[CONTRIBUTION FROM THE OFFICE OF CEREAL INVESTIGATIONS, BUREAU OF PLANT INDUSTRY, UNITED STATES DEPARTMENT OF AGRICULTURE, AND THE DEPARTMENT OF AGRICULTURAL CHEMISTRY, UNIVERSITY OF WISCONSIN]

EFFECTS OF THE METHOD OF DESICCATION ON THE CARBOHYDRATES OF PLANT TISSUE[1]

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Introduction

The investigations reported in this paper are a continuation of earlier studies on the effect of desiccation on the soluble carbohydrates of various plant tissues.[3] This paper deals particularly with the changes taking place in the sugar content of various tissues dried at temperatures over a range of 32° to 98°. The drying and analyses were conducted simultaneously with the study of the effects of desiccation on the nitrogenous constituents of beet, corn and barberry leaves and corn ears reported recently.[4]

Method of Analysis

The details of the method of sampling, preparation of the tissues for drying, desiccation data and the extraction used with the various tissues are given in the previous paper.[4] The water extract obtained from the tissues by the method described therein contained all of the free sugars, dextrins and soluble starch. Reducing sugars, sucrose and the total polysaccharides were determined in all of the water extracts from the various samples according to the following procedure.

One g. of calcium carbonate was added to a 500cc. aliquot portion of the water extract to neutralize the acids present. The solution was then heated to boiling to stop enzyme action. After cooling, the solution was clarified with neutral lead acetate and the precipitate filtered off and washed with 300 cc. of hot water to remove the sugar held by occlusion. The excess of lead was then precipitated, filtered off and washed with 100 cc. of hot water, making a total volume of 1 liter. The green tissues and the dried samples were subjected to the same treatment throughout.

Reducing Sugars were determined by the Shaffer and Hartmann method on aliquot portions of the clarified extract and calculated as glucose.[5]

Total Sugars were determined directly on the beet, corn and barberry leaves by the Herzfeld inversion method on 100cc. aliquot portions of the clarified extract, as these

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[2] The writer is indebted to Dr. E. R. Schulz of the Office of Cereal Investigations for assisting in the analyses. The writer also wishes to express his indebtedness to Dr. James G. Dickson and Professor W. E. Tottingham for suggestions and help given throughout the work.

[3] Link and Tottingham, THIS JOURNAL, **45**, 439 (1923).

[4] Link and Schulz, *ibid.*, **46**, 2044 (1924).

[5] Shaffer and Hartmann, *J. Biol. Chem.*, **45**, 365 (1921).

tissues did not contain maltose, dextrins or starch at the time of sampling.[6] After inversion the reducing power of the solution was determined as described above and calculated in terms of glucose. The water extract of the corn ears contained dextrins as well as maltose and glucose; therefore, the 2.5% sulfuric acid hydrolysis for two hours in a water-bath was used. The reducing sugars were determined before this hydrolysis and again after the hydrolysis and calculated as glucose. Sucrose was not present, as no increase in reducing power occurred after the Herzfeld inversion.

Experimental Data and Discussion of Results

Since the enzymes present in the water extract from the green tissues were destroyed within 45 minutes from the time of sampling, the analysis of the green tissue can be taken as the basis of comparison for the changes in sugar content occurring during the drying. The results expressed in percentage of reducing sugar and total sugar, based upon the dry weight of the tissues are presented in Fig. 1 and Table 1.

Beet Leaves.—The significant features of this series are the decrease in free reducing sugars at the lower temperatures and the decided loss in total sugar in the sample dried at 98°. Drying the leaves at room temperature and at 45° permitted the synthesis of sucrose to take place with a consequent decrease in free reducing sugars. There was, however, no appreciable change in the total sugar. Drying the samples at 65° and 80° produced little change in the sugar content, as the variations were all small enough to be within the limits of experimental error. The tissues dried at 98° showed a decided loss of free reducing and total sugar, about 44.0% of the latter having been lost.

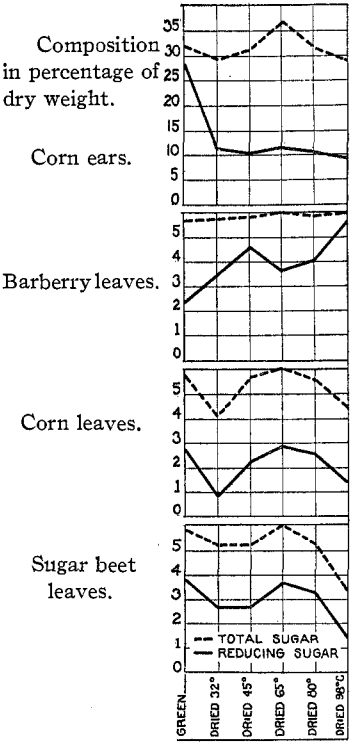

Fig. 1.—The effects of desiccation temperatures on the sugar content of corn ears and leaves, barberry leaves and beet leaves.

