the dairy bacteriology section of the Iowa Agricultural Experiment Station and, before being used, were plated on beef infusion agar (the plates being incubated at 21°C. for at least 48 hours) to detect any contamination. The sources of these organisms, their characteristics in milk, and the flavor of the cultures secured when they were combined with S. lactis are given in table X.

Flasks of milk , with and without chemicals added, were inoculated with cultures of the associated organisms. The flasks were incubated at 21°C. for ten days, frequently cultured on beef infusion agar slopes to detect contamination, and then steam distilled. The first 25 cc. fraction of distillate from each culture was collected and tested for the presence of diacetyl, while the remainder of the distillate was used for the preparation of barium salts.

The amounts of volatile acids and diacetyl produced by various cultures, and the barium and Duclaux values obtained on these acids, are given in table XI. The addition of 0.3 % sterile citric acid to the milk cultures always gave increased amounts of volatile acids. This increase was much greater with cultures of S. paracitrovorus than with cultures of S. citrovorus. The addition of 0.6 % sterile citric acid, in the majority of cases, produced either slightly more or about the same amounts of volatile acids as 0.3 % citric acid.

In a few instances, however, the addition of 0.6 % sterile citric acid gave amounts of volatile acids lower than those obtained with 0.3 % due, presumably, to the inhibiting

TABLE X.

Some of the Characteristics of the Associated

Designation No.	Isolated from	+cc. of N/10 acid distilled from 250 cc. of: Milk	Milk plus 0.4 % citric acid	Final acidity calculated as % lactic acid	++cc. of N/10 … distilled f… 1000 cc. … Milk A	B
Culture 1	Butter culture 9-20-'29	23.5	63.5	0.25	39	40
2	Sour cream	23.0	63.2	0.31	50	50
3	Sour cream	23.8	43.6	0.42	46	47
4	Sour cream 11-27-'29	29.5	62.8	0.63	78	82
5	Sour cream 12-4-'29	25.1	70.8	0.21	18	11
6	Sour cream	28.0	57.5	0.23	18	14
7	Sour cream 12-7-'29	19.5	47.0	0.27	20	18
8	Sour cream 1-4-'30	15.0	43.2	0.27	26	22
9	Sour cream 6-22-'30	24.2	88.1	0.47	53	48
10	Sour cream 7-13-'30	18.5	69.7	---	--	--
11	Sour cream 7-13-'30	----	----	---	--	--
12	Butter 8-21-'30	----	----	0.52	51	51
13	Sour cream	----	----	0.28	42	43

TABLE X.

teristics of the Associated Organisms Used.

of: ... ld	Final acidity calcu- lated as % lactic acid	++ cc. of N/10 acid distilled from 1000 cc. of Milk A	Milk B	Milk plus 0.3 % citric acid	Possible distinction	Flavor of culture secured when combined with S. lactis
	0.25	39	40	86	S. citrovorus	Good
	0.31	50	50	144	S. paracitrovorus	Lacking
	0.42	46	47	127	S. paracitrovorus	Lacking
	0.63	78	82	146	S. paracitrovorus	Good
	0.21	18	11	72	S. citrovorus	Good
	0.25	18	14	74	S. citrovorus	Good
	0.27	20	18	70	S. citrovorus	Good
	0.27	26	22	68	S. citrovorus	Fair to good
	0.47	53	48	126	S.paracitrovorus	Fair to good
	---	--	--	---	S. citrovorus	Fair to good
	---	--	--	---	-------------	Fair to good
	0.52	51	51	151	S. paracitrovorus	Fair to good
	0.28	42	43	73	S. paracitrovorus	Good

[illegible]	Sour cream	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
3	Sour cream	23.8	43.6	0.42	46	47
4	Sour cream 11-27-'29	29.5	62.8	0.63	78	82
5	Sour cream 12-4-'29	25.1	70.8	0.21	18	11
6	Sour cream	28.0	57.5	0.23	18	14
7	Sour cream 12-7-'29	19.5	47.0	0.27	20	18
8	Sour cream 1-4-'30	15.0	43.2	0.87	86	88
9	Sour cream 6-22-'30	24.2	88.1	0.47	53	48
10	Sour cream 7-13-'30	18.5	69.7	---	--	--
11	Sour cream 7-13-'30	----	----	---	--	--
12	Butter 8-21-'30	----	----	0.52	51	51
13	Sour cream 12-31-'30	----	----	0.28	42	43
14	Sour cream 12-31-'30	------	----	0.45	44	44
15	Sour cream	26.6	52.0	---	--	--
16	Sour cream	26.6	54.3	---	--	--
17	--------	----	----	---	--	--

+ These titration values were obtained immediately after the is inoculated in sterile milk, incubated at 21 °C. for 7 days, 25 the addition of 15 cc. N/1 H_2SO_4 ; the first liter of distill and the results expressed as the volatile acid values.

++ These values were secured with the usual method about one or isolation of the organisms.

