

# Flavor Development in Salted Butter by Pure Cultures of Bacteria

## Preliminary Results

BY W. H. HOECKER AND B. W. HAMMER

AGRICULTURAL EXPERIMENT STATION  
IOWA STATE COLLEGE OF AGRICULTURE  
AND MECHANIC ARTS

DAIRY INDUSTRY SECTION

Dhananjayarao Gadgil Library



GIPE-PUNE-047341

7311:73-4.

H1

47341

## CONTENTS

	Page
Summary .....	320
Introduction .....	323
Methods .....	324
Sources of cultures .....	324
Preparation of cultures for butter .....	324
Preparation of cream .....	325
Churning of cream .....	325
Holding of butter .....	325
Analytical procedures .....	325
Experimental .....	326
Diacetyl and acetylmethylcarbinol in milk cultures of the various organisms .....	326
Diacetyl and acetylmethylcarbinol in cream to which no culture was added .....	327
Diacetyl and acetylmethylcarbinol in cream plus cul- ture .....	331
Diacetyl and acetylmethylcarbinol in cream plus culture immediately after mixing .....	331
Diacetyl and acetylmethylcarbinol in cream plus culture after 16 hours holding .....	333
General flavor of butter and diacetyl and acetylmethyl- carbinol in butter and in buttermilk .....	334
Changes in diacetyl and acetylmethylcarbinol in butter during holding .....	335
Relationship of diacetyl and acetylmethylcarbinol con- tents to flavor of the butter .....	337
Discussion .....	341
Literature cited .....	344

## SUMMARY

Pure cultures of various streptococci produced relatively large amounts of diacetyl and acetylmethylcarbinol in milk containing added citric acid. These included *S. citrovorus* or *S. paracitrovorus*, *S. diacetilactis*, *S. citrophilus* and an unidentified organism H28. *S. aromaticus*, which does not ferment citric acid, produced diacetyl and small amounts of acetylmethylcarbinol in milk. With each of the species the ratios of diacetyl to acetylmethylcarbinol varied in the different trials; frequently, the diacetyl was much higher in proportion to the acetylmethylcarbinol than with butter cultures.

The diacetyl contents of cream plus culture immediately after mixing were both higher and lower than the theoretical amounts calculated from the diacetyl contents of the cream and culture; the carbinol contents were about the same as the theoretical values in most trials, but were higher in some instances.

In cream plus culture held 16 hours at approximately 40°F., the diacetyl and acetylmethylcarbinol contents increased when butter culture, *S. diacetilactis* or *S. citrophilus* was used; decreases often occurred with *S. citrovorus* or *S. paracitrovorus*; usually, little or no change occurred with organism H28 or *S. aromaticus*.

Butter made without the use of culture or with *S. aromaticus* contained only small amounts of diacetyl and acetylmethylcarbinol, whereas butter made with the other cultures commonly contained appreciable amounts of these compounds.

Only small percentages of the diacetyl and acetylmethylcarbinol present in cream plus culture were retained in the butter, the remainder being in the buttermilk; the percentage retention was essentially the same with the different cultures, although with each culture there was considerable variation from one churning to another.

Both increases and decreases in diacetyl occurred in butter held 1 day at 40°F. and then 2 and 4 weeks at 35° or 0°F., the larger changes usually occurring with butter held at 35°F. Occasionally, increases in diacetyl contents after 2 weeks were followed by decreases after 4 weeks. Except in a few instances, the acetylmethylcarbinol contents did not change appreciably.

Butter made with butter culture, *S. citrovorus*, *S. paracitrovorus* or *S. citrophilus* usually contained relatively large amounts of diacetyl and acetylmethylcarbinol and commonly placed high in a series of churnings. However, butter containing exceptionally large amounts of diacetyl and acetylmethylcarbinol sometimes placed low and was criticized as being coarse, sour, oily or containing some other objectionable flavor. Butter made without culture or with *S. aromaticus* contained only small amounts of diacetyl and acetylmethylcarbinol and usually placed low.

# Flavor Development in Salted Butter by Pure Cultures of Bacteria

## Preliminary Results<sup>1</sup>

BY W. H. HOECKER AND B. W. HAMMER

The cultures commonly used in the development of flavor in butter contain two types of bacteria, one of which (*Streptococcus lactis*) primarily produces lactic acid from lactose, while the other (*Streptococcus citrovorus* or *Streptococcus paracitrovorus*), under the conditions established by *S. lactis*, produces diacetyl, acetylmethylcarbinol and other compounds from citric acid.

Matuszewski, Pijanowski and Supińska (7, 8) isolated and described an aroma-producing organism (*Streptococcus diacetilactis*) and suggested its use in the manufacture of butter, either alone or in combination with a usual butter culture. The essential characteristics of this organism are the production of diacetyl, acetylmethylcarbinol and volatile acids from citric acid along with considerable lactic acid from lactose. van Beynum and Pette (19) isolated and described two species of aroma-producing streptococci. One of these (*Streptococcus citrophilus*) produces essentially the same compounds as *S. diacetilactis*, while the other (*Streptococcus aromaticus*) produces diacetyl, acetylmethylcarbinol and some lactic acid from carbohydrate. An unidentified, citric acid-fermenting *Streptococcus* (organism H28), isolated from swiss cheese at the Iowa Agricultural Experiment Station, produces diacetyl, acetylmethylcarbinol and volatile acids from citric acid and some lactic acid from lactose.

Difficulties often are encountered in maintaining the proper balance between the two types of organisms in a butter culture, and then the culture may lack flavor. An organism which combines the characteristics of the two types of organisms in the usual butter cultures should be less variable from day to day than the mixed cultures so that certain of the aroma-producing streptococci are of interest from the standpoint of butter manufacture.

<sup>1</sup> Projects 120 and 127 of the Iowa Agricultural Experiment Station.

Preliminary comparisons of pure cultures of various streptococci and the usual butter cultures for the development of flavor in butter are reported herein. Because of the number of analyses to be made for each churning and the number of churnings in each series, the work was done in the laboratory, using a hand churn, rather than in the butter plant. The data deal with: 1. diacetyl and acetylmethylcarbinol in milk cultures of the various organisms, 2. diacetyl and acetylmethylcarbinol in cream, 3. diacetyl and acetylmethylcarbinol in cream plus culture immediately and after holding, 4. distribution of diacetyl and acetylmethylcarbinol between butter and buttermilk, 5. changes in diacetyl and acetylmethylcarbinol in butter during holding and 6. whether there is a correlation between amounts of diacetyl and acetylmethylcarbinol present and the desirable flavor of butter.

## METHODS

### SOURCES OF CULTURES

Pure cultures of the various streptococci were obtained from the laboratories making the original isolations, and the butter cultures were from the collection maintained at the Iowa Agricultural Experiment Station.

### PREPARATION OF CULTURES FOR BUTTER

For each culture, 1 liter of whole milk was pasteurized in a 2-liter erlenmeyer flask by heating in a water bath to 180°F. for 30 minutes, cooling to 72°F. and inoculating. By means of the following procedures, relatively large amounts of diacetyl and acetylmethylcarbinol were produced in the various cultures; when citric acid was used, it was added to the milk as a concentrated aqueous solution.

*Butter culture:* 0.15 percent citric acid was added to the milk at the time of inoculation; the cultures were incubated at 72°F. for 24 hours and then held at about 40°F. for an additional 24 hours.

*S. citrovorus* or *paracitrovorus:* After the cultures had been incubated at 72°F. for 40 hours, 0.9 percent citric acid was added and the cultures incubated at 72°F. for an additional 48 hours (11).

*S. diacetylactis* or *S. citrophilus:* At the time of inoculating the milk, 0.15 percent citric acid was added and the cultures incubated at 72°F. for 24 to 40 hours.

*Organism H28:* After the cultures were incubated at 72°F. for 30 hours, 0.30 to 0.35 percent citric acid was added and the cultures incubated at 72°F. for an additional 48 hours.

*S. aromaticus*: The cultures were incubated at 72°F. for 48 hours without addition of citric acid.

