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# Determination of Acetylmethylcarbinol and Diacetyl in Dairy Products<sup>1</sup>

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**S**TUDIES carried on by various investigators in recent years have definitely proved that a good quality of butter culture usually contains comparatively large quantities of acetylmethylcarbinol and some diacetyl; consequently, the determination of these compounds in dairy products has become the usual practice in dairy laboratories. Often, however, certain irregularities have been encountered with the technique employed in the determination of these compounds.

Attempts to determine the nature of these irregularities and, if possible, to find a working remedy herein are reported.

## METHODS

Acetylmethylcarbinol and diacetyl are commonly determined together (3) because ordinarily small amounts of diacetyl also are present with the carbinol. To 200 gm. or 200 ml. of the sample for analysis 40 ml. of ferric chloride solution (40 gm. made up to 100 ml. with distilled water) were added to oxidize the carbinol to diacetyl and then steam distilled. The distillate was carried by means of an adapter on the end of the condenser into a mixture of one part of a solution of hydroxylamine hydrochloride (20 gm. made up to 100 ml.) and two parts of sodium acetate solution (20 gm. made up to 100 ml.). The distillation was continued until the volume of the distillate collected was approximately three-fourths of the original sample used. The mixture, consisting of distillate, hydroxylamine hydrochloride and sodium acetate, was heated to

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80°-90° C., and nickel chloride solution (10 gm. made up to 100 ml.) was added. The quantities of reagents used depended on the amount of diacetyl expected, always an excess being used. van Niel (7) has shown that the concentration of the reagents can fluctuate between wide limits; for about 100 mg. diacetyl he suggested 2 ml. hydroxylamine hydrochloride (20 percent solution), 3 to 5 ml. sodium acetate (20 percent solution) and 1 to 2 ml. nickel chloride (10 percent solution). The distillate with the reagents added was allowed to stand at room temperature at least two days, and preferably longer, in order to obtain complete crystallization. This can be accomplished also by heating the distillate containing hydroxylamine hydrochloride and sodium acetate to 80°-90° C. and, after adding nickel chloride, allowing it to stand overnight at 70°-80° C. (1). The nickel dimethylglyoximate was filtered into a weighed crucible, and the filtrate occasionally treated with more reagents to assure complete crystallization. The nickel salt was washed with distilled water, dried to constant weight at 105°-110° C. and the results recorded as grams of nickel dimethylglyoximate equivalent to acetylmethylcarbinol plus diacetyl per 200 gm. or 200 ml. of material.

Diacetyl was determined by the method used for the acetylmethylcarbinol plus diacetyl, except that ferric chloride was not added and, in the procedure preferred, the distillation was carried out after the sample had been saturated with an inert gas, such as carbon dioxide or nitrogen, to prevent the formation of diacetyl from acetylmethylcarbinol during the distillation process.

The volatile acid was determined by steam distilling a 200 ml. sample and the distillate collected (by means of an adapter on the end of the condenser) into a known amount of  $n/20$  sodium hydroxide in a flask immersed in ice water; 200 ml. of the distillate were collected and the excess alkali titrated with  $n/20$  hydrochloric acid, using phenolphthalein as the indicator. The results are expressed as milliliters of  $n/20$  sodium hydroxide required to neutralize the acid in the distillate.

The pH determinations were made electrometrically, using quinhydrone.

#### RESULTS OBTAINED

When the filtrates from nickel dimethylglyoximate equivalent to diacetyl or acetylmethylcarbinol plus diacetyl are saved and allowed to stand for some time, often new crystals of the nickel salt appear. This is particularly true with the determination of diacetyl.

Acetylmethylcarbinol is fairly volatile (2, 5, 6); therefore, it is probable that a considerable fraction of the carbinol distills over with the diacetyl when both are present in the material being analyzed. During the preliminary trials of this investigation it was found that, when ferric chloride was added to the material and then steam distilled, some of the carbinol also passed over into the distillate with the diacetyl.

TABLE 1. *Nickel dimethylglyoximate equivalent to acetylmethylcarbinol recovered in successive filtrates*

Determination for	Grams nickel dimethylglyoximate obtained			
	Butter culture 232 <sup>1</sup>	Cultures of citric acid fermenting streptococci		Aqueous acetyl-methylcarbinol solution
		9 <sup>2</sup>	29 <sup>3</sup>	
Acetylmethylcarbinol + diacetyl	0.1143	0.1980	0.0911	0.1297
1st filtrate	0.0048	0.0221	0.0037	0.0105
2nd filtrate	0.0016	0.0050	0.0019	0.0009
3rd filtrate	none	0.0007	none	none
Diacetyl	0.0139		0.0025	0.0087
1st filtrate	0.0298		0.0310	0.0355
2nd filtrate	0.0067		0.0071	0.0081
3rd filtrate	none		trace	none
Residue from diacetyl determination + 40 ml. FeCl <sub>3</sub>	0.0607		0.0473	0.0643
1st filtrate	0.0042		0.0032	none
2nd filtrate	0.0012		0.0008	
3rd filtrate	none		none	

<sup>1</sup> Butter culture made from skim milk to which 0.15 percent citric acid had been added.

<sup>2</sup> Skim milk culture of a citric acid fermenting *Streptococcus* to which 0.85 percent citric acid had been added.

<sup>3</sup> Skim milk culture of a citric acid fermenting *Streptococcus* to which 0.15 percent citric acid and 0.3 percent sulfuric acid had been added.

To determine the amount of acetylmethylcarbinol that passed over into the distillate with the diacetyl, the filtrate from nickel dimethylglyoximate was acidified with concentrated sulfuric acid, 40 ml. ferric chloride added and the mixture steam distilled in the usual way.

