Free from glucose: Determine by accurate rotation. Levulose when made from inulin should be uncontaminated with glucose; when made from invert sugar, glucose is removed only with the greatest difficulty

MALTOSE (HYDRATE):

Disaccharide, $C_{12}H_{22}O_{11} + H_2O$

Specific rotation $[\alpha]_D^{20}$	+131° (26 g. per 100 cc.)
Ash.....	0.05 per cent
Moisture.....	0.15 per cent

Free from dextrin: Determine by accurate rotation. Free from glucose: Test with Barford's solution, which is not reduced to any extent by maltose *d*-MANNITOL:

Hexahydric alcohol, $C_6H_{12}O_6$

Specific rotation (with borax) $[\alpha]_D^{20}$	+22.5° (10 g. mannitol + 12.8 borax per 100 cc.)
M. p.....	166.5°
Ash.....	0.05 per cent
Moisture.....	0.10 per cent

Free from reducing sugars

d-MANNOSE:

Aldohexose, $C_6H_{12}O_6$

Specific rotation $[\alpha]_D^{20}$	+14.25° (10.000 g. per 100 cc.)
Ash.....	0.05 per cent
Moisture.....	0.10 per cent

PERBITOL:

Heptahydric alcohol, $C_7H_{14}O_7$

Rotation (0.4 g. substance in 5 cc. saturated borax solution in a 1-dm. tube) $[\alpha]_D^{20}$	+ 0.39°
M. p.....	188° C.
Ash.....	0.10 per cent
Moisture.....	0.10 per cent

RAFFINOSE (HYDRATE):

Trisaccharide, $C_{18}H_{32}O_{16} + 5H_2O$

Specific rotation $[\alpha]_D^{20}$	+104.5° (26.00 g. per 100 cc.)
Ash.....	0.10 per cent
Moisture.....	0.15 per cent

RIHAMNOSE (HYDRATE):

Methyl pentose, $C_6H_{12}O_5 + H_2O$

Specific rotation $[\alpha]_D^{20}$	+8.37° (6.948 g. per 100 cc.)
Ash.....	0.05 per cent
Moisture.....	0.10 per cent

SORBITOL:

Hexahydric alcohol, $C_6H_{12}O_6$

M. p. (1 molecule H_2O)	55°
(0.5 molecule H_2O)	75°
(anhydrous)	110°
Ash.....	0.05 per cent
Moisture.....	0.15 per cent

Free from reducing sugars

SUCROSE:

Disaccharide, $C_{12}H_{22}O_{11}$

Specific rotation $[\alpha]_D^{20}$	+66.5° (4.275 g. per 100 cc.)
Ash.....	0.05 per cent
Moisture.....	0.05 per cent

Free from reducing sugars

XYLOSE:

Aldopentose, $C_5H_{10}O_5$ (called by Fischer *L*-xylose, more properly *d*-xylose.)

Specific rotation $[\alpha]_D^{20}$	+18.5° (10.230 g. per 100 cc.)
Ash.....	0.05 per cent
Moisture	0.10 per cent

The Standardization of Rare Sugars¹

By Howard T. Graber

RESEARCH LABORATORY, DIGESTIVE FERMENTS CO., DETROIT, MICHIGAN

It is not the object of this paper to present anything new in discussing the standardization of sugars, but rather to apply our present knowledge and arrange it as a systematic procedure.

ROUTINE ANALYTICAL PROCEDURE

In the routine examination of all sugars in this laboratory, particular attention is paid to sampling to insure the selection of a sample representative of the whole lot. Since all of the rare sugars are made in relatively small quantities and receive a very thorough mixing after coming from the vacuum driers, sampling is not a difficult procedure.

The properly labeled samples are brought to the laboratory in 1-oz., amber, salt-mouth bottles, tightly stoppered with a paraffined cork. The labels bear the name, date of manufacture, and Rx. number, and a 3 × 5 index card accompanies the sample, which passes to the filing clerk who registers it in the record book kept for that purpose, the necessary assays to be made are placed on the card, and the sample and card then go to the chemist who makes the assays.

The routine analysis comprises a determination of specific rotation, color of the powder, moisture, ash, and solubility, as well as a determination of the presence of alcohol, chlorides, sulfates, calcium, barium, and heavy metals, and finally the reaction to the three indicators—litmus, methyl red, and phenolphthalein.

