ESTIMATION OF NITRATE NITROGEN IN PLANT JUICE: A STUDY OF THE EXPRESSION AND CLARIFICATION OF THE JUICE[1, 2]

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(WITH ONE FIGURE)

Introduction

Since the publication by GILBERT (3) of the colorimetric method for the determination of nitrate nitrogen, phosphate, and potash in plant juices, several workers have presented criticisms and modifications of this method as applied to nitrates. These include particularly HOLTZ and LARSON (7) and EMMERT (2). Considerable work has been done in this laboratory on this particular determination and the purpose of this paper is to present the modifications which have evolved here during the past four years.

The GILBERT method is essentially as follows: The fresh plant tissue is ground and the juice expressed through fine mesh cloth. This juice is decolorized with carbon black and finally clarified by the addition of solutions of $AgSO_4$, $CuSO_4$, and a mixture of solid $Ca(OH)_2$ and $MgCO_3$ as recommended by HARPER (5). It was originally the plan to determine nitrate nitrogen, phosphorus, and potash on aliquots of the original juice. As the phosphate determination does not permit the use of alkaline reagents for clarification, the treatment with carbon black was extremely important. Later it was believed that heating would coagulate much of the colloidal material, allowing its separation with the carbon black on the filter. This was adopted shortly after publication of the original method, but has never been described in the literature.

HOLTZ and LARSON (7) have published a criticism of the recovery of nitrate nitrogen by the GILBERT method, stating that, using the procedure as published, they were only able to recover 40–45 per cent. of the nitrate added to extracts of wheat plants. This extremely low recovery they attribute to the use of $MgCO_3$ in the final clarification, which prolonged the final evaporation. These workers have suggested a modified procedure

[1] Contribution no. 390 of the Rhode Island Agricultural Experiment Station.

[2] The product collected when plant tissues have been subjected to pressure has been variously termed sap, juice, plant solution, tissue fluid, expressed plant tissue fluid, etc. In order to reduce this confusion of names the author suggests that the word "juice" be used as defined in the Oxford Dictionary; "The watery or liquid part of vegetables or fruits which can be expressed or extracted." The New Webster's International Dictionary defines juice: "The extractable fluid contents of plant cells or plant structures, consisting of water holding sugars or other substances in solution."

in which the $AgSO_4$ and $CuSO_4$ solutions, the carbon black, and the $Ca(OH)_2$ are added at the same time, and filtered without heating. HOLTZ and LARSON further report that this method gives them 90–100 per cent. recovery of added nitrate nitrogen, but their data show no higher results on the original sample of extract than those obtained by the GILBERT method.

The use of carbon black has been criticized by EMMERT (**2**), who cites the theoretical possibility of reduction of nitrates in the solution in the presence of the carbon black. In addition, he mentions the difficulty in obtaining suitable carbon black, and the possibility of relatively great adsorption and occlusion with some brands.

The GILBERT method as it was originally described gave results for nitrate nitrogen, phosphorus, and potash in the juice of plants that correlated with the amounts of these elements applied to the substrate upon which the plants were growing, and with crop yields. The method has then fulfilled the requirements which were imposed upon it, and it still is to be considered sufficiently accurate to indicate any large differences which may exist in crop juices. The modified method presented in this paper includes several refinements in technic that have improved the accuracy of the method so that it more nearly measures the true quantity of nitrate nitrogen in the plant.

Modifications in the method

COMPARISON OF METHODS FOR OBTAINING THE JUICE

Considerable difficulty was experienced in obtaining sufficient juice by the grinding and straining procedure, especially from small samples. There is some question whether the juice so obtained is a representative aliquot of the juice present in the plant, and it was felt that a more suitable method for securing samples of juice was desirable. Several workers (GORTNER and HARRIS (**4**), KORSTIAN (**8**), NEWTON, BROWN and MARTIN (**12**), etc.), have used an ice-salt bath to freeze the plant tissue. This process was used in this laboratory with considerable success, but in some cases the plant tissue was extremely resistant to freezing at the temperature resulting (−10 to −15° C.). Later, following the method of HARVEY (**6**), MEYER (**11**), LEWIS and TUTTLE (**9**), and others, solid carbon dioxide was used as a freezing medium with greater success. The temperature was so low that there was no question of the thoroughness of the freezing when the tissue was placed in contact with the solid CO_2. There has recently been placed on the market a machine which manufactures small cakes of the solid CO_2 from the compressed gas in drums. This eliminates purchasing large quantities of the solid CO_2, and the cost of freezing individual samples is low.