The data in table 1 show that nearly all of the carbinol in the filtrate was oxidized by ferric chloride to diacetyl and thus recovered as nickel dimethylglyoximate, while a small amount of the carbinol again passed over into the distillate. The data indicate also that the carbinol was present in the second and sometimes even in the third successive filtrate.

Attempts to recover all of the carbinol in the filtrate with one distillation by heating it with steam to 95°-99° C. and holding it at that point for about five minutes with a small flame or by occasionally letting steam in were fairly successful. Almost all of the carbinol in the filtrate was oxidized to diacetyl with this treatment. The recovery of the carbinol in filtrates reported in this work was done in the manner described above.

Table 2 shows that a considerable fraction of acetylmethylcarbinol also distills over with the diacetyl during the determination of diacetyl

TABLE 2. Recovery of acetylmethylcarbinol from filtrates of diacetyl or acetylmethylcarbinol plus diacetyl determinations

Material used	Trial number	Determination for	Grams nickel dimethylglyoximate obtained		
			Recovered from distillate	filtrate	Total recovered
Butter culture made from skim milk	1	Ac <sub>2</sub> <sup>1</sup>	0.0026	0.0178	0.0697
		Residue + FeCl <sub>3</sub>	0.0470	0.0023	
		Amc <sup>2</sup> + ac <sub>2</sub>	0.0669	0.0059	0.0728
	2	Ac <sub>2</sub>	0.0019	0.0154	0.0650
		Residue + FeCl <sub>3</sub>	0.0452	0.0025	
		Amc + ac <sub>2</sub>	0.0680	0.0054	0.0734
Butter culture made from skim milk to which 0.15% citric acid had been added	1	Ac <sub>2</sub>	0.0019	0.0157	0.0588
		Residue + FeCl <sub>3</sub>	0.0399	0.0013	
		Amc + ac <sub>2</sub>	0.0622	0.0042	0.0671
	2	Ac <sub>2</sub>	none	0.0418	0.1116
		Residue + FeCl <sub>3</sub>	0.0642	0.0056	
		Amc + ac <sub>2</sub>	0.1154	0.0128	0.1282
	3	Ac <sub>2</sub>	none	0.0433	0.1072
		Residue + FeCl <sub>3</sub>	0.0574	0.0065	
		Amc + ac <sub>2</sub>	0.1003	0.0106	0.1109
Skim milk cultures of citric acid fermenting streptococci to which citric acid had been added	1	Ac <sub>2</sub>	0.0018	0.0274	0.1012
		Residue + FeCl <sub>3</sub>	0.0679	0.0041	
		Amc + ac <sub>2</sub>	0.0975	0.0079	0.1054
	2	Ac <sub>2</sub>	0.0083	0.1230	0.2512
		Residue + FeCl <sub>3</sub>	0.1130	0.0069	
		Amc + ac <sub>2</sub>	0.2708	0.0260	0.2968
Aqueous solution of commercial acetylmethylcarbinol	1	Ac <sub>2</sub>	0.0043	0.0184	0.0600
		Residue + FeCl <sub>3</sub>	0.0329	0.0044	
		Amc + ac <sub>2</sub>	0.0591	0.0073	0.0664
Aqueous solution of purified acetylmethylcarbinol <sup>3</sup>	1	Ac <sub>2</sub>	0.0023	0.0637	0.1823
		Residue + FeCl <sub>3</sub>	0.1090	0.0073	
		Amc + ac <sub>2</sub>	0.1909	0.0204	0.2113
Commercial diacetyl added to skim milk and acidified with sulfuric acid	1	Ac <sub>2</sub>	none	0.0789	0.2434
		Residue + FeCl <sub>3</sub>	0.1460	0.0185	
		Amc + ac <sub>2</sub>	0.2319	0.0258	0.2577
Aqueous solution of commercial diacetyl	1	Ac <sub>2</sub>	0.0406	none	0.0406
		Residue + FeCl <sub>3</sub>	none	none	
		Amc + ac <sub>2</sub>	0.0375	none	0.0375
Aqueous solution of commercial diacetyl	1	Ac <sub>2</sub>	0.1419	0.0024	0.1443
		Residue + FeCl <sub>3</sub>	none	none	
		Amc + ac <sub>2</sub>	0.0969	0.0012	0.0981

<sup>1</sup> Ac<sub>2</sub> = diacetyl.<sup>2</sup> Amc = acetylmethylcarbinol.<sup>3</sup> Commercial acetylmethylcarbinol was washed with cold anhydrous ether (6).

alone. Runs made on butter cultures or pure cultures of citric acid fermenting streptococci or solutions of commercial acetylmethylcarbinol consistently show that larger quantities of carbinol were recovered from the filtrates of nickel dimethylglyoximate equivalent to diacetyl than from that of acetylmethylcarbinol plus diacetyl. The carbinol present

in the distillate may cause some error in the determination of diacetyl unless air is excluded. When nickel dimethylglyoximate is filtered into a crucible by suction, air mixes well with the filtrate and when the latter is held for some time the acetylmethylcarbinol is oxidized slowly to diacetyl which reacts with the excess reagents and forms new crystals of nickel dimethylglyoximate.