0.42	46	47	127	S. paracitrovorus	Lacking
0.63	78	82	146	S. paracitrovorus	Good
0.21	18	11	72	S. citrovorus	Good
0.23	18	14	74	S. citrovorus	Good
0.27	20	18	70	S. citrovorus	Good
0.27	20	22	68	S. citrovorus	Fair to good
0.47	53	48	126	S.paracitrovorus	Fair to good
---	--	--	---	S. citrovorus	Fair to good
---	--	--	---	-------------	Fair to good
0.52	51	51	151	S. paracitrovorus	Fair to good
0.28	42	45	73	S. paracitrovorus	Good
0.45	44	44	142	S. paracitrovorus	Good
---	--	--	---	S. paracitrovorus	Lacking
---	--	--	---	S. paracitrovorus	Fair to lacking
---	--	--	---	S. paracitrovorus	------

ed immediately after the isolation of organisms which were ted at 21°C. for 7 days, 250 cc. steam distilled following the first liter of distillate was titrated with N/10 NaOH, olatile acid values.

usual method about one or two years after the effect on the high total acidity. The addition of 0.3 % sterile lactic acid to S. citrovorus cultures gave increased amounts of volatile acidity, while with S. paracitrovorus cultures the increase was not appreciable. The addition of 0.6% sterile lactic acid had about the same general inhibiting effect as an equal amount of citric acid. The addition of sterile sulfuric, phosphoric, or tartaric acid to the milk cultures increased the amount of volatile acids in almost the same manner as sterile lactic acid.

None of the associated organisms produced diacetyl in milk alone, and only a few were capable of producing it when non-volatile organic or inorganic acids were added to the milk. The amount of diacetyl produced varied, however, with the amount and kind of organic or inorganic acid added, and with the strains of associated organisms inoculated. The addition of sterile citric acid to cultures capable of producing diacetyl, always caused greater increases in the amounts of diacetyl produced than other chemicals. The increased volatile acidities in cultures to which amounts of sterile organic or inorganic acid other than citric acid had been added, was not directly proportional to the amounts of diacetyl produced. the addition of 0.3 % sterile lactic, tartaric,or phosphoric, or 0.2 % sulfuric acid to cultures, usually gave almost the same amounts of volatile acids as 0.6 % or 0.4 %, respectivelty, of these acids; but the larger amounts caused

TABLE XI.

Amounts and Types of Volatile Acids Formed
and
with Chemicals Added by Various Cultures of A

Designation of run	Chemical added		Associated organisms inoculated	Final acidity calculated as % lactic acid	cc. of culture distilled	cc. of N/10 alk. for one liter of distillate	Diacetyl produced in 25 cc. distillat
	Kind	Amount in %					
a	None	----	Culture 1	0.25	1000	39	None
1b	None	----	" "	0.245	1000	40	"
56 a	None	----	" "	0.32	1000	42	"
56 b	None	----	" "	0.32	1000	42	"
2 a	Citric acid	0.3	" "	0.42	1000	86	+Small amount
2 b	Citric acid	0.3	" "	0.42	950	82	Small amount
3 a	Lactic acid	0.3	" "	0.53	1000	64	Very slight amount
3 b	Lactic acid	0.3	" "	0.54	1000	59	Very slight amount
44 a	Lactic acid	0.6	" "	0.71	1000	50	Very small amount
44 b	Lactic acid	0.6	" "	0.68	950	52	Trace
44 c	Lactic acid Citric acid	0.3 0.3	" "	0.66	950	86	Small amount
56 c	Conc. H_2SO_4	0.2	" "	0.58	1000	57	Trace
56 d	Conc. H_2SO_4	0.4	" "	0.85	1000	58	Small

TABLE XI.

[…]f Volatile Acids Formed in Fresh Milk Alone
and
[…]y Various Cultures of Associated Organisms.

[…] of […] led	cc. of N/10 alk. for one liter of distillate	Diacetyl produced in 25 cc. of distillate	% barium in salt A	B	Av.	Results of Duclaux
…00	39	None	52.74	52.79	52.765	Acetic plus small amount of propionic
…00	40	"				
…00	42	"	53.16	53.13	53.145	Acetic plus trace of propionic
…00	42	"				
…00	86	+Small amount	53.23	53.18	53.205	Acetic plus trace of propionic
…50	82	Small amount				
…00	64	Very slight amount	52.82	52.78	52.80	Acetic plus trace of propionic
…00	59	Very slight amount				
…00	50	Very small amount	53.01	52.99	53.0	Acetic plus small amount of propionic
…50	52	Trace				
…0	86	Small amount	53.04	53.03	53.035	Acetic plus trace of propionic
…0	57	Trace	53.48	53.31	53.395	Acetic plus trace of propionic
…0	58	Small amount				

56 b	None	----	" "	0.32	1000	42	"
2 a	Citric acid	0.3	" "	0.42	1000	86	+Small amount
2 b	Citric acid	0.3	" "	0.42	950	82	Small amount
3 a	Lactic acid	0.3	" "	0.53	1000	64	Very slight amount
3 b	Lactic acid	0.3	" "	0.54	1000	59	Very slight amount
44 a	Lactic acid	0.6	" "	0.71	1000	50	Very small amount
44 b	Lactic acid	0.6	" "	0.68	950	52	Trace
44 c	Lactic acid Citric acid	0.3 0.3	" "	0.66	950	85	Small amount
56 c	Conc. H_2SO_4	0.2	" "	0.58	1000	57	Trace
56 d	Conc. H_2SO_4	0.4	" "	0.85	1000	58	Small amount
56 e	H_3PO_4	0.3	" "	0.72	1000	54	None
56 f	H_3PO_4	0.6	" "	1.16	1000	60	Slight amount
56 g	Tartaric acid	0.3	" "	0.49	1000	54	None
56 h	Tartaric acid	0.6	" "	0.64	1000	60	Small amount
39	None	---	None	0.185	1050	2	None
40	Citric acid Lactic acid	0.3 0.3	None ~~None~~	0.82	1000	5	None

+ Comparative grading of the amounts from a trace to a fa

is given in "Methods" on page 18.

