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EFFECTS OF THE METHOD OF DESICCATION ON THE NITROGENOUS CONSTITUENTS OF PLANT TISSUE[1]

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Introduction

In a previous paper[3] data were presented showing the effects of desiccation at different temperatures upon the soluble carbohydrates of different plant tissues. The present paper gives the results of further experiments in which the effects of desiccation at different temperatures on the nitrogenous constituents of the same type of plant tissues have been determined.

In the study of nitrogen and protein metabolism in plants, little attention has been given to the method of treatment prior to chemical analysis. In many instances, each investigator has employed the method best suited to his particular conditions of experimentation, with little attempt at standardizing conditions in order to secure results comparable with those of others.

Suzuki,[4] in his attempt to find the change in diurnal nitrogen in the leaves of several plants, air-dried the tissue. Jodidi and his co-workers[5] in their studies on the nitrogenous constituents in mosaic and mosaic-free spinach and cabbage, air-dried the tissue first, and finally dried it in a vacuum oven at 50° for 24 hours. In other work the fresh tissue was dried at higher temperatures. Schulze and Schütz,[6] in studying the seasonal and diurnal changes in the protein nitrogen of the box elder, dried the tissue rapidly at 90°. Considerable work has been done in which the methods of desiccation have been compared over a limited range of temperatures and conditions of drying. Wagner reported experiments with leaves from fodder and sugar beets, dried at 60° and 105°. Neither crude protein nor pure protein was measurably affected by drying at those temperatures, but the digestible proportion in each case decreased considerably, especially when dried at the higher temperature.[7]

Couperot[8] found that a considerable portion of the nitrates and hydrogen cyanide were lost by slow desiccation at room temperature. The nitrates decreased as much as

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[2] We desire to express our appreciation to Dr. James G. Dickson of the Office of Cereal Investigations, United States Department of Agriculture, and to Professor W. E. Tottingham of the University of Wisconsin for valuable suggestions contributed to this work.

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

[4] Suzuki, *Bull. Coll. Agric. Tokyo*, **3**, 241 (1897).

[5] Jodidi and others, THIS JOURNAL, **42**, 1061, 1883 (1920).

[6] Schulze and Schütz, *Landw. Vers. Sta.*, **71**, 299 (1909).

[7] Wagner, *Z. Ver. deut. Zucker-Ind.*, **71**, 251 (1901).

[8] Couperot, *J. pharm. chim.*, [6] **29**, 100 (1909).

20 to 25% of the initial amount present when dried at room temperatures, whereas practically no loss of either form of nitrogen occurred when the drying was done rapidly at 60°.

Waterman[9] found that the percentages of total nitrogen and water-soluble nitrogen were unaffected when potatoes were dried at or below 55°.

Osborne and Wakeman[10] found that the proportion of water-soluble nitrogen of spinach leaves was a little higher in the leaves dried at 60° than in the green leaves. They found both protein and non-protein substances contributing to this small increase. An increase of proteose nitrogen indicated a slight autolysis during the drying process. From the close similarity of nitrogenous extracts recovered from the dried leaves by successive extractions with various solvents as compared with the green leaves, they concluded that the nitrogenous constituents of green leaves are altered only to a slight degree by proper drying.

Chibnall[11] in studying the effect of drying at low temperature on the distribution of nitrogen in the leaves of the Runner bean, showed that considerable proteolysis takes place resulting in an increase in the simple water-soluble nitrogenous products, chiefly ammonia, amides and mono-amino acids. He found the amide and ammoniacal nitrogen increased from 1.0 to 6.0% of the total nitrogen. From his experimental results, he points out the unreliability of certain earlier work on the occurrence of asparagine and amino acids in plants, due to changes occurring under the different conditions of drying.

Tottingham and his co-workers[12] in their work on the efficiency of various methods of desiccation and extraction as related to the proportions of total soluble nitrogen and soluble protein in the leaves of the barberry and sugar beet, found that drying at 40° to 72° brought about coagulation of some of the soluble protein.

Experimentation

The analytical studies here recorded were conducted on four lots of plant tissues varying considerably in physical condition and chemical composition: first, leaves of the sugar beet (*Beta vulgaris*), collected from nearly mature plants on a cool, cloudy day in September; second, leaves; and third, ears of corn (*Zea mays*) collected simultaneously from plants in the "early milk stage," at noon on a hot, dry day in August; fourth, leaves of common barberry (*Berberis vulgaris*) collected during a hot, dry period in August from bushes growing in a wood.