The sum of nickel dimethylglyoximate obtained from (1) diacetyl determinations and its filtrate, (2) the residue from (1) to which ferric chloride had been added and its filtrate was always less than the sum of nickel dimethylglyoximate obtained from the regular determination of acetylmethylcarbinol plus diacetyl and its filtrate on aliquot samples of material containing both the carbinol and diacetyl. This was particularly true with the cultures and also with the solutions of commercial acetylmethylcarbinol used in table 2. This situation was reversed, however, when commercial diacetyl solutions were used. Table 2 also shows that when ferric chloride was added to a solution of commercial diacetyl and steam distilled, the nickel dimethylglyoximate obtained was considerably less than that obtained when no ferric chloride was used. Apparently the ferric chloride destroyed some of the diacetyl during the distillation. It is therefore probable that ferric chloride also destroyed some of the diacetyl formed during the determination of acetylmethylcarbinol plus diacetyl and likewise during the recovery of the carbinol from the filtrates. When attempts were made to recover commercial diacetyl as nickel dimethylglyoximate, traces of acetylmethylcarbinol also were consistently recovered (not all data are presented) in the filtrates. This suggests the possibility that a small amount of carbinol may exist as a result of chemical equilibrium between the carbinol and diacetyl, or that traces of the carbinol exist as an impurity with the commercial diacetyl.

Some attempts to recover commercial acetylmethylcarbinol as nickel

TABLE 3. *Commercial acetylmethylcarbinol recovered as nickel dimethylglyoximate in distillates and filtrates*

Grams amc <sup>1</sup> used	Grams amc found in distillate	Percentage recovered	Grams amc found in filtrate	Percentage in filtrate	Total percentage recovered
0.2020	0.1706	84.45	0.0097	4.80	89.25
0.2020	0.1627	80.54	0.0167	8.26	88.80
0.2020	0.1654	81.88	0.0162	8.02	89.90
0.1029	0.0844	82.02	0.0057	5.54	87.56
0.2018	0.1654	81.96	0.0163	8.08	90.04
0.0943	0.0804	85.25	0.0064	6.78	92.03

<sup>1</sup> Amc = acetylmethylcarbinol.

dimethylglyoximate (6) have been successful to the extent of about 84 percent only. This percentage recovery was materially increased as is indicated in table 3.

In order to obtain the acetylmethylcarbinol in a pure state, the commercial product was washed with cold anhydrous ether (6). This purified carbinol was weighed quantitatively and recovered as nickel dimethylglyoximate by steam distillation and then calculated in terms of grams of acetylmethylcarbinol recovered.

Some of the determinations in table 3 were carried out by holding the material in the distillation flask at about the boiling point for 5 to 10 minutes and then distilling by steam.

The percentage carbinol recovered in the distillates in table 3 ranged from 80.54 to 85.25, while that in the filtrates ranged from 4.80 to 8.26. The total carbinol recovered ranged from 87.56 to 92.03 percent.

In table 4 are given the results of some attempts to oxidize more completely the acetylmethylcarbinol to diacetyl in the distillation flask, thus preventing the carbinol from passing over into the distillate, which in turn would eliminate the additional step of recovering any carbinol from the filtrate of nickel dimethylglyoximate.

A stock solution of acetylmethylcarbinol in distilled water was prepared. To 200 ml. portions of the carbinol solution in the distillation flasks, 40 ml. ferric chloride were added, the flasks were stoppered, held at room temperature for various periods and the determinations were made. The data in table 4 indicate that the holding of this mixture at room temperature from  $\frac{1}{2}$  to 75 hours had no significant effect on the total recovery of the carbinol.

Assuming that the oxidation of acetylmethylcarbinol by ferric chloride is more intense at higher temperatures, some trials were undertaken under various conditions. The temperature of the material in the distillation flask was raised to 95°-99° C. with steam and held there either by letting in steam occasionally or by keeping a small flame under the flask.

The results obtained in table 4 indicate that holding the material at 95°-99° C. for 5 to 10 minutes seemed to give the maximum yield of the carbinol in the distillate. Refluxing acetylmethylcarbinol solutions for 5 minutes and a skim milk culture of a citric acid fermenting *Streptococcus* for 5 to 10 minutes also seemed to yield the highest recovery of the carbinol in the distillate. However, as the period of holding the material at 95°-99° C. or refluxing it was further increased the recovery of the carbinol from the filtrates decreased and the total amount of nickel dimethylglyoximate equivalent to acetylmethylcarbinol decreased also. This probably indicates that the longer some of the diacetyl, formed from the oxidation of the carbinol by ferric chloride in the distillation flask, is held at this range of temperature, the more will be destroyed.

Holding the material at 90°-95° C. for 20 minutes gave essentially the same result as holding it at 95°-99° C. for 5 minutes. Similar results

TABLE 4. Attempts to affect the complete oxidation of acetylmethylcarbinol to diacetyl