.000	42	"	53.16	53.13	53.145	Acetic plus trace of propionic
.000	86	+Small amount	53.23	53.18	53.205	Acetic plus trace of propionic
950	82	Small amount				
.000	64	Very slight amount	52.82	52.78	52.80	Acetic plus trace of propionic
.000	59	Very slight amount				
.000	50	Very small amount	53.01	52.99	53.0	Acetic plus small amount of propionic
950	52	Trace				
950	86	Small amount	53.04	53.03	53.035	Acetic plus trace of propionic
.000	57	Trace	53.48	53.31	53.395	Acetic plus trace of propionic
.000	58	Small amount				
1000	54	None	53.20	53.04	53.12	Acetic plus trace of propionic
1000	60	Slight amount				
1000	54	None	53.58	53.56	53.57	Acetic plus trace of propionic
1000	60	Small amount				
1050	2	None	---	---	----	
1000	5	None				

unts from a trace to a fairly large quantity

ods" on page 18.

TABLE XI (continu

Amounts and Types of Volatile Acids Formed in I
and
with Chemicals Added by Various Cultures of Ass

Designation of run	Chemical added: Kind	Chemical added: Amount in %	Associated organisms inoculated	Final acidity calculated as % lactic acid	cc. of culture distilled	cc. of N/10 alk. for one liter of distillate	Diacetyl produced in 25 cc.c distillate
4 a	None	----	Culture 4	0.63	900	78	None
4 b	None	----	" 4	0.62	1000	82	None
5 a	Citric acid	0.3	" "	0.91	1000	146	None
5 b	Citric acid	0.3	" "	0.82	950	173	None
6 a	Lactic acid	0.3	" "	0.82	950	75	None
6 b	Lactic acid	0.3	" "	0.82	1000	74	None
57 a	Conc. H_2SO_4	0.2	" "	0.89	950	80	None
57 b	Conc. H_2SO_4	0.4	" "	0.95	1000	68	None
57 c	H_3PO_4	0.3	" "	0.95	950	66	None
57 d	H_3PO_4	0.6	" "	1.25	1000	70	None
57 e	Tartaric acid	0.3	" "	0.75	950	76	None
57 f	Tartaric acid	6.6	" "	0.79	950	71	None

TABLE XI (continued)

/olatile Acids Formed in Fresh Milk Alone
and
)y Various Cultures of Associated Organisms.

c. of ul- ure is- lled	cc. of N/10 alk. for one liter of distillate	Diacetyl produced in 25 cc. of distillate	% barium in salt			Results of Duclaux
			A	B	Av.	
)00	78	None	53.18	53.19	53.185	Acetic plus trace of propionic
)00	82	None				
)00	146	None	53.46	53.41	53.435	Acetic plus trace of propionic
)50	173	None				
)50	75	None	53.03	53.08	53.055	Acetic plus trace of propionic
)00	74	None				
)50	80	None	53.76	53.80	53.78	Acetic plus trace of propionic
)00	68	None				
)50	66	None	53.45	53.52	53.485	Acetic plus trace of propionic
)00	70	None				
)50	76	None	53.67	53.67	53.67	Acetic plus trace of propionic
)50	71	None				

	Kind	Amount in %	inoculated	as % lactic acid	dis- tilled	liter of dis- tillate	in 25 cc. distillat
4 a	None	----	Culture 4	0.63	900	78	None
4 b	None	----	" 4	0.69	1000	82	None
5 a	Citric acid	0.3	" "	0.91	1000	146	None
5 b	Citric acid	0.3	" "	0.82	950	173	None
6 a	Lactic acid	0.3	" "	0.82	950	75	None
6 b	Lactic acid	0.3	" "	0.82	1000	74	None
57 a	Conc. H_2SO_4	0.2	" "	0.89	950	80	None
57 b	Conc. H_2SO_4	0.4	" "	0.95	1000	68	None
57 c	H_3PO_4	0.3	" "	0.95	950	66	None
57 d	H_3PO_4	0.6	" "	1.25	1000	70	None
57 e	Tartaric acid	0.3	" "	0.75	950	76	None
57 f	Tartaric acid	0.6	" "	0.79	950	71	None
19	None	---	None	0.21	1000	3.2+	None
20	Citric acid	0.3	None	0.56	1000	4.9+	None
21	Lactic acid	0.3	None	0.51	1000	5.4+	None

+Values obtained when the entire one liter of distillate was ti… of calculating from the value for 50 cc.

ed	liter of distillate	in 25 cc. of distillate	A	B	Av.	Results of Duclaux
)	78	None	53.18	53.19	53.185	Acetic plus trace of propionic
)	82	None				
)	146	None	53.46	53.41	53.435	Acetic plus trace of propionic
)	173	None				
)	75	None	53.03	53.08	53.055	Acetic plus trace of propionic
)	74	None				
)	80	None	53.76	53.80	53.78	Acetic plus trace of propionic
)	68	None				
)	66	None	53.45	53.52	53.485	Acetic plus trace of propionic
)	70	None				
)	76	None	53.67	53.67	53.67	Acetic plus trace of propionic
)	71	None				
)	3.2+	None				
)	4.9+	None				
)	5.4+	None				

r of distillate was titrated instead
r 50 cc.

