The iron division precipitate is treated directly with nitric acid and potassium chlorate to remove the manganese. (At this point phosphates may be removed if present.) Iron is removed by adding a large excess of ammonium hydroxide. The filtrate from the iron precipitate is evaporated until only a faint odor of ammonia remains and nickel is tested for by adding dimethyl glyoxime directly to $^1/_5$ of this solution, no adjustment of hydrogen ion being necessary.

The remainder of the solution is further evaporated to a sirup and transferred to a test-tube; enough water is then added to dissolve the precipitated salts, and cobalt is tested for by a modified Vogel's test; 3 cc. of 10% potassium thiocyanate solution is added, followed by 3 cc. of a mixture of amyl alcohol and ether (1 vol. : 3 vol.) and the tube gently shaken. Cobalt causes the ether layer to become blue-green. If the color is not distinct, the careful addition of a few drops of 12 *N* hydrochloric acid to the ether layer will give the blue-green color if cobalt is present, or a straw-colored or colorless layer, if it is absent. This test is extremely sensitive and can be applied to the original solution if iron is absent.

The writer wishes to thank Professor J. H. Reedy of the University of Illinois and Professor J. F. G. Hicks of the University of Nevada for testing the above method with their students.

Contribution from DePauw University
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[Contribution from the Department of Agricultural Chemistry, University of Wisconsin, and the Office of Cereal Investigation, United States Bureau of Plant Industry]

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

By Karl Paul Link and W. E. Tottingham

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Introduction

In determining the composition of plant tissue it is essential that the material undergo a minimum of possible chemical changes from the time the plant is harvested to the time actual analysis is begun. When a large number of samples are involved simultaneously, various mechanical difficulties encountered in extraction render it almost impossible to analyse the fresh tissue directly. Therefore, it is practically necessary to resort to some method of preserving the sample for subsequent analysis.

Two methods have been used to preserve such tissues, namely: (1) desiccation by heating at 85° to 105°, and (2) immersion in conc. alcohol.

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

The first method is most generally used. The second has been employed especially in work which involved the determination of carbohydrates only.

In connection with analytical studies on the tissues of maize in various stages of growth the writers have found that when a sample was dried at 98° in a large unventilated oven, or at temperatures as low as 90° in a vacuum oven at 35 mm. pressure, most of it was much discolored. Often especially when the sugar content was high, the odor of the dried tissue gave decided evidence of caramelization. This experience appeared to justify a more extensive study of desiccation. Confining the investigation to the direct use of heat, we have attempted to determine what temperature will quickly check enzymatic and respiratory activity in the plant tissue without appreciably altering its chemical composition.[2]

In the earliest study on the carbohydrates of the leaf, Brown and Morris[3] resorted to drying the tissue rapidly on trays maintained at temperatures between 80° and 90°. In their work on the diastase of the leaf they found that if special precautions were not taken, metabolic changes continued in the leaf cells during the slow process of drying at the low temperature of 30° to 35° which was necessary to avoid reducing the activity of the enzyme. They pointed out that such continuation of the vital processes results in marked diminution in starch and sugars before the leaf tissue is dry enough to arrest further change. Therefore, they resorted to the higher temperatures mentioned.

Parkin,[4] in an investigation of the sugars of the snow-drop, dried leaves at a temperature sufficiently low to prevent discoloration. He does not specify the temperature, but merely mentions that the material was air-dried rapidly. His analytical results on air-dried samples checked with those obtained by killing the leaves in liquid air followed by plunging into hot water to inactivate the enzymes. It seems rather remarkable that one should have obtained the same results by these widely different treatments. From the work of Brown and Morris, one would expect respiration and enzymatic action to continue, and the starch and disaccharides to be hydrolyzed into simple sugars, for some time after the leaves were plucked.

Willstätter[5] in his work on the chemistry of chlorophyll and related pigments used leaves dried at 50° to 55° in a current of air. He found no chemical change in chlorophyll extracted from the dried leaves as compared with that extracted directly from the green tissue.

Davis, Daish and Sawyer[6] consider heat treatment unsatisfactory, especially in the case of moderately thick leaves such as those of the mangold, where the internal temperature of the tissue may rise slowly. In such cases some enzymatic action is possible before the enzymes are destroyed. They therefore adopted the following method in their work. One kg. of freshly plucked leaf material is dropped in successive small quantities into 2 liters of boiling 95% alcohol, to which a little ammonium hydroxide is added to neutralize the acids of the tissue. After it has boiled for half an hour, the

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[2] The writers are greatly indebted to Dr. James G. Dickson of the Office of Cereal Investigations, U. S. Department of Agriculture, for valuable suggestions contributed to this work.