SOLUBILITY—In making the solubility test, a 30 per cent solution of the sugar in recently boiled and cooled neutral distilled water is used for sucrose, lactose, and dextrose, and comparison is always made with a standard dextrose of extreme purity. In this solubility test a notation is made of:

1—The color of the solution, which must be water-white.

2—The clearness, which must be brilliant without the slightest trace of haze.

3—The absence of foreign matter.

For the rarer sugars, a 10 per cent solution is used as above.

SPECIFIC ROTATION—For the saccharimetric readings, we use the Schmidt and Haensch half-shade saccharimeter bearing the certificate of the Bureau of Standards. The rays of white light, supplied by an incandescent light with etched bulb, are filtered through a dichromate absorption cell¹, with an inner separation of the glass plate walls of 15 mm., and filled with a 6 per cent solution of $K_2Cr_2O_7$. In designing the laboratory for polarimetric measurements, as well as in choosing the necessary apparatus, the specifications given on pages 122 and 123 of Bureau of Standards Circular 44 were followed. All measurements are taken at a temperature of 20° C. In all cases the measurements must be that of the accepted constant for the sugar at hand.

The actually determined saccharimetric normal weight of sucrose² and dextrose,³ together with the calculated normal weights of lactose, maltose, raffinose,⁴ fructose and invert sugar, as determined by the Bureau of Standards, are adopted.

In the case of those sugars which show mutarotation we prefer to let the solution stand over night at room temperature to come to equilibrium, rather than to add alkali.

COLOR—In the estimation of color, comparison is always made with a highly refined sample of pure white dextrose which reads 100 Red, 95 Green, 93 Blue-Violet, on the Palo tintometer. The standard and unknown are placed side by side on a piece of plate glass, blackened on the under side with lampblack and shellac. By this means, any difference of color in the two sugars is readily detected. In cases of doubt, the sugar under examination is also read on the tintometer.

ASH—The ash is determined in fused quartz dishes. Five grams of the sugar are weighed into a previously tared quartz dish, heated carefully over a low flame until the mass

¹ Bureau of Standards, Circular 44, 20.

² *Ibid.*, Scientific Paper 268.

³ *Ibid.*, 293.

⁴ *Ibid.*, Circular 44, 81.

¹ Presented before the Section of Sugar Chemistry and Technology at the 61st Meeting of the American Chemical Society, Rochester, N. Y., April 26 to 29, 1921.

is charred and ceases to froth, and then placed in a muffle furnace, the muffle of which is quartz. The heat is evenly distributed over the bottom of the muffle by means of Fisher burners. A thermocouple, protected by a vitreous shell, rests on the bottom of the muffle, and connects with a Brown recording pyrometer. The temperature of the muffle is held at dull red heat, or between 1100° to 1200° F., for 2 hrs. The amount of sugar taken for the determination of ash is always uniform, and by means of the above equipment the temperature and time of ashing are accurately controlled. The attempt is made to have our sugars as near ashless as it is possible to make them.

MOISTURE—The moisture determinations of these rare sugars are not entirely satisfactory, on account of four factors:

- 1—The hygroscopicity of some of the sugars.
- 2—The retention of water by occlusion, especially when crystallized in large grains.
- 3—The extreme sensitiveness of a few of the sugars to decomposition at temperatures much below 100° C., notably levulose and rhamnose.
- 4—The fact that such sugars as maltose, lactose, and raffinose retain variable amounts of water of crystallization under different conditions of drying.

On this account, whether the drying is to be accomplished under vacuum or in the open, each sugar must be considered by itself.

Five grams of the sugar are accurately weighed in a tared aluminium dish, approximately 55 mm. in diameter and 15 mm. high, fitted with an airtight cover of the same material. The sugar and dish are placed in an electrically controlled drying oven and held for 1 hr. at about 50° C. with proper ventilation to carry off the moisture. After this preliminary drying the door of the oven is closed, the temperature adjusted to 100° C., and the drying continued for an additional 4 hrs., or over night.

When the sugar is to be removed from the oven it is securely covered and transferred to the desiccator, cooled and weighed as usual, replaced in the oven, dried an additional 2 hrs., weighed again, and this method is continued until the weight is constant. In the case of levulose (m. p. 95° C.) and rhamnose (m. p. 92.8° C.) the sugar must be dried over night to constant weight at about 5° below the melting point.

