CCLII. THE USE OF SOME MICRO-ORGANISMS IN SUGAR ANALYSIS.

II. THE QUANTITATIVE DIFFERENTIATION OF FRUCTOSE AND MANNOSE.

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IN a previous paper [Harding & Nicholson, 1933] a system of carbohydrate analysis by the use of certain micro-organisms was described. The method made possible the quantitative analysis in dilute solutions of mixtures of glucose, galactose, sucrose, maltose, lactose and fructose and/or mannose. Unfortunately the differentiation of fructose from mannose was not possible by the use of the biological reagents employed at that time.

Kendall [1923] reported *Micrococcus tetragenus* (*Gaffkya tetragena*) as producing acid from glucose, fructose and galactose but not from mannose, lactose or saccharose.

*Gaffkya tetragena*¹ was found to be suitable for use as an agent for the removal of glucose and fructose. By the use of *G. tetragena*, *P. vulgaris* and *M. krusei* it was possible to differentiate glucose, fructose and mannose.

The use of G. tetragena in the estimation of fructose and mannose.

The experimental methods used were the same as those used in the previous paper [Harding & Nicholson, 1933].

Fermentative properties. *G. tetragena* ferments glucose, fructose and galactose with the production of acid but no gas. The fermentation of galactose is very slow, but little acid being produced at the end of 72 hours' fermentation.

Removal power. 1 g. wet weight of the packed organisms removed 1 mg. of glucose or fructose from 10 ml. of solution. No removal of mannose, maltose, sucrose, lactose, arabinose or xylose occurs under the conditions employed. Although the organism ferments galactose, no removal of this sugar by *G. tetragena* takes place during the incubation period used for the removal of glucose and fructose.

Culture. The bacteria are grown on Hartley's broth for 23 hours at 38°, then transferred to the surface of a neutral 2% glucose-glycerol agar and incubated at 38° for 48 hours. The methods for separating, washing and keeping the cultures of *G. tetragena* are the same as those used for *P. vulgaris* [Harding & Nicholson, 1933].

Estimation of fructose. 2.0 ml. of a 50% suspension of *G. tetragena* are placed in an aluminium tube and centrifuged in the Lundgren angle centrifuge. The supernatant fluid is poured off and the adherent moisture removed from the tube by filter paper. The solution to be tested is adjusted to pH 6.5-7.0 and 10 ml.,

¹ Obtained from the American Type Culture Collection, the John McCormick Institute for Infectious Diseases, Chicago.

containing not more than 1.0 mg. of glucose and fructose, are added to the tube, the bacteria are thoroughly mixed with the solution by means of a thin glassrod and the mixture is incubated at 38° for 30 min. The organisms are then removed from the solution, by the methods described for *P. vulgaris* [Harding & Nicholson, 1933]. Ammonium salts if present are removed as $\text{Mg}(\text{NH}_4)\text{PO}_4$ by the addition of KH_2PO_4 and MgO [Harding & Downs, 1933]. The reducing value of the solution before and after the action of *G. tetragena* is determined using the requisite water controls. In the absence of glucose the difference between the total and residual sugar represents fructose. In the presence of glucose the removal figure represents the sum of the glucose and fructose. The glucose is then determined [Harding & Nicholson, 1933] and the difference between the reducing value of the sugars removed by *G. tetragena* and the reducing value of the glucose represents fructose.

Estimation of mannose. Since *G. tetragena* removes glucose and fructose and *M. krusei* removes glucose, fructose and mannose [Harding & Nicholson, 1933], the reducing value of the mannose present in a solution may be obtained by subtracting the reducing value of the residual sugar left after the action of *M. krusei* from that left after the action of *G. tetragena*. The details of the analysis of a known mixture of glucose, fructose and mannose are shown in Table I.

G. tetragena can be used directly on Folin-Wu blood filtrates, in the same manner as outlined above for aqueous solutions of glucose, fructose and mannose.