TABLE XI (continu

Amounts and Types of Volatile Acids Formed in
and
with Chemicals Added by Various Cultures of A

Designation of run	Chemical added: Kind	Chemical added: Amount in %	Associated organisms inoculated	Final acidity calculated as % lactic adid	cc. of culture distilled	cc. of N/10 alk. for one liter of distillate	Diacetyl produced in 25 cc. distillat
7 a	None	---	Culture 5	0.2	1000	18	None
7 b	None	---	" "	0.21	1000	11	None
42 a	None	---	" "	0.26	550	12	None
42 b	None	---	" "	0.25	1000	13	None
58 a	None	---	" "	0.23	1000	15	None
58 b	None	---	" "	0.25	1000	18	None
8 a	Citric acid	0.3	" "	0.47	1000	72	Large amount
8 b	Citric acid	0.3	" "	0.47	1000	68	Large amount
9 a	Lactic acid	0.3	" "	0.54	950	60	None
9 b	Lactic acid	0.3	" "	0.51	1000	60	None
43 a	Lactic acid	0.6	" "	0.675	900	55	None
43 b	Lactic	0.6	" "	0.685	1000	60	None

TABLE XI (continued)

olatile Acids Formed in Fresh Milk Alone
and
y Various Cultures of Associated Organisms.

of - e - ed	cc. of N/10 alk. for one liter of distillate	Diacetyl produced in 25 cc. of distillate	% barium in salt			Results of Duclaux
			A	B	Av.	
0	18	None	51.44	51.46	51.45	Acetic plus trace of propionic
0	11	None				
0	12	None	---	---	---	------------
0	13	None				
0	15	None	---	---	---	------------
0	18	None				
0	72	Large amount	53.32	52.90	53.11	Acetic plus trace of propionic
0	68	Large amount				
0	60	None	53.28	53.35	53.315	Acetic plus trace of propionic
0	60	None				
0	55	None	53.55	53.44	53.495	Acetic plus trace of propionic
0	60	None				

42 a	None	---	"	"	0.26	550	12	None
42 b	None	---	"	"	0.25	1000	13	None
58 a	None	---	"	"	0.23	1000	15	None
58 b	None	---	"	"	0.25	1000	18	None
8 a	Citric acid	0.3	"	"	0.47	1000	72	Large amount
8 b	Citric acid	0.3	"	"	0.47	1000	68	Large amount
9 a	Lactic acid	0.3	"	"	0.54	950	60	None
9 b	Lactic acid	0.3	"	"	0.51	1000	60	None
43 a	Lactic acid	0.6	"	"	0.675	900	55	None
43 b	Lactic acid	0.6	"	"	0.685	1000	60	None
43 c	Lactic acid Citric acid	0.3 0.3	"	"	0.72	1000	98	Large amount
58 c	Conc. H_2SO_4	0.2	"	"	0.6	1000	57	None
58 d	Conc. H_2SO_4	0.4	"	"	0.84	1000	55	Slight amount
58 e	" H_3PO_4	0.3	"	"	0.75	1000	51	None
58 f	H_3PO_4	0.6	"	"	1.21	1000	58	Very slight amount
61 a	None	---	None		0.19	1000	1.1+	None
61 b	None	---	None		0.19	1000	1.6+	None

+ Values obtained when the entire one liter of distillate wa
calculating from the value for 50 cc.

50	12	None	---	---	---	------------
00	13	None				
00	15	None	---	---	---	------------
00	18	None				
00	72	Large amount	53.32	52.90	53.11	Acetic plus trace of propionic
00	68	Large amount				
0	60	None	53.28	53.35	53.315	Acetic plus trace of propionic
00	60	None				
00	55	None	53.55	53.44	53.495	Acetic plus trace of propionic
00	60	None				
00	98	Large amount	53.57	53.58	53.575	Acetic plus trace of propionic
00	57	None	53.65	53.59	53.62	Acetic plus trace of propionic
00	55	Slight amount				
00	51	None	53.32	53,45	53.385	Acetic plus trace of propionic
00	58	Very slight amount				
00	1.1+	None				
00	1.6+	None				

e liter of distillate was titrated instead of
50 cc.

TABLE XI (continued

Amounts and Types of Volatile Acids Formed in
and
with Chemicals Added by Various Cultures of As

Designation of run	Chemical added: Kind	Chemical added: Amount in %	Associated ORGANISMs inoculated	Final acidity calculated as % lactic acid	cc. of culture distilled	cc. of N/10 alk. for one liter of distillate	Diacetyl produced in 25 cc. o distillate
16 a	None	---	Culture 6	0.23	1000	18	None
16 b	None	---	" "	0.23	1000	14	None
45	None	---	" "	0.22	1000	10	None
59 a	None	---	" "	0.22	1000	16	None
59 b	None	---	" "	0.22	1000	13	None
17	Citric acid	0.3	" "	0.54	1000	74	small amount
18	Lactic acid	0.3	" "	0.52	1000	58	None
46 a	Lactic acid	0.6	" "	0.65	1000	44	Very small amount
46 b	Lactic acid	0.6	" "	0.62	1000	46	slight amount
59 c	Conc. H_2SO_4	0.2	" "	0.56	1000	42	slight amount
59 d	Conc. H_2SO_4	0.4	" "	0.76	1000	57	Small amount
59 e	H_3PO_4	0.3	" "	0.71	1000	44	slight

TABLE XI (continued)

s of Volatile Acids Formed in Fresh Milk Alone
and
dded by Various Cultures of Associated Organisms.