#### PREPARATION OF CREAM

The cream was from vats of sweet cream prepared for commercial churnings. It had been pasteurized at 150°F. for 30 minutes in a 300-gallon vat and neutralized with sodium sesqui-carbonate to approximately 0.10-percent acid, the neutralizer being added after the cream had been cooled to 120°F. When the cream reached approximately 36° to 40°F., some of it was transferred to a thoroughly steamed can from which 2.5-liter portions were placed in sterile 6-liter erlenmeyer flasks. One of the milk cultures, equivalent to 10 percent of the volume of cream, was added to each flask and the different flasks held at approximately 40°F. for 16 hours before churning.

#### CHURNING OF CREAM

One-gallon, glass dazey churns were sterilized by holding a strong sodium hypochlorite solution in them for 20 hours. The churns were rinsed with cold sterile water before use. The cream was churned at a low enough temperature so that the butter granules were firm.

Each churning of butter was washed in a sterile mixing bowl, using three portions of cold sterile water of approximately 250 ml. each. The butter was worked with a sterile wooden paddle. After the addition of salt, the amount of which varied somewhat from one comparison to another but was constant for the churnings in a comparison, the butter was worked until it appeared dry. An effort was made to work the different churnings approximately the same.

#### HOLDING OF BUTTER

Each churning of butter was divided into five portions of approximately 125 gm. each and placed in sterile glass jars. Chemical determinations were made immediately on one portion, and the others were held at 40°F. for 1 day; two portions from each churning then were placed at 35°F. and two at 0°F.

#### ANALYTICAL PROCEDURES

Diacetyl was determined colorimetrically, using essentially the method of Prill and Hammer (15). A spiral glass condenser was employed in place of the one described in the original method, the 500-ml. distillation flask was not placed at an angle, and the gas above the sample was not displaced with steam before heating. The colors were read



with a colorimeter and the results expressed as parts per million (p.p.m.).

Acetylmethylcarbinol plus diacetyl also was determined colorimetrically. The milk cultures were distilled by the method described by Michaelian and Hammer (9). Small aliquots of the distillates were taken, 0.5 ml. of hydroxylamine acetate solution (solution A (15)) added, the volume brought to 6 ml. with distilled water and the usual colorimetric method for diacetyl then followed. For the acetylmethylcarbinol plus diacetyl determinations on cream and culture mixtures, buttermilk and butter, 15 ml. of a 40-percent ferric chloride solution was added to a sample of appropriate size. The material was refluxed for 10 minutes after which the water was drained from the reflux condenser and approximately 5 ml. of distillate collected in 1 ml. of hydroxylamine acetate solution by slowly steam distilling for 10 to 15 minutes. The distillates were treated in the same manner as the distillates in diacetyl determinations.

Since recovery of acetylmethylcarbinol by oxidation with ferric chloride to diacetyl is not complete and diacetyl makes up such a small percentage of the total acetylmethylcarbinol plus diacetyl, the results obtained in acetylmethylcarbinol plus diacetyl determinations are considered to represent acetylmethylcarbinol.

pH determinations were made electrometrically, using a quinhydrone electrode, and the results recorded to the nearest 0.1 pH unit.

## EXPERIMENTAL

Six series of churnings were made. Table 1 gives all the original data except those on pH values of the cultures which are given in table 2, together with the ratios of diacetyl to acetylmethylcarbinol in the cultures.

### DIACETYL AND ACETYLMETHYLCARBINOL IN MILK CULTURES OF THE VARIOUS ORGANISMS

The milk cultures of the various organisms were examined for aroma and analyzed immediately before adding them to the cream; aromas rather than flavors were considered because of the quantities of citric acid added with certain cultures.

*Butter culture:* The cultures had typical, mild, pleasing aromas suggestive of diacetyl. Contents of diacetyl ranged from 0.77 to 1.20 p.p.m. and of acetylmethylcarbinol from 170.0 to 266.0 p.p.m.; ratios of diacetyl to acetylmethylcarbinol varied from 1:185 to 1:234. pH values of the cultures were 4.4 and 4.3.

*S. citrovorus* or *S. paracitrovorus*: The aromas closely resembled those of butter cultures. Diacetyl and acetylmethylcarbinol contents ranged from 4.48 to 11.84 p.p.m. and from 90.8 to 1110.0 p.p.m., respectively; the variations seemed to depend on the strain of organism used and the pH to which the culture was adjusted by addition of citric acid. Ratios of diacetyl to acetylmethylcarbinol ranged from 1:8 to 1:200 and pH values of the cultures from 4.5 to 3.2.

*S. diacetilactis*: The aromas resembled those of green butter cultures and, while not disagreeable, lacked the suggestion of diacetyl that is found in good butter cultures. Although the aromas did not suggest diacetyl, amounts in the cultures ranged from 0.16 to 1.65 p.p.m. Carbinol contents differed widely, ranging from 456.0 to 493.0 p.p.m. in three cultures and from 10.9 to 31.3 p.p.m. in the other three. Ratios of diacetyl to acetylmethylcarbinol varied from 1:50 to 1:421 and pH values from 4.6 to 4.2.

*S. citrophilus*: The aromas closely resembled those of cultures of *S. diacetilactis*. Contents of diacetyl varied from 0.76 to 1.27 p.p.m. and of carbinol from 104.5 to 526.0 p.p.m.; ratios of diacetyl to acetylmethylcarbinol ranged from 1:138 to 1:550. pH values were 4.6 or 4.5.

*Organism H28*: The odor of diacetyl was conspicuous in the cultures, but the aromas were not the same as those of butter cultures. Diacetyl contents varied from 2.16 to 10.60 p.p.m., which is higher than with butter cultures, and acetylmethylcarbinol values varied from 56.8 to 142.5 p.p.m., which is somewhat lower than with butter cultures. Ratios of diacetyl to carbinol ranged from 1:13 to 1:53 and pH values of the cultures from 5.3 to 4.5.

*S. aromaticus*: The aromas (only three cultures were studied) were not especially pleasing and did not resemble the aromas of butter cultures, although diacetyl contents were approximately the same as those of butter cultures. Diacetyl varied from 0.78 to 1.54 p.p.m. and acetylmethylcarbinol from 12.9 to 23.9 p.p.m.; the latter values were much lower than with butter cultures. Ratios of diacetyl to carbinol ranged from 1:8 to 1:28 and pH values of the cultures from 5.3 to 5.0.

#### DIACETYL AND ACETYLMETHYLCARBINOL IN CREAM TO WHICH NO CULTURE WAS ADDED

Diacetyl and acetylmethylcarbinol contents of the cream immediately after neutralization and pasteurization varied from 0.10 to 0.23 p.p.m. and from 5.2 to 14.5 p.p.m., respectively. After holding 16 hours at about 40°F., the diacetyl contents ranged from 0.10 to 0.29 p.p.m. and the carbinol contents from 4.9 to 14.0 p.p.m.

TABLE 1. DIACETYL (AC<sub>2</sub>) AND ACETYLMETHYL CARBINOL (AMC) CONTENTS OF CREAM, MILK CULTURE, CREAM PLUS CULTURE, BUTTERMILK AND BUTTER; ALSO PLACINGS OF THE BUTTER ON BASIS OF FLAVOR.