It is unlikely that the substitution of solid CO_2 for the ice-salt bath would affect the recovery of nitrate nitrogen from the resulting juice, since MEYER (**11**) cites evidence that there is little difference in osmotic pressure between samples of juice expressed after freezing with liquid air, solid CO_2, and ice-salt mixture. No direct comparison has been attempted in this laboratory.

After allowing the sample of plant tissue to remain in contact with the solid CO_2 for four hours or more, it is thawed, and pressed with mechanical pressure. An especially designed press cage has been used with good results in a hydraulic press maintaining 1000 pounds per square inch pressure on the tissue.

The cage used was designed to give the greatest ease of cleaning together with the lowest cost of manufacture. Three parts make up the entire assembly: The *sleeve, A,* is a six inch length of cold drawn seamless steel tubing with an outside diameter of three inches, and one-eighth inch walls. The *base plate, B,* is machined from a three-inch cast iron disc three-fourths inch thick to a diameter one sixty-fourth inch less than the inside diameter of the sleeve, leaving a one-quarter inch flange to allow the plate to extend only one-half inch into the sleeve. The *plunger, C,* is machined from a three inch bar of cast iron, six and one-half inches long, leaving a half-inch flange at one end to facilitate removal. This plunger is machined to 0.001 inch of the inside diameter of the sleeve. (See figure 1).

The dimensions given may be varied to suit the individual requirements: A cage made from one-inch tubing two and one-half inches long is very convenient for small samples. These cages can be readily taken apart and cleaned, a convenient feature when a large number of determinations are to be made.

There is a small hydraulic press on the market which is convenient, satisfactory, and relatively inexpensive, and which is designed for the expression of juices and oils. The juice as it is expressed from the tissue flows from the lower end of the cage and into a gutter in the press plate of the press, from which it discharges into a beaker.

The physical character of the juice from the above procedure is markedly different from that obtained when the tissue is ground. The juice from frozen tissue is practically free from cell débris and contains only small quantities of protoplasm, whereas the juice from ground tissue contained much colloidal material.

CARBON BLACK AS A DECOLORIZING AGENT

The treatment with carbon black was necessary in the original preparation of the juice, since, as has been noted above, two other determina-

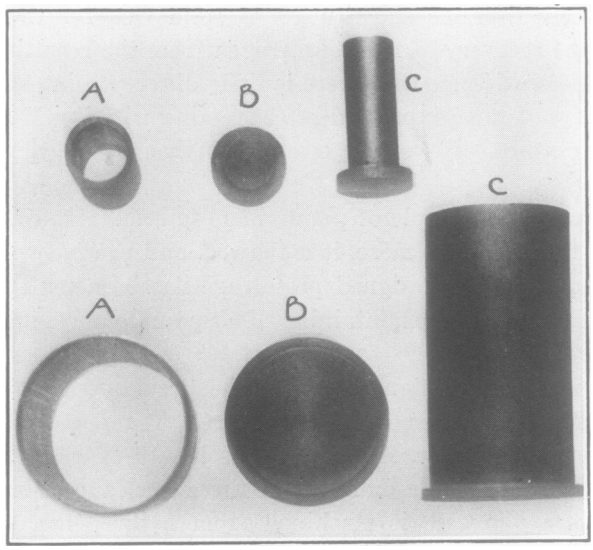

FIG. 1. Press cages used in the expression of plant juices: *A*, sleeve; *B*, base plate; *C*, plunger.

tions were to be made on aliquots of the sample. Since this work is directly concerned only with the determination of nitrate nitrogen, it was found that the reagents recommended by HARPER (5), with slight modifications, removed all of the coloring matter without the preliminary carbon black decolorization. In addition to this, there is considerable possibility

TABLE I

RECOVERY OF NITRATE NITROGEN FROM SYNTHETIC SOLUTION WHEN DIFFERENT BRANDS OF CARBON BLACK WERE USED. TWENTY-FIVE CC. OF A SOLUTION CONTAINING 100 PPM. NITRATE NITROGEN PLUS 2.5 GM. OF CARBON BLACK

BRAND	NITRATE NITROGEN RECOVERED
	ppm.
None	100
A	95
B	105
C	109
D	99
E	14

that the carbon black will adsorb or occlude some of the nitrate nitrogen present in solution. Some carbon blacks, on the other hand, contain nitrates and perhaps free nitric acid. A trial of five brands of carbon blacks is shown in table I.

The fact that the determination could be made on juices without carbon black made possible a study of the amounts actually removed from beet juices by the black itself (table II).

