

ethylene and propylene in the ratio approximately of 9:3:2:2, as well as small amounts of acetylene, methylacetylene and allene.

EVANSTON, ILLINOIS

[CONTRIBUTION FROM THE KANSAS AGRICULTURAL EXPERIMENT STATION]

INDICATIONS OF GLUCOSE IN MILK¹

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The work here reported was started by a request from the Dairy Department to determine if a large increase in the blood sugar of cows would cause the appearance of glucose in the milk. The samples of milk were furnished by the Dairy Department in connection with various projects in which the Department of Chemistry is cooperating.

The milk of lot number one was a composite from all herds supplying milk to the Dairy Department. That of lot number seven was taken separately from three of these herds. The other lots were individual milkings from each of three cows over the intervals indicated.

The official polarimetric method² for estimating milk sugar was modified to facilitate the estimation of small amounts of glucose in milk. Fifty cc. of milk was clarified, without further dilution, with 2.5 cc. of official mercuric nitrate solution. The mixture was filtered after standing for five minutes and the rotation of the filtrate measured at 20° in a 20-cm. water-jacketed tube.

Fermentation with yeast, as applied by Raymond and Blanco³ to other material, was tried as a method of removing any glucose in the milk. To determine whether this method was practicable, 0.5% of glucose was added to a sample of milk, the rotation of which was previously shown not to be changed by treatment with yeast. The extra rotation due to the added glucose was completely removed from 50-cc. samples of this sweetened milk by contact with 3 g. of yeast at 30° in between fifteen and thirty minutes, or with 1 g. of yeast in less than two hours. Samples treated with 3 g. of yeast showed no further change on standing for two hours with the yeast. It was therefore concluded that all the glucose and none of the lactose was removed, and that one hour would be a suitable time for the fermentation.

The yeast was prepared from Fleischmann's baker's yeast by washing and centrifuging five times.⁴ The last wash water was found to be clear and free from reducing sugars.

Analyses of 275 samples of milk showed decrease of rotation, after treating 50-cc. portions with 3 g. of yeast for one hour, varying from zero, in 20 cases, to a decrease corresponding to 0.35% of glucose in the milk. In eleven other samples the rotation was observed to increase from 0.01 to 0.07% of the original rotation on treatment with yeast. It is, at present, uncertain whether these apparent increases should be accepted

¹ Contribution No. 156 from the Department of Chemistry.

² Method of Analysis of the Association of Official Agricultural Chemists, 1925.

³ A. L. Raymond and J. G. Blanco, *J. Biol. Chem.*, 79, 649 (1928).

⁴ M. Somoji, *ibid.*, 75, 33 (1927).

as real and what significance should be given them. The sensitiveness and reliability of the determinations are indicated by the fact that of 566 duplicate determinations 443 checked as closely as the polariscope could be read, *i. e.*, less than 0.002% sugar while the average variation in all duplicates was only 0.008%. Thus the method seems sensitive enough to make significant all the measurements reported.

Removal of glucose on treatment with yeast is considered to be the cause of decrease in rotation; first because glucose is generally considered⁵ to be the precursor in the blood of the lactose in the milk, and second, because glucose is known to be readily fermented by yeast⁶ either in aqueous solutions or in blood.