Material analyzed	Series 1			Series 2			Series 3			Series 4			Series 5			Series 6		
	Ac <sub>2</sub> p.p.m.	Amc p.p.m.	Plac- ing	Ac <sub>2</sub> p.p.m.	Amc p.p.m.	Plac- ing	Ac <sub>2</sub> p.p.m.	Amc p.p.m.	Plac- ing	Ac <sub>2</sub> p.p.m.	Amc p.p.m.	Plac- ing	Ac <sub>2</sub> p.p.m.	Amc p.p.m.	Plac- ing	Ac <sub>2</sub> p.p.m.	Amc p.p.m.	Plac- ing
No culture																		
Raw cream				0.07	5.1		0.07	4.8		0.08	7.5		0.08	6.4				
Pasteurized cream																		
(immediately)	0.23	14.5		0.13	6.2		0.10	6.0		0.11	7.8		0.14	6.1		0.10	5.2	
(16 hrs.)	0.29	14.0		0.11	6.7		0.10	5.6		0.20	7.9		0.13	6.2		0.13	4.9	
Buttermilk	0.23	12.4		0.15	9.2		0.10	7.3		0.19	13.5		0.11	9.2		0.15	7.8	
Butter																		
(immediately)	0.08	2.3	5	0.06	1.2	7	trace	0.9	6	0.07	2.1	6	0.07	1.6	6	trace	1.2	6
(2 wks. @ 35° F.)	0.07	2.1	6	trace	1.9	6	trace	0.8	6	trace	1.7	6	0.09	1.7	6	trace	1.4	6
(4 wks. @ 35° F.)	0.08	2.1	7	trace	0.9	7	trace	0.5	5	trace	1.6	4	trace	1.5	6	trace	1.2	6
(2 wks. @ 0° F.)	0.08	2.9	6	trace	1.4	7	trace	0.7	6	trace	1.7	6	0.08	1.5	6	trace	1.3	6
(4 wks. @ 0° F.)	trace	2.2	7	trace	1.0	6	trace	0.9	6	trace	1.6	6	trace	1.4	6	trace	1.4	5
Butter culture																		
Milk culture	0.82	192.0		0.99			0.77	178.5		1.04	217.0		0.92	170.0		1.20	266.0	
Cream + culture																		
(immediately)	0.33	32.5		0.24	16.8		0.15	19.9		0.26	39.5		0.18	21.9		0.29	29.6	
(16 hrs.)	0.83	36.2		0.66	22.2		0.66	31.8		1.12	46.3		0.82	30.8		0.56	48.1	
Buttermilk	1.32	69.5		0.83	29.1		1.75	49.4		1.92	103.0		1.04	56.6		0.91	66.6	
Butter																		
(immediately)	0.49	10.0	1	0.26	3.7	3	0.34	5.8	1	0.69	15.5	4	0.30	6.6	5	0.40	12.0	1
(2 wks. @ 35° F.)	0.92	10.4	2	0.48	2.3	1	0.31	5.4	3	0.64	13.1	4	0.39	7.0	4	0.35	12.6	4
(4 wks. @ 35° F.)	1.08	9.1	4	0.22	1.9	4	0.36	4.6	3	0.68	14.0	3	0.37	6.4	1	0.37	13.2	3
(2 wks. @ 0° F.)	0.59	9.1	1	0.54	3.4	1	0.34	5.4	1	0.63	13.4	4	0.34	6.8	1	0.34	12.1	3
(4 wks. @ 0° F.)	0.59	9.1	3	0.27	3.9	1	0.38	5.7	1	0.64	13.1	3	0.40	6.8	2	0.36	12.1	4
<i>S. citrovorus</i> or																		
<i>S. paracitrovorus</i>																		
Milk culture	11.70	446.0		11.84	97.5					4.48	90.8		11.30	555.0		5.55	1110.0	
Cream + culture																		
(immediately)	1.35	67.7		1.15	12.9					1.24	15.8		1.60	75.0		0.64	125.0	
(16 hrs.)	0.98	52.6		0.94	14.2					0.62	15.7		1.79	74.0		0.56	131.0	
Buttermilk	1.44	68.5		1.13	17.0					0.57	30.1		1.46	100.0		0.67	175.0	
Butter																		
(immediately)	0.54	9.1	3	0.25	3.8	1				0.18	3.6	1	0.52	17.3	1	0.17	20.0	2
(2 wks. @ 35° F.)	0.40	9.4	1	0.36	1.9	4				0.16	3.2	3	0.46	14.7	2	0.20	19.5	2
(4 wks. @ 35° F.)	0.46	9.7	1	0.18	1.3	3				0.17	2.9	5	0.41	14.8	3	0.21	19.5	4
(2 wks. @ 0° F.)	0.40	12.4	2	0.42	2.3	4				0.18	3.3	1	0.46	15.4	2	0.21	20.5	1
(4 wks. @ 0° F.)	0.48	9.8	2	0.24	2.0	3				0.18	2.9	5	0.42	15.6	3	0.16	19.6	3
<i>S. diacetylactis</i>																		
Milk culture	1.20	485.0		1.65	456.0		0.25	21.6		1.17	493.0		0.16	10.9		0.62	31.3	
Cream + culture																		
(immediately)	0.17	72.6		0.64	62.1		0.14	8.0		0.69	60.5		0.14	6.4		0.15	9.5	
(16 hrs.)	0.96	96.0		1.19	108.0		0.28	20.0		1.28	96.8		0.42	22.4		0.40	25.8	

Material analyzed	Series 1			Series 2			Series 3			Series 4			Series 5			Series 6		
	Ac <sub>2</sub> p.p.m.	Amc p.p.m.	Plac- ing	Ac <sub>2</sub> p.p.m.	Amc p.p.m.	Plac- ing	Ac <sub>2</sub> p.p.m.	Amc p.p.m.	Plac- ing	Ac <sub>2</sub> p.p.m.	Amc p.p.m.	Plac- ing	Ac <sub>2</sub> p.p.m.	Amc p.p.m.	Plac- ing	Ac <sub>2</sub> p.p.m.	Amc p.p.m.	Plac- ing
Buttermilk	1.99	157.0		3.64	124.0		0.75	33.7		2.26	171.5		0.79	41.6		0.56	37.6	
Butter																		
(immediately)	0.63	29.6	7	0.69	21.3	2	0.10	3.7	4	1.00	28.4	3	0.23	5.1	4	0.20	5.9	3
(2 wks. @ 35° F.)	0.92	27.0	7	1.32	36.9	7	0.17	3.9	4	1.25	30.3	5	0.26	6.2	3	0.31	6.9	3
(4 wks. @ 35° F.)	1.21	33.8	3	0.30	36.8	1	0.11	3.6	4	1.32	30.9	1	0.27	6.0	2	0.30	7.0	2
(2 wks. @ 0° F.)	0.71	22.8	7	1.29	33.6	3	0.15	3.4	4	1.02	27.2	5	0.22	5.8	5	0.24	6.2	5
(4 wks. @ 0° F.)	0.73	24.7	5	0.93	30.5	7	0.19	3.4	2	0.88	27.4	1	0.23	5.5	4	0.27	6.8	1
<i>S. citrophilus</i>																		
Milk culture	0.88	485.0		0.76	104.5		1.05	526.0		1.27	493.0		1.12	432.0		1.07	509.0	
Cream + culture																		
(immediately)	0.26	69.0		0.26	16.9		0.35	60.2		0.50	61.6		0.34	52.4		0.36	49.1	
(16 hrs.)	0.98	81.6		0.52	54.4		1.11	96.7		1.04	96.8		0.88	84.5		0.67	71.4	
Buttermilk	1.54	138.0		1.00	52.0		1.17	138.0		2.13	174.0		1.28	126.0		0.97	125.0	
Butter																		
(immediately)	0.49	23.4	4	0.29	8.3	4	0.38	18.5	2	0.74	28.6	2	0.46	20.1	2	0.24	16.6	4
(2 wks. @ 35° F.)	0.65	18.9	3	0.47	13.3	2	0.48	15.2	1	0.81	25.6	2	0.47	18.5	1	0.52	19.2	1
(4 wks. @ 35° F.)	0.70	19.5	5		8.6	2	0.41	15.4	2	0.79	26.7	2	0.54	19.1	4	0.59	19.0	1
(2 wks. @ 0° F.)	0.45	16.5	5	0.43	10.5	6	0.46	14.3	3	0.63	26.6	2	0.46	17.7	3	0.41	18.5	2
(4 wks. @ 0° F.)	0.51	18.5	1	0.24	7.1	5	0.46	16.2	4	0.67	24.9	2	0.37	18.8	1	0.54	17.0	2
Organism H28																		
Milk culture	2.76	56.8		10.60	133.8		3.83	142.5		3.36	59.6		4.86	70.0		2.16	114.9	
Cream + culture																		
(immediately)	0.42	20.4		0.74	21.5		0.38	16.7		0.30	14.9		0.41	12.9		0.41	17.4	
(16 hrs.)	0.47	21.7		0.74	22.5		0.58	22.4		0.45	16.4		0.44	12.9		0.44	18.0	
Buttermilk	0.56	36.7		1.29	27.0		0.70	27.3		0.66	22.7		0.62	18.6		0.53	24.5	
Butter																		
(immediately)	0.22	4.1	2	0.27	3.9	6	0.13	3.9	3	0.16	3.8	5	0.22	2.9	3	0.18	3.4	5
(2 wks. @ 35° F.)	0.23	4.0	4	0.40	4.1	3	0.18	3.5	2	0.22	3.6	1	0.22	3.2	5	0.23	3.8	5
(4 wks. @ 35° F.)	0.26	4.7	2	0.26	3.7	6	0.17	3.3	1	0.21	3.5	6	0.25	3.3	5	0.26	3.7	5
(2 wks. @ 0° F.)	0.20	3.9	3	0.45	3.9	2	0.20	3.8	2	0.20	3.4	3	0.22	2.9	4	0.22	3.8	4
(4 wks. @ 0° F.)	0.22	4.3	6	0.26	3.6	2	0.23	3.1	3	0.17	3.4	4	0.24	3.2	5	0.20	3.6	6
<i>S. aromaticus</i>																		
Milk culture	0.78	21.8		1.54	12.9		0.85	23.9										
Cream + culture																		
(immediately)	0.29	23.6		0.18	10.3		0.16	8.9										
(16 hrs.)	0.19	16.9		0.29	15.5		0.24	11.6										
Buttermilk	0.37	30.0		0.31	16.6		0.26	14.0										
Butter																		
(immediately)	0.09	3.0	6	0.06	1.9	5	0.07	1.6	5									
(2 wks. @ 35° F.)	0.16	3.1	5	0.31	2.1	5	0.11	1.6	5									
(4 wks. @ 35° F.)	0.17	3.8	6	0.09	1.9	5	0.11	1.4	6									
(2 wks. @ 0° F.)	0.15	2.6	4	0.17	1.9	5	0.09	1.6	5									
(4 wks. @ 0° F.)	0.16	3.6	4	0.10	2.0	4	0.09	1.6	5									

