THE EXTRACTION OF PLANT TISSUE FLUIDS AND THEIR UTILITY IN PHYSIOLOGICAL STUDIES[1]

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(WITH ONE FIGURE)

Introduction

The value of studies of the physico-chemical properties of expressed plant tissue fluids, as indices of physiological and ecological relationships, has long been recognized. Such studies have been confined for the most part to the measurement of osmotic pressure, electrical conductivity, and, more recently, hydrogen-ion concentration. In 1922, NEWTON and GORTNER (**7**) proposed a method for the determination of "bound water," which gives a measure of the relative concentration of hydrophilic colloids. Later, NEWTON (**6**) further extended the series of determinations to include analyses of the press-juice and the parent tissues, from which could be calculated the quantitative distribution of various substances between the tissue fluids and the aplastic or structural portions of the plant.

Both the "bound water" method and the analytical method have been extensively employed in this laboratory during the last few years, in physiological studies of cereals, grasses, and a few other plants. In the course of the work some of the possible errors connected with the extraction of the tissue fluids, and the chances of successfully duplicating results, have been examined. It is believed that the presentation of the results obtained in studying the method of extraction, together with a few considerations as to the utility of the extracted fluids in physiological investigations, will be of interest to a number of physiologists.

Methods

The standard method adopted in the collection of plant material and the preparation of press-juice will first be described. The plant tissue as collected in the field is placed directly in quart Mason jars, packed in crushed ice. When it is difficult to obtain material free from dead leaves, or dead portions of leaves, the jars, after removal to the laboratory, are sorted over one at a time, the plants being removed in small quantities, and the sound portions placed as quickly as possible in another jar, also packed

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in ice and kept covered except at the moment of transferring material. In the grinding and pressing which follows, all apparatus which comes in contact with the tissues and juice is chilled before use to about 0° C. A portion of 350 to 400 grams of the sound tissue is ground in an ordinary meat-grinder, set to cut as finely as possible. The pulp is wrapped in a single thickness of heavy cotton (previously boiled and rinsed in distilled water), placed in a steel press-bowl 12.5 cm. in diameter, with perforated walls and a gutter base, and hydraulic pressure applied very gradually, just enough to keep the juice flowing slowly but not enough to move the pointer of the pressure gauge away from the zero stop. The pointer actually does not move until a pressure of about half a ton has been reached, equal in terms of unit area of the piston to about 3 or 4 atmospheres. When the pointer does begin to move, no further pressure is applied, nor is any attempt made

TABLE I.

SHOWING AGREEMENT IN SOLID CONTENT AND FREEZING POINT DEPRESSION OF FLUIDS OBTAINED FROM THREE SEPARATE PORTIONS OF ONE COLLECTION OF MINHARDI WHEAT LEAVES

Sample	Solids by refractometer	Freezing-point depression
1	19.1	a 1.379
		b* 1.380
2	19.1	a 1.380
		b* 1.379
3	19.1	a 1.384
		b* 1.380
		c* 1.375

* Replicate determinations with fresh portions of juice.

to secure the fluid remaining in the pulp. From the gutter base of the press-bowl the juice flows through a short length of rubber tubing into a flask packed in ice. The last operation is to whirl the juice 4 minutes in centrifuge tubes jacketed with ice slush, with the rheostat governing the speed of the centrifuge (International No. 2) set at the 8th stop. This removes soil particles and other solids in suspension.

When the conditions under which the fluids are obtained are thus standardized in detail, experimental results may be duplicated without difficulty. An experiment to test this point is reported in table I. Three portions of one collection of winter wheat leaves, collected from a field plot, October 6, 1924, were ground and pressed out separately, and the solid content and freezing-point depression of the juice determined. There was no measurable difference in the three samples.