TABLE II

RECOVERY OF NITRATE NITROGEN FROM BEET JUICES WITH AND WITHOUT CARBON BLACK
TWENTY-FIVE CC. OF JUICE PLUS 2.5 GM. OF CARBON BLACK

SAMPLE NUMBER	PLUS CARBON BLACK	NO CARBON BLACK
1	563	667
2	526	602
3	641	735
4	379	757

These figures indicate that carbon black introduces the possibility of serious error. This agrees with the findings of EMERSON (1) and LIPMAN and SHARP (10), who worked with carbon black and animal charcoal. No attempt has been made to determine the way in which the carbon black operates to remove nitrate nitrogen.

EMMERT (2) states that there is the possibility of serious error through the continuation of reduction of nitrate nitrogen in the extract after the addition of carbon black. To the author the possibility of this taking place seems very slight. While there is no direct evidence to be offered to prove that no reduction takes place, table III gives several results which indicate that change in the quantity of nitrate nitrogen recovered is small

TABLE III

NITRATE NITROGEN RECOVERED BY GILBERT METHOD FROM LEAVES AFTER STANDING

CROP (LEAVES)	NITRATE NITROGEN DETERMINED ONE-HALF HOUR AFTER PICKING	TEMPERATURE OF ROOM	NITRATE NITROGEN DETERMINED IN LATER DETERMINATION	TIME ELAPSING BETWEEN DETERMINATIONS
	ppm.	*degrees*	*ppm.*	*hours*
Beet (midribs removed) ...	649	25	641	7
Beet (midribs removed) ...	58	25	70	5
Cabbage (midribs removed)	285	27	273	6.5

even though the juice is allowed to stand for a considerable time. These results were obtained by halving each leaf immediately after collection,

determining nitrate nitrogen in one-half at once and allowing the other half to remain at the temperature shown for the time indicated.

The juice is in contact with the carbon black for a very short time, probably not over five minutes in most cases. While the theoretical possibility that the reduction of nitrates may continue, due to catalytic action of the carbon black or plant reducing substances is acknowledged, it has been the experience of the author that this is so slight under normal conditions that it may be disregarded. It is to be noted that EMMERT secured considerable reduction only when he used metallic zinc with NaOH, a much more powerful reducing agent than normally occurs in plant tissues.

The use of carbon black is not advised without an extremely careful investigation of the effect of the carbon black chosen on nitrates in solution. In this procedure the use of carbon black is eliminated.

CLARIFICATION REAGENTS

The quantities of reagents used in the final clarification of the juice and removal of the chlorides have been changed considerably from the amounts recommended by HARPER in his original procedure. The quantity of saturated $AgSO_4$ solution used to precipitate chlorides has been reduced from 10 cc. to 5 cc., since it has been observed that the latter amount is sufficient to precipitate the chlorides present in normal juices. EMMERT recommends that the treatment with $AgSO_4$ be omitted when the chlorine content is below 20 ppm. It has been noted in this laboratory that most of the juices under examination contain more than the above designated quantity. Any considerable excess of silver, however, leaves a brown or black mirror when the solution is evaporated preliminary to the treatment with phenoldisulphonic acid.

GILBERT has recommended that 0.5 cc. of N $CuSO_4$ be used to precipitate the proteins present in the juice. This quantity is sufficient in most instances, but the use of 1.0 cc. of N $CuSO_4$ is more likely to satisfy all conditions. Table IV shows the results obtained when clarification reagents are omitted or substituted in various ways.

These data substantiate the findings of several workers, that some basic material is necessary to precipitate the silver and copper as the hydroxides. Undoubtedly the greater part of the clarification is brought about by these hydroxides, and it is essential to have them in the state of division which will give the maximum adsorption of coloring material. It is evident that $MgCO_3$ is not suited to be used alone in this connection, as there remained a dark residue after evaporation. Sodium hydroxide gave a finely divided precipitate of colloidal hydroxides which could not be removed by filtering. The use of $Ca(OH)_2$ proved to be as efficacious as the mixture with

TABLE IV

INFLUENCE OF AMOUNT AND KIND OF CLARIFICATION REAGENTS ON THE PHYSICAL PROPERTIES OF THE RESIDUE FROM EVAPORATION OF A FILTERED ALIQUOT AND THE FINAL COLOR DEVELOPED BY PHENOLDISULPHONIC ACID REAGENT