TABLE 2. RATIOS OF DIACETYL (AC<sub>2</sub>) TO ACETYLMETHYLCARBINOL (AMC) AND THE pH VALUES IN THE VARIOUS MILK CULTURES.

Series	Butter culture		<i>S. citrovorus</i> or <i>S. paracitrovorus</i>		<i>S. diacetylactis</i>		<i>S. citrophilus</i>		Organism H28		<i>S. aromaticus</i>	
	Ratio of ac <sub>2</sub> to amc	pH	Ratio of ac <sub>2</sub> to amc	pH	Ratio of ac <sub>2</sub> to amc	pH	Ratio of ac <sub>2</sub> to amc	pH	Ratio of ac <sub>2</sub> to amc	pH	Ratio of ac <sub>2</sub> to amc	pH
1	1:234	4.4	1:38	4.1	1:404	4.3	1:550	4.5	1:21	4.6	1:28	5.0
2			1:8	3.2	1:277	4.3	1:138	4.5	1:13	4.5	1:8	5.1
3	1:232	4.3			1:88	4.2	1:500	4.5	1:37	4.6	1:28	5.3
4	1:209	4.4	1:20	3.2	1:421	4.6	1:388	4.6	1:18	4.5		
5	1:185	4.4	1:49	3.8	1:69	4.4	1:386	4.5	1:14	4.6		
6	1:222	4.4	1:200	4.5	1:50	4.2	1:475	4.5	1:53	5.3		

## DIACETYL AND ACETYLMETHYLCARBINOL IN CREAM PLUS CULTURE

Diacetyl and acetylmethylcarbinol determinations were made on cream plus culture immediately after mixing and again after holding 16 hours at about 40°F.

## DIACETYL AND ACETYLMETHYLCARBINOL IN CREAM PLUS CULTURE IMMEDIATELY AFTER MIXING

Provided no transformation to or from the compounds occurs, diacetyl and acetylmethylcarbinol contents of the cream plus culture immediately after mixing should consist of the diacetyl and acetylmethylcarbinol present in the original cream plus the amounts added with the culture. The theoretical amounts of diacetyl and acetylmethylcarbinol in the mixtures, as calculated from the amounts contributed by the cream and cultures, and also the determined amounts, are given in table 3.

*Butter culture:* The theoretical amounts of diacetyl ranged from 0.16 to 0.28 p.p.m. and the determined amounts from 0.15 to 0.33 p.p.m.; during mixing small increases occurred in three trials, whereas little change was noted in the others. The theoretical amounts of acetylmethylcarbinol varied from 21.0 to 30.6 p.p.m. and the determined amounts from 19.9 to 39.5 p.p.m.; there was an appreciable gain in one trial during mixing and no significant changes in the others.

*S. citrovorus* or *S. paracitrovorus:* The theoretical amounts of diacetyl varied from 0.51 to 1.27 p.p.m., and the determined amounts varied from 0.64 to 1.60 p.p.m.; increases occurred during mixing in three trials, whereas there was no significant change in the others. The theoretical amounts of acetylmethylcarbinol ranged from 14.5 to 106.0 p.p.m. and the determined amounts from 12.9 to 125.0 p.p.m.; during mixing increases occurred in three trials while no changes were noted in the others.

*S. diacetylactis:* With diacetyl the theoretical and determined amounts ranged from 0.11 to 0.32 p.p.m. and from 0.14 to 0.69 p.p.m., respectively; there were increases during mixing in two trials, a decrease in one trial and little change in the others. With acetylmethylcarbinol the theoretical and determined amounts ranged from 6.5 to 57.2 p.p.m. and from 6.4 to 72.6 p.p.m., respectively; during mixing the carbinol increased in three trials, whereas there was little change in the others.

*S. citrophilus:* The theoretical and determined amounts of diacetyl varied from 0.19 to 0.29 p.p.m. and from 0.26

TABLE 3. THEORETICAL AND DETERMINED AMOUNTS OF DIACETYL AND ACETYL METHYLCARBINOL IN CREAM PLUS CULTURE IMMEDIATELY AFTER MIXING.

Series	Culture used	Diacetyl (p. p. m.)				Acetylmethylcarbinol (p. p. m.)			
		Past. cream	Culture	Cream + culture		Past. cream	Culture	Cream + culture	
				Theoretical	Determined			Theoretical	Determined
1	Butter culture	0.23	0.82	0.28	0.33	14.5	192.0	30.6	32.5
2		0.13	0.99	0.21	0.24				
3		0.10	0.77	0.16	0.15	6.0	178.5	21.7	19.9
4		0.11	1.04	0.20	0.26	7.8	217.0	26.8	39.5
5		0.14	0.92	0.21	0.18	6.1	170.0	21.0	21.9
6		0.10	1.20	0.20	0.29	5.2	266.0	28.9	29.6
1	<i>S. citrovorus</i> or <i>S. paracitrovorus</i>	0.23	11.70	1.27	1.36	14.5	446.0	53.7	67.7
2		0.13	11.84	1.19	1.16	6.2	97.5	14.5	12.9
3									
4		0.11	4.48	0.51	1.24	7.8	90.8	15.3	15.8
5		0.14	11.30	1.15	1.60	6.1	565.0	56.9	75.0
6		0.10	5.56	0.60	0.64	5.2	1110.0	106.0	125.0
1	<i>S. diacetylactis</i>	0.23	1.20	0.32	0.17	14.5	485.0	57.2	72.6
2		0.13	1.65	0.27	0.64	6.2	456.0	47.1	62.1
3		0.10	0.25	0.11	0.14	6.0	21.6	7.4	8.0
4		0.11	1.17	0.21	0.69	7.8	493.0	51.9	60.5
5		0.14	0.16	0.14	0.14	6.1	10.9	6.5	6.4
6		0.10	0.62	0.15	0.15	5.2	31.3	7.6	9.5
1	<i>S. citrophilus</i>	0.23	0.88	0.29	0.26	14.5	485.0	57.2	69.0
2		0.13	0.76	0.19	0.26	6.2	104.5	15.1	16.9
3		0.10	1.05	0.19	0.35	6.0	526.0	53.3	60.2
4		0.11	1.27	0.22	0.50	7.8	493.0	51.9	61.6
5		0.14	1.12	0.23	0.34	6.1	432.0	45.0	52.4
6		0.10	1.07	0.19	0.36	5.2	509.0	51.0	49.1
1	Organism H28	0.23	2.76	0.46	0.42	14.5	56.8	18.3	20.4
2		0.13	10.60	1.08	0.76	6.2	133.8	17.8	21.5
3		0.10	3.83	0.44	0.38	6.0	142.5	18.4	16.7
4		0.11	3.36	0.41	0.30	7.8	59.6	12.5	14.9
5		0.14	4.86	0.57	0.41	6.1	70.0	11.9	12.9
6		0.10	2.16	0.29	0.41	5.2	114.9	15.1	17.4
1	<i>S. aromaticus</i>	0.23	0.78	0.28	0.29	14.5	21.8	15.1	23.6
2		0.13	1.54	0.26	0.18	6.2	12.9	6.8	10.3
3		0.10	0.85	0.17	0.16	6.0	23.9	7.6	8.9