Material used	Material treated prior to distillation	Grams nickel dimethylglyoximate $\rightleftharpoons$ acetylmethylcarbinol			
		Recovered from		Total	Percentage recovered from filtrate
		distillate	filtrate		
Aqueous solution of purified com- mercial acetyl- methylcarbinol	200 ml. + 40 ml. FeCl <sub>3</sub>	0.2319	0.0258	0.2577	10.01
	" " " " " " held at R. T. <sup>1</sup> for 30 min.	0.2375	0.0265	0.2640	10.04
	" " " " " " held at R. T. for 60 min.	0.2374	0.0254	0.2628	9.66
	" " " " " " held at 95-99° C. for 5 min.	0.2409	0.0214	0.2623	8.16
	" " " " " " held at 95-99° C. for 10 min.	0.2447	0.0155	0.2602	5.95
	" " " " " " distilled without steam	0.2472	0.0114	0.2586	4.41
	" " " " " " "	"	"	"	"
Aqueous solution of commercial acetylmethyl- carbinol	200 ml. + 40 ml. FeCl <sub>3</sub>	0.1909	0.0204	0.2113	9.65
	" " " " " " held at 95-99° C. for 5 min.	0.2085	0.0104	0.2189	4.75
	" " " " " " held at 95-99° C. for 10 min.	0.1989	0.0109	0.2098	5.19
	" " " " " " held at 95-99° C. for 30 min.	0.1912	0.0048	0.1960	2.42
	" " " " " " refluxed 5 min.	0.2127	0.0079	0.2206	3.58
	" " " " " " refluxed 10 min.	0.1994	0.0034	0.2028	1.67
	" " " " " " refluxed 20 min.	0.1906	0.0031	0.1937	1.60
	" " " " " " refluxed 30 min.	0.1717	0.0002	0.1717	.12
Aqueous solution of purified com- mercial acetyl- methylcarbinol	200 ml. + 40 ml. FeCl <sub>3</sub>	0.1764	0.0213	0.1977	10.77
	" " " " " " held at R. T. for ½ hr.	0.1769	0.0192	0.1961	9.79
	" " " " " " held at R. T. for 1 hr.	0.1797	0.0169	0.1966	8.59
	" " " " " " held at R. T. for 2 hrs.	0.1759	0.0212	0.1971	10.75
	" " " " " " held at R. T. for 8 hrs.	0.1762	0.0207	0.1969	10.51
	" " " " " " held at R. T. for 24 hrs.	0.1769	0.0189	0.1958	9.65
	" " " " " " held at R. T. for 72 hrs.	0.1792	0.0097	0.1889	5.13
	" " " " " " distilled at a very slow rate	0.1864	0.0028	0.1892	1.48
	" " " " " " refluxed 2 min.	0.1778	0.0189	0.1967	9.61
	" " " " " " refluxed 5 min.	0.1995	0.0064	0.2059	3.11
	" " " " " " held at 95-99° C. for 5 min.	0.1853	0.0158	0.2011	7.86
	" " " " " " held at 90-95° C. for 5 min.	0.1801	0.0169	0.1970	8.58
	" " " " " " held at 90-95° C. for 10 min.	0.1820	0.0129	0.1949	6.62
	" " " " " " held at 90-95° C. for 20 min.	0.1886	0.0077	0.1963	3.92
	" " " " " " "	"	"	"	"

TABLE 4. Continued

Material used	Material treated prior to distillation	Grams nickel dimethylglyoximate $\rightleftharpoons$ acetylmethylcarbinol			
		Recovered from		Total	Percentage recovered from filtrate
		distillate	filtrate		
Aqueous solution of purified com- mercial acetyl- methylcarbinol	200 ml. + 40 ml. FeCl <sub>3</sub>	0.2554	0.0321	0.2875	11.16
	" " " " " " held at 95-99° C. for 5 min.	0.2711	0.0266	0.2977	8.94
	150 ml. + 50 ml. water + 40 ml. FeCl <sub>3</sub>	0.1936	0.0231	0.2167	10.66
	100 ml. + 100 ml. " " " " "	0.1289	0.0153	0.1442	10.61
	50 ml. + 150 ml. " " " " "	0.0638	0.0061	0.0699	8.73
	25 ml. + 175 ml. " " " " "	0.0311	0.0041	0.0352	11.65
	25 ml. + 175 ml. " " " " "	0.0340	0.0036	0.0376	9.57
Skim milk cul- ture of a citric acid fermenting Streptococcus to which 0.85 per- cent citric acid had been added	200 gm. + 40 ml. FeCl <sub>3</sub>	0.1849	0.0212	0.2061	10.29
	" " " " " " distilled at a slow rate	0.2012	0.0174	0.2186	7.96
	" " " " " " distilled at a slow rate	0.1961	0.0110	0.2071	5.31
	" " " " " " held at 95-99° C. for 5 min.	0.1915	0.0148	0.2063	7.17
	" " " " " " held at 95-99° C. for 10 min.	0.1933	0.0113	0.2046	5.52
	" " " " " " refluxed for 5 min.	0.1914	0.0223	0.2137	10.44
	" " " " " " refluxed for 10 min.	0.1907	0.0135	0.2042	6.61
	" " " " " " refluxed for 30 min.	0.1719	0.0041	0.1760	2.33
	200 gm. + 60 ml. FeCl <sub>3</sub>	0.1968	0.0160	0.2128	7.52
	" " " " " " held at 95-99° C. for 5 min.	0.2005	0.0104	0.2109	4.93
	200 gm. + 80 ml. FeCl <sub>3</sub>	0.1976	0.0173	0.2149	8.05

<sup>1</sup> Room temperature.



TABLE 5. *The effect of hydrogen ion concentration on the oxidation of acetylmethylcarbinol to diacetyl during the distillation*