cc.of culture distilled	cc. of N/10 alk. for one liter of distillate	Diacetyl produced in 25 cc.of distillate	% barium in salt A	% barium in salt B	% barium in salt Av.	Results of Duclaux
1000	18	None	52.01	52.03	52.02	Acetic plus trace of propionic
1000	14	None				
1000	10	None	---	---	---	---------
1000	16	None	---	---	---	---------
1000	13	None	---	---	---	---------
1000	74	small amount	53.12	53.17	53.145	Acetic plus large amt. of propionic
1000	58	None	53.32	53.39	53.355	Acetic plus small amount of propionic
1000	44	Very small amount	52.73	53.06	52.895	Acetic plus small amount of propionic
1000	46	slight amount				
1000	42	slight amount	53.59	53.68	53.635	Acetic plus trace of propionic
1000	57	Small amount				
1000	44	slight amount				

		in %					
16 a	None	---	Culture 6	0.23	1000	18	None
16 b	None	---	" "	0.23	1000	14	None
45	None	---	" "	0.22	1000	10	None
59 a	None	---	" "	0.22	1000	16	None
59 b	None	---	" "	0.22	1000	13	None
17	Citric acid	0.3	" "	0.54	1000	74	small amount
18	Lactic acid	0.3	" "	0.52	1000	58	None
46 a	Lactic acid	0.6	" "	0.65	1000	44	Very small amount
46 b	Lactic acid	0.6	" "	0.62	1000	46	slight amount
59 c	Conc. H_2SO_4	0.2	" "	0.56	1000	42	slight amount
59 d	Conc. H_2SO_4	0.4	" "	0.76	1000	57	Small amount
59 e	H_3PO_4	0.3	" "	0.71	1000	44	slight amount
59 f	H_3PO_4	0.6	" "	1.18	1000	54	small amount
19	None	---	None	0.21	1000	3.2+	None
55 e	Lactic acid cit. acid	0.3 0.3	None	0.82	1000	5.2+	None

+ Values obtained when the entire one liter of distilla
calculating from the value for 50 cc.

1000	18	None	52.01	52.03	52.02	Acetic plus trace of propionic
1000	14	None				
1000	10	None	---	---	---	---------
1000	16	None	---	---	---	---------
1000	13	None	---	---	---	---------
1000	74	small amount	53.12	53.17	53.145	Acetic plus large amt. of propionic
1000	58	None	53.32	53.39	53.355	Acetic plus small amount of propionic
1000	44	Very small amount	52.73	53.06	52.895	Acetic plus small amount of propionic
1000	46	slight amount				
1000	42	slight amount	53.59	53.68	53.635	Acetic plus trace of propionic
1000	57	Small amount				
1000	44	slight amount	53.66	53.66	53.66	Acetic plus trace of propionic
1000	54	small amount				
1000	3.2[+]	None				
1000	5.2[+]	None				

entire one liter of distillate was titrated instead of
om the value for 50 cc.

TABLE XI (continued

Amounts and Types of Volatile Acids Formed in F
and
with Chemicals Added by Various Cultures of Ass

Designation of run	Chemical added: Kind	Chemical added: Amount in %	Associated organisms inoculated	Final acidity calculated as % lactic acid	cc. of culture distilled	cc. of N/10 alk for one liter of distillate	Diacetyl produced in 25 cc. of distillate
31 a	None	---	Culture 13	0.28	1000	42	None
31 b	None	---	" "	0.28	1000	43	None
60 a	None	---	" "	0.24	1000	40	None
32 a	Citric acid	0.3	" "	0.49	1000	73	Small amount
32 b	Citric acid	0.6	" "	0.97	1000	30	Small amount
48 a	Citric acid	0.6	" "	0.81	1000	51	Large amount
33 a	Lactic acid	0.3	" "	0.46	1000	48	None
33 b	Lactic acid	0.6	" "	0.75	1000	52	Very small amount
34	Lactic acid citric acid	0.3 0.3	" "	0.80	1000	108	Large amount
48 b	Conc. H_2SO_4	0.3	" "	0.63	1000	48	Slight amount

TABLE XI (continued)

Volatile Acids Formed in Fresh Milk Alone
and
by Various Cultures of Associated Organisms.

cc. of culture distilled	cc. of N/10 alk. for one liter of distillate	Diacetyl produced in 25 cc. of distillate	% barium in salt			Results of Duclaux
			A	B	Av.	
1000	42	None	52.92	52.82	52.87	Acetic plus trace of propionic
1000	43	None				
1000	40	None	---	---	---	---------------
1000	73	Small amount	53.27	53.26	53.265	Acetic plus trace of propionic
1000	30	Small amount	52.29	52.29	52.29	Acetic plus trace of propionic
1000	51	Large amount	53.27	53.24	53.255	Acetic plus trace of propionic
1000	48	None	52.64	52.63	52.635	Acetic plus trace of propionic
1000	52	Very small amount	52.61	52.69	52.65	Acetic plus trace of propionic
1000	108	Large amount	53.22	53.25	53.235	Acetic plus trace of propionic
1000	48	Slight amount	53.16	[illegible]	53.14	Acetic plus trace

60 a	None	---	" "	0.24	1000	40	None
32 a	Citric acid	0.3	" "	0.49	1000	73	Small amount
32 b	Citric acid	0.6	" "	0.97	1000	30	Small amount
48 a	Citric acid	0.6	" "	0.81	1000	51	Large amount
33 a	Lactic acid	0.3	" "	0.46	1000	48	None
33 b	Lactic acid	0.6	" "	0.75	1000	52	Very small amount
34	Lactic acid citric acid	0.3 0.3	" "	0.80	1000	108	Large amount
48 b	Conc. H_2SO_4	0.3	" "	0.63	1000	48	slight amount
48 c	Conc. H_2SO_4	0.3	" "	0.62	1000	50	Very slight amount
60 c	Conc. H_2SO_4	0.2	" "	0.55	1000	48	None
60 d	Conc. H_2SO_4	0.4	" "	0.74	1000	57	Very slight amount
60 e	H_3PO_4	0.3	" "	1.18	1000	71	None
60 f	H_3PO_4	0.6	" "	1.12	1000	55	Trace
60 g	Tartaric acid	0.3	" "	0.46	1000	60	None
60 h	Tartaric acid	0.6	" "	0.59	1000	56	Very slight amount
10	None	---	None	0.16	1000	2	None
11	Citric acid	0.3	None	0.53	1000	2	None
12	Lactic acid	0.3	None	0.51	1000	5	None