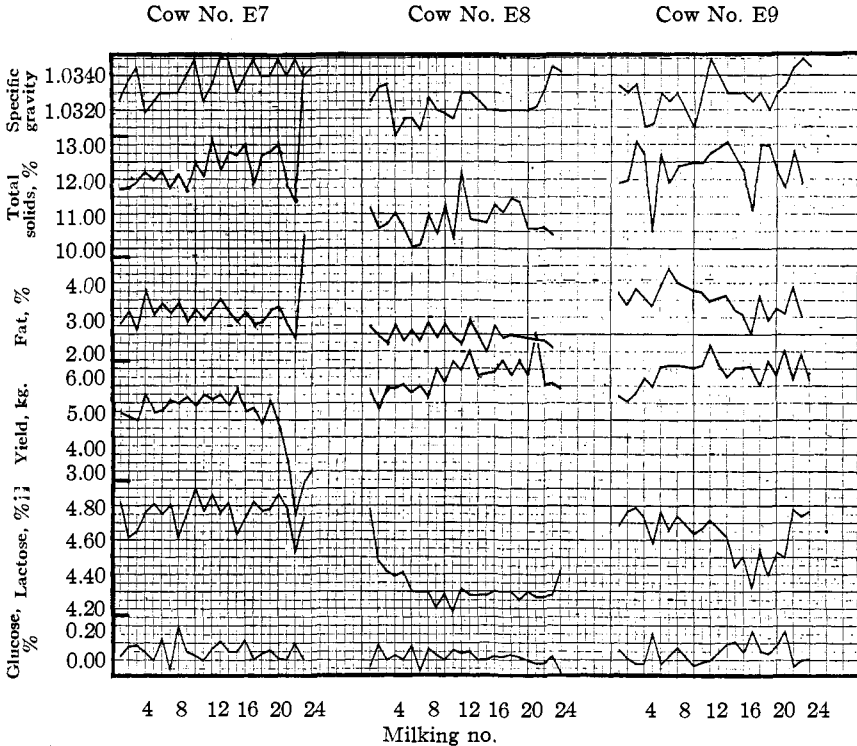


Fig. 1.

Table I shows variations in the glucose content of milk calculated from changes in specific rotation due to fermentation with yeast. The third lot is the only long interval in which all samples apparently contained glucose. This was in the spring shortly after the cows had been turned out to pasture. The observations in the following three lots were on cows receiving dry feed only.

Table II and Fig. 1 indicate simultaneous values in percentage of apparent glucose, percentage of lactose, yield, percentage of fat, percentage of

⁵ E. B. Meigs, *Physiol. Rev.*, 2, 204-211 (1922).

⁶ E. Schmidt, F. Trefz and H. Schnegg, *Ber.*, 59B, 2635 (1926).

TABLE I
APPARENT GLUCOSE IN COW'S MILK

Lot no.	Dates, 1930	Rotation changes as per cent. glucose		Number of milkings where rotation shows		
		Range	Average	Decrease	No change	Increase
1	3/27	0.000	0.000		1 (herd)	
2	3/31-4/4	0.02- .19	.094	32		
		.000	.000		3	
3	4/25-5/13	.07- .35	.195	143		
4	6/5-6/16	.01- .20	.066	45		
		.000	.000		12	
		— .01- .07	— .040			11
5	7/22-7/26	.03- .22	.086	30		
6	8/6	.000	.000		3	
7	8/8	.01- .08	.045	5 (herd)		
		.000	.000		1 (herd)	

total solids, and specific gravity during twenty-four consecutive milkings of three cows. The presence of apparent glucose seems to be independent of any of the other characteristics of the milk which were measured.

TABLE II
APPARENT GLUCOSE CONTENT AND OTHER CHARACTERISTICS OF COW'S MILK
Cow No. E-7

Milking number	Glucose, %	Lactose, %	Yield, kg.	Fat, %	Solids, %	Specific gravity
1	0.02	4.82	4.700	2.80	11.70	1.0325
2	.08	4.61	4.582	3.18	11.76	1.0336
3	.09	4.65	4.481	2.62	11.90	1.0345
4	.05	4.76	5.245	3.80	12.20	1.0318
5	.00	4.81	4.700	3.07	11.97	1.0325
6	.13	4.75	4.770	3.40	12.27	1.0330
7	— .06	4.81	5.075	3.10	11.74	1.0330
8	.20	4.61	4.975	3.44	12.15	1.0330
9	.05	4.76	5.165	2.85	11.66	1.0340
10	.03	4.90	4.907	3.24	12.52	1.0350
11	.00	4.77	5.236	2.90	12.08	1.0325
12	.07	4.87	5.083	3.20	13.19	1.0335
13	.11	4.76	5.243	3.55	12.25	1.0350
14	.05	4.82	4.819	3.14	12.80	1.0350
15	.05	4.63	5.434	2.87	12.45	1.0330
16	.12	4.73	4.750	3.15	13.02	1.0340
17	.00	4.82	4.854	2.79	11.83	1.0350
18	.04	4.77	4.340	2.85	12.70	1.0340
19	.06	4.78	5.110	3.19	12.81	1.0340
20	.01	4.87	4.313	3.30	13.04	1.0350
21	.00	4.78	3.365	2.77	11.86	1.0340
22	.10	4.53	1.689	2.37	11.34	1.0350
23	.00	4.68	2.686	5.40	15.25	1.0340
24			3.120			1.0345

TABLE II (Continued)

