LXVIII. THE IODIMETRIC METHOD OF DETERMINING LACTOSE IN MILK.

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IODIMETRIC determination of an aldehyde was first carried out by Romijn [1897], who utilised the oxidation of formaldehyde to formic acid by iodine in alkaline solution for this purpose. The application of the method to aldoses was investigated by Willstätter and Schudel [1918], who found that reaction proceeded quantitatively only when (1) the iodine added was twice the amount required theoretically; (2) the alkali was $1\frac{1}{2}$ times the equivalent of the iodine added; (3) the reaction was terminated in 15–20 minutes.

The quantitative conditions have been confirmed by subsequent workers, but there has been disagreement as to the time required to complete the reaction. Willstätter and Schudel's data were with reference to aldoses in a concentration of 1 %: Judd [1920] also found 15–20 minutes necessary for the reaction, but Baker and Hulton [1920] found that 5 minutes sufficed.

My own findings are that 5 minutes suffice for 0.5 to 1 % solutions of aldoses, whilst 20 minutes are required for 0.1 % solutions. Macleod and Robison [1929] obtained a similar result for 0.1 % aldose solutions.

Application to lactose in milk.

This was first carried out by Kolthoff [1923], who clarified the milk with freshly precipitated zinc ferrocyanide. The choice of a suitable clarifying agent is a matter of great importance in the iodimetric method, and I have found the ordinary precipitants (alumina cream, acid mercuric nitrate) unsuitable owing to fluctuations caused in the results by the presence of excess reagent in the filtrate. I finally adopted "colloidal iron" (B.D.H.), added in the ratio of 7 cc. to 10 cc. milk. It was added drop by drop from a burette, with constant stirring of the milk. The resulting mixture filtered rapidly through coarse filter-paper (Chardin), and contained no inorganic constituent derived from the clarifying reagent.

Accuracy of iodimetric method.

(1) *Comparison with gravimetric copper method.* Using the O'Sullivan [1909] modification of the gravimetric estimation of lactose the following results were obtained.

No. of sample milk	% lactose	
	Iodimetric	Gravimetric
1	5.09	5.10
2	5.13	5.19

(2) *Comparison with polarimetric method.* Kolthoff found the iodimetric method to give higher results than the polarimetric. This discrepancy is usually assumed to be due to non-sugar substances in milk-serum reacting with iodine and giving too high a result. This assumption has not been proved, and it is interesting that a similar discrepancy between polarimetric and gravimetric methods in the case of human milk lactose has been recently explained by the presence of two aldoses other than lactose, *i.e.* gynolactose (laevorotatory) and allolactose (dextrorotatory). The presence of these sugars would clearly produce a higher result by reduction methods than by optical methods [Polnovski, Michel and Lespagnol, 1931].

(3) *Comparison with chloramine-T method.* The chloramine-T method has been fully investigated by Hinton and Macara [1927], and has been favourably commented on by the Milk-Products Sub-Committee to the Standing Committee on uniformity of analytical methods [1930].

Hinton and Macara considered their method superior to the iodimetric one, which was stated to be less accurate because (1) it is more difficult to control owing to the rapidity of the reaction (5 minutes as compared with 1–1½ hours for the chloramine-T method); (2) non-sugar substances are more liable to be oxidised by the alkaline iodine solution.

No comparative figures were given to substantiate these criticisms and my own conclusions, based on the following results, show that the two methods agree very closely, and that any oxidation of non-sugars occurs in the chloramine-T rather than the iodimetric method.

In comparing the two methods the following techniques were adopted.

Iodimetric method. 50 cc. milk in a litre beaker are diluted with 250 cc. distilled water and treated from a burette with 35 cc. "colloidal iron," at the rate of a few drops a second, mixing the contents constantly by a whirling movement; 165 cc. distilled water are added and the mixture filtered through coarse filter-paper. The filtrate is a 10 % milk-serum.

To 25 cc. of the filtrate in a conical flask are added 25 cc. *N*/10 iodine and 37.5 cc. *N*/10 NaOH. The solution is shaken and after 5 minutes treated with 37.5 cc. *N*/10 H₂SO₄ and titrated with *N*/20 sodium thiosulphate.

25 cc. *N*/10 iodine are titrated with *N*/20 sodium thiosulphate. If the difference between the two titrations = *X* then % lactose in milk-serum = 0.036 *X* (% in milk = 0.36 *X*).

Chloramine-T method. To 25 cc. milk-serum prepared as above are added 3 cc. NaOH, 20 cc. 10 % KI and finally 50 cc. *N*/20 chloramine-T: after 1½ hours the solution is treated with 10 cc. 2*N* HCl and titrated with *N*/20 sodium thiosulphate.

Similar reagents are added to a control flask containing 25 cc. distilled

water. After $1\frac{1}{2}$ hours the solution is acidified and titrated as before. If the difference between the two titrations = X cc. then lactose % = $0.036 X$.

No. of milk sample	% lactose	
	Iodimetric method	Chloramine-T
1	4.95	4.97
2	5.00	4.99
3	5.22	5.27
4	5.25	5.28
5	5.27	5.33
6	5.06	5.15
7	5.30	5.40
8	5.20	5.24
1 g. lactose added to 100 cc. of sample 8	6.20	6.30

Range of accuracy of iodimetric method. 1 % hydrated lactose solution was accurately prepared and varying quantities were taken from 5 cc. up to 25 cc., distilled water being added in each case to make the volume up to 25 cc.

The solutions were then analysed for lactose by the method already described, and the following results obtained:

Vol. of 1 % lactose taken (cc.)	Water added (cc.)	cc. N/20 iodine absorbed in 5 mins. (X)	% lactose $0.036 X$	Theoretical result	Error (%)
25.0	0.0	26.50	0.954	1.0	-4.6
22.5	2.5	24.35	0.877	0.9	-2.6
20.0	5.0	22.00	0.792	0.8	-1.0
17.5	7.5	19.25	0.693	0.7	-1.0
15.0	10.0	16.60	0.598	0.6	-0.3
12.5	12.5	14.00	0.504	0.5	+0.8
10.0	15.0	11.15	0.401	0.4	+0.3
7.5	17.5	8.40	0.302	0.3	+0.7
5.0	20.0	5.70	0.205	0.2	+2.5
0.0	25.0	0.00	—	—	—

Hence between the limits of 0.3 % and 0.8 % lactose there is a maximum error of 1 %. Milk-lactose has a value ranging from 4 % to 6 %, and therefore the 10 % serum obtained from milk usually varies between 0.4 % and 0.6 % in its lactose content. Hence the iodimetric method is admirably adapted to the estimation of lactose in milk when a maximum error of 1 % is not objected to. When a result exceeding 8 % is obtained, the 10 % serum should be diluted with an equal volume of water and the resulting 5 % serum analysed.

CONCLUSIONS.

1. The iodimetric method of estimating lactose in milk gives results closely agreeing with the gravimetric copper method.

2. It is at least as accurate as the chloramine-T method, and is much more convenient when a rapid estimation is required, the reaction taking 5 minutes as against $1\frac{1}{2}$ hours in the chloramine-T method.

3. "Colloidal iron" is the best clarifying agent to use for the iodimetric method.

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