COPPER SOLUTIONS FOR MICROSCOPICAL DETECTION OF GLUCOSE AND FRUCTOSE*

ELIZABETH S. BRETZ

PROBLEM

A number of copper reagents have been proposed for distinguishing glucose from fructose. Since the distinction depends upon the relative rate of oxidation of the two sugars, the tests will necessarily be influenced by concentration of sugar, time, temperature, and concentration of reagent. For a microscopical detection of these sugars, the problem therefore is to find the temperature and time of reaction at which these sugars may be most readily distinguished when they occur at different concentrations. Methyl fructosazone may be used to distinguish glucose from fructose; but because the copper tests are much more sensitive for microscopical work, it seems important to have more definite knowledge about their possibilities and limitations.

REAGENTS

The copper reagents proposed by Ost, Pieraerts, and Benedict for macroscopical tests and the one proposed by Flückiger for microscopical tests for sugars in plant cells were employed. A brief account of the composition and of the results reported for these reagents follows.

1. *Flückiger's copper tartrate reagent* (2, p. 272). Copper tartrate is prepared by mixing a solution of 30 gm. copper sulfate in 300 cc. hot water with a solution of 70 gm. potassium sodium tartrate in 200 cc. hot water. The precipitate is filtered, dried in a desiccator, and kept in a brown bottle. For each separate test a few crystals of copper tartrate are placed on a slide in a drop of 20 per cent sodium hydroxide. When the copper tartrate has dissolved, a drop or small section of the material to be tested is added. According to Flückiger (2, p. 272), reduction by fructose occurs immediately at room temperature and by glucose very soon with slight warming.

In order to have a uniform reagent for comparative tests the procedure may be modified by preparing standard solutions. If the latter procedure is followed, the solution should be renewed daily, as autoreduction slowly occurs in the solution. Since autoreduction increases with an increase in concentration of copper tartrate in the reagent, the amount of copper tartrate present should not greatly exceed the amount necessary to oxidize the sugar in the test object.

*Papers from the Department of Botany, The Ohio State University, No. 389.

Lampe (6, p. 347), by using a "sky-blue" solution, obtained the maximum amount of cuprous oxide with fructose in 7 minutes at room temperature and with glucose in 3 minutes at 45° C. The solutions used in the present tests were 0.05 gm. copper tartrate in 10 cc. of 20 per cent sodium hydroxide and 0.05 gm. copper tartrate in 20 cc. of 20 per cent sodium hydroxide.

2. *Ost's copper carbonate reagent* (7, p. 840, footnote 2). To 700 cc. boiling water 250 gm. potassium carbonate is gradually added. To that solution 100 gm. potassium bicarbonate is slowly added until it completely dissolves. A solution of 17.5 gm. copper sulfate in 100 to 150 cc. water is then added under vigorous agitation. After cooling, the solution is made up to 1000 cc. and filtered. Klein (4, p. 779) says this reagent reduces with gentle warming only ketoses. Zerban and Sattler (8, p. 307) made macrochemical tests using Nijns' modification of the above solution. This contained 15 gm. copper sulfate instead of the 17.5 gm. used by Ost. They placed 20 cc. sugar solution—containing not over 0.3 per cent fructose—in 50 cc. of the copper reagent previously warmed in a small Erlenmeyer flask to 48.5° C. to 49° C. After 2.5 hours at that temperature the cuprous oxide obtained was weighed. Zerban and Sattler (8, p. 308) found that with about equal quantities of glucose and fructose, 1 mg. of glucose reduces about one-thirteenth the amount of copper reduced by 1 mg. of fructose. The reducing effect per mg. of glucose decreases as the concentration of glucose increases, and also as the concentration of fructose in the mixture increases.

3. *Pieraerts' modification of Ost's reagent* (7, p. 840). The solution is prepared in the same way as Ost's reagent, but the amounts of the salts are changed to 140 gm. potassium carbonate, 100 gm. potassium bicarbonate, and 15 gm. copper sulfate. The reagent deposits basic copper carbonate after 24 hours. It must be renewed weekly (7, p. 851). Tests made by Pieraerts in the cold (13° C. to 16° C.) were examined every 15 minutes during the first 6 hours, thereafter on the ninth, twelfth, and twenty-fourth hours (7, p. 837). According to Pieraerts, in the cold glucose does not reduce the reagent in 24 hours, while 4 to 5 per cent fructose reduces it in 1 hour, 3 per cent in 1.5 hours, 2 per cent in 2 hours, and 1 per cent in 2.25 hours. Pentoses cause reduction in 4 hours in the cold (7, p. 842). Tests made by Pieraerts on a water bath were examined as reduction became evident. At the end of 1 hour the tests were concluded (7, p. 837). He states that glucose gives no precipitate of cuprous oxide at 30° C., 35° C., 40° C., or 45° C. Above 50° C. there is reduction (7, p. 842). At 30° C. 1 to 5 per cent fructose reduces the reagent by the end of 1 hour. Pentoses cause no reduction at this temperature in 1 hour, but at 35° C. they do reduce the reagent in 1 hour (7, p. 843). Pieraerts summarizes (7, pp. 846-847) the use of his modification of Ost's solution by saying that it can distinguish 1 to 5 per cent free fructose in the presence of other natural sugars, pentoses excepted, by heating 1 hour at 35° C. If pentoses are present, it is still possible to identify free fructose in 2.5 hours in the cold, or in 1 hour at 30° C. One is surprised, then, to read Pieraerts' statement (7, p. 847) that the diagnosis of fructose is