Methods

Preparation of Samples for Drying.—The procedure described by Link and Tottingham[3] was followed in the preparation of the tissue for the samples placed in the respective ovens. Moisture determinations were made on the sample by drying them for 20 hours at 100°, and all analytical data were calculated on the dry matter thus determined.

Method of Drying.—The samples were dried at a room temperature of 32°, at 45° and 65° with forced ventilation, and at 80° and 98°, respec-

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

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

[11] Chibnall, *Biochem. J.*, **14**, 598 (1922).

[12] Tottingham and others, THIS JOURNAL, **46**, 203 (1924).

tively. Drying at room temperatures (32°) was facilitated by means of an electric fan so placed as to circulate air over and under wire screens containing single layers of tissue. Drying at 45° was accomplished in wire-bottomed trays of a drying cabinet heated by steam coils. At 65°, the drying was done in the ventilated drying room described by Link and Tottingham.[3] Drying at 80° was done in an oven without ventilation. At 98° the method described by Spoehr was used.[13] Not more than 50 g. of green tissue was placed in previously heated wide-mouthed bottles of 1 liter capacity and left stoppered for 30 minutes to insure rapid killing, and thereby prevent enzyme action. The drying was then continued for 24 hours in an unventilated oven at 98°.

Preparation of Green Samples for Analysis.—Samples of 100 g. of the green tissue were chopped into fine pieces and transferred to a large mortar in which they were crushed with a pestle. A small quantity of ether was then added to plasmolyze the cells. Angular quartz sand previously washed was then added and the grinding continued by means of a large pestle until the tissue was reduced to a fine pulp. The pulp was then transferred to a cloth on a funnel, and extracted with water until all the soluble nitrogenous constituents were removed. Preliminary experiments showed that between 2 and 3 liters of water per 100 g. of green tissue was necessary for complete extraction. The extract was then filtered through paper pulp on a Büchner funnel and made up to a definite volume.[14] As the time required for the whole procedure did not exceed one hour in any case, there was little opportunity for change in composition.

Method of Analysis.—Total nitrogen was determined on all samples. Total water-soluble nitrogen, coagulable nitrogen, proteose nitrogen, protein and proteose-free water-soluble nitrogen, and amino nitrogen were determined in the water extract from the samples. The Kjeldahl-Gunning method was used for all nitrogen determinations as follows.

Total N, directly on 5g. samples of the green tissue and 1g. samples of the dried tissue.

Total water-soluble N, on 250cc. aliquot portions from the original water extract.

Coagulable N. 500 cc. of the original solution was neutralized with 50% acetic acid using litmus paper as indicator, then acidified with 2 cc. of 10% acetic acid, the mixture heated to boiling and boiled for 3 minutes. The coagulum was then filtered on a fluted filter, washed with 250 cc. of hot water and the nitrogen determined.

Proteose N. The filtrate from the coagulum was acidified with sulfuric acid and saturated with zinc sulfate. The precipitated proteose was

[13] Spoehr, *Carnegie Inst. Rept.*, **287**, 31 (1919).

[14] The fraction of nitrogenous constituents that came through the filter as outlined above is called "water soluble N," but may not be a true solution.

filtered, washed with saturated zinc sulfate solution, then with 200 cc. of cold water and the nitrogen determined.

Protein-proteose-free water-soluble N. The simple water-soluble nitrogenous constituents were determined on aliquot portions from the filtrate of the proteose separation.

α-Amino N was determined by the Van Slyke method on aliquot portions from the protein-proteose-free solution.[15]

All the analyses given in the subsequent tables are expressed in percentages based on the moisture-free samples.

Preparation of the Dried Samples for Analysis.—The samples dried under the various conditions were pulverized in a drug mill before subjecting them to the method of grinding and extraction applied to the green tissue. It was necessary to dry the samples desiccated at room temperature for 24 hours in the drying cabinet at 45° before they could be run through the mill. The time required for drying the respective samples and the final moisture content after drying are given in Table I.