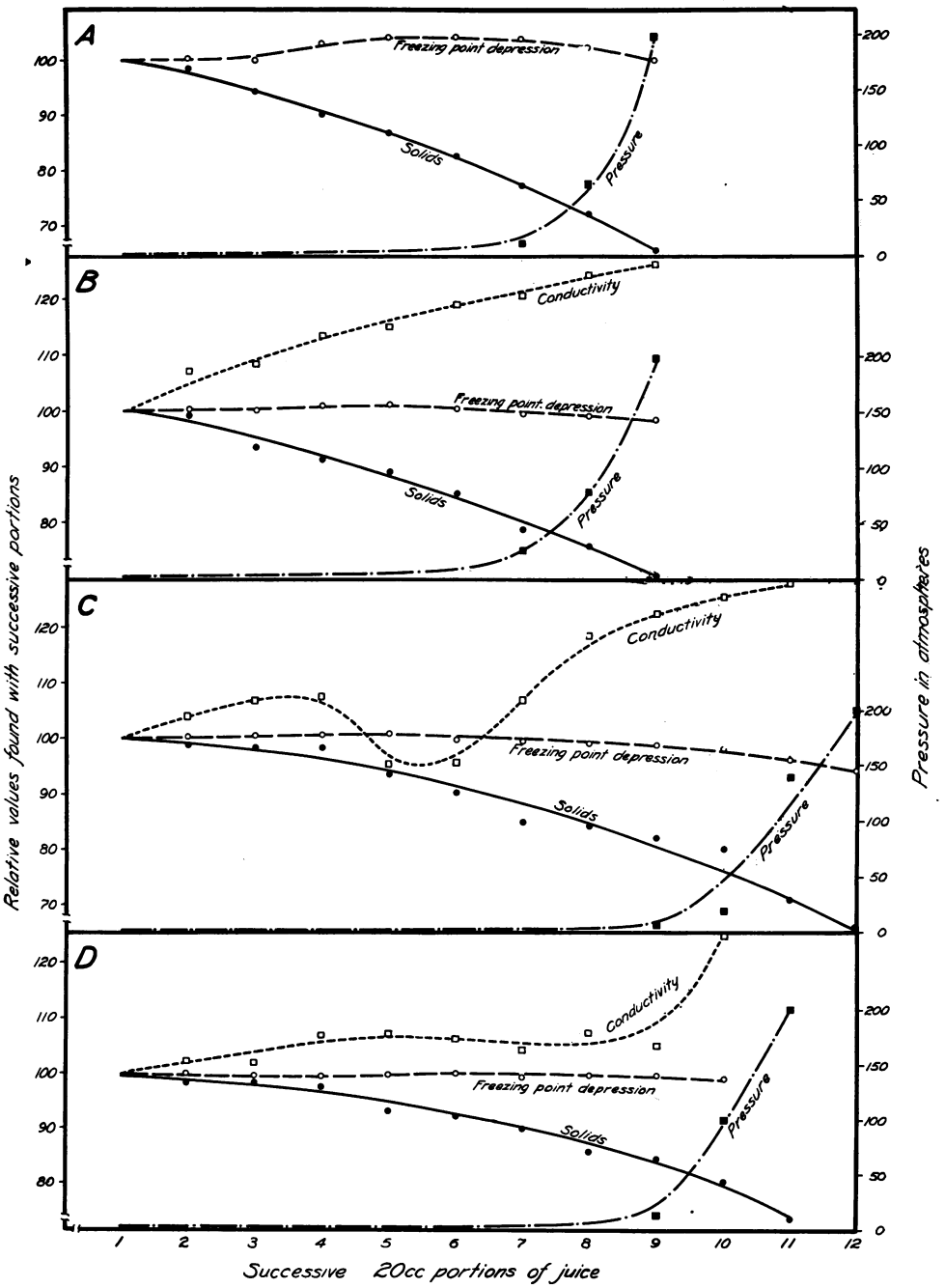

FIGURE 1.—Relative solid content, freezing-point depression, and electrical conductivity of successive 20 cc. portions of juice, collected at gradually increasing pressures. Actual data in corresponding sections A, B, and C–D, table II.

In all the work reported in this paper, the solid content of the juice was determined by the refractometric method proposed by GORTNER and HOFFMAN (3), the freezing-point depression by the Beckmann method, and the electrical conductivity and hydrogen-ion concentration (the latter in one series only) with a highly accurate bridge and potentiometer. The water bath used in the conductivity work maintains its temperature (in these experiments 25° C.) constant within .035° C.

Results

Experimental data on the effect of the pressure used in extracting the fluids are recorded in table II and fig. 1. The standard method was followed in pressing out, except that successive 20 cc. portions of juice were collected separately, and the procedure was extended up to the maximum pressure available. The various constants reported were then determined separately on each 20 cc. portion. In constructing the graph, the pressures are plotted in actual units, but in all other measurements the figures shown in the table for the "1st. 20 cc." have been assigned an arbitrary value of 100, and the values for succeeding portions of juice have been calculated and plotted as percentages of the first figures. The origin of the curve in one case, namely, the freezing-point depression in series C, was fixed by extrapolation, since, as will be observed in the table, the experimental value of the first 20 cc. in this one case was so far out of harmony with all other results that an error seemed probable, and it was considered misleading to base the curve on the uncorrected value.