Table I. *Determination of glucose, fructose and mannose.*

Amounts taken

5 mg. of glucose in 100 ml. solution:	2 ml. require	0.86 ml. 0.005 N iodine
5 mg. of fructose	" "	0.84 ml. "
5 mg. of mannose	" "	0.42 ml. "
Total reduction: 2 ml. require		2.12 ml. "

ml. 0.005 N I
for 2.0 ml. solution

Total reduction found	2.10
Reduction after <i>P. vulgaris</i>	1.26
$2.10 - 1.26 = 0.84 = 4.88$ mg. glucose removed by <i>P. vulgaris</i>	
Reduction after <i>G. tetragena</i>	0.43
$1.26 - 0.43 = 0.83 = 4.94$ mg. fructose removed by <i>G. tetragena</i>	
Reduction after <i>M. krusei</i>	0.02
$0.43 - 0.02 = 0.41 = 4.82$ mg. mannose removed by <i>M. krusei</i>	

The amounts of wet washed organisms used and the time of incubation are those given in Table I [Harding & Nicholson, 1933] and the paragraph "Removal power" in this paper.

* Data from Table I [Harding & Downs, 1933.]

The estimation of fructose and mannose added to urine. Although *G. tetragena* cannot be used on urines cleared with Lloyd's reagent, basic lead acetate or $\text{HgSO}_4\text{-BaCO}_3$ [West *et al.*, 1929] as the action of the organisms produced a definite increase in the reducing value of the filtrates, satisfactory results may be obtained if the urines are cleared with basic lead acetate followed by $\text{HgSO}_4\text{-BaCO}_3$ [West *et al.*, 1929] and the analysis carried out on solutions of the sugars precipitated from such filtrates by a modification of Salkowski's [1879] copper-lime method [Archibald, 1935; Harding *et al.*, 1936].

Table II shows the recovery of added fructose and mannose from fasting human urines treated in the manner just described, the sugars having been added to the urines before the clearing processes were started.

Table II. *The recovery of fructose and mannose added to "fermented" urine specimens.*

Two-hour fasting specimens were treated with *S. marxianus* to remove the "fermentable sugar". A sufficient amount of a solution containing 100 mg./100 ml. of fructose and 200 mg./100 ml. of mannose to give the desired percentage of sugar was then added. This urine + sugar was taken as the urine specimen and dilutions of the final solution were calculated to it.

Urine	Dilution of final solution	Sugar in final solution, mg./100 ml.					
		Sugar added to original urine, mg./100 ml.		Fructose		Mannose	
		Fructose	Mannose	Theoretical	Estimated	Theoretical	Estimated
1	1:0.61	2.5	5	4.0	3.9	8.0	7.8
2	1:1.66	7.7	15.5	4.7	4.6	9.3	9.6
3	1:1.17	2.5	5	2.2	2.1	4.3	4.2
4	1:1	5	10	5.0	5.1	10.0	10.4

SUMMARY.

The use of *G. tetragena* affords a ready means of separating fructose and mannose when present in dilute solutions.

Combining its use with that of *P. vulgaris* and *M. krusei* it is possible to analyse mixtures of glucose, fructose and mannose and to recover added fructose and mannose from blood and urine filtrates.

The use of *G. tetragena* necessitates a slight revision in the scheme of analysis for sugar mixtures published by Harding and Nicholson [1933].

A summary of the steps in the revised analysis follows:

- A. Estimate glucose, fructose and mannose by *M. krusei*.
- B. Estimate galactose in residual fluid from A by *S. marxianus*.
- C. Estimate glucose and fructose by *G. tetragena*

A - C = mannose.

- D. (In absence of galactose) estimate glucose by *P. vulgaris*

C - D = fructose.

- E. (In presence of galactose) estimate fructose and mannose in residual fluid from D by *M. krusei*

A - E = glucose; E - mannose = fructose or C - glucose = fructose.

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