TABLE I

DATA ON THE EFFECTS OF VARIOUS TEMPERATURES IN DRYING PLANT TISSUES ON THE RATE OF DESSICATION AND THE FINAL MOISTURE PERCENTAGE

	Beet leaves		Corn leaves		Corn ears		Barberry leaves	
Drying temperature	Period of drying in hours	Moisture 81.39% in fresh tissue. Dried sample %	Period of drying in hours	Moisture 73.36% in fresh tissue. Dried sample %	Period of drying in hours	Moisture 86.99% in fresh tissue. Dried sample %	Period of drying in hours	Moisture 62.49% in fresh tissue. Dried sample %
24 hrs. at room temp. 32°, then at 45°	10	4.95	20	4.01	48	2.45	24	6.45
Oven 45°	14	5.74	20	6.04	24	11.00	20	9.24
Oven 65°	8	5.40	12	5.99	20	10.37	12	10.61
Oven 80°	8	4.81	12	5.74	20	10.52	12	9.60[a]
Oven 98°	24	3.12	24	4.41	24	5.44	24	5.74

[a] Tissue dried at 80° but not analyzed for nitrogenous constituents.

Experimental Data and Discussion

The data resulting from the analyses are presented graphically in Figs. 1 and 2.

There was no loss in total nitrogen in the four different types of tissues used. All series show a constant agreement within the range of experimental error of the total nitrogen recovered in the form of coagulable protein, proteose, and protein-proteose-free extract, as compared with the direct determination of the total water-soluble nitrogen.

Beet Leaves.—This series shows a marked decrease of the total water-soluble nitrogen, especially at the high temperatures (Fig. 1). The necessary calculations show that with the exception of the tissue dried at 32°,

[15] Ammonia and amide nitrogen were not removed from the solution.

the loss of coagulable nitrogen is practically equivalent to the loss of soluble nitrogen. The increases in proteose and simpler forms of nitrogen at 32°, indicate hydrolytic decomposition of some of the protein. (Fig. 2.) The slight variations of proteose nitrogen at the other temperatures cannot be considered significant. Values for the lower forms of nitrogen contained in the protein-proteose-free water extract show considerable regularity, but indicate that hydrolytic changes took place at 32°, while at 98° the reverse may have occurred. Increase of α-amino nitrogen at 32° to 65° indicates hydrolytic change of the higher nitrogen compounds under those conditions.

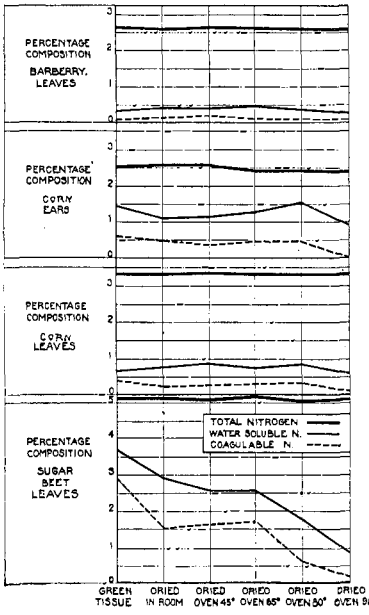

Fig. 1.—The effects of desiccation temperatures on the total nitrogen, water-soluble nitrogen and coagulable nitrogen of common barberry leaves, corn ears and leaves and sugar beet leaves. (Expressed in percentage based on dry weight.)

Corn Leaves.—The results in this series differ distinctly from those obtained with beet leaves. The proportion of the total nitrogen in the fresh tissue removed by water is very low compared with that in the beet leaf. Of this nitrogen 47% is α-amino nitrogen as compared with 7.0% in the latter case (Fig. 1). The total water-soluble nitrogen increased with desiccation in all cases excepting at 98° where it decreased. The coagulable nitrogen decreased at the two extremes of desiccation temperatures, namely, 32° and 98°. The data indicate that this loss is not due to coagulation, because the water-soluble nitrogen increased. At 98° the decrease doubtless is due to coagulation. The amount of proteose nitrogen throughout was too small for serious consideration. The α-amino nitrogen remained nearly constant at all temperatures except 32° and 98°, at which the loss observed would seem to be due to synthetic processes and to mechanical occlusion in the coagulum at the respective temperatures.

