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THE FATE OF ACETYLMETHYLCARBINOL AND DIACETYL IN DAIRY PRODUCTS

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Butter cultures normally contain two distinct types of bacteria, (a) a typical lactic acid forming type (*Streptococcus lactis*) and (b) a type which is important from the standpoint of the production of satisfactory flavor and aroma and includes *Streptococcus citrovorus* and *Streptococcus paracitrovorus.*

Michaelian, Farmer and Hammer (1) found that butter cultures having a satisfactory flavor and aroma contain comparatively large quantities of acetylmethylcarbinol plus diacetyl while cultures lacking in flavor and aroma contain small amounts or none. *Streptococcus citrovorus* or *Streptococcus paracitrovorus* when grown in milk whose acidity is adjusted to a pH between 4 and 4.5, produces acetylmethylcarbinol ($CH_3 . CO . CHOH . CH_3$). The yield of the carbinol is greatly increased by adding citric acid to the milk culture.

In the early studies on the presence of acetylmethylcarbinol and diacetyl in butter cultures it was noted that the quantities of these compounds commonly decreased on continued holding of the cultures. The citric acid fermenting streptococci appeared to be responsible for the destruction.

Some recent investigations carried out in this laboratory with *Aerobacillus polymyxa* have shown that acetylmethylcarbinol may be reduced to 2, 3-butylene glycol by these bacteria. This change may be represented by the equation:

$$CH_3 . CO . CHOH . CH_3 . + 2H \longrightarrow CH_3 . CHOH . CHOH . CH_3$$

Kluyver (2) and O'Mera (3) assumed that this reduction occurs in fermentations in which acetylmethylcarbinol is formed but gave no data to prove their assumption. These considerations suggest that the disappearance of acetylmethylcarbinol in a butter culture under the usual conditions of holding may be due to the reduction of this compound to the corresponding glycol. The work reported here was undertaken to test this suggestion.

Two distinct types of media were used: (a) tomato bouillon and (b) milk, to some of which citric acid was added.

TRIALS WITH TOMATO BOUILLON

The method used was to inoculate the medium with one of the citric acid fermenting streptococci, incubate at 21°C to allow the organisms to multiply, add a sterile aqueous solution of acetylmethylcarbinol or diacetyl, and then determine the carbinol or diacetyl and 2, 3 - butylene glycol at once and again after holding at 21°C for various periods. Typical results are given in Table I.

Table I — Reduction of Acetylmethylcarbinol to 2, 3 - Butylene Glycol

ORGANISM	Hours incubation before adding A. M. C.	Hours elapsed between adding A. M. C. and analysis	Molarity	
			A. M. C.*	2, 3-B.G.*
Streptococcus paracitrovorus No. 29	48	0	0.0052	0.0006
		48	0.0004	0.0056
		72	0.0003	0.0058
Streptococcus paracitrovorus No. 49	24	0	0.0041	0.0004
		24	0	0.0049
		48	0	0.0049
		72	0	0.0047
		120	0	0.0049

* A. M. C. = acetylmethylcarbinol; 2, 3 – B. G. = 2, 3 – butyleneglycol.

A rapid decrease in the molarity of acetylmethylcarbinol and a corresponding increase of 2, 3-butylene glycol is evident.

When diacetyl was added to a tomato bouillon culture, it likewise was reduced to 2, 3-butylene glycol.

The reduction of both acetylmethylcarbinol and diacetyl was less rapid in those cultures whose acidity had been raised to about pH 4 than in those which were approximately neutral.

TRIALS WITH MILK

A large volume of skim milk was inoculated and incubated for 24 hours before acetylmethylcarbinol or diacetyl was added. Analyses showed that a considerable part of the carbinol was reduced to the glycol. Diacetyl was partially reduced but largely to acetylmethylcarbinol. Various portions of the skim milk culture, after addition of acetylmethylcarbinol, were treated with different reagents to test their effect on the reduction. Potassium nitrate had little, if any, effect. Hydrogen peroxide in low concentrations had little effect but 0.5 ml. of 30 per cent hydrogen peroxide per liter of culture markedly inhibited the reduction.

A few experiments were conducted on regular commercial butter cultures in order to make the problem directly applicable to dairy practice. To determine the effect of acidity on the rate of reduction, the butter culture was divided into two parts one of which was

neutralized with sodium hydroxide. Some results obtained at a holding temperature of 21°C are given in Table II.

Table II — Effect of Acidity on Reduction of Acetylmethylcarbinol In Butter Cultures

Trial number	Hours elapsed	Culture not Neutralized			Culture Neutralized		
		Original acidity percent *	Molarity		Acidity after neutralization percent	Molarity	
			A. M. C.	2, 3-B.G.		A. M. C.	2, 3-B.G.
1	0	0.89	0.0026	0.0035	0.15	0.0026	0.0035
	24		0.0022	0.0049		0.0006	0.0066
	96		0.0014	0.0058		0.0005	0.0076
2	0	0.91	0.0017	0.0044	0.14	0.0017	0.0044
	24		0.0014	0.0052		0.0006	0.0067
	96		0.0008	0.0059		0	0.0075

* Percentage of the culture by weight calculated as lactic acid.

The reduction was more rapid in the samples of butter culture which were neutralized than in the unneutralized samples when both were held at 21°C. It should be observed that the formation of acetylmethylcarbinol + 2, 3-butylene glycol continued after the first analysis was made.

Studies also were made with butter cultures to determine the effect of the addition of various substances such as sodium fumarate, potassium nitrate, hydrogen peroxide, and sodium chloride on the reduction of the carbinol. The first three are hydrogen acceptors and it was thought might compete with acetylmethylcarbinol for hydrogen. The sodium chloride inhibits the growth of many bacteria. The effect of the temperature of holding was also studied. Some data obtained under these different conditions are presented in Table III.

Table III — The Effect of Different Reagents and Holding Temperatures On the Rate of Reduction of Acetylmethylcarbinol

Original acidity of B.C.%	Holding conditions	Molarity					
		At Once		After 48 hrs.		After 96 hrs.	
		A. M. C.	2, 3-B.G.	A. M. C.	2, 3-B.G.	A. M. C.	2, 3-B.G.
0.93	21°C						
	Without modification	0.0022	0.0051	0.0015	0.0059	0.0015	0.0069
	With 0.5ml H_2O_2 per liter	0.0022	0.0051	0.0020	0.0053	0.0022	0.0053
	With 1% sodium fumarate	0.0022	0.0051	0.0023	0.0055	0.0023	0.0055
	With 12% NaCl	0.0022	0.0051	0.0019	0.0055	0.0017	0.0056
	After neut. (NaOH) to 0.21%	0.0022	0.0051	0	0.0079	0	0.0083
	0°C						
	Without modification	0.0022	0.0051	0.0020	0.0056	0.0022	0.0058
	After neut. (NaOH) to 0.21%	0.0022	0.0051	0.0016	0.0063	0.0011	0.0066

The least reduction occurs when sodium fumarate or hydrogen peroxide are added or when the culture is held at 0°C. Reduction is fairly rapid with 12 per cent sodium chloride, holding at 21°C or when the culture is neutralized and held at 0°C. Very rapid reduction results from neutralizing the culture and then holding at 21°C.

LITERATURE CITED

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