to 0.50 p.p.m., respectively; during mixing increases occurred in five trials with no appreciable change in the other. The theoretical and determined amounts of acetylmethylcarbinol ranged from 15.1 to 57.2 p.p.m. and from 16.9 to 69.0 p.p.m., respectively; the carbinol increased during mixing in four trials, but there was little change in the others.

*Organism H28:* With diacetyl the theoretical amounts ranged from 0.29 to 1.08 p.p.m. and the determined amounts from 0.30 to 0.76 p.p.m.; during mixing there was an increase in one trial, a decrease in four trials and little change in the other. With acetylmethylcarbinol the theoretical amounts varied from 11.9 to 18.4 p.p.m. and the determined amounts from 12.9 to 21.5 p.p.m.; no significant changes in acetylmethylcarbinol contents occurred during mixing.

*S. aromaticus:* The theoretical and determined amounts of diacetyl ranged from 0.17 to 0.28 p.p.m. and from 0.16 to 0.29 p.p.m., respectively; during mixing there was a decrease in one trial and little change in the others. The theoretical and determined amounts of acetylmethylcarbinol varied from 6.8 to 15.1 p.p.m. and from 8.9 to 23.6 p.p.m., respectively; small increases in carbinol regularly occurred during mixing.

DIACETYL AND ACETYLMETHYLCARBINOL IN CREAM PLUS  
CULTURE AFTER 16 HOURS HOLDING

*Butter culture:* Increases in diacetyl during holding of the cream ranged from 0.27 to 0.86 p.p.m., while increases in carbinol ranged from 3.7 to 18.5 p.p.m. Usually, increases in diacetyl were proportionately larger than in acetylmethylcarbinol.

*S. citrovorus* or *S. paracitrovorus:* Changes in diacetyl contents during holding of the cream varied from a decrease of 0.62 p.p.m. to an increase of 0.17 p.p.m., while the changes in carbinol varied from a decrease of 15.1 p.p.m. to an increase of 6.0 p.p.m. Diacetyl usually decreased proportionately more than carbinol.

*S. diacetilactis:* Increases in diacetyl during holding of the cream ranged from 0.13 to 0.79 p.p.m. and were approximately the same as for the mixtures containing butter cultures. However, the increases in acetylmethylcarbinol ranged from 12.1 to 45.9 p.p.m. and were larger than the increases with butter cultures.

*S. citrophilus:* Increases in diacetyl and in acetylmethylcarbinol during holding of the cream ranged from 0.26 to 0.76 p.p.m. and from 12.6 to 37.5 p.p.m., respectively, and closely resembled the increases obtained with *S. diacetilactis* cultures.



*Organism H28:* Diacetyl and acetylmethylcarbinol contents showed little change during holding of the cream, the increases varying from 0 to 0.20 p.p.m. and from 0 to 5.7 p.p.m., respectively.

*S. aromaticus:* Changes in diacetyl during holding of the cream varied from a decrease of 0.10 p.p.m. to an increase of 0.11 p.p.m., while the changes in carbinol varied from a decrease of 6.7 p.p.m. to an increase of 5.2 p.p.m.

GENERAL FLAVOR OF BUTTER AND DIACETYL AND ACETYLMETHYLCARBINOL IN BUTTER AND IN BUTTERMILK

The butter was examined for flavor after holding at 40°F. for 1 day. Diacetyl and acetylmethylcarbinol were determined on the butter and the buttermilk as soon as the churnings were complete.

*Control butter:* Butter made from sweet cream to which no culture was added lacked the mild, pleasing aroma which is characteristic of butter made with butter culture. The butter contained relatively little diacetyl and acetylmethylcarbinol, the amounts ranging from a trace to 0.08 p.p.m. and from 0.9 to 2.3 p.p.m., respectively. With the buttermilk diacetyl contents varied from 0.10 to 0.23 p.p.m. and acetylmethylcarbinol contents from 7.3 to 13.5 p.p.m.

*Butter culture:* The flavor of the butter was full, mild and pleasing and suggestive of diacetyl. Diacetyl and acetylmethylcarbinol contents of the butter ranged from 0.26 to 0.69 p.p.m. and from 3.7 to 15.5 p.p.m., respectively. In the buttermilk diacetyl contents varied from 0.83 to 1.92 p.p.m. and carbinol contents from 29.1 to 103.0 p.p.m.

*S. citrovorus* or *S. paracitrovorus:* The flavor was very similar to that of butter made with butter culture, although possibly slightly coarse. Diacetyl and acetylmethylcarbinol contents of the butter ranged from 0.17 to 0.54 p.p.m. and from 3.6 to 20.0 p.p.m., respectively. The buttermilk had diacetyl contents from 0.57 to 1.46 p.p.m. and carbinol contents from 17.0 to 175.0 p.p.m.

*S. diacetilactis:* The flavor of the butter varied considerably, one churning definitely suggesting fishiness (series 1) while the flavor of some of the others was criticized as being coarse and sour. Diacetyl and acetylmethylcarbinol contents of the butter varied widely and ranged from 0.10 to 1.00 p.p.m. and from 3.7 to 29.6 p.p.m., respectively. With the buttermilk diacetyl and acetylmethylcarbinol contents ranged from 0.56 to 3.64 p.p.m. and from 33.7 to 171.5 p.p.m., respectively.

*S. citrophilus:* The flavor was very much like that of butter made with butter culture. Diacetyl contents of the

butter varied from 0.24 to 0.74 p.p.m. and acetylmethylcarbinol contents from 8.3 to 28.6 p.p.m. The contents of the buttermilk ranged from 0.97 to 2.13 p.p.m. and from 52.0 to 174.0 p.p.m., respectively.

*Organism H28:* The flavor of the butter often was criticized as flat, although some samples were considered good. Diacetyl and acetylmethylcarbinol contents of the butter ranged from 0.13 to 0.27 p.p.m. and from 2.9 to 4.1 p.p.m., respectively. With the buttermilk diacetyl contents varied from 0.53 to 1.29 p.p.m. and carbinol contents from 18.6 to 36.7 p.p.m.

*S. aromaticus:* The butter lacked flavor, and little or no differences in flavor could be detected between butter made with this organism and butter made without culture. Diacetyl and acetylmethylcarbinol contents of the butter ranged from 0.06 to 0.09 p.p.m. and from 1.6 to 3.0 p.p.m., respectively, while for the buttermilk the values varied from 0.26 to 0.37 p.p.m. and from 14.0 to 30.0 p.p.m., respectively.