[3] Brown and Morris, *J. Chem. Soc.*, **63**, 604 (1893).

[4] Parkin, *Biochem. J.*, **6**, 1 (1911).

[5] Willstätter and Stoll, "Untersuchungen über Chlorophyll," Julius Springer, Berlin, **1916**.

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

material is placed in suitable jars until ready for analysis. This method of preserving plant tissue has found somewhat extended use. Kraus and Kraybill[7] used it in their studies on "Vegetation and Reproduction." Analytical results presented later in this paper show that this method preserves maximum value of polysaccharide and sugar contents of the tissues, as compared with various methods of desiccation by heat. The alcohol method was here used as a control in the analysis of tissue desiccated under various conditions, because there is little doubt that the method is efficient in checking enzymatic action and respiration.

Experimentation

In order that the work might cover a wide range of material, 5 different types of tissue were selected for study, namely, beet leaves, corn ears, corn stalks, corn seedlings (top and roots) and potato tubers. The beet leaves came from plants which were half grown, and were harvested at 1 P. M. on a clear day. They were chosen to correspond with tissue used by Brown and Morris and by Parkin; the moisture content was 87.0%. The corn ears were in the well advanced "milk" stage; they were harvested at 12:30 P. M. on a clear day. They were selected because of the comparatively large amount of sugar which they contain, and because they represent tissue which is not readily cut into small pieces, without loss of liquid and, therefore, might dry with difficulty; moisture content, 61.0%. The corn stalks came from plants in the early stage of ear formation, and were collected at 11:15 A. M. on a clear day. They were chosen because of high sugar content, so that effects of heat upon these constituents might be determined; moisture content, 88.2%. The corn seedlings were roots and tops from young plants which were germinated in grain sprouter. They were 7 days old when separated from the endosperm for desiccation; moisture content, 90.0%. Potatoes were selected as representative of tissues high in starch; moisture content, 78.4%. Of these materials, the corn ears and beet leaves were also subjected to the method of preserving in alcohol.

Preparation of Samples

A large quantity of each type of tissue was selected in the field. It was taken immediately to the laboratory and rapidly reduced to smaller fragments as follows: beet leaves were cut into pieces 10 to 12 mm. square; corn ears were split once longitudinally and cut into pieces 60 to 80 mm. long; corn stalks were split 6 times longitudinally and cut into pieces 20 to 25 mm. long; corn seedlings were cut into pieces 10 to 12 mm. long; potato tubers were sliced transversely into pieces 6 to 7 mm. thick.

Equal quantities, approximating 50 g. in dry matter, were placed in the respective ovens, which had previously been regulated to the desired temperatures. In no case did more than 15 minutes elapse between the time of harvesting and the time when the tissue was placed in the ovens. The drying process was continued until constant weight of the samples

[7] Kraus and Kraybill, *Oregon Agr. Coll. Expt. Sta. Bull.*, 149 (1918).

was attained The dried tissues were passed through a drug mill and finally ground in a Dreef mill[8] until all particles could pass through a 100-mesh sieve. Moisture determinations were made on all samples by drying at 100° and all analytical data are based upon the dry matter thus determined.

For the alcohol method of preservation a convenient quantity, depending upon the moisture content, was placed in enough 95% alcohol to insure a concentration of 75% alcohol after dilution by the water from the tissue. A little calcium carbonate was added, in order to neutralize any acids which might have been present, and the mixture was heated for 1 hour on a steam-bath at 78°.

Methods of Analysis

A sample of 5 g. of actual dry matter of each tissue was freed from lipoids and soluble pigments by percolation with anhydrous, alcohol-free ethyl ether. After expulsion of the ether the residue of the sample was extracted with 90% alcohol at 55° for the removal of sugars. After the extract was filtered and washed, it was concentrated to about 50 cc. at 55° and 18 mm. It was then diluted with water, clarified with neutral lead acetate, freed from excess of lead by sodium carbonate and finally made up to a volume of 300 cc.

Reducing Sugars.—Reducing sugars were determined by the Munson-Walker method.[9]

Total Sugars.—Aliquot portions from the original sugar extract were hydrolyzed by 2.5% hydrochloric acid in the usual manner. Total sugars were then determined by the Defren-O'Sullivan method.[10]

Dextrins.—The residue from the sugar extraction was further extracted with 10% alcohol at a temperature of 50°. The filtered extract was concentrated for removal of alcohol and clarified by the same methods applied to the sugar extract. After hydrolysis and analysis for total sugars, the glucose value obtained was computed to the equivalent value for dextrin.