The decrease in invert sugar at the lower temperatures is probably due to the synthetic action of the enzyme invertase reported to be present in the leaf of the sugar beet by Robertson, Irvine and Dobson[7] and by Traegel.[8] The desiccation proceeded rapidly enough at 65° and 80° to prevent enzyme action. The decrease in the sugar content in the sample dried at 98° can hardly

[6] Herzfeld, *Z. Ver. deut. Zucker-Ind.*, **38**, 699 (1888).

[7] Robertson, Irvine and Dobson, *Biochem. J.*, **4**, 258 (1909).

[8] Traegel, *Z. Ver. deut. Zucker-Ind.*, **73**, 158 (1923); *C. A.*, **17**, 2792 (1923).

be attributed to either enzymatic or respiratory processes. Data presented later show that leaching from the tissue and caramelization probably cause this loss at the higher temperatures.

Corn Leaves.—This tissue shows a decided loss of both reducing and total sugars in the sample dried at room temperature. O'Sullivan reported the presence of invertase in the corn plant.[9] The hydrolysis of the sucrose at the lower temperatures was undoubtedly due to the invertase present. At 45° there was a slight change, while at 65° and 85° the analysis checked closely with that of the green tissue. At 98° losses occurred in reducing and total sugar, due probably to leaching and caramelization.

Barberry Leaves.—In this series of samples no drying temperature employed permitted desiccation without materially changing the amount of reducing sugar. There was no loss in total sugar in any case, the slight variations being within the limits of experimental error. At the lower temperatures, the inversion of the sucrose was probably due to enzyme action. At 80° and 98°, heat together with the high concentration of organic acids undoubtedly caused the inversion of the sucrose present. Many workers have reported the presence of considerable quantities of acetic, malic and various aromatic acids in the common barberry.[10]

Corn Ears.—The samples of corn ears dried very slowly at all temperatures. The results show that none of the samples was dried without a change in the content of reducing sugars. The analysis of the green tissue shows that 85.0% of the total sugar was reducing sugar. There was no sucrose present,[11] the increase in reducing power after hydrolysis with 2.5% sulfuric acid being due to the presence of maltose and dextrins. Since drying decreased the free reducing sugar in all of the samples, synthesis of dextrin must have taken place and the reverse action, the respiration of free sugars, did not occur. The rather constant value of the total sugar present after hydrolysis except in the sample dried at 65° strengthens this view. At 65° conditions must have been favorable for the hydrolysis of starch with the formation of dextrin.

Davis[12] showed that the ordinary diastatic enzymes are active[13] at 65°. He found that the maltase is destroyed at 50°, but that the enzyme that hydrolyzes starch to maltose is active up to 80°. At higher temperatures the enzyme is destroyed.

Waterman found that when potatoes were dried at temperatures rang-

[9] O'Sullivan, *Proc. Chem. Soc.*, **16**, 61 (1900).

[10] Wehmer, "Die Pflanzenstoffe," Gustav Fischer, Jena, **1911**, p. 206.

[11] The absence of sucrose appears to be abnormal. Sucrose is usually present during ear formation.

[12] Davis, *J. Soc. Dyers Colour.*, **30**, 249 (1914).

[13] Davis, *J. Agr. Sci.*, **7**, 355 (1915).

ing from 35° to 55°, part of the starch was transformed into sucrose, the amount produced decreasing as the temperature increased.[14]

At 65° both the synthesis of dextrins from the initial maltose and glucose and the hydrolysis of starch to dextrin took place. The process can be represented as follows,

$$\underset{C_6H_{12}O_6}{\text{Glucose}} \rightleftharpoons \underset{C_{12}H_{22}O_{11}}{\text{Maltose}} \rightleftharpoons \underset{(C_6H_{10}O_5)_n}{\text{Dextrin}} \rightleftharpoons \underset{(C_6H_{10}O_5)_n}{\text{Starch}}$$

the reaction taking place in the direction of the lower arrow from starch to dextrin and at the same time in the direction of the upper arrow from glucose to maltose, thence to dextrin. In the case of the samples dried at the other temperatures, the reaction was apparently confined to the direction of the upper arrow to the dextrin stage. The slight losses in total sugar in the samples dried at room temperature and at 98° are probably not significant. In the former sample the loss is probably due to respiration, while at 98° some caramelization occurred.

Appleman and Arthur[15] showed that this synthetic action of the enzymes in immature corn ears can occur. They found that the decrease in the percentage of sugar is due to the formation of polysaccharides, chiefly starch and dextrin, the synthesis proceeding until a definite equilibrium is reached, which is about the same for all temperatures up to 45°.

