		Purity from dry substance by		
No.	Per cent. sugar.	Drying.	Refractometer.	Spec. gravity.
5503	44.6	58.18	59.27	57.29
5504	39.0	51.05	51.66	49.87
5505	44.0	57.77	58.95	...
5506	47.9	64.66	65.76	...
5507	32.2	42.52	43.20	41.15
5508	46.7	62.14	63.20	...
5509	26.9	37.65	38.35	...

Purity of *beet molasses*, coming from using actual dry substance and dry substance by refractometer in half dilution, also Brix from specific gravity.

		Purity from dry substance by		
No.	Per cent. sugar.	Actual drying.	Refraction.	Spec. gravity.
4685	42.09	60.92	61.28	56.4
4686	50.54	61.23	61.78	58.3
4633	48.15	67.00	66.50	...
4634	52.15	67.50	67.12	...
4637	52.56	65.03	64.81	62.0
4638	48.90	62.83	63.34	...
4649	50.15	63.39	64.30	...
4650	49.94	66.10	65.80	...
4652	45.96	61.94	62.36	...
4682	51.43	64.03	65.34	...
4685*a*	39.39	57.31	57.34	...
4686*a*	50.77	61.65	62.06	59.9
4812	41.57	57.17	57.82	...
4813	52.19	66.31	67.08	57.4
5579	48.20	59.98	59.80	56.5

The purity by Brix determination is much lower than by the other two methods of determination. This fact is well known by all sugar chemists and introduces considerable error into factory control work. With the use of the refractometer, theoretically this error will be present to a slight extent, but in actual practice, I doubt whether there would be as large an error using dry substance by the refractometer as when using dry substance by actual drying, because the methods for actual drying as practiced are so crude.

[CONTRIBUTIONS FROM THE HAVEMEYER LABORATORIES OF COLUMBIA UNIVERSITY, No. 156.]

THE DETECTION AND IDENTIFICATION OF CERTAIN REDUCING SUGARS BY CONDENSATION WITH *p*-BROMBENZYLHYDRAZIDE.

BY E. C. KENDALL AND H. C. SHERMAN.

Kahl[1] investigated the action of various hydrazides upon reducing

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[1] Ueber die Paarung von Säurehydraziden mit Zuckerarten. Inaug. Diss. Fireburg, 1904.

sugars. The hydrazides of *p*-brom- and *p*-chlorbenzoic, and of salicylic and palmitic acids were tested and the results obtained with the first of these seemed to us to warrant a further study of its behavior with special reference to its possible use in the detection and identification of reducing sugars.

The hydrazide is prepared by heating on a water bath a mixture of 10 grams of the ethyl ester of *p*-brombenzoic acid, 8.2 grams of a 50 per cent. water solution of hydrazine hydrate, and 12 cc. of 95 per cent. alcohol. After 4 hours' heating, the excess of alcohol is distilled off and the residue poured into a beaker and allowed to cool and crystallized into a solid mass. In our work the hydrazine hydrate was prepared from the sulphate as required and the crystals obtained as above were drained on a Büchner funnel, washed with ether and recrystallized from alcohol, when a pure product was obtained. The hydrazide crystallizes in white needles melting at 164°. It is soluble in alcohol, slightly soluble in hot water and in ether; insoluble in cold water and chloroform.

Kahl carried out his condensations in alcohol and in acetic acid solutions. In the former case he employed 3.8 grams of sugar, 4 grams of the hydrazide, 45 cc. of alcohol. On boiling for 2 hours a white paper-like crust of hydrazone separated upon the flask at the surface of the liquid. In this way were formed the hydrazones of glucose, galactose, mannose, and arabinose. Xylose hydrazone was also obtained, but the yield was very small. No corresponding product could be obtained from levulose, maltose or lactose.

The hydrazide, therefore, appeared to react only with mono-aldose sugars.

In the case of acetic acid solutions Kahl used 4.2 grams of sugar in 15 cc. of water, 5 grams of hydrazide, and 25 cc. of acetic acid. The sugar solution was placed in a test tube and the hydrazide added, then by addition of acetic acid the hydrazide was brought into solution and the mixture was allowed to stand for 12 hours at room temperature.

The object of the present investigation was to ascertain whether the reaction of *p*-brombenzylhydrazide is sufficiently sensitive and characteristic with small amounts of sugars to make this a useful means of detection and identification.

In confirmation of Kahl's work the reaction was carried out as described above both in alcohol and in acetic acid solutions. The glucose hydrazone obtained, after purification with boiling alcohol, melted at 206–207° (uncorr.). It was slowly soluble in boiling water and in cold glacial acetic acid. Its solubility in acetic acid appeared to be proportional to the concentration of the acid. Since acetic acid was thus found to hold the hydrazone in solution, only alcohol was used as a solvent in the subsequent experiments.

Experiments with Pure Sugars.

Glucose.—Thirty mg. glucose, 60 mg. hydrazide and 10–15 cc. of alcohol were heated in a test tube and allowed to evaporate to dryness on a water bath. Addition of alcohol and evaporation to dryness were repeated three or four times to facilitate the reaction. There resulted a white crust of hydrazone on the bottom and sides of the test tube, which also contained excess of hydrazide and probably some unchanged sugar. Many experiments were made in which different solvents were tried, in the attempt to dissolve away the hydrazide and sugar without dissolving the hydrazone, with the result that a mixture of water and chloroform was found to give the best results. Boiling chloroform will dissolve the hydrazide up to 30 mg. per cc. without exerting any appreciable solvent action upon the hydrazone. Water at 100° dissolves, with the unchanged sugar, a considerable amount of the hydrazone, but water at 61° (the boiling point of chloroform) does not dissolve the hydrazone even when the latter is present in very small quantity. Hence in order to use this hydrazide condensation as a working test for the detection of a reacting sugar, it is only necessary to add to the test tube containing the dry residue above mentioned, about 5–6 cc. of chloroform and 4–5 drops of water and heat with thorough shaking until the chloroform boils. Under this treatment the crust is detached and more or less disintegrated, the residual hydrazide and sugar removed, and the hydrazone remains suspended in the liquid as flakes or crusty particles.[1]

Galactose, mannose, and arabinose gave positive results under the same treatment, while *maltose and lactose* yielded clear solutions and *levulose* only rendered the water layer milky without yielding any distinct flakes or crust of hydrazone.