Starch.—The residue from the dextrin extraction was boiled with 250 cc. of water. Boiling was continued for 10 minutes in the case of tissues poor in starch, but in the other cases the time was extended to 30 minutes to insure complete gelatinization of the starch granules. After digestion with fresh saliva and filtration, the solution was hydrolyzed and the glucose determined in the usual manner. The results are expressed as percentages of starch.

Analysis of Alcohol-Preserved Samples.—The mechanical difficulties of grinding the samples when the sugar content is high render it unfeasible to unite the extract and residue in this method of treatment. The alcohol extract was therefore decanted and filtered. It was then distilled at 50° under 18 mm. pressure to remove the alcohol. The residue was taken up with water, clarified with neutral lead acetate in the usual manner and finally diluted to standard volume. Reducing sugars and total sugars were determined as usual. The residue from the extraction with alcohol was transferred to a large beaker and the alcohol allowed to evaporate at 60°. The residue was then

[8] Wiley, "Principles and Practice of Agricultural Analysis," 2nd Ed., vol. 2, p. 12, Chemical Publishing Co., 1914.

[9] Munson and Walker, This Journal, **28**, 663 (1906).

[10] Defren and O'Sullivan, *ibid.*, **18**, 749 (1896); *J. Chem. Soc.*, **30**, 130 (1876).

dried at 80° under 35 mm. pressure. It was finally ground and analyzed in the same manner as the samples that were dried in the ovens directly.

Discussion

Series I. Beet Leaves.—Based upon the tissue preserved in alcohol as a control, the analytical data of this series show that heating at 65° in a vacuum did not check enzymatic and respiratory activities rapidly enough to prevent some loss of sugars. Since the total sugar content of the tissues was the same in all other cases it appears that the higher temperatures here employed did inhibit respiratory loss of sugars. The results obtained after drying at 98° without either reduced pressure or ventilation indicate considerable hydrolysis of disaccharides to have taken place without destruction of the free sugars. The results here obtained at the lowest temperature agree with those of Brown and Morris but are contrary to those of Parkin, who reported no loss of sugars from mangold leaves dried in a current of air at room temperature.

Series II. Corn Ears.—An attempt was made to dry this tissue at 65° in a vacuum, but the moisture content was not reduced with sufficient rapidity to inhibit the initiation of mold growth. Only the sample so dried at 80° following preliminary treatment in the autoclave, gave results which agreed well with those of the alcohol method. This method of desiccation therefore proves favorable for this tissue, and probably for similar bulky and succulent tissue, provided it is previously heated to the death point. Without such preliminary treatment, however, the carbohydrates underwent some hydrolytic changes and respiratory loss. These changes, especially the hydrolytic loss of starch, were still more marked following desiccation at 98° without either vacuum or ventilation service. In this case probably some of the apparent loss of carbohydrates should be ascribed to caramelization of the free sugars which accumulated by hydrolyis.

Series III. Corn Stalks.—The results of this series indicate clearly that the temperature of 65° in a vacuum is unsatisfactory for such tissue. They also indicate that at 98° the loss due to caramelization was very high. Using the sample dried at 80° as a control in this case, the results indicate destruction of $^1/_2$ the total sugars at the highest temperature, together with some hydrolysis of starch and a very large disappearance of disaccharides. The loss of total carbohydrates was less serious at 65° than at 98°. In the former case the increase of simple sugars shows that the formation of these by hydrolysis was more rapid than their actual destruction by respiration due to enzyme action.

Series IV. Corn Seedlings.—In this case, the combination of free ventilation with heating at a temperature of 65° gave a greater content of determined carbohydrates than at 80°. This difference is greater with top than with roots and appears to depend chiefly upon variations in sucrose.

The low results obtained by drying at 98° indicate excessive hydrolysis and caramelization at that temperature. Apparently similar effects resulted to a less extent at 80°, even in a vacuum. In this respect the corn seedlings seem to be peculiarly sensitive.