1000	40	None	---	---	---	---------------
1000	73	Small amount	53.27	53.26	53.265	Acetic plus trace of propionic
1000	30	Small amount	52.29	52.29	52.29	Acetic plus trace of propionic
1000	51	Large amount	53.27	53.24	53.255	Acetic plus trace of propionic
1000	48	None	52.64	52.63	52.635	Acetic plus trace of propionic
1000	52	Very small amount	52.61	52.69	52.65	Acetic plus trace of propionic
1000	108	Large amount	53.22	53.25	53.235	Acetic plus trace of propionic
1000	48	slight amount	53.16	53.12	53.14	Acetic plus trace of propionic
1000	50	Very slight amount				
1000	48	None	53.47	53.49	53.48	Acetic plus trace of propionic
1000	57	Very slight amount				
1000	71	None	53.72	53.65	53.685	Acetic plus trace of propionic
1000	55	Trace				
1000	60	None	53.29	53.32	53.305	Acetic plus trace of propionic
1000	56	Very slight amount				
1000	2	None	---	---	---	-----------
1000	2	None	---	---	---	-----------
1000	5	None	---	---	---	-------------

TABLE XI (continued)

Amounts and Types of Volatile Acids Formed in
and
with Chemicals Added by Various Cultures of As

Designation of run	Chemical added: Kind	Chemical added: Amount in %	Associated organisms inoculated	Final acidity: calculated as % lactic acid	cc. of culture distilled	cc. of N/10 alk. for one liter of distillate	Diacetyl produced in 25 cc. of distillate
13_a	None	---	Culture 2	0.315	1000	50	None
13b	None	---	" "	0.31	1000	50	None
14	Citric acid	0.3	" "	0.73	1000	144	None
15	Lactic acid	0.3	" "	0.61	1000	65	None
22 a	None	---	Culture 7	0.27	1000	20	None
22b	None	---	" "	0.27	1000	18	None
23	Citric acid	0.3	" "	0.59	1000	70	Small amount
24	Lactic acid	0.3	" "	0.53	1000	56	None
25a	None	---	Culture 8	0.27	1000	26	None
25b	None	---	" "	0.26	1000	22	None

TABLE XI (continued).

Volatile Acids Formed in Fresh Milk Alone
and
by Various Cultures of Associated Organisms.

c.of ul- ure is- lled	cc. of N/10 alk. for one liter of distillate	Diacetyl produced in 25 cc. of distillate	% barium in salt			Results of Duclaux
			A	B	Av.	
000	50	None	53.26	53.29	53.275	Acetic plus trace of propionic
000	50	None				
000	144	None	53.65	53.65	53.65	Acetic plus trace of propionic
000	65	None	53.29	53.23	53.26	Acetic plus trace of propionic
000	20	None	52.08	52.08	52.08	Acetic plus trace of propionic
000	18	None				
000	70	Small amount	53.26	53.21	53.235	Acetic plus trace of propionic
000	56	None	53.19	53.23	53.21	Acetic plus trace of propionic
000	26	None	52.49	52.86	52.675	Acetic plus trace of propionic
000	22	None	52.49	52.86	52.675	
		Small	[illegible]	[illegible]	[illegible]	Acetic plus trace

22a	None	---	Culture 7	0.27	1000	20	None
22b	None	---	" "	0.27	1000	18	None
23	Citric acid	0.3	" "	0.59	1000	70	Small amount
24	Lactic acid	0.3	" "	0.53	1000	56	None
25a	None	---	Culture 8	0.27	1000	26	None
25b	None	---	" "	0.26	1000	22	None
26	Citric acid	0.3	" "	0.54	1000	68	Small amount
27	Lactic acid	0.3	" "	0.56	1000	56	None
47	Lactic acid	0.6	" "	0.67	1000	57	None
28a	None	---	Culture 12	0.52	1000	51	None
28b	None	---	" "	0.53	1000	51	None
29	Citric acid	0.3	" "	0.93	1000	151	None
30	Lactic acid	0.3	" "	0.78	1000	61	None
55a	None	---	None	0.2	1000	2.7+	None
55b	None	---	None	0.205	1000	2.6+	None
55c	Lac. acid Cit. acid	0.3 0.3	None	0.82	1000	5.2+	None

+Values obtained when the entire one liter of distillate wa
calculating from the value for 50 cc.

000	20	None	52.08	52.08	52.08	Acetic plus trace of propionic
000	18	None				
000	70	Small amount	53.26	53.21	53.235	Acetic plus trace of propionic
000	56	None	53.19	53.23	53.21	Acetic plus trace of propionic
000	26	None	52.49	52.86	52.675	Acetic plus trace of propionic
000	22	None	52.49	52.86	52.675	
000	68	Small amount	53.0	53.04	53.02	Acetic plus trace of propionic
000	56	None	53.42	53.44	53.43	Acetic plus trace of propionic
000	57	None	53.38	53.39	53.385	Acetic plus trace of propionic
000	51	None	53.01	52.99	53.0	Acetic plus trace of propionic
000	51	None				
000	151	None	53.58	53.64	53.61	Acetic plus trace of propionic
000	61	None	53.28	53.31	53.295	Acetic plus small amount of propionic
1000	2.7+	None				
1000	2.6+	None				
1000	5.2+	None				

one liter of distillate was titrated instead of
for 50 cc.