Of the sugars which react, only small amounts are required for detection by this method. Positive tests were obtained with the following quantities: Glucose, 5 mg.; mannose, 15 mg.; galactose, 1.5 mg.; arabinose, 1 mg. This condensation, therefore, while not so general a test as the ordinary osazone reaction, compares favorably with it in delicacy in certain cases.

Experiments with Sugar Mixtures.

In view of the interference of maltose and lactose with the formation and precipitation of glucosazone,[2] it was of interest to determine whether

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[1] If a large excess of hydrazide is present, some of this may separate *on cooling*, but dissolves again on heating to the boiling point of the chloroform.

[2] Sherman and Williams, This Journal, **28**, 629.

the reaction of glucose with the hydrazide would be influenced by the presence of sugars which do not react.

When a mixture of glucose with four times its weight of maltose, lactose, or levulose was tested with hydrazide in alcoholic solution and the alcohol evaporated as described above, there resulted, instead of a flaky crust, a sirupy mass which on addition of 4–6 drops of water dissolved and on heating with water and chloroform showed no flakes of hydrazone even though there had been used as much as 30 mg. of glucose, which in pure solution would have been sufficient to yield a heavy flaky precipitate of hydrazone. The same amount of glucose in the presence of twice its weight of maltose, lactose, or levulose, usually gave indecisive results; with an equal weight of any one of these non-reacting sugars the precipitate of glucose-hydrazone could be obtained, but was not nearly so abundant or characteristic as when the same weight of glucose was tested alone.

Identification of Reacting Sugars.

Glucose, galactose, mannose and arabinose, either individually or when present together, give flakes of hydrazone insoluble in the chloroform-water mixture. In seeking a means of distinguishing between the hydrazones of these four sugars it was found that when not more than 30 mg. of glucose were tested as described above the hydrazone formed was completely dissolved when pulverized and boiled for 5 minutes with 20 cc. of 95 per cent. alcohol. Mannose and arabinose under the same conditions gave hydrazones which were partly soluble, while that of galactose was insoluble.

Galactose can therefore be distinguished by the insolubility of its hydrazone in boiling alcohol under the conditions obtaining in these experiments.

It was further found that the three remaining sugars might be distinguished under favorable conditions as follows: To the mixture of chloroform and water containing the flakes of hydrazone resulting from the test as described above, add alcohol until the chloroform and water mix to form a homogeneous liquid; now boil the mixture and the glucose hydrazone present will completely dissolve if not more than 20 to 30 mg. of glucose were present; the hydrazone of arabinose remains insoluble under this treatment; that of mannose is partly dissolved. Hence a hydrazone which under these conditions dissolves completely represents only (or possibly a very small amount of mannose); one which is insoluble, arabinose; one which is partly soluble, mannose or a mixture.

These solubility-differences may be tabulated as follows:

Treat 30 mg. sugar with 60 mg. hydrazide in alcoholic solution; evaporate to dryness 4 times; add 20 cc. alcohol and boil:

Hydrazone insoluble *Galactose.*	Hydrazone partly dissolved *Mannose, arabinose.*	Hydrazone dissolved *Glucose.*
	Filter, evaporate off the alcohol and take up with chloroform and water. Add alcohol till solvents mix; boil:	

Hydrazone insoluble *Arabinose.*	Partly dissolved *Mannose.*	Dissolved *Glucose.*

It is probable that the behavior here described and utilized for identification of the reacting sugars could not be depended upon, if the conditions were varied too greatly from those given. If several times the required amount of hydrazide were used, the excess might not be completely dissolved by the 5 cc. of boiling chloroform and the insoluble portion might be mistaken for hydrazone, and if the test as here described were applied to much more than 30 mg. of glucose the hydrazone would not be completely soluble in alcohol under the conditions suggested above.

Summary.

In confirmation of Kahl it is found that *p*-brombenzylhydride condenses to form an insoluble hydrazone with glucose, galactose, mannose, and arabinose; not with levulose, maltose or lactose; and to only a slight extent with xylose.

When one of the reacting sugars is mixed with twice its weight of hydrazide (say 30 and 60 mg. respectively) and evaporated to dryness three or four times in alcohol solution and the resulting residue is taken up with boiling chloroform and a little water (at the same temperature) in the manner and within the limits of quantity above described, characteristic flakes of hydrazone insoluble in the chloroform-water mixture are obtained.

The non-reacting sugars interfere with the formation and separation of the glucose hydrazone to an extent comparable with the interference of maltose and lactose in the ordinary osazone reaction for glucose.

The hydrazones formed by glucose, galactose, mannose and arabinose differ markedly in their solubilities in alcohol whether treated directly in the dry state or while suspended in the chloroform-water mixture. Under carefully regulated conditions these differences in solubility may suffice for the identification of the particular reacting sugar present.

Since condensation with *p*-brombenzylhydrazide shows new differ, ences in behavior among the reducing sugars and since, as here developed- it constitutes a qualitative test, which for certain of the reacting sugars compares favorably in delicacy with the reactions in common use, it may readily prove a useful addition to the existing methods for the detection and identification of the reducing sugars.

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