impossible if less than 20 per cent of that hexose is present, especially if pentoses are also in the mixture.

4. *Benedict's copper citrate reagent* (1, p. 486). A solution is made of 173 gm. sodium citrate and 100 gm. anhydrous sodium carbonate by heating in 600 cc. water. This is filtered if necessary and made up to 850 cc. A solution of 17.3 gm. copper sulfate in 100 cc. water is diluted to 150 cc. The copper sulfate solution is poured slowly with constant stirring into the carbonate-citrate solution. The mixture is ready for use and does not deteriorate on standing. It is not caustic and may be kept in cork or glass stoppered bottles. Upon reduction the solution is apt to yield green or yellow hydrated cuprous oxide, rather than the red anhydrous oxide.

Kolthoff (5, p. 888), working with 1 cc. sugar solution in 5 cc. of Benedict's reagent, could distinguish 1 per cent fructose from glucose and lactose after heating 0.5 hour at 37° C. to 40° C., but with 0.5 per cent the distinction was doubtful. At room temperature for 24 hours 0.5 per cent fructose was distinctly evident, 0.2 per cent gave a very slight reaction, and 0.1 per cent gave no apparent test. According to Klein (4, p. 780), copper solutions containing citric or tartaric acid are extremely photosensitive. Before beginning a test it should be determined whether autoreduction has occurred.

5. *Pieraerts' copper-glycocol reagent* (7, p. 841). To a solution of 12 gm. glycocol in hot water one adds little by little 6 gm. freshly prepared cupric hydroxide, which dissolves almost completely after 15 minutes on a steam bath. The solution, cooled to 60° C., is added to 50 gm. potassium carbonate, made up of 1000 cc., and filtered. The liquid keeps very well. If the potassium carbonate is added at a temperature higher than 60° C. there will be a precipitate of carbonic anhydride, which greatly diminishes the sensitivity of the reaction.

In the preparation of cupric hydroxide for the above reagent certain precautions are necessary to prevent its immediate oxidation. Add 5 per cent of ammonium chloride to 5 per cent copper sulfate solution. Pour this into 20 per cent sodium hydroxide. Wash the precipitate on a Buchner funnel with cold water. Keep the funnel covered to prevent oxidation. Drying the precipitate in an oven will hasten oxidation. It is best to dry the precipitate on a suction filter, weigh, and use it immediately.

Pieraerts (7, p. 845) says that free fructose forms an abundant precipitate of cuprous oxide in the cold after 24 hours and is the only natural sugar which does so. The results are uncertain with heat. One cannot trust the indications at 30° C. for an hour, if more than 3 per cent fructose is present.

METHODS

The concentrations of glucose and fructose used were 0.1 per cent, 0.5 per cent, 1 per cent, 5 per cent, and 10 per cent. The three temperatures used were room temperature, 40° C., and that of a steam bath. Slides at room temperature were kept in a moist chamber to prevent drying. One drop of sugar solution was thoroughly mixed with two drops of copper reagent on a microscopic slide.

Accurate counts were made of the cuprous oxide crystals obtained in each test. A description of the method of counting is not included in this paper, since the objective is chiefly to indicate with which reagents differentiation between glucose and fructose can be obtained.

In Flückiger's reagent it was not known what proportions of copper tartrate and sodium hydroxide would give the best results. Two solutions were tried, 0.05 gm. copper tartrate in 10 cc. of 20 per cent sodium hydroxide solution, and 0.05 gm. copper tartrate in 20 cc. of 20 per cent sodium hydroxide solution. The first solution did not give differentiation between 10 per cent glucose and fructose. The solution of 0.05 gm. in 20 cc. gave differentiation at all concentrations at room temperature.

DISCUSSION

The following paragraphs point out discrepancies in the results of the present microscopic tests and the results of the macroscopic tests mentioned in the section on reagents.

Ost's copper carbonate reagent was not found to reduce "only ketoses" with gentle warming, as reported by Klein, although at 40° C. there was good differentiation between the sugars at all concentrations.

Pieraerts' modification of Ost's reagent was found to be reduced by glucose to some extent at room temperature and at 40° C., contrary to the macroscopical observations of Pieraerts. It should be noted, however, that Pieraerts' room temperature was 13° C. to 16° C., while that of the present tests was about 25° C.