Material used	Material treated prior to distillation	Grams nickel dimethylglyoximate $\rightleftharpoons$ acetylmethylcarbinol			
		Recovered from		Total	Percentage recovered from filtrate
		distillate	filtrate		
Aqueous solution of purified acetylmethyl- carbinol	200 ml. + 40 ml. FeCl <sub>3</sub>	0.1764	0.0213	0.1977	10.77
	200 ml. + 40 ml. FeCl <sub>3</sub>	0.1769	0.0192	0.1961	9.79
	200 ml. + 0.5 ml. conc. H <sub>2</sub> SO <sub>4</sub> + 40 ml. FeCl <sub>3</sub>	0.1865	0.0158	0.2023	7.81
	200 ml. + 0.5 ml. conc. H <sub>2</sub> SO <sub>4</sub> + 40 ml. FeCl <sub>3</sub>	0.1885	0.0110	0.1995	5.51
Aqueous solution of purified acetylmethyl- carbinol + 0.04% K <sub>2</sub> HPO <sub>4</sub> . pH 7.80	200 ml. + 40 ml. FeCl <sub>3</sub>	0.2622	0.0195	0.2817	6.92
	200 ml. + 0.05 ml. conc. H <sub>2</sub> SO <sub>4</sub> , pH 2.21, + 40 ml. FeCl <sub>3</sub>	0.2740	0.0191	0.2931	6.52
	200 ml. + 0.10 ml. conc. H <sub>2</sub> SO <sub>4</sub> , pH 1.90, + 40 ml. FeCl <sub>3</sub>	0.2749	0.0213	0.2962	7.19
	200 ml. + 0.25 ml. conc. H <sub>2</sub> SO <sub>4</sub> , pH 1.56, + 40 ml. FeCl <sub>3</sub>	0.2779	0.0163	0.2942	5.54
	200 ml. + 0.5 ml. conc. H <sub>2</sub> SO <sub>4</sub> , pH 1.32, + 40 ml. FeCl <sub>3</sub>	0.2796	0.0159	0.2955	5.38
	200 ml. + 0.75 ml. conc. H <sub>2</sub> SO <sub>4</sub> , pH 1.14, + 40 ml. FeCl <sub>3</sub>	0.2805	0.0228	0.3033	7.52
	200 ml. + 1.0 ml. 5n. NaOH, pH 9.60, + 40 ml. FeCl <sub>3</sub>	0.2590	0.0266	0.2856	9.31
Skim milk cul- ture of a citric acid fermenting Streptococcus to which 0.85% citric acid had been added. pH 4.45	200 gm. + 40 ml. FeCl <sub>3</sub>	0.1980	0.0271	0.2251	12.04
	200 gm. + 0.5 ml. conc. H <sub>2</sub> SO <sub>4</sub> , pH 3.36, + 40 ml. FeCl <sub>3</sub>	0.1746	0.0306	0.2052	14.91
	200 gm. + 0.5 ml. conc. H <sub>2</sub> SO <sub>4</sub> , pH 3.36, + 40 ml. FeCl <sub>3</sub>	0.1740	0.0318	0.2058	15.45
	200 gm. + 0.5 ml. conc. H <sub>2</sub> SO <sub>4</sub> , pH 3.36 + 60 ml. FeCl <sub>3</sub>	0.1987	0.0198	0.2185	9.06
	200 gm. + 1.0 ml. conc. H <sub>2</sub> SO <sub>4</sub> , pH 2.29, + 40 ml. FeCl <sub>3</sub>	0.1576	0.0318	0.1894	16.79
	200 gm. + 2.0 ml. conc. H <sub>2</sub> SO <sub>4</sub> , pH 1.12, + 40 ml. FeCl <sub>3</sub>	0.1585	0.0343	0.1928	17.79
	200 gm. + 5.0 ml. conc. H <sub>2</sub> SO <sub>4</sub> , + 40 ml. FeCl <sub>3</sub>	0.1520	0.0344	0.1864	18.45
	200 gm. + 1.0 ml. 5n. NaOH, pH 4.91, + 40 ml. FeCl <sub>3</sub>	0.2011	0.0180	0.2191	8.22
	200 gm. + 3.5 ml. 5n. NaOH, pH 6.52 + 40 ml. FeCl <sub>3</sub>	0.1953	0.0197	0.2150	9.16
	200 gm. + 5.0 ml. 5n. NaOH, pH 9.20, + 40 ml. FeCl <sub>3</sub>	0.2003	0.0136	0.2139	6.36

were obtained when the distillation was carried out at a relatively slow rate (about 80 to 100 minutes as compared with 30 to 50 minutes for the regular steam distillation) and also when the material was distilled without steam.

The use of smaller samples of the acetylmethylcarbinol solution did not reduce the recovery of the carbinol in the filtrates.

The addition of 60 or 80 ml. of ferric chloride to 200 gm. of skim milk culture of a citric acid fermenting *Streptococcus* increased the recovery of the carbinol in the distillate as compared with that of 40 ml. This increase, however, was not essentially greater than when 40 ml. were used and distilled slowly or when it was held at 95°-99° C. for 5 minutes and then distilled in the usual way. When 60 ml. of ferric chloride were added and the mixture held at 95°-99° C. for 5 minutes, the recovery of the carbinol in the distillate and the total recovery was about the same as when 40 ml. were added and it was distilled slowly or refluxed for 5 to 10 minutes and then distilled in the usual way.

The use of various amounts of acids, to facilitate a more complete oxidation of the carbinol in the distillation flask, looked encouraging with commercial acetylmethylcarbinol solutions. The results obtained from the trials on the above solution and on a skim milk culture of a citric acid fermenting *Streptococcus* are given in table 5.

A solution of commercial acetylmethylcarbinol with distilled water was adjusted to pH 7.80 by adding 0.04 percent  $K_2HPO_4$ . Portions from this adjusted carbinol solution were taken out, various amounts of concentrated (c.p.)  $H_2SO_4$ , or 5n. NaOH added and pH determinations made. To 200 ml. of this solution (after the pH had been adjusted) 40 ml. of ferric chloride were added and the mixture steam distilled.

From a lot of skim milk culture of a citric acid fermenting *Streptococcus* (0.85 percent citric acid had been added) with a pH 4.45, portions were taken out and treated as above. To 200 gm. of the treated culture 40 ml. ferric chloride were added and the mixture steam distilled.

The additions of 0.05 ml. up to 0.75 ml. of concentrated sulfuric acid to the commercial acetylmethylcarbinol solution brought about a gradual increase in the recovery of the carbinol in the distillate as is evident in table 5.

The percentage recovery of the carbinol in the filtrates apparently was not influenced by the pH of the solution.

The addition of sulfuric acid to a skim milk culture of a citric acid fermenting *Streptococcus* tended to decrease the recovery of the carbinol in the distillates and increase it in the filtrates.

When 0.5 ml. of concentrated sulfuric acid and 60 ml. of ferric chloride were added to the culture, the recovery of the carbinol in the filtrate decreased but the total recovery was less than that obtained when 40 ml. of ferric chloride alone were used.