TREATMENT	COLOR OF RESIDUE BEFORE ADDITION OF PHENOLDISULPHONIC ACID	COLOR OF FINAL SOLUTION
2 cc. of juice made to 100 cc. final volume including:		
1. 5 cc. saturated $AgSO_4$: 0.5 cc. N $CuSO_4$ 0.2 gm. $Ca(OH)_2$ + 0.5 gm. $MgCO_3$	White	Yellow
2. 5 cc. saturated $AgSO_4$	Black	Black
3. 5 cc. saturated $AgSO_4$ + 1.0 cc. N $CuSO_4$	Green	Black
4. 5 cc. saturated $AgSO_4$ + 1.0 cc. N $CuSO_4$ + 0.2 gm. $Ca(OH)_2$	White	Yellow
5. 5 cc. saturated $AgSO_4$ + 1.0 cc. N $CuSO_4$ + 0.4 gm. $MgCO_3$	Brown	Black
6. 5 cc. saturated $AgSO_4$ + 0.5 cc. N $CuSO_4$ + 0.2 gm. $Ca(OH)_2$	White	Yellow
7. 5 cc. saturated $AgSO_4$ + 0.5 cc. N $CuSO_4$ + 1 cc. 20 per cent. NaOH	Black	Black
8. Same as 7, but with 2 cc. 20 per cent. NaOH	Black	Black
9. Same as 7, but with 5 cc. 20 per cent. NaOH	Black	Black

$MgCO_3$. This substantiates the work of HOLTZ and LARSON, who found that the $Ca(OH)_2$ alone, was sufficient in the clarification. However, it was not found that the addition of $MgCO_3$ (although it reduced the solubility of the basic precipitant) influenced the time of evaporation, as these workers indicate.

The use of $Ca(OH)_2$ alone, to replace the mixture of $Ca(OH)_2$ and $MgCO_3$ gave slightly higher results, although the magnitude of this increase appears to be within the experimental error of the method (table V).

TABLE V

NITRATE NITROGEN RECOVERED, USING 5 CC. SATURATED $AgSO_4$ AND 1.0 CC. N $CuSO_4$ WITH 0.2 GM. $Ca(OH)_2$ ALONE AND IN COMBINATION WITH 0.5 GM. $MgCO_3$

CROP (LEAVES)	NITRATE NITROGEN			DIFFERENCE
	REAGENTS + $Ca(OH)_2$ + $MgCO_3$	REAGENTS + $Ca(OH)_2$	DIFFERENCE	
	ppm.	*ppm.*	*ppm.*	*per cent.*
Tomato	371	386	+15	+4.0
"	379	390	+11	+2.9
Spinach	140	147	+ 7	+5.0
"	144	148	+ 4	+2.7

From the results it is recommended that 0.2 gram $Ca(OH)_2$ be used in place of the mixture of $Ca(OH)_2$ and $MgCO_3$.

The original procedure recommended by GILBERT directs that after the addition of the clearing reagents the solution shall be heated. It seemed possible that the heating would alter the adsorption equilibrium which exists between the colloidal precipitate and the nitrates in solution. When the solution containing the clearing reagents was allowed to stand for one hour at room temperature clarification was as complete as when heated. A series of determinations was made to compare recovery of nitrate by the two treatments (table VI).

TABLE VI

NITRATE NITROGEN RECOVERED FROM JUICES. HEATING AFTER THE ADDITION OF CLEARING REAGENTS COMPARED WITH ALLOWING TO STAND AT ROOM TEMPERATURE

CROPS (LEAVES)	NITRATE NITROGEN			DIFFERENCE
	HEAT	NO HEAT	DIFFERENCE	
	ppm.	*ppm.*	*ppm.*	*per cent.*
	227	231	+ 4	+ 1.8
	259	333	+ 74	+ 28.6
Beet (midribs removed)	323	398	+ 75	+ 23.2
	83	92	+ 9	+ 10.8
	270	300	+ 30	+ 11.1
	229	291	+ 62	+ 27.1
Cabbage (midribs removed)	377	321	− 56	− 14.8
	371	411	+ 40	+ 10.8
	286	244	− 42	− 14.7
Celery	360	360	0	0.0
	85	103	+ 18	+ 21.2
Mangels (midribs removed)	94	85	− 9	− 9.6
Spinach	125	140	+ 15	+ 12.0
	138	144	+ 6	+ 4.3
	296	285	− 11	− 3.7
	379	393	+ 14	+ 3.7
	76	76	0	0.0
	92	90	− 2	− 2.2
Tomato	83	111	+ 28	+ 33.7
	138	118	− 20	− 14.5
	70	74	+ 4	+ 5.7
	28	36	+ 8	+ 28.6
	353	366	+ 13	+ 3.7
	185	200	+ 15	+ 8.2
Potassium nitrate solutions	185	199	+ 14	+ 7.6
	189	195	+ 6	+ 3.2
	192	183	− 9	− 4.7
Averages			+ 10.6	+ 6.7

The data given in this table indicate that, while the variation in the different individual determinations is great, by far the greater number of results show a higher value for nitrates when no heat is used in the clarification. Since the heating and cooling of the sample consumed considerable time, and the results indicate a less complete recovery of nitrates, it was decided to dispense with the heating.