Benedict's copper citrate reagent gave differentiation between glucose and fructose at 0.1 per cent and 0.5 per cent at room temperature and at 40° C. when used in microscopical mounts, whereas Kolthoff, working macroscopically, failed to find differentiation at these low concentrations, except in the case of 0.5 per cent at room temperature.

Pieraerts' copper-glycocol reagent, recommended by Pieraerts for use at room temperature in distinguishing fructose from glucose, failed to do so in the present tests at 0.5 per cent and at 1 per cent. Even more cuprous oxide was reduced by glucose than by fructose in some cases. Differences in the size of the crystals obtained made it difficult to compare the amount of precipitate.

SUMMARY OF RESULTS

1. Five copper reagents were tested at the temperature of a steam bath, at 40° C., and at room temperature as to their use in distinguishing between 0.1 per cent, 0.5 per cent,

1 per cent, 5 per cent, and 10 per cent glucose and fructose in microscopical mounts.

2. None of the reagents gave positive tests only for fructose. Positive tests for glucose varied from zero to a copious precipitate, depending upon the temperature and concentration of the sugar.

3. Each of the reagents may be used for distinguishing glucose from fructose under certain conditions of temperature and concentration of the sugars.

4. All except Pieraerts' copper-glycocol reagent gave differentiation between glucose and fructose at room temperature at all concentrations. None of the reagents gave differentiation with steam at all concentrations. Flückiger's copper tartrate reagent and Pieraerts' copper-glycocol reagent failed to give differentiation at 40° C. at all concentrations.

5. Table I indicates the time interval in relation to different temperatures at which the reagents are useful in distinguishing between glucose and fructose at different concentrations.

TABLE I

DIFFERENTIATION BETWEEN GLUCOSE AND FRUCTOSE

REAGENT AND TEMPERATURE	TIME IN MINUTES AT WHICH DIFFERENTIATION APPEARS				
	CONCENTRATION OF SUGAR SOLUTION				
	0.1 per cent	0.5 per cent	1 per cent	5 per cent	10 per cent
Flückiger's Room Temp.	15–60..*	4–15	4–60..	4–60..	4–60..
Ost's Copper Carbonate					
Steam bath	¼–2..	¼–2..	¼–2..		
40° C.	30–60	30–60	15–60	5–1440..	5–1440..
Room Temp.	120	1440..	120-1440..	60–1440..	60–1440..
Pieraerts' Modification of Ost's.					
Steam bath	½–1..	½			
40° C.	30–60..	15–60..	10–60..	5–60..	2–60..
Room Temp.	30–120..	15–120..	15–120..	15–120..	7–120..
Benedict's Copper Citrate.					
40° C.	10–60..	5–60..	5–60..	5–60..	5–60..
Room Temp.	60..	60..	60..	60..	30–60..
Pieraerts' Copper-Glycocol.					
40° C.		60	30–60	10–60	10–60
Room Temp.	1440..			60	10–60

*Two periods (..) after a figure indicate that no tests were made for a longer time interval. Where there are no periods the last figure given is the longest time interval in which there was good differentiation, as shown by further tests.

The writer wishes to express her appreciation to Dr. H. C. Sampson, of the Department of Botany of The Ohio State University, for his assistance in the direction of the experimental work and in the preparation of this paper.

LITERATURE CITED

(1) **Benedict, Stanley R.** A Reagent for the Detection of Reducing Sugars. Journal of Biological Chemistry, V: 485–487. 1908–09.
(2) **Flückiger, F. A. and Tschitch, A.** The Principles of Pharmacognosy. Power, F. B., tr. New York: William Wood and Co. 1887.
(3) **Haar, A. W. van der.** Anleitung zum Nachweis, zur Trennung und Bestimmung der reinen und aus Glukosiden usw. erhaltenen Monosaccharide und Aldehydsäuren. Berlin: Gebrüder Borntraeger. 1920.
(4) **Klein, G.** Handbuch der Pflanzenanalyse II. Berlin: Julius Springer. 1932.
(5) **Kolthoff, I. M.** De reduceerende werking der suikers. Chemisch Weekblad XIII: 887–895. 1916.
(6) **Lampe, L.** A Microchemical and Morphological Study of the Developing Endosperm of Maize. Botanical Gazette, XCI: 337–376. 1931.
(7) **Pieraerts, M. J.** Diagnose du d-fructose en presence d'autres sucres naturels. Bulletin de l'Association des Chimistes de Sucrerie et de Distillerie de France et des Colonies. July, 1907–June, 1908: 830–848.
(8) **Zerban, B. W. and Sattler, Louis.** Effect of Dextrose and Sucrose on the Determination of Levulose by Nijns' Method. Industrial and Engineering Chemistry, Analytical Edition II: 307–309. 1930.