TABLE 6. Attempts to increase the recovery of acetylmethylcarbinol by aeration

Material used	Material treated prior to distillation	Grams nickel dimethylglyoximate $\rightleftharpoons$ acetylmethylcarbinol			
		Recovered from		Total	Percentage recovered from filtrate
		distillate	filtrate		
Aqueous solution of purified acetylmethyl- carbinol	200 ml. + 40 ml. FeCl <sub>3</sub>	0.2677	0.0247	0.2924	8.45
	" " " " " " aerated	0.2683	0.0208	0.2891	7.19
	" " " " " " aerated	0.2890	0.0123	0.3013	4.08
	" " " " " " held at 95-99° C. for 5 min., aerated	0.2933	0.0092	0.3025	3.04
Skim milk cul- ture of a citric acid fermenting Streptococcus to which 0.85% citric acid had been added	200 gm. + 40 ml. FeCl <sub>3</sub>	0.1849	0.0212	0.2061	10.29
	" " " " " " aerated	0.1890	0.0220	0.2110	10.43
	" " " " " " aerated	0.1784	0.0307	0.2091	14.68
	" " " " " " aerated during slow rate distillation	0.2066	0.0081	0.2147	3.77

The recovery of the carbinol in the distillate decreased and in the filtrate increased with the addition of 1.0 ml. 5*N.* NaOH to the carbinol solution (pH 9.6). With the skim milk culture of a citric acid fermenting *Streptococcus* the addition of 5*N.* NaOH in amounts of 1.0 ml., 3.5 ml. or 5.0 ml. gave results about the same as the control.

It seems probable that the destruction of some of the diacetyl is one of the factors in preventing the complete recovery of the carbinol in cultures or in solutions of commercial acetylmethylcarbinol.

Some attempts to accelerate the removal of diacetyl formed in the distillation flask by aeration are reported in table 6. The receiving flask containing the mixture of hydroxylamine hydrochloride and sodium acetate was immersed in ice water. By means of a pump, attached to the receiving flask, a slow stream of air was bubbled into the distillation flask and passed through the system during the entire period of steam distillation.

The aeration definitely increased the recovery of the carbinol in the distillate in one of the trials in which a solution of commercial acetylmethylcarbinol was employed, but the duplicate run of the same solution was about the same as the control. The holding of the same carbinol solution at 95°-99° C. for 5 minutes and then aerating during the steam distillation significantly increased the recovery of the carbinol in the distillate. The aeration, however, did not seem to have any appreciable effect on the recovery of the carbinol in the distillate when a skim milk culture of a citric acid fermenting *Streptococcus* was used. The aeration during a slow distillation of this culture increased the recovery of the carbinol in the distillate. Similar increases with the same culture without aeration were reported in table 4.

It was previously mentioned that when 60 or 80 ml. of ferric chloride were used during the distillation there was some increase in the recovery of the carbinol in the distillate. This led to studies to determine how much acetylmethylcarbinol could be oxidized by 40 ml. or more of ferric chloride solution (40 gm. made up 100 ml. with distilled water). The results are given in table 7.

When to 200 ml. of a relatively strong solution of commercial acetylmethylcarbinol 40 ml. ferric chloride were added, 576 mg. of nickel dimethylglyoximate equivalent to acetylmethylcarbinol were obtained in the distillate. When 60, 100 or 120 ml. of ferric chloride were used, the recovery of the carbinol in the distillates increased accordingly, while the percentage recovery in the filtrates decreased. However, when 200 ml. of ferric chloride were used, the recovery in the distillate decreased, the recovery in the filtrate was the lowest and the total recovery was lower than when 120 ml. of ferric chloride were used. Again it seems probable that this decrease is caused by the destruction of some diacetyl formed from the oxidation of the carbinol by the ferric chloride in the distillation flask.

TABLE 7. *The effects of various amounts of ferric chloride on the recovery of acetylmethylcarbinol*

Ml. ferric chloride used	Grams nickel dimethylglyoximate $\rightleftharpoons$ acetylmethylcarbinol			
	Recovered from		Total	Percentage recovered from filtrate
	distillate	filtrate		
40	0.5760	0.0725	0.6485	11.18
60	0.6215	0.0561	0.6776	8.28
100	0.6444	0.0430	0.6874	6.25
120	0.6601	0.0336	0.6937	4.84
200	0.6539	0.0254	0.6793	3.74

The attempts, discussed so far, to oxidize acetylmethylcarbinol completely to diacetyl in the distillation flask and thus eliminate the recovery of carbinol from the filtrate were only partly successful. Holding the material in the distillation flask at 95°-99° C. for 5 to 10 minutes or refluxing it for about 5 minutes seemed to yield the best recovery of the carbinol in the distillate; however, a small amount of the carbinol regularly passed over into the distillate. When the period for this type of treatment was prolonged, the recovery in the distillate tended to decrease because of a possible destruction of some of the diacetyl.

Other attempts to prevent the carbinol from passing over into the distillate were: (1) Using various oxidizing reagents along with ferric chloride, (2) increasing or decreasing the concentration of the latter compound, (3) using superoxal in the distillate, and (4) using various devices such as a fractionating column with or without glass beads to hold back the unoxidized carbinol. None of these attempts were successful.

The data shown in table 8 suggest the possibility that a correction factor may be used in place of recovering acetylmethylcarbinol from the filtrate. The percentage carbinol recovered from the filtrates of the regular steam distillations (table 8) ranged from 7.36 to 12.04 and averaged 9.66. In the majority of trials the recovery ranged between 9 and 11 percent. These percentage recoveries of the carbinol from the filtrates may vary with the conditions under which the distillations are performed. The percentage recoveries of the carbinol from the filtrates, therefore, ought to be determined by making several runs and then the average recovery applied as a correction factor for other determinations.