Experiments with the Spoehr Method.—Inasmuch as there was a loss in total sugars in some of the samples dried at 98° by the Spoehr method, it seemed advisable to study the method by further experiments. Cabbage leaves, containing 90% of moisture were chopped fine and weighed in 100-, 50- and 25g. samples. These samples were then placed in wide-mouthed bottles of 1 liter capacity—previously heated to 98°, tightly stoppered and replaced in the oven. A 50g. sample was placed in a fourth bottle and left unstoppered, while a fifth bottle was stoppered without a sample and replaced. All bottles had previously been equipped with thermometers standardized to within 0.5°. The temperatures were recorded every five minutes for one hour by reading through the glass door of the oven. After the bottles had been in the oven for 40 minutes[16] the stoppers were removed from the three bottles containing samples. The temperature curves are given in Fig. 2.

The temperature rose very rapidly in the closed bottles, the maximum being reached in the bottle containing the smallest amount of tissue. After the stoppers were removed the temperature dropped, the largest drop occurring in the bottle containing the smallest sample.

The results show clearly that the method is very efficient in bringing

[14] Waterman, *Chem. Weekblad*, **12**, 48 (1915).

[15] Appleman and Arthur, *J. Agr. Research*, **17**, 4, 137 (1919).

[16] In the original Spoehr method the stoppers are removed after 30 minutes' heating. [See *Carnegie Inst. Rept.* No. **287**, p. 27 (1919)]. The extra 10-minute period was allowed because of the large quantity of tissue used.

the tissue to a killing temperature, as the tissue is subjected to considerable pressure due to the vaporized water while the bottles are closed. Succulent tissue, like beet and immature corn leaves become limp and flaccid in a very short time and their contents leach out due to the slow removal of water from the partially closed system.[17] After the prolonged drying at 98°, the tissue adheres to the sides and bottom of the bottles so that it can be removed only with the aid of a spatula. That leaching of sugar from the beet and corn ears occurred was proved by washing the bottles with hot water and then determining the reducing properties of the water with Fehling's solution. A positive test was obtained in each case. No leaching occurred from the barberry leaves and corn ears.

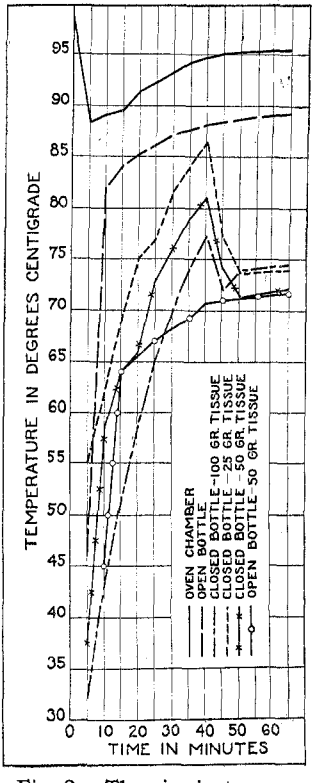

Fig. 2.—The rise in temperature in an open bottle (empty), open bottle with 50 g. of tissue, and closed bottles with 25, 50 and 100 g. of tissue, respectively, when placed in an oven at 98°. The stoppers of the closed bottles were removed after 40 minutes' heating.

It hardly seems necessary to continue drying the tissue at 98° for 24 hours after the preliminary killing. It would be better to dry the tissue at a lower temperature, 65° to 80°, in an oven with good ventilation and thereby prevent losses by leaching and caramelization.[18] Drying at 98° is too drastic treatment for green tissue, since some darkening and discoloration always occur. When the drying temperature is too high, the possibility of the complete inversion of the sucrose in acid tissue like that of the common barberry is quite evident.[19] Browne reports that sucrose in perfectly neutral solution when heated for a few hours at 100° begins to undergo decomposition as a result of caramelization and inversion, the decomposition proceeding with increasing rapidity in a very short time.[20]

The writer has conferred with Dr. Spoehr

[17] Spoehr used shallow dishes, which would minimize the danger of leaching due to better circulation.

[18] Caramelization seems to be more common in tissue with a neutral or alkaline reaction. Practically no caramelization occurs in acid tissue such as common barberry or oxalis leaves.

[19] Separate experiments made by Spoehr on the cactus showed that no appreciable inversion of the disaccharides took place in that tissue when subjected to this drying process.

[20] Browne, "Handbook of Sugar Analysis," John Wiley and Sons, **1912**, p. 656.

of the Carnegie Institution on the results obtained by the use of the method of killing and drying at 98°. In justice to Dr. Spoehr it should be stated that he did not recommend the method of killing and drying at 98° for universal application, and that he realizes its limitations with certain tissues. The writer thought it advisable to determine the application of this method under practical working conditions with large samples, and the experiments were conducted with that object in view.