Discussion

Since three collections of different varieties are involved, the results of the different series, from the point of view of the plant materials used, are not directly comparable. This however makes all the more interesting the considerable resemblance between the curves obtained in all series, as it is evident that their characteristic shapes must be due to the pressures applied. The total solid content decreases from the outset, the rate of decline increasing with the pressure. The freezing-point depression is most nearly constant in value, but nevertheless shows a tendency to rise towards the middle of the extraction process, and fall again when the pressure begins to climb. The conductivity curves, in the three series in which they are available, while they are more irregular than the others, still agree in rising markedly with heavy pressure, and, save for the unaccountable dip in the curve in series C, might almost be said to rise continuously. The tendency for the values of all these properties to change more rapidly after the pressure becomes appreciable, is the justification of our standard practice of

TABLE II

PROPERTIES OF SUCCESSIVE PORTIONS OF JUICE EXPRESSED AT GRADUALLY INCREASING PRESSURES

		1st. 20 cc.	2nd. 20 cc.	3rd. 20 cc.	4th. 20 cc.	5th. 20 cc.	6th. 20 cc.	7th. 20 cc.	8th. 20 cc.	9th. 20 cc.	10th. 20 cc.	11th. 20 cc.	12th. 20 cc.
A. *Einkorn wheat leaves collected June 24, 1924.*													
Pressure in atmospheres		Too small to be recorded on gauge dial						13.	66.	200.	.....	.....	.....
Solids by refractometer		14.6	14.4	13.8	13.2	12.7	12.1	11.3	10.6	9.6	.....	.....	.....
Freezing-point depression		0.993	0.996	0.994	1.028	1.038	1.039	1.036	1.020	0.995	.....	.....	.....
B. *Minhardi wheat leaves collected May 27, 1925.*													
Pressure in atmospheres		Too small to be recorded on gauge dial						27.	80.	200.	.....	.....	.....
Solids by refractometer		22.1	22.0	20.7	20.2	19.7	18.8	17.4	16.7	15.5	.....	.....	.....
Freezing-point depression		1.417	1.420	1.419	1.430	1.435	1.424	1.412	1.405	1.396	.....	.....	.....
Conductivity $\times 10^3$		14.38	15.41	15.60	16.35	16.59	17.17	17.39	17.90	18.13	.....	.....	.....
pH		5.64	5.66	5.51	5.48	5.49	5.50	5.50	5.52	5.52	.....	.....	.....
C–D. *Kharkov wheat leaves collected June 12, 1925.*													
Pressure in atmospheres	C*	Too small to be recorded on gauge dial								7.	20.	140.	200.
	D	Too small to be recorded on gauge dial								13.	100.	200.	.....
Solids by refractometer	C	24.0	23.7	23.6	23.6	22.5	21.7	20.4	20.2	19.7	19.2	17.0	15.8
	D	23.9	23.5	23.5	23.3	22.3	22.1	21.5	20.5	20.2	19.2	17.5	.....
Freezing-point depression	C	1.244	1.287	1.290	1.292	1.295	1.282	1.280	1.275	1.270	1.260	1.232	.....
	D	1.296	1.293	1.290	1.290	1.296	1.300	1.291	1.294	1.292	1.283	.....	.....
Conductivity $\times 10^3$	C	10.11	10.46	10.81	10.89	9.66	9.70	10.84	12.01	12.42	12.67	12.98	.....
	D	10.61	10.86	10.82	11.36	11.40	11.32	11.08	11.43	11.15	13.25	.....	.....

* C – Leaves not frozen before grinding.
D – Leaves frozen with carbon dioxide snow before grinding.

using for analyses only the fluid obtained at low pressure. The use of heavy pressure brings ultrafiltration through the closely packed pulp into play, reducing the colloidal content of the expressed fluid. Possibly some sugar may also be screened out, though our experience with the water-binding capacity of sap colloids suggests that the diminution in the concentration of these substances may be sufficient to account for the observed decline in the freezing-point values. The continued increase in conductivity with pressure shows that the outward passage of electrolyte molecules is not impeded.

Series C and D relate particularly to the influence of pre-freezing of the tissues on the properties of the juice obtained. Different portions of the same collection of wheat leaves were used in the two cases, that used for series C being treated in the usual way, but that for series D before grinding being frozen with a stream of carbon dioxide from a cylinder of the liquified gas until the mass of leaves was filled with carbon dioxide snow. Since the work of DIXON and ATKINS (**2**) this has been a common method of rendering the cell membranes permeable before expressing the fluids. A comparison of the tabular data and curves pertaining to these two series will show that pre-freezing lessened appreciably the deviation of the properties of successive portions of press-juice from those of the first portion pressed out. Nevertheless, the difference in the results of the two series is one of degree rather than of kind. The same characteristic trends are observable in both cases.