As previously mentioned, with regular steam distillations about 80 to 84 percent of the commercial acetylmethylcarbinol could be recovered as nickel dimethylglyoximate in the distillates, and about 9 to 11 percent in the filtrates. However, there still remains some carbinol that cannot be accounted for but which probably is oxidized to diacetyl and then destroyed during the distillation. Data supporting this assumption are given in table 9.

TABLE 8. *Percentage acetylmethylcarbinol recovered from filtrates of regular steam distillations*

Material used	Trial number	Grams nickel dimethylglyoximate $\rightleftharpoons$ acetylmethylcarbinol + diacetyl			
		Recovered from		Total	Percentage recovered from filtrate
		distillate	filtrate		
Aqueous solution of purified acetylmethylcarbinol	1	0.2319	0.0258	0.2577	10.01
	2	0.2375	0.0265	0.2640	10.04
	3	0.2374	0.0254	0.2628	9.66
Aqueous solution of commercial acetylmethylcarbinol	1	0.1909	0.0204	0.2113	9.65
Aqueous solution of purified acetylmethylcarbinol	1	0.1764	0.0213	0.1977	10.77
	2	0.1769	0.0192	0.1961	9.79
	3	0.1797	0.0169	0.1966	8.59
	4	0.1759	0.0212	0.1971	10.75
	5	0.1762	0.0207	0.1969	10.51
	6	0.1769	0.0189	0.1958	9.65
Butter culture made from skim milk	1	0.0669	0.0059	0.0728	8.10
	2	0.0680	0.0054	0.0734	7.36
Three butter cultures made from skim milk to which 0.15% citric acid had been added	1	0.1154	0.0128	0.1282	9.98
	2	0.1003	0.0106	0.1109	9.56
	3	0.0975	0.0079	0.1054	7.49
Skim milk cultures of citric acid fermenting streptococci to which about 0.85% citric acid had been added	1	0.2708	0.0260	0.2968	8.76
	2	0.1980	0.0271	0.2251	12.04
	3	0.1953	0.0197	0.2150	9.16
	4	0.0591	0.0073	0.0664	10.99
	5	0.1849	0.0212	0.2061	10.29
				Average	9.66

TABLE 9. *Destruction of diacetyl by ferric chloride during steam distillation*

Material used	Lot no.	Material treated prior to distillation	Grams Ni dimethylglyoximate $\rightleftharpoons$ diacetyl	Percentage destroyed
Aqueous solutions of commercial diacetyl	1	200 ml. + no $\text{FeCl}_3$	0.1419	0.00
		200 ml. + 40 ml. $\text{FeCl}_3$	0.0969	31.71
		200 ml. + 40 ml. $\text{FeCl}_3$ , held at 95-99° C. for 15 min.	0.0883	37.77
	2	200 ml. + no $\text{FeCl}_3$	0.1589	0.00
		" " " " " " held at 95-99° C. for 30 min.	0.1586	0.00
		" " " " " " held at 95-99° C. for 30 min.	0.1446	8.99
		200 ml. + 40 ml. $\text{FeCl}_3$	0.1055	33.61
		" " " " " " held at 95-99° C. for 30 min.	0.1065	32.98
		" " " " " " held at 95-99° C. for 30 min.	0.0974	38.70
		200 ml. + 80 ml. $\text{FeCl}_3$	0.1033	34.99
		200 ml. + 40 ml. $\text{FeCl}_3$ , refluxed for 20 min.	0.0902	43.24
	3	200 ml. + no $\text{FeCl}_3$	0.0322	0.00
		200 ml. + 40 ml. $\text{FeCl}_3$	0.0181	43.78
		" " " " " "	0.0178	44.72
	4	200 ml. + no $\text{FeCl}_3$	0.1421	0.00
		200 ml. + 40 ml. $\text{FeCl}_3$	0.0936	34.13
	5	200 ml. + no $\text{FeCl}_3$	0.1930	0.00
		200 ml. + 40 ml. $\text{FeCl}_3$	0.1215	37.04
		" " " " " "	0.1202	37.72
Commercial diacetyl added to skim milk	1	200 gm. + no $\text{FeCl}_3$	0.0291	0.00
		" " " " " "	0.0298	0.00
		200 gm. + 40 ml. $\text{FeCl}_3$	0.0168	43.62
		" " " " " "	0.0165	44.63
Commercial diacetyl added to skim milk and acidified with sulfuric acid	1	200 gm. + no $\text{FeCl}_3$	0.3351	0.00
		200 gm. + 40 ml. $\text{FeCl}_3$	0.2960	11.66
	2	200 gm. + no $\text{FeCl}_3$	0.0431	0.00
		200 gm. + 40 ml. $\text{FeCl}_3$	0.0375	12.99

The addition of 40 ml. of ferric chloride to aqueous solutions of commercial diacetyl caused the destruction, of the latter compound, which ranged from 31.71 to 44.72 percent with regular steam distillations. Holding this mixture at 95°-99° C. for 15 or 30 minutes or refluxing 20 minutes increased the percentage destruction to some extent. However, when a diacetyl solution without ferric chloride was held at 95°-99° C. for 30 minutes, there was also a reduction of 8.99 percent in the recovery of diacetyl.

When diacetyl was added to skim milk and steam distilled, the destruction by ferric chloride was about the same as in aqueous solutions. This destruction was only 11.66 and 12.99 percent in two separate trials with acidified mixtures of skim milk and diacetyl.