The analyses show that the butter contained less of the diacetyl and acetylmethylcarbinol present in the cream plus culture than the buttermilk. In computing the actual percentages of diacetyl and acetylmethylcarbinol retained in the butter, the cream was considered to contain 30 percent fat and the butter 80 percent fat. Since the amounts of diacetyl and acetylmethylcarbinol change during the churning (17), the quantities present in the mixture just prior to draining the buttermilk were calculated from the diacetyl and acetylmethylcarbinol in the butter and the buttermilk. The percentages of diacetyl and acetylmethylcarbinol present in the cream plus culture which were retained in the butter ranged from 7.4 to 38.7 percent for diacetyl and from 5.4 to 11.8 percent for acetylmethylcarbinol. There were no significant differences in the percentages of diacetyl or acetylmethylcarbinol retained in the butter made with the various organisms.

#### CHANGES IN DIACETYL AND ACETYLMETHYLCARBINOL IN BUTTER DURING HOLDING

Diacetyl and acetylmethylcarbinol were determined on the butter after holding at 35° and 0°F. for 2 weeks and 4 weeks; as already noted, the butter was held at 40°F. for 1 day before being placed at 35° or 0°F. during which time changes in diacetyl and acetylmethylcarbinol may have occurred.

*Control butter:* Diacetyl and acetylmethylcarbinol contents at once and also after holding were very low so that no definite changes were detected.

*Butter culture:* With one churning there was an in-

crease in diacetyl after 2 weeks at either 35° or 0°F., while after 4 weeks the diacetyl content was about the same as after 2 weeks; at 35°F. the increase was greater than at 0°F. With another churning the diacetyl content showed an increase after 2 weeks at either temperature followed by a decrease after 4 weeks. With still another churning there was an increase in diacetyl after 2 weeks at 35°F., with no further change, and an increase after 4 weeks at 0°F. For the remaining churnings no differences were noted in diacetyl contents at once and after holding 2 and 4 weeks at either temperature. Acetylmethylcarbinol contents showed little or no change during holding.

*S. citrovorus* or *S. paracitrovorus*: Two churnings showed decreases in diacetyl during holding at either 35° or 0°F.; with two churnings there were practically no differences between diacetyl contents at once and after holding at either temperature; while for another churning there was an increase in diacetyl after 2 weeks followed by a decrease after 4 weeks at either temperature. There was little or no change in the acetylmethylcarbinol.

*S. diacetylactis*: With three churnings increases in diacetyl occurred after 2 and 4 weeks at 35°F., while at 0°F. there was an increase in diacetyl with two churnings and a decrease in the other; the largest increases occurred at 35°F. In one churning diacetyl increased greatly after 2 weeks at 35° or 0°F. (from an original of 0.69 p.p.m. to 1.82 p.p.m. at 35°F. and to 1.29 p.p.m. at 0°F.); these increases were followed by large decreases after 4 weeks, the larger decrease occurring in butter held at 35°F. With two churnings there was no definite difference between the diacetyl contents at once and after holding. Acetylmethylcarbinol increased appreciably during holding with one churning, the increase being larger at 35° than 0°F.; with the remaining churnings there was no definite change in acetylmethylcarbinol.

*S. citrophilus*: With one churning there was an increase in diacetyl after 2 and 4 weeks at either 35° or 0°F. Two churnings showed small increases after 2 weeks at either temperature; after 4 weeks one of these churnings showed a decrease in diacetyl at 0°F., and the other showed no further change at either temperature. With still another churning the diacetyl increased after 2 and 4 weeks at 35°F. while no change occurred at 0°F. For the remaining churnings no significant change in diacetyl occurred. Acetylmethylcarbinol contents showed little or no change.

*Organism H28*: With one churning a significant increase

in diacetyl occurred after 2 weeks at either 35° or 0°F., while after an additional 2 weeks there was a decrease; for the remaining churnings only small increases or no changes occurred at either temperature. There were no definite changes in acetylmethylcarbinol.

*S. aromaticus*: One churning showed an increase in diacetyl after 2 weeks at either 35° or 0°F.; this increase was followed by a decrease after an additional 2 weeks. With the other two churnings there were no definite changes in diacetyl. Acetylmethylcarbinol was approximately the same after holding as in the original butter.

#### RELATIONSHIP OF DIACETYL AND ACETYLMETHYLCARBINOL CONTENTS TO FLAVOR OF THE BUTTER

The samples of butter were placed on the basis of flavor after 1 day and also when they were analyzed for diacetyl and acetylmethylcarbinol after 2 and 4 weeks. The difference between two samples often was very slight so that a sample placed just ahead of another may have differed from it very little. Changes in the placing of the various churnings in a series may have been influenced both by deterioration in some churnings and improvement in flavor in others. Figures 1 and 2 show the diacetyl contents and placings of the butter after 1 day and again after holding for 2 and 4 weeks at 0° and 35°F.

Butter containing a relatively large amount of diacetyl usually was considered to have a desirable flavor and given a high placing, the flavor being full, mild, pleasing and definitely suggestive of diacetyl, while butter containing only a small amount of diacetyl was flat and lacking in flavor and commonly was given a low placing. In a few instances samples of butter containing exceptionally large amounts of diacetyl also were given low placings. Butter made with butter culture, a culture of *S. citrovorus* or *S. paracitrovorus* or a culture of *S. citrophilus* usually contained relatively large amounts of diacetyl and commonly was given a high placing.

The four samples placing highest in each series usually had a satisfactory flavor and would be classed as good butter. Among them there was no close correlation between diacetyl content and placing. In some cases a sample containing one third to one half as much diacetyl as another sample was given a higher placing. This suggests that compounds other than diacetyl may be important in accounting for the desirable flavor of butter made with culture.

Butter made without culture (control) contained very small amounts of diacetyl; it was flat and lacked the char-

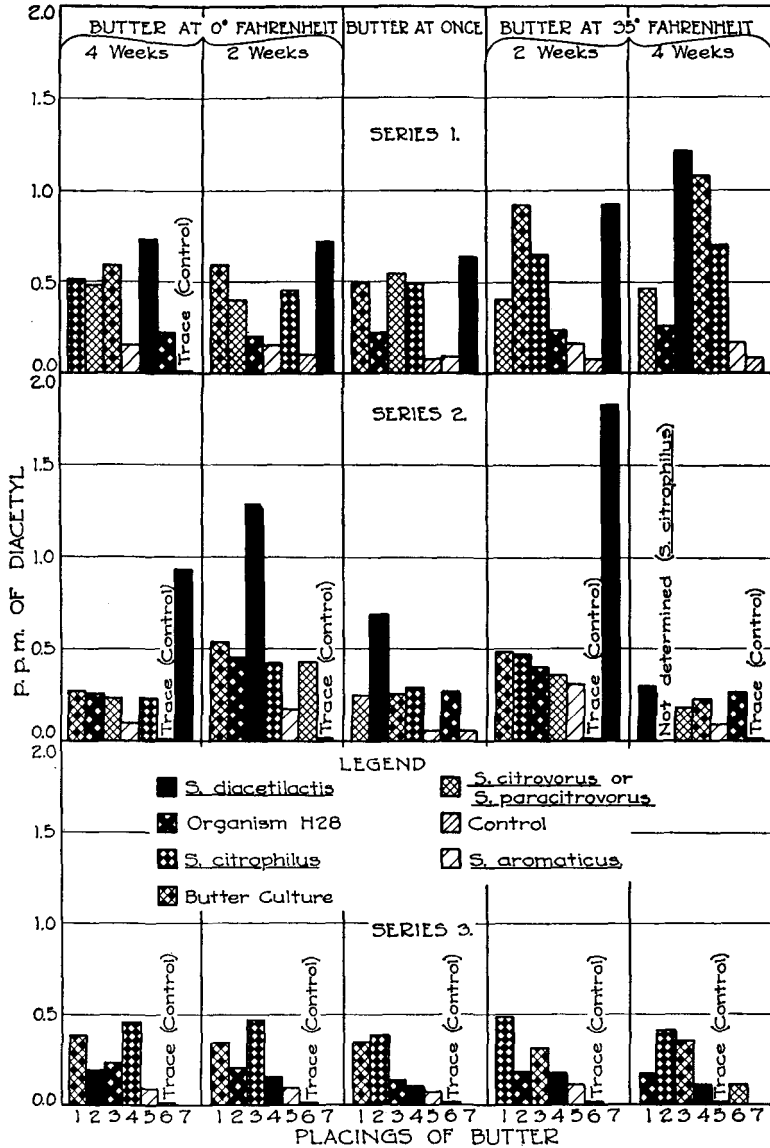


Fig. 1. Diacetyl contents and placings of butter at once and after holding 2 and 4 weeks at 0° and 35° F.; (series 1, 2 and 3).