At times a white precipitate will be formed when the solution containing the phenoldisulphonic acid is neutralized with NaOH. This may be removed, as can a brown precipitate which is rarely encountered at this point by allowing the precipitates to flocculate and filtering.

Method recommended

Place in a cheesecloth bag a sample of fresh plant tissue sufficient to yield a minimum of 10 cc. of juice, and freeze thoroughly for at least two hours. Either an ice-salt bath or solid CO_2 may be used, although the latter is preferable. Remove from refrigerating medium, allow to thaw, and press at once in any apparatus which will give sufficient mechanical pressure. It is best to have arrangements for duplication of this pressure on comparative samples. A hydraulic press with pressure gauge is the most satisfactory equipment. Collect the expressed juice, which should be free from cell residues, and pipette an aliquot (usually 2 cc.) into a volumetric flask (100-cc.).

To the juice in the flask, add about 20 cc. of nitrate-free distilled water; 5 cc. saturated $AgSO_4$ solution, 1 cc. N $CuSO_4$ solution, and 0.2 gram finely divided C. P. $Ca(OH)_2$, shaking after each addition. Shake thoroughly, make to volume with nitrate-free distilled water and filter after standing at least one hour. Discard first portion of the filtrate. Take a suitable aliquot (10 to 50-cc.) of the clear, colorless filtrate, evaporate to dryness on a water bath without overheating, and determine nitrate nitrogen by the phenoldisulphonic acid method, using NaOH to neutralize the acid according to Harper (5). If a precipitate forms at this point, allow to flocculate and filter.

Recovery of added nitrates by the modified method

The recovery of nitrate nitrogen by the above method was determined by adding a known amount of KNO_3 to the juices of different crops. Measured amounts of a standardized solution of KNO_3 were placed in evaporating dishes, evaporated to dryness, and the residue dissolved in a definite quantity of juice. This procedure obviated any change in the adsorption relation of the juice caused by dilution (table VII).

It is to be noted that in only three cases does the recovery exceed 100 per cent. The lowest recovery in the data is 78.1 per cent. and the average

TABLE VII

RECOVERY BY MODIFIED METHOD OF NITRATE NITROGEN ADDED TO PLANT JUICES

CROPS (LEAVES)	NITRATE NITROGEN			RECOVERY
	ORIGINALLY IN JUICE	ADDED TO JUICE	RECOVERED IN JUICE	
	ppm.	*ppm.*	*ppm.*	*per cent.*
Beet (midribs removed)	163	400	488	86.7
	163	1400	1538	98.4
	227	50	240	86.6
	231	100	333	100.6
	231	200	398	92.3
Cabbage (midribs removed)	229	100	377	114.6
	229	200	371	86.5
	291	100	321	82.1
	291	200	411	83.7
	230	50	229	81.8
	230	100	286	86.7
	230	200	360	83.7
Mangels (midribs removed)	42	50	75	81.5
Spinach	30	400	353	82.1
	30	1000	822	79.8
	30	2000	1935	95.3
Tomatoes	76	25	90	89.1
	76	50	111	88.1
	76	75	118	78.1
	214	50	233	88.3
	214	100	296	94.3
	214	200	379	91.5
	270	100	390	105.4
	270	200	448	95.3
	270	250	513	98.7
Average				90.05

of 25 results is 90.05 per cent. This average recovery equals that of HOLTZ and LARSON (7) in one of their two trials.

Comparison of the two methods

When the GILBERT method was compared with the procedure outlined above the differences found were not consistent but were too great in some instances to be disregarded (table VIII).

The discrepancies may be attributed in part to the fact that the grinding of the sample did not accomplish a complete maceration of all the plant cells, and that probably in many cases the juice secured was not a representative aliquot of the juice of the plant as a whole. As has been mentioned previously, many workers have found that the juice obtained

TABLE VIII

NITRATE NITROGEN DETERMINED BY THE GILBERT METHOD COMPARED WITH THAT BY