While a large percentage of commercial diacetyl is destroyed by ferric chloride during steam distillation, it is probable that the extent of this destruction is much less during the oxidation of acetylmethylcarbinol to diacetyl by the ferric chloride.

Attempts to determine the products formed from the destruction of diacetyl by ferric chloride are given in table 10.

Data in table 10 indicate that while small amounts of volatile acids were obtained from the steam distillation of diacetyl solution alone, these amounts were significantly increased when 40 ml. ferric chloride were added to the same solution. Refluxing the latter mixture for 30 minutes, before steam distillation, gave volatile acid values which were higher than those obtained by regular steam distillation. The addition of 1 ml. of concentrated sulfuric acid to diacetyl solutions with or without 40 ml. of ferric chloride gave volatile acid values which were higher than the controls. These values were greater when 40 ml. of ferric chloride were used along with 1 ml. of sulfuric acid. The addition of 1 ml. of 5n. NaOH along with 40 ml. of ferric chloride to the diacetyl solution also increased the volatile acidity. When 4 ml. of concentrated sulfuric acid were added to the residues from various volatile acid determinations of the diacetyl solution, volatile acid values were obtained which were higher than those obtained when the same amount of sulfuric acid was added to boiled distilled water and steam distilled. The values obtained from various other volatile acid determinations on boiled distilled water were insignificant as compared with those obtained from diacetyl solutions under identical conditions.

Trials to determine the presence of carbon dioxide, due to complete oxidation of diacetyl, were negative. It seems probable that volatile acids were the chief products formed from the destruction of diacetyl by ferric chloride; therefore, attempts were made to determine the nature of the volatile acids formed.

To a strong aqueous solution of diacetyl some ferric chloride was added and steam distilled. Other trials, such as refluxing the above mixture for 30 minutes or adding concentrated sulfuric acid or 5n. sodium



TABLE 10. Volatile acid formed from the destruction of diacetyl by ferric chloride

Material used	Trial no.	Material treated prior to distillation	Volatile acid <sup>1</sup>	
			Detn. 1	Detn. 2
Aqueous solution of commercial diacetyl	1	No FeCl <sub>3</sub>	5.1	3.0
	2	No FeCl <sub>3</sub>	4.6	
		Residue + 4 ml. conc. H <sub>2</sub> SO <sub>4</sub>	6.6	
	3	No FeCl <sub>3</sub> , refluxed 30 min.	3.6	6.6
		Residue + 4 ml. conc. H <sub>2</sub> SO <sub>4</sub>	8.9	6.6
	4	40 ml. FeCl <sub>3</sub>	13.5	14.6
		Residue + 4 ml. conc. H <sub>2</sub> SO <sub>4</sub>		11.6
	5	40 ml. FeCl <sub>3</sub> , refluxed 30 min.	17.1	23.9
		Residue + 4 ml. conc. H <sub>2</sub> SO <sub>4</sub>		11.7
	6	No FeCl <sub>3</sub> + 1 ml. conc. H <sub>2</sub> SO <sub>4</sub>	10.4	7.4
Boiled, distilled water	1	No FeCl <sub>3</sub>	1.2	
		Residue + 4 ml. conc. H <sub>2</sub> SO <sub>4</sub>	1.2	
	2	No FeCl <sub>3</sub> , refluxed 30 min.	1.5	
		Residue + 4 ml. conc. H <sub>2</sub> SO <sub>4</sub>	1.6	
	3	40 ml. FeCl <sub>3</sub>	2.1	
		Residue + 4 ml. conc. H <sub>2</sub> SO <sub>4</sub>	2.0	
	4	40 ml. FeCl <sub>3</sub> , refluxed 30 min.	3.3	
		Residue + 4 ml. conc. H <sub>2</sub> SO <sub>4</sub>	1.8	
	5	No FeCl <sub>3</sub> + 4 ml. conc. H <sub>2</sub> SO <sub>4</sub>	1.8	
	6	No FeCl <sub>3</sub> + 1 ml. 5n. NaOH	0.8	

<sup>1</sup> Milliliters n/20 NaOH required to neutralize the acid in 200 ml. distillate.

hydroxide along with ferric chloride and then steam distilling, were undertaken also.

In four different trials it was possible to obtain enough volatile acids to make four different identification tests by the partition method (4). In all four trials the only volatile acid identified was acetic acid, and the tests for formic acid were negative.

#### SUMMARY

1. During the determination of diacetyl, in a material containing both diacetyl and acetylmethylcarbinol, the carbinol also distilled over with the diacetyl. This caused erroneous results, because of the oxidation of the carbinol to diacetyl, unless air was excluded from the distillate.

2. During the determination of acetylmethylcarbinol plus diacetyl some of the unoxidized carbinol also passed over into the distillate with the diacetyl. This carbinol which was recovered from the filtrates of the regular determinations ranged from 7.36 to 12.04 and averaged 9.66 percent in 20 trials.

3. The percentage recovery of commercial acetylmethylcarbinol as nickel dimethylglyoximate ranged from 80.54 to 85.35 in the distillates and 4.80 to 8.26 in the filtrates. The total carbinol recovered ranged from 87.56 to 92.03 percent.

4. Attempts to bring about the complete oxidation of acetylmethylcarbinol to diacetyl, and thus to eliminate the necessity of recovering the carbinol from the filtrates, were only partly successful.

5. The data obtained support the assumption that some of the diacetyl, formed from the oxidation of acetylmethylcarbinol by ferric chloride, was destroyed during distillation.

6. The percentage destruction of the commercial diacetyl by ferric chloride ranged from 31.71 to 44.72 with regular steam distillations in 16 trials.

7. Volatile acid seemed to be the chief product formed from the destruction of commercial diacetyl by ferric chloride. Acetic acid was present, but tests for formic acid were negative.

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