In this laboratory, sugars are dried in large vacuum shelf dryers for 12 to 18 hrs., with a vacuum of 29 in. and running water with thermostatic control at not over 120° F. We attempt to make the residual moisture nil.

In the drying of invert sugar sirup and similar products, the usual technique of drying upon washed and ignited quartz sand is observed. Five grams of the sirup are weighed accurately, diluted to 250 cc. in a volumetric flask, and 50 cc. of this dilution taken from the moisture assay.

IMPURITIES—In testing for impurities, the procedure is as follows:

Alcohol—When 1 cc. of iodine T.S. and 1 cc. of potassium hydroxide T.S. are added to 5 cc. of a 20 per cent solution of the sugar in distilled water, no odor of iodoform should be perceptible after shaking.

Chlorides—When 3 drops of dilute nitric acid and 10 drops of silver nitrate T.S. are added to 5 cc. of a 10 per cent solution of the sugar in distilled water, and the mixture allowed to stand after shaking, the sugar should show not more than a very slight trace of chloride.

Sulfates—When 3 drops of dilute hydrochloric acid and 10 drops of barium chloride T.S. are added to 5 cc. of a 10 per cent solution of the sugar in distilled water, the sugar should show not more than a very slight trace of sulfates.

Calcium—When to the above solution, made alkaline with

ammonium hydroxide, ammonium oxalate T.S. is added, no haziness or turbidity should develop.

Heavy Metals—When 2 cc. of hydrogen sulfide T.S. are added to 5 cc. of a 10 per cent solution of the sugar, and the mixture heated, no discoloration results. Also, when 1 g. of hops is added to 160 cc. of a 10 per cent boiling solution of the sugar and the mixture boiled for 20 min., no greenish or black color, due to iron tannate, should be formed.

REACTION—In testing the reaction of our sugars, 4 g. of the sugar are dissolved in 50 cc. of distilled water, 2 drops of phenolphthalein are added, and 1 drop of 0.1 N sodium hydroxide must change the color of the indicator, showing the absence of excess acidity.

SPECIAL TESTS

The above tests are made on all sugars. In addition there are a few special cases to be considered.

GALACTOSE—In the routine analysis of galactose, we test for the presence of barium in 5 cc. of a 10 per cent solution of the sugar.

INVERT SUGAR SIRUP—In invert sugar sirup, the determination of reducing sugars is made by the Defren-O'Sullivan method and it shows complete conversion on the dry basis. Nonreducing sugars are absent.

LACTOSE—In the analysis of lactose the sugar must be free from nitrogen and fat.

It must further show purity from dextrose by two separate tests:

1—Lack of acidity in culture media, under the influence of the *B. typhosus*.

2—Lack of gas formation in 96 hrs. incubation of 10 cc. of a 20 per cent solution of the sugar in the presence of an excess of yeast.

RAFFINOSE AND RHAMNOSE—Raffinose (5 H₂O) and rhamnose (1 H₂O) are put on the market as hydrates. Raffinose is also marketed as the anhydride.

SUCROSE—Sucrose is tested very carefully for reducing substances, according to the Bureau of Standards test,¹ with Striegler's solution. Under the condition of this test, if 10 g. of sucrose in 50 cc. of distilled water are added to 50 cc. of Striegler's solution (which has been boiled 1 min.) and the mixture boiled exactly 5 min., not more than 0.007 g. Cu₂O are precipitated. Immediately after 5 min. of boiling any possible further hydrolysis of the sucrose is eliminated by diluting the mixture with 100 cc. of cold, recently boiled, distilled water.

CONCLUSION

In conclusion it should be stated that in our work on standardizing these products, we aim at an ultimate degree of purity that will warrant the designation of "C. P." In our opinion, however, this designation should be reserved for chemicals in which present methods of analysis will reveal no traces of impurities. We cannot pretend that these rare sugars are susceptible of this perfection, except by such repetitions of crystallization that the yield is absurdly small, and the resulting cost prohibitive. Therefore we refrain from using the term C. P., although constantly striving for it. In the present uses of these products, the main consideration is that each one shall be free from other carbohydrates. This we are able to achieve with an encouraging amount of success.

If research should now be directed to finding new uses for the so-called rare sugars, especially of commercial or less purified grade, it would be a great help to the development of the C. P. line, salable at a price that is reasonable.

¹ Leach, "Food Inspection and Analysis," 594.

² Bureau of Standards, *Scientific Paper* 258, 80.