Cow No. E-8						
Milking number	Glucose, %	Lactose, %	Yield, kg.	Fat, %	Solids, %	Specific gravity
1	-0.04	4.78	5.401	2.74	11.18	1.0325
2	.09	4.48	4.813	2.44	10.57	1.0333
3	.00	4.42	5.456	2.25	10.72	1.0335
4	.03	4.39	5.436	2.80	11.04	1.0305
5	.00	4.41	5.534	2.32	10.60	1.0315
6	.09	4.30	5.297	2.62	10.05	1.0315
7	-.07	4.30	5.493	2.29	10.10	1.0308
8	.07	4.30	5.145	2.84	11.01	1.0327
9	.03	4.20	6.000	2.40	10.40	1.0320
10	.00	4.29	5.585	2.82	11.28	1.0318
11	.06	4.17	6.210	2.42	10.28	1.0315
12	.04	4.32	5.944	2.20	12.25	1.0330
13	.05	4.28	6.530	2.95	10.86	1.0330
14			5.800			
15	.00	4.28	5.865	2.00	10.76	1.0320
16	.02	4.30	5.890	2.75	11.28	1.0320
17	.02	4.30	6.256	2.38	11.07	1.0320
18	.03	4.30	5.810	2.48	11.48	1.0320
19	.02	4.25	6.250	2.45	11.37	1.0320
20	.00	4.30	5.826	2.40	10.58	1.0320
21	-.02	4.27	7.086	2.36	10.58	1.0322
22	-.02	4.27	5.563	2.32	10.61	1.0332
23	.02	4.28	5.587	2.11	10.38	1.0345
24	-.08	4.44	5.425			1.0342
Cow No. E-9						
1	0.06	4.68	5.191	3.70	11.90	1.0334
2	.01	4.77	5.056	3.36	11.96	1.0330
3	-.02	4.79	5.310	3.78	13.12	1.0335
4	-.02	4.74	5.700	4.00	12.68	1.0310
5	.16	4.58	5.478	3.30	10.50	1.0312
6	-.03	4.77	6.050	3.88	12.72	1.0330
7	.02	4.66	6.100	4.44	11.90	1.0325
8	.08	4.74	6.095	4.01	12.37	1.0335
9			6.082			
10	-.03	4.64	6.034	3.78	12.50	1.0315
11	-.01	4.67	6.100	3.74	12.47	
12	.00	4.72	6.700	3.45	12.76	1.0350
13			6.160			
14	.10	4.62	5.783	3.62	13.07	1.0330
15	.11	4.44	6.015	3.20		
16	.05	4.51	6.040	3.09	12.18	1.0330
17	.17	4.32	6.063	2.52	11.12	1.0325
18	.05	4.55	5.554	3.65	13.00	1.0330
19	.04	4.39	6.252	2.90	13.00	1.0320
20	.09	4.53	5.800	3.25	12.20	1.0330

TABLE II (Concluded)

Milking number	Glucose, %	Lactose, %	Yield, kg.	Fat, %	Solids, %	Specific gravity
21	.17	4.50	6.565	3.11	11.76	1.0335
22	.04	4.78	5.641	3.87	12.82	1.0345
23	.00	4.74	6.444	3.01	11.86	1.0350
24	.01	4.77	5.680			1.0340

Summary

A method for estimating small amounts of glucose in milk has been developed, which depends on selective fermentation of the glucose and measurement of the rotary power of fermented and unfermented milk.

Changes in rotary power corresponding to glucose percentages varying from zero to 0.35% of glucose have been found in normal cow's milk.

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[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

THE IDENTIFICATION OF PHENOLS

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Reagents which have been recommended for the identification of phenols are diphenylcarbamine chloride,¹ *p*-nitrobenzyl bromide² and 3,5-dinitrobenzoyl chloride.³ Since none of these reagents in the hands of the inexperienced student has given entirely satisfactory results, the characterization of phenols by the use of chloro-acetic acid has been developed in this Laboratory.

Chloro-acetic acid reacts smoothly with phenols in aqueous sodium hydroxide giving good yields of the sodium salts of aryloxyacetic acids. The acids themselves are crystalline solids easily purified by recrystallization from water. One gram of a phenol furnishes an amount of the derivative sufficient for the determination of its neutral equivalent, often a valuable aid in identification, in addition to its melting point.

The experimental procedure is quite simple. To a mixture of 1.0 g. of the phenol with 3.5 ml. of 33% sodium hydroxide is added 2.5 ml. of a 50% chloro-acetic acid solution; if necessary, a little water is added to dissolve the sodium salt of the phenol. The test-tube containing the solution is stoppered loosely and heated for one hour in a gently boiling water-bath. The solution is cooled, diluted, acidified to congo red with a mineral acid, and extracted once with ether. The ether extract is washed once with a little water, and the aryloxyacetic acid is removed by washing with dilute

¹ Herzog, *Ber.*, **40**, 1831 (1907).

² Reid, *THIS JOURNAL*, **39**, 304 (1917); Lyman and Reid, *ibid.*, **42**, 615 (1920).

³ Brown and Kremers, *J. Am. Pharm. Assocn.*, **11**, 607 (1922).