TABLE XI (conclud

Amounts and Types of Vo atile Acids Formed in
and
with Chemicals Added by Various Cultures of As

Designation of run	Chemical added		Associated organisms inoculated	Final acidity calculated as % lactic acid	cc. of culture distilled	cc. of N/10 alk. for one liter of distillate	Diacetyl produced in 25 cc. of distillate
	Kind	Amount					
35a	None	---	Culture 14	0.45	1000	44	None
35b	None	---	" "	0.44	1000	44	"
36a	Citric acid	0.3	" "	0.79	1000	142	"
36b	Citric acid	0.6	" "	0.93	1000	188	"
37a	Lactic acid	0.3	" "	0.73	1000	58	"
37b	Lactic acid	0.6	" "	1.08	1000	10	"
41	Lactic acid	0.6	" "	0.93	1000	58	"
38	Cit. acid lac. acid	0.3 0.3	" "	0.90	1000	154	"
49a	None	---	Culture 3	0.42	1000	46	"
49b	None	---	" "	0.45	1000	47	"
50a	Citric acid	0.3	" "	0.81	1000	127	"
50b	Citric acid	0.6	" "	0.91	1000	172	"
51a	Lactic acid	0.6	" "	0.82	1000	66	"
51b	Lactic acid	0.6	" "	0.82	1000	63	"

TABLE XI (concluded)

f Vo atile Acids Formed in Fresh Milk Alone
and
d by Various Cultures of Associated Organisms.

cc. of culture distilled	cc. of N/10 alk. for one liter of distillate	Diacetyl produced in 25 cc. of distillate	% barium in salt			Results of Duclaux
			A	B	Av.	
1000	44	None	53.05	53.11	53.08	Acetic plus trace of propionic
1000	44	"				
1000	142	"	53.67	53.62	53.645	Acetic acid
1000	188	"	53.63	53.61	53.62	Acetic plus trace of propionic
1000	58	"	53.15	53.01	53.08	Acetic acid
1000	10	"	---	---	---	--------------
1000	58	"	53.11	53.01	53.06	Acetic plus trace of propionic
1000	154	"	53.39	53.38	53.385	Acetic plus trace of propionic
1000	46	"	53.30	53.32	53.31	Acetic plus trace of propionic
1000	47	"				
1000	127	"	53.72	53.71	53.715	Acetic acid
1000	172	"	53.85	53.79	53.82	Acetic acid
1000	66	"	53.56	53.62	53.59	Acetic plus trace of propionic
1000	63	"				

	acid						
37a	Lactic acid	0.3	" "	0.73	1000	58	"
37b	Lactic acid	0.6	" "	1.08	1000	10	"
41	Lactic acid	0.6	" "	0.95	1000	58	"
38	Cit. acid lac. acid	0.3 0.3	" "	0.90	1000	154	"
49a	None	---	Culture 3	0.42	1000	46	"
49b	None	---	" "	0.45	1000	47	"
50a	Citric acid	0.3	" "	0.81	1000	127	"
50b	Citric acid	0.6	" "	0.91	1000	172	"
51a	Lactic acid	0.6	" "	0.82	1000	66	"
51b	Lactic acid	0.6	" "	0.82	1000	63	"
51c	Lactic acid citric acid	0.3 0.3	" "	0.87	1000	130	"
52a	None	---	Culture 9	0.47	1000	53	"
52b	None	---	" "	0.465	1000	48	"
53a	Citric acid	0.3	" "	0.82	1000	126	"
53b	Citric acid	0.6	" "	0.95	1000	136	"
54a	Lactic acid	0.3	" "	0.74	1000	66	"
54b	Lactic acid	0.6	" "	0.85	1000	66	"
54c	Lactic acid	0.6	" "	0.84	1000	60	"
39	None	---	None	0.185	1000	2	"
40	Citric acid Lactic acid	0.3 0.3	"	0.82	1000 "	5	"

[illegible]	[illegible]		[illegible]	[illegible]	[illegible]	of propionic
1000	58	"	53.15	53.01	53.08	Acetic acid
1000	10	"	---	---	---	-------------
1000	58	"	53.11	53.01	53.06	Acetic plus trace of propionic
1000	154	"	53.39	53.38	53.385	Acetic plus trace of propionic
1000	46	"	53.30	53.32	53.31	Acetic plus trace of propionic
1000	47	"				
1000	127	"	53.72	53.71	53.715	Acetic acid
1000	172	"	53.85	53.79	53.82	Acetic acid
1000	66	"	53.56	53.62	53.59	Acetic plus trace of propionic
1000	63	"				
1000	130	"	53.70	53.69	53.695	Acetic plus trace of propionic
1000	53	"	53.56	53.54	53.55	Acetic plus trace of propionic
5 1000	48	"				
1000	126	"	53.62	53.57	53.595	Acetic plus trace of propionic
1000	136	"	53.72	53.70	53.71	Acetic plus trace of propionic
1000	66	"	53.22	53.15	53.185	Acetic plus trace of propionic
1000	66	"	53.39	53.25	53.32	Acetic plus trace of propionic
1000	60	"				
5 1000	2	"	---	---	-----	----------
1000	5	"	---	---	-----	----------

greater increases in the diacetyl production than the smaller amounts. Moreover, in some instances, the addition of 0.6 % sterile lactic, tartaric or phosphoric, or 0.4 % sulphuric acid, caused a decrease in the volatile acidities but an increase in diacetyl production. These results suggest that a certain range of p^H, variable with different organisms, is an important factor in diacetyl production.

S. citrovorus cultures in sterile fresh milk alone, usually produced small amounts of volatile acids which consisted of acetic plus a small or a large amount of propionic acid, while S. paracitrovorus cultures produced comparatively larger quantities of volatile acidities made up of acetic plus a trace or a small amount of propionic acid. The barium and Duclaux values on the volatile acids formed in organic or inorganic acid added cultures, indicated acetic plus a trace (seldom a small amount) of propionic acid.

SUMMARY OF RESULTS

1- Lots of uninoculated sterile phosphate-yeast-beef infusion bouillon, with and without addition of chemicals, gave considerable quantities of volatile acids when steam distilled. These acids were propionic, or propionic with a small or a large amount of acetic acid.

2- S. citrovorus, in phosphate-yeast-beef infusion bouillon with no chemicals added, produced considerable amounts of volatile acids which consisted of a mixture of about equal quantities of acetic and propionic acids.

3- The addition of sterile citric acid to phosphate-yeast-beef infusion bouillon inoculated with associated organisms, increased the amount of volatile acids. The barium and Duclaux values on these acids indicated acetic plus a trace, a small amount, or a large amount of propionic acid.

4- The addition of sterile lactic, beta hydroxy propionic, tartaric, succinic, malic, or glycollic acid to phosphate-yeast-beef infusion bouillon inoculated with associated organisms did not increase the volatile acidity. The barium values on the acids secured indicated propionic, or propionic with a small or a large amount of acetic acid.

5- The results obtained using fermented milk (free from citric and volatile acids) were essentially the same as those obtained with phosphate-yeast-beef infusion bouillon.

6- S. citrovorus cultures in sterile fresh milk usually produced small amounts of volatile acids made-up of acetic plus large amounts of propionic acid, while S. paracitrovorus cultures produced larger quantities of volatile acids, which consisted of acetic plus a trace or a small amount of propionic acid.

7- The addition of sterile citric acid to sterile fresh milk inoculated with associated organisms always increased the volatile acid production. The addition of sterile lactic acid gave a marked increase with S. citrovorus cultures, while with S. paracitrovorus cultures this increase was unappreciable. The barium and Duclaux values on volatile acids secured when citric or lactic acid had been added, indicated acetic plus a trace (seldom a small amount) of propionic acid.

8- The addition of sterile sulphuric, phosphoric or tartaric acid to fresh milk inoculated with associated organisms gave results essentially the same as those secured with lactic acid.

9- None of the associated organisms produced diacetyl in fresh sterile milk, while only a few of them were capable of producing it when non-volatile organic or inorganic acids had been added to the milk. A greater production of diacetyl resulted from the addition of sterile citric acid to cultures, than from the addition of other organic or inorganic acids. An increased amount of volatile acidity was not necessarily accompanied by an increase in diacetyl production. Apparently, a certain range of p^H, variable with different organisms, was an important factor in the production of diacetyl when sterile lactic, tartaric, phosphoric or sulphuric acid had been added to the cultures.

DISCUSSION OF RESULTS

The results reported show that the addition of sterile citric acid to phosphate-yeast-beef infusion bouillon, fermented milk (free from citric acid) or fresh milk inoculated with one of the associated organisms, always gave large increases in the volatile acidities, while the addition of sterile lactic, beta hydroxy propionic, tartaric, succinic, malic or glycollic acid did not give significant increases. Furthermore, the addition of sterile inorganic acids (sulphuric or phosphoric) to fresh milk inoculated with the organisms, gave essentially the same amounts of volatile acids as the addition of sterile lactic or tartaric acid.

Apparently these latter organic acids function, in milk cultures, in the same manner as sulphuric or phosphoric acid. Since the inorganic acids presumably cannot be changed into volatile acids, it appears probable that citric acid is the actual source of volatile acids formed by the associated organisms in milk, and that these organisms are not capable of fermenting lactic acid into volatile acids, as was suggested by Hammer (7). The lactic acid may free the citrates, naturally present in milk, into an easily available form which is readily changed to volatile acid. The fact that <u>S. paracitrovorus</u> is capable of producing a small amount of lactic acid in milk while <u>S. citrovorus</u> is not (7), partially explains the reason for the larger volatile acid production by the former than by the latter type. The availability of the citric acid may have a bearing on the types of acids formed. S. citrovorus cultures produced types of volatile acids in milk with no chemicals added,which consisted of acetic plus large amounts of propionic acid, while with S. paracitrovorus cultures these acids were primarily acetic with a trace of propionic acid.

Since the type of volatile acid in milk cultures containing diacetyl practically always indicated acetic acid, it may be that acetic acid directly is the source of the diacetyl formed. Furthermore, since the addition of increased amounts of organic or inorganic acids to the cultures nearly always increased the amount of diacetyl present, it appears probable that a certain range of p^H is an essential factor in the maximum production of diacetyl by the associated organisms. This agrees with the idea held by certain investigators, that a high aroma and flavor in a butter culture cannot be secured without considerable total acidity.

CONCLUSIONS

1- The organisms associated with *S. lactis* in butter cultures are capable of fermenting citric acid with the production of volatile acids which consist primarily of acetic with a trace or a small or a large amount of propionic acid.

2- These associated organisms alone are not capable of fermenting lactic acid. It merely functions to free the citrates, naturally present in milk, into easily available citric acid, which in turn is changed into forms of volatile acids by these organisms.

3- Citric acid indirectly, and acetic acid directly, are possible sources of the diacetyl formed by the associated organisms.

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