Undoubtedly pre-freezing of the tissues is desirable when only the osmotic pressure and conductivity of the fluids are to be determined, though clearly it does not obviate the necessity of standardizing the procedure followed in pressing out. When, however, the object is to obtain the cell contents in a condition as nearly as possible unchanged, pre-freezing is impracticable. Specifically, its precipitating effect on proteins necessitates its omission when the juice is to be used for the study of colloidal properties or protein distribution.

The usefulness of many studies of changes in plant composition, whether seasonal or experimentally induced, has been curtailed by confining the analyses to the entire tissues, whereas it is the tissue fluids which are mainly concerned in physiological activity. When an analysis of the fresh tissue is supplemented by an analysis of the press-juice prepared as described in our standard method, it is possible to calculate the distribution of any desired constituent between the fluid cell contents and the inert supporting framework.

Methods for the complete extraction of leaf cell proteins have been proposed by CHIBNALL (**1**) and by TOTTINGHAM and co-workers (**8**). These,

however, are open to serious objections from our point of view: CHIBNALL'S method involves the use of ether, which will bring about changes in the colloids, and both methods require protracted manipulations during which enzyme activity is unchecked. In the method we have adopted, the grinding of the fresh tissues and pressing out of the juice may be completed in a few minutes, and by carrying out these manipulations at a temperature close to 0° C., the conditions are made particularly favorable to the securing of the cell contents in an unchanged condition. It is not attempted to extract all the soluble proteins, but merely to obtain a representative portion of the tissue fluids. Given the composition of the press-juice, including its total solid content, together with the dry matter content of the original tissue, a calculation may readily be made of the total quantity of any fluid constituent in the original tissue.

The assumption that the press-juice has the same composition as the original tissue fluids is of course difficult to prove. An attempt was made to test the point by comparing the freezing-point depression of the leaf tissues, and of the fluid obtained from them. The leaves were wrapped around the bulb of the Beckmann thermometer, and secured in place with rubber bands, or the bulb was inserted in a test-tube containing the ground leaf pulp. The use of a thermocouple, though more convenient in some respects for solids, had been shown in earlier work (**4**) to be less accurate, particularly in ascertaining the degree of undercooling. It was found that determinations with leaves and pulp could not be made with the same precision as with juice, but fortunately the differences proved to be so large as to make probable errors of minor importance. The results are given in table III, the series letters B and C–D identifying the plant materials used with those reported upon in the corresponding sections of table II. The press-juice values in table III are the means of the successive readings in table II up to the point at which pressures begin to be recorded, and beyond which our standard method precludes the further collection of juice.

TABLE III

COMPARISON OF FREEZING-POINT DEPRESSIONS OF FRESH AND PRE-FROZEN LEAVES, GROUND LEAF-PULP, AND PRESS-JUICE

		LEAVES	PULP	PRESS-JUICE
B	Fresh	2.192	1.686	1.424
C–D	Fresh	2.127	.....	1.286
	Pre-frozen	1.805	.....	1.294

The freezing-point depression of the press-juice was in all cases markedly less than that of the leaves, a fact which, considered by itself, would

indicate a lower concentration in the juice than in the original tissue fluids. But it will be noticed that in series B the grinding of the leaves to pulp, and in series C–D the pre-freezing of the leaves, presumably with no change in concentration of the fluids in either case, produced a marked diminution in the freezing-point depression. Evidently the physical organization of the fluids exercises an important influence on the freezing-point. This is not surprising in view of earlier results (**5, 7**) with plant colloids. Also it is our common experience that determinations of the freezing point of a number of fresh portions of the same lot of juice will check quite closely (*cf.* table I), whereas a second freezing of one portion generally shows a changed value owing to the change in colloidal state produced by the first freezing. When plant tissue is ground and the juice pressed out, the vacuolar sap and protoplasmic colloids, which are separate in the cell, become intimately mixed. Equilibria must be altered, and probably hydrogen-ion concentration changed, with resulting changes in bound water and freezing-point depression. The experimental results given in table III can not therefore be held either to prove or disprove the assumption that the press-juice has the same composition as the original tissue fluids. The point must await further investigation.

Summary

1. By fine grinding of fresh plant tissue and pressing out at low pressure, these operations being carried out close to 0° C., a fluid is obtained which is believed to be of substantially the same composition as the original tissue fluids.

2. The need for careful standardization of the extraction procedure, particularly with regard to the pressure employed, is shown by experimental data.

3. By supplementing ordinary tissue analyses with analyses of the expressed fluids, the distribution of any constituent between the physiologically active and inert portions of the plant may be conveniently determined.

4. The important influence of the physical state of the tissue fluids on their freezing point, and the difficulty of proving the identity of the press-juice with the actual fluids in the plant, are illustrated experimentally.

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