Corn Ears.—The data indicate the difficulty of sampling the ear tissue by the slight variation in the total nitrogen content (Fig. 1). These variations are not sufficiently consistent with the desiccation treatment to indicate actual loss of nitrogen by the liberation of ammonia. One would expect such losses to become conspicuous at the lower temperatures. At 65° and also 80° the amount of total nitrogen extracted with water agrees closely with that from the fresh tissue, but at the other temperatures,

especially at 98°, some decrease in solubility occurred. The proportion of the total coagulable nitrogen decreased throughout, with the exception of its striking disappearance at 98°. In the case of proteose nitrogen, the variations occurred irregularly and the small amounts involved hardly justify interpretive treatment. The simple forms of nitrogen in the protein-proteose-free extract increased slightly at 80° and 98°, at which temperatures the proteose nitrogen decreased. This might indicate hydrolysis of the latter compounds. At room temperature the decrease of α-amino nitrogen might be due to synthetic processes occurring in the early stages of desiccation. The distribution of nitrogenous constituents in immature corn ears appears to be especially subject to disturbance by desiccation treatment. It seems probable that at the lower desiccation temperatures synthetic and hydrolytic processes progressed with such counterbalancing effects that the analytical results do not indicate what actual changes took place.

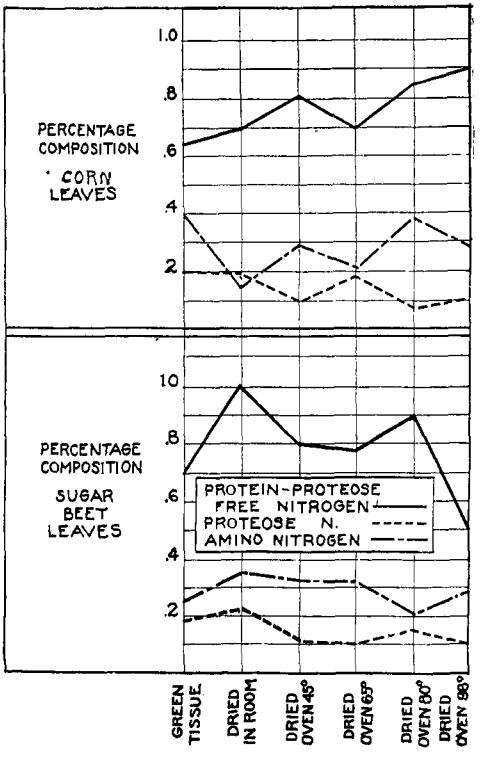

Fig. 2.—The effects of desiccation temperatures on the proteose nitrogen, protein-proteose-free nitrogen and α-amino nitrogen of beet leaves and corn ears. (Expressed in percentage based on dry weight.)

Common Barberry Leaves.—This tissue, under the conditions of sampling, represents a type very low in water-soluble nitrogen. It should be observed that the moisture content of the leaves was reduced far below normal and that consequently the tissue was in a metabolically inactive state (Fig. 1). The proportion of total water-soluble nitrogen was very small. In contrast to results with the other tissues, the nitrogenous constituents remained practically undisturbed by desiccation. In no case was either proteose nitrogen or α-amino nitrogen present. These results show little modification of nitrogen distribution by desiccation treatment.

General Discussion

A general survey of the data presented indicates that one must take into account the nature of the tissue involved and the temperature selected

in considering the effects of desiccation in analyzing for the nitrogenous constituents of plants. Each tissue represents a separate case, and no general conclusions applying to all types of tissue can be drawn. It is evident that at high temperatures, 80° to 100°, great diminution of soluble nitrogen takes place by coagulation, while at low temperatures, 32° to 45°, this effect may occur simultaneously with proteolytic changes (Figs. 1 and 2). It is apparent that in some cases these opposing factors may balance each other with a redistribution of the simple nitrogenous constituents so that no change may be discoverable by analysis. The initial water content and the rate of water removal determine the extent to which enzymatic action and metabolic processes may occur in fresh tissue subjected to desiccation. The nature and the quantity of nitrogenous constituents in the tissue together with the specific enzymatic activity determine to a great extent the nature of the above changes. These relations are well illustrated by the wide difference in results with beet leaves and corn ears, on the one hand, and common barberry and corn leaves, on the other.

Summary

1. Final moisture contents and nitrogen composition as compared with fresh material are given for four different types of tissues dried under various conditions.

2. Drying at different temperatures had no effect on the total nitrogen content of the tissues.

3. Drying at high temperatures, 80° and 98°, caused coagulation of the soluble nitrogenous constituents in all the tissues employed. This was more marked in the case of beet leaves and corn ears than in the corn and common barberry leaves.

4. Drying at low temperatures, 32° and 45°, allowed proteolytic decomposition to take place, but in some instances this effect appears to be counterbalanced by coagulation of the soluble nitrogenous constituents.

5. The general effect of all methods of desiccation was a decrease in the amount of soluble nitrogen, due to coagulation. At 65°, this was the only significant alteration.

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