General Discussion

A survey of the results shows that a drying temperature below 65° is not sufficient to check enzyme action and respiration in green, succulent tissue. Beet and corn leaves were dried successfully at 65° and 80°. In the case of the barberry leaves, drying at 65° to 80° prevented a loss in total sugar, but the free reducing sugars increased, due to the inversion of sucrose. A temperature of 65° proved to be poor for drying corn ears, as apparently both an increase in dextrins by the hydrolysis of starch and a decrease in free reducing sugars by their synthesis to dextrins occurred at this temperature. The latter process also occurred at 80°.

The Spoehr method of killing and drying at 98° caused an appreciable loss of sugar in the beet and corn leaves due to leaching and caramelization. The barberry leaves were dried without a loss in total sugar, but with complete inversion of the sucrose. In the corn ears the synthesis of dextrins occurred with a consequent reduction of total sugar. There was also some loss due to caramelization at this high temperature.

The results show that no universal method for drying plant tissues can be relied upon to give accurate results. Each tissue presents a specific problem governed by the chemical and physical nature of the tissue[21] and

TABLE I

THE EFFECTS OF DESICCATION TEMPERATURE ON THE SUGAR CONTENT OF VARIOUS PLANT TISSUES[a]

Degree Drying	Sugar beet leaves		Corn leaves		Barberry leaves		Corn ears	
	Reducing sugars	Total sugars	Reducing sugars	Total sugars	Reducing sugars	Total sugars	Reducing sugars	Total sugars[b]
Green tissue.....	3.80	5.85	2.87	5.85	2.39	5.67	28.00	33.00
At room temperature, 32°..	2.70	5.22	0.80	4.07	3.48	5.73	12.14	29.86
At oven temperature, 45°..	2.71	5.24	2.23	5.67	4.54	5.89	10.99	32.71
At oven temperature, 65°..	3.74	6.00	2.95	6.15	3.52	6.00	13.29	37.17
At oven temperature, 80°..	3.33	5.30	2.55	5.62	4.01	5.90	11.15	32.81
At 98°, Spoehr method.....	1.45	3.30	1.43	4.39	5.63	6.00	9.26	29.30

[a] All the data presented in this table are expressed in percentages based on the moisture free samples and are calculated in terms of glucose.

[b] Includes dextrins soluble in water.

[21] For the relation of the physical and chemical nature of the tissue to the rate of dehydration see Bosworth, *Botan. Gaz.*, **75**, 86 (1923); also Rosa, *Missouri Agr. Exp. Sta. Res. Bull.*, **48**, 58 (1921).

the enzyme content. It is impossible to determine without actual experimentation which drying temperature should be employed.

The data show that it is impossible to dry certain tissues without changing their sugar content. In order to analyze such tissues, the alcohol method of preservation recommended by Davis, Daish and Sawyer must be adopted.[22] This method was tried by the writer[3] with beet leaves and corn ears and was found to be effective. It is interesting to note that Appleman and Arthur[15] used the alcohol method in preserving their corn samples for analysis.

Summary

1. Drying at temperatures below 65° changed the sugar content of beet, corn and barberry leaves and corn ears.

2. Drying at 65° and 80° proved successful with beet and corn leaves, which are tissues that dry rapidly.

3. Killing and drying for 24 hours at 98° lowered the sugar content by caramelization and leaching in beet and corn leaves and corn ears and caused hydrolysis of the sucrose in barberry leaves.

4. The Spoehr method of heating the tissue in a closed vessel for 30 minutes at 98° raises the temperature to the killing point more quickly than direct heating at the same temperature in an open system. It is suggested that subsequent drying should be at a reduced temperature in a well-ventilated oven, thereby minimizing losses by leaching and caramelization.

5. It has been shown that the method of preservation by heat is inapplicable with certain tissues. In such cases the method of preservation with alcohol is the only alternative.

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THE RELATION BETWEEN THE STRUCTURE OF ORGANIC HALIDES AND THE SPEEDS OF THEIR REACTION WITH INORGANIC IODIDES. II. A STUDY OF THE ALKYL CHLORIDES

BY J. B. CONANT AND R. E. HUSSEY

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The first paper of this series[1] outlined a method of comparing the reactivities of the halogen atoms in organic compounds by measuring the rates at which the organic halides reacted with potassium iodide in acetone. This general reaction ($RX + KI \longrightarrow RI + KX$) is free from side reactions such as hydrolysis and formation of unsaturated or cyclic compounds and, therefore, significant measurements can be obtained with

[22] Davis, Daish and Sawyer, *J. Agr. Sci.*, **7**, 255 (1916).

[1] THIS JOURNAL, **46**, 232 (1924).