Series V. Potato Tubers.—The results with this succulent tissue agree in several details with those obtained from corn ears. Because of its high moisture content a temperature of 65° in a vacuum did not prevent hydrolysis of starch as compared with the sample dried at 80° in a vacuum after autoclaving. As in the case of corn ears, the use of the unventilated oven at 98° entailed a still greater loss of starch. The destructive action of the higher temperature upon free sugars is distinct here. The results

TABLE I

EXPERIMENTAL DATA

Desiccation				Composition of dry tissue				
Oven temp. °C.	Condition	Drying Hours	Condition after drying	Reducing sugars %	Total sugars %	Dextrines %	Starch %	Glucose equivalent[e]
			SERIES I. BEET LEAVES					
65	35 mm.	12	shriveled; slightly darkened	1.06	2.46	..	...	...
80	35 mm.	14	normal[a]	0.97	3.76	..	...	...
98	unvent. oven	10	brown	2.14	3.76	..	...	...
		Alcohol preservation		1.00	3.80	..	...	...
			SERIES II. CORN EARS					
65	35 mm.	..	mold growth initiated	...	...	..	...	...
80	35 mm. (A)	21	normal	3.30	4.50	2.17	37.08	48.10
80	35 mm. (B)[b]	21	normal	2.00	4.20	3.22	38.50	50.60
98	unvent. oven	18	kernels darkened	5.80	6.50	4.35	31.90	46.80
		Alcohol preservation		2.25	7.25[c]	...	38.70	50.60[d]
			SERIES III. CORN STALKS					
65	35 mm.	16	normal	11.05	13.20	..	1.30	14.64
80	35 mm.	12	normal	7.30	15.96	..	2.56	18.80
98	unvent. oven	13	much darkened; odor of caramel	6.65	8.28	..	1.97	10.46
			SERIES IV. CORN SEEDLINGS					
						Roots		
65	steam-heated trays with fan vent.	10	normal	3.90	11.00	1.97	2.98	16.50
80	35 mm.	12	sl. dark	2.50	10.30	1.68	2.27	14.70
98	unvent. oven	7	tissue blackened; odor of caramel	2.90	6.20	1.78	2.08	10.50
						Tops		
65				2.90	11.62	0.53	...	12.20
80				3.57	7.50	0.49	...	8.00
98				2.60	6.82	0.44	...	7.30

SERIES V. POTATO TUBERS

65	35 mm.	16	normal	1.84	3.35	2.22	50.70	62.20
65	heated trays with fan vent.	10	normal	0.92	3.39	2.20	51.43	63.00
80	35 mm. (A)	12	normal	0.95	2.60	2.22	53.55	64.60
80	35 mm. (B)[b]	12	normal	0.92	2.40	2.90	52.20	63.60
98	unvent. oven	29	darkening; hard, brittle	...	1.37	3.18	46.80	57.00

[a] "Normal" indicates that the tissue suffered no apparent discoloration and its odor gave no evidence of caramelization.

[b] Treated before drying to inhibit enzyme action. Placed in a tightly covered pail and autoclaved for 15 minutes at a temperature of 116°.

[c] The concentration of the alcohol was below 70% after extraction; hence, the dextrins went into solution with the sugars, and were included in the determination of total sugars.

[d] Assuming total sugars to be 4.0%.

[e] Total sugars and polysaccharides as glucose equivalent.

with the potato differ from those with corn ears in showing no beneficial effect of the autoclaving treatment preceding drying at 80° in a vacuum. This may have been due to the possibility of reducing the tissue to thin sections. Furthermore, the losses by enzymatic and respiratory activity would probably be more pronounced in the developing corn kernels than in potato tubers taken from storage. It is possible that potato tubers in the process of sprouting would require autoclave treatment for successful desiccation by heat.

Suggested Method of Desiccation

The present investigation included desiccation of masses of fresh plant tissue equivalent to approximately 80 to 450 g. of water content. As sources of heat the following were used: partially ventilated ovens 20 × 20 × 45 cm. in dimensions with a pressure of 35 mm. at both 65° and 80°, unventilated ovens 58 × 30 × 40 cm. at 98° and trays of wire netting at the center of a drying room 2.4 × 1.8 × 1.2 meters at 65°, ventilated by means of a 30cm. electric blower.

The vacuum oven proved successful, that is, inhibited metabolic changes of the tissue without causing caramelization only at the higher temperature. In the case of unventilated ovens poor results were probably due to the retarding effect of the vapor pressure of water expelled from the tissue in the fore period of desiccation. Furthermore, the destructive effect of caramelization upon sugars was excessive at 98°.

Upon comparison of the results here obtained it appears advisable to recommend the ventilated trays at 65° for all tissues which can be reduced to thin sections for rapid desiccation. The removal of water vapor as expelled is doubtless an important feature of this method. For more watery tissues and those not reducible to thin sections the vacuum oven at

80° seems preferable. Apparently some such discrimination should be used in adapting type of tissue to method of desiccation.