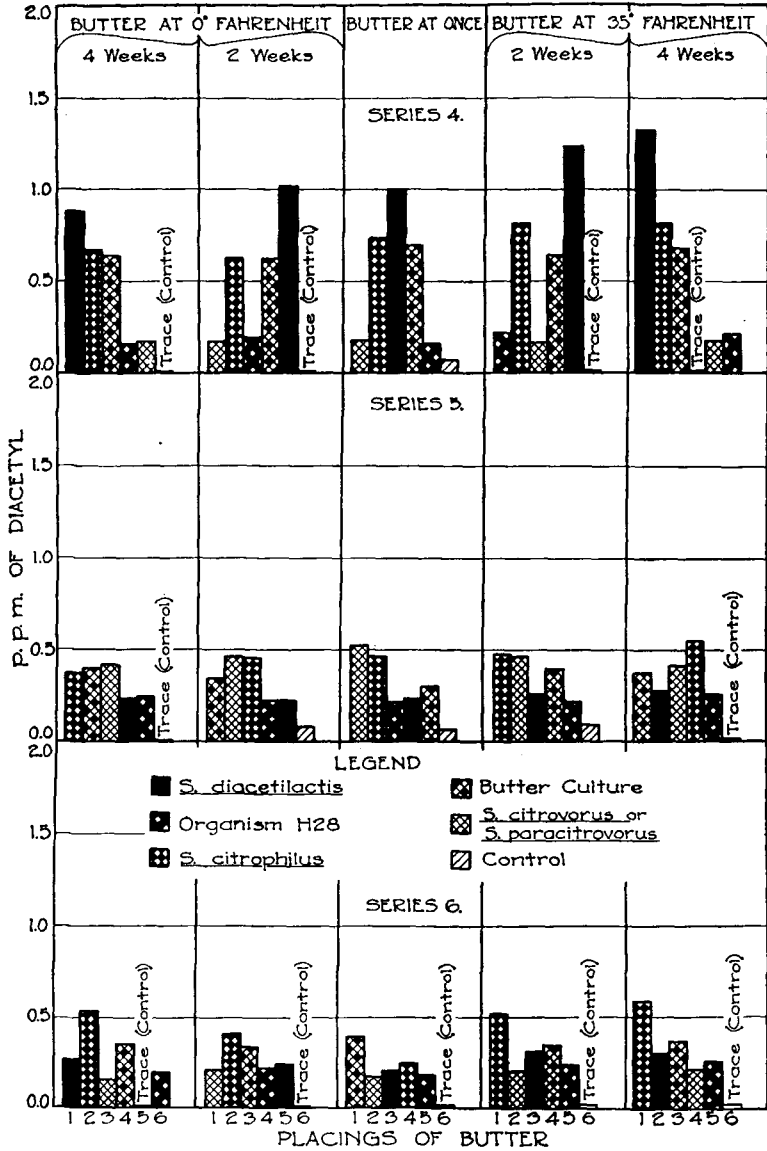


Fig. 2. Diacetyl contents and placings of butter at once and after holding 2 and 4 weeks at 0° and 35° F.; (series 4, 5 and 6).

acteristic pleasing flavor of good butter. Usually it placed last in the series. Butter made with *S. aromaticus* also contained only small amounts of diacetyl and often was placed just ahead of the control.

Butter containing an exceptionally large amount of diacetyl often was given a low placing and criticized as being coarse, sour, oily or containing some other objectionable flavor.

In series 1, butter made with *S. diacetilactis* contained large amounts of diacetyl and commonly was sour and fishy. Butter made with butter culture originally contained 0.49 p.p.m. diacetyl and after 1 day was very desirable in flavor, but after 4 weeks at 35°F. the diacetyl had increased to 1.08 p.p.m., and the butter was coarse and sour. Butter made with *S. citrophilus* originally contained 0.49 p.p.m. diacetyl and after 1 day had a satisfactory flavor, but after 4 weeks at 35°F. the diacetyl content had increased to 0.70 p.p.m., and the flavor was oily and sour.

In series 2, butter made with *S. diacetilactis* originally contained 0.69 p.p.m. diacetyl, and after 1 day the flavor was good. After 2 weeks at 35° or 0°F. the diacetyl contents were 1.82 and 1.29 p.p.m., respectively, and the butter was sour. Upon further holding at 35°F. the diacetyl content decreased to 0.30 p.p.m., and the flavor was desirable, while butter held for 4 weeks at 0°F. showed only a small decrease in diacetyl, and the flavor was sour.

Again in series 4, butter made with *S. diacetilactis* contained large amounts of diacetyl and was sour and oily, except after holding for 4 weeks at either 35° or 0°F. when the butter was considered to be very desirable, although the diacetyl contents had not changed appreciably. Butter made with butter culture contained considerably less diacetyl but also was criticized as coarse and sour.

Although butter that contained large amounts of diacetyl also usually contained large amounts of carbinol, the correlation between the acetylmethylcarbinol contents and placings of the butter was less close than that between the diacetyl contents and the placings.

Butter made with butter culture, a culture of *S. citrovorus* or *S. paracitrovorus* or a culture of *S. citrophilus* usually contained a relatively large amount of acetylmethylcarbinol and commonly was given a high placing. Butter made with *S. diacetilactis* also usually contained a large amount of acetylmethylcarbinol but often was placed low because of off flavors present. Butter made without culture (control) and butter made with *S. aromaticus* contained small amounts of acetylmethylcarbinol and were given low placings.

## DISCUSSION

The production of diacetyl and acetylmethylcarbinol in appreciable amounts by pure cultures of streptococci suggests that such cultures may be useful in the manufacture of butter. This also is suggested by the flavor development obtained in butter with some of the cultures. Since cultures of *S. citrovorus*, *S. paracitrovorus*, organism H28 and *S. aromaticus* do not produce acid rapidly enough to quickly limit growth of contaminating organisms, satisfactory propagation of these species in pasteurized milk may be difficult. However, no unusual difficulties should be encountered with *S. diacetylactis* and *S. citrophilus*, although temperatures other than those commonly used for butter cultures may be advisable. Further comparisons of butter cultures and cultures of *S. diacetylactis*, *S. citrophilus* and perhaps other species for flavor development in butter should be made, using commercial churnings. Matuszewski *et al.* (8) suggested the use of *S. diacetylactis* in butter manufacture.

The higher concentration of diacetyl in proportion to the carbinol with some of the cultures than with butter cultures may prove to be of significance. However, the proportion between the two compounds is delicately balanced and so influenced by the oxygen in the cultures, pH and presumably other factors (3, 10, 16, 20, 21) that these may be of more importance than the particular organism used.

The variations in the determined and calculated diacetyl contents of cream plus culture immediately after mixing probably were related to the delicate balance between diacetyl and acetylmethylcarbinol and perhaps also the balance between acetylmethylcarbinol and 2,3-butylene glycol. Prill and Hammer (17) noted an increase in diacetyl and little or no change in acetylmethylcarbinol during mixing of cream and butter culture. During the holding of the mixtures the increases in diacetyl and acetylmethylcarbinol undoubtedly were influenced by the oxygen incorporated by the mixing. Virtanen (21) found that increasing the oxygen during ripening of butter cultures or pure cultures of betacocci greatly stimulated the production of acetylmethylcarbinol. Fabricius and Hammer (5), as well as other investigators (6, 13, 17), reported increases in diacetyl and acetylmethylcarbinol during holding of cream plus culture before churning.

Cultures containing relatively large amounts of diacetyl and acetylmethylcarbinol tended to produce butter that was relatively high in these compounds, but the correlation was not close. The relationships between diacetyl and acetylmethylcarbinol contents of the culture and the butter made



with it are influenced by various factors, such as the method of holding the cream plus culture and the churning procedure (5). Barnicoat (1) concluded that most of the flavor-producing substances in butter made from slightly ripened cream are not developed during the ripening process but are added pre-formed with the culture and that the final concentration of acetylmethylcarbinol plus diacetyl in butter is dependent on the amount of buttermilk retained. Wiley, Cox and Whitehead (22) found "that starters which were lacking in aroma imparted as much 'brightness' to the butter as did starters which possessed a high aroma."

A higher percentage retention of diacetyl than of acetylmethylcarbinol in butter agrees with previous reports (4, 6, 13, 17). The variations in retentions of diacetyl and of acetylmethylcarbinol noted by different investigators may be due partly to different procedures used in manufacture of the butter and to different analytical methods. Mohr and Wellm (13) and Krenn (6) attributed the low retention of diacetyl and carbinol in butter to the removal of some of these compounds by the wash water, whereas Prill and Hammer (17) found only very small quantities in wash water.

Changes in the diacetyl contents of butter held at relatively low temperatures suggest that conditions which permit the active multiplication of organisms are not necessary for changes in the diacetyl. Prill and Hammer (16) held ripened butter cultures in ice water for 12 to 15 hours and noted that the diacetyl increased even when no additional citric acid was added. Wiley, Cox and Whitehead (23) incubated butter cultures at 44.6°F. and found little if any carbinol produced while the diacetyl increased. Barnicoat (2) stated that the increase in diacetyl in butter is not caused by oxidation with air, but probably is due to the dehydrogenase activity of the "starter" organisms or their enzymes. Mohr, Schrimpl and Arbes (12) found that during the storage of salted and unsalted butter the diacetyl content may decrease or remain constant.

The importance of diacetyl from the standpoint of the desirable flavor in butter made with cultures is confirmed by the general relationship between comparatively high diacetyl contents and high placings. The samples which had high diacetyl contents and placed low emphasize that diacetyl content is not the only consideration and that off flavors readily outweigh high diacetyl contents. Presumably, in most cases, the off flavors were due to chemical changes rather than to action of organisms. The chemical changes may have been influenced by products of the growth of the organisms added to the cream; these would include not only diacetyl

but acids and other products and, on the basis of certain studies, the acids probably were of most importance in this connection.

The lack of a close correlation between diacetyl contents and placings even with samples having no definite off flavors suggests the importance of compounds other than diacetyl as flavor contributants. Volatile acids undoubtedly are of significance in this connection. Pien, Baisse and Martin (14) found no close correlation between flavor and diacetyl content of butter and suggested that possibly some other substance may be partly responsible for the flavor. Prill, Fabricius and Hammer (18) found that when higher homologs of diacetyl were worked into salted butter the flavor definitely was suggestive of diacetyl; however, these homologs could not be detected in butter cultures.

## LITERATURE CITED

- (1) Barnicoat, C. R. Diacetyl in cold-stored butters. Jour. Dairy Res., 6: 397-406. 1935.
- (2) Barnicoat, C. R. Diacetyl in cold-stored butters. II. Jour. Dairy Res., 8: 15-30. 1937.
- (3) Brewer, C. R., Werkman, C. H., Michaelian, M. B. and Hammer, B. W. Effect of aeration under pressure on diacetyl production in butter culture. Iowa Agr. Exp. Sta., Res. Bul. 233. 1938.
- (4) Davies, W. L. The development of aroma in butter. Proc. 11th World's Dairy Congr., Berlin, 2: 76-78. 1937.
- (5) Fabricius, N. E. and Hammer, B. W. The influence of the type of butter culture and its method of use on the flavor and keeping quality of salted butter. Iowa Agr. Exp. Sta., Res. Bul. 221. 1937.
- (6) Krenn, J. Über die Bildung von Diacetyl bei der Reifung des Raumes und dessen Schicksal bei der Butterung. Proc. 11th World's Dairy Congr., Berlin, 2: 78-80. 1937.
- (7) Matuszewski, T., Pijanowski, E. and Supińska, J. *Streptococcus diacetilactis* n. sp. i jego zastosowanie przy wyrobie masła. Roczniki Nauk Rolniczych i Leśnych 36: 25-28. 1936.
- (8) Matuszewski, T., Pijanowski, E. and Supińska, J. A new lactic *Streptococcus* as a constituent of butter starters. Proc. 11th World's Dairy Congr., Berlin, 2: 80-85. 1937.
- (9) Michaelian, M. B. and Hammer, B. W. Studies on acetylmethylcarbinol and diacetyl in dairy products. Iowa Agr. Exp. Sta., Res. Bul. 179. 1935.
- (10) Michaelian, M. B. and Hammer, B. W. The oxidation of acetylmethylcarbinol to diacetyl in butter cultures. Iowa Agr. Exp. Sta., Res. Bul. 205. 1936.
- (11) Michaelian, M. B., Hoecker, W. H. and Hammer, B. W. Effect of pH on the production of acetylmethylcarbinol plus diacetyl in milk by the citric acid fermenting streptococci. Jour. Dairy Sci., 21: 213-218. 1938.
- (12) Mohr, W., Schrimpl, E. and Arbes, A. Die Änderung des Aromas von Dauerbutter während der Lagerung und bei der Auslagerung. Molkerei-Ztg., 53: 1501-1503. 1939.
- (13) Mohr, W. and Wellm, J. Der Diacetylgehalt in deutscher Butter und Einfluss des Herstellungsverfahrens auf den Diacetylgehalt der Butter. Proc. 11th World's Dairy Congr., Berlin, 2: 89-97. 1937.
- (14) Pien, J., Baisse, J. and Martin, R. Le dosage du diacétyle dans les beurres. Lait, 17: 675-698. 1937.
- (15) Prill, E. A. and Hammer, B. W. A colorimetric method for the microdetermination of diacetyl. Iowa State Col. Jour. Sci., 12: 385-395. 1938.
- (16) Prill, E. A. and Hammer, B. W. Production of diacetyl from citric acid in butter cultures. Jour. Dairy Sci., 22: 67-77. 1939.
- (17) Prill, E. A. and Hammer, B. W. Changes in the diacetyl and the acetylmethylcarbinol contents during the manufacture of butter. Jour. Dairy Sci., 22: 79-88. 1939.
- (18) Prill, E. A., Fabricius, N. E. and Hammer, B. W. Diacetyl and other *alpha*-dicarbonyl compounds with special reference to the flavor of butter. Iowa Agr. Exp. Sta., Res. Bul. 268. 1939.

- (19) van Beynum, J. and Pette, J. W. De beleekenis der melkzuurbacteriën bij de boteraromavorming. Netherlands. Directie Landbouw. Verslag. Landbouwk. Onderzoek., 42. p. 360. 1936.
- (20) van Beynum, J. and Pette, J. W. De ontleding van citroenzuur door boteraromabacteriën. Netherlands. Directie Landbouw. Verslag. Landbouwk. Onderzoek., 44. 1938.
- (21) Virtanen, A. J. The influence of oxygen on the formation of butter aroma. Proc. 11th World's Dairy Congr, Berlin, 2: 121-123. 1937.
- (22) Wiley, W. J., Cox, G. A. and Whitehead, H. R. The formation of diacetyl by starter cultures. I. A comparison of single strain and mixed culture starters for butter manufacture. Jour. Council Sci. and Ind. Res., 12: 232-238. 1939.
- (23) Wiley, W. J., Cox, G. A. and Whitehead, H. R. The formation of diacetyl by starter cultures. II. Rate of diacetyl production by lactic streptococci. Jour. Council Sci. and Ind. Res., 12: 239-249. 1939.