Neither of the above methods may be applicable when one wishes to determine the soluble protein content of tissues. However, as Osborne[11] and his co-workers have shown, leaves dried at 60° with ventilation are satisfactory in this respect. In the case of this tissue, therefore, it appears possible to preserve by one method a sample suitable for both protein and carbohydrate analyses.

Direct desiccation in place of alcoholic treatment simplifies the analytical procedure. Due to the frequent necessity of analyzing extract and residue separately the latter method is also more subject to error. It is inapplicable, too, when soluble protein is to be determined, due to precipitating effect. Doubtless conditions will exist in some cases, such as those necessitating sampling of growing plants at long distances from laboratory facilities, which will necessitate use of the alcoholic method. The method is not satisfactory when cold alcohol is used, as pointed out by Brown and Morris.[3] Thus another limitation presents itself when heating facilities cannot be obtained near the place of sampling. Of course, a gasoline stove will do here, whereas a complete oven, less portable, would be required by the other method.

Summary and Conclusions

1. Carbohydrate determinations have been reported for 5 different types of fresh plant tissue preserved by desiccation in several different ways.

2. The results from all 5 series indicate that the temperature of 98° should not be employed for drying fresh plant tissue when the sugar and starch contents are high.

3. Temperatures as low as 65° in an air current, at atmospheric pressure, can be used safely with tissue that can be dried rapidly. These temperatures cannot be used safely with tissue not readily reducible to thin sections.

4. The temperature of 80° in a vacuum is decidedly superior to 65° in a vacuum. In the case of coarsely divided tissues, such as corn ears, 80° in a vacuum gives improved results when the tissue has been previously heated in an autoclave to inhibit enzymatic and respiratory processes.

5. Well-ventilated ovens, especially those in which a large volume of air is circulated continuously over the tissue, permit the use of low drying temperatures, with minimum changes of tissue composition.

6. The alcohol method of preservation gave the same analytical results for carbohydrates as desiccation at 80° in a vacuum, in the cases of beet leaves and corn ears. The methods are, therefore, equally efficient in checking enzyme action and stopping respiration.

[11] Osborne and Wakeman, *J. Biol. Chem.*, **42**, 1 (1920).

7. None of the preservative methods here tested is recommended for use when the determination of soluble protein is required.

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THE DECOMPOSITION OF FORMIC ACID BY SULFURIC ACID[1]

BY ERNEST R. SCHIERZ

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Introduction

That formic acid decomposes in the presence of sulfuric acid with the liberation of carbon monoxide was first reported by Doebereiner.[2] This reaction has been studied by Meyer,[3] Veley,[4] Morgan,[5] and Okaya.[6] The last mentioned author observed that it proceeded with the rythmic evolution of carbon monoxide if no precautions were taken to prevent supersaturation. Sabatier and Maihle[7] showed that in presence of sulfur trioxide below 100° and anhydrous oxalic above 105° the decomposition products are the same as with sulfuric acid. That the reaction is a quantitative one has been established by Wegner.[8]

The present investigation was undertaken in order to study the kinetics of the decomposition of formic acid by sulfuric acid and the effects of various soluble substances on the rate of the reaction.

Experimental Part

Preparation and Purification of Materials.—Formic acid was prepared by dehydrating an 85% acid by warming to about 50° and then adding an amount of anhydrous oxalic acid[9] sufficient to combine with the water present. The liquid when cooled was decanted from the crystals of hydrated oxalic acid and distilled. This very concentrated distillate was completely dehydrated by the method proposed by Schlesinger and Martin.[10] The resulting acid distilled at 31.0–31.5° at 37–39 mm.; d^{25} 1.217.

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[1] The work included in this paper is from the thesis presented by Ernest R. Schierz in partial fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Wisconsin. This investigation was conducted under the supervision of Professor J. H. Walton.

[2] Doebereiner, *Schweiger-Meinecke's J. Chem. Phys.*, **32**, 345 (1821); *Gilbert's Ann.*, 71, 107 (1822).

[3] Meyer, *Z. Elektrochem.*, **14**, 506 (1909).

[4] Veley, *Phil. Mag.*, [6] **6**, 271 (1903).

[5] Morgan, *J. Chem. Soc.*, **109**, 274 (1916).

[6] Okaya, *Proc. Phys. Math. Soc. Japan*, [3] 1, 43 (1919).

[7] Sabatier and Maihle, *Compt. rend.*, **152**, 1212 (1911).

[8] Wegner, *Z. anal. Chem.*, **42**, 427 (1903).

[9] Beilstein, *Handbuch der organischen Chemie*, **1893**, 3rd ed., I, **p.** 393.

[10] Schlesinger and Martin, THIS JOURNAL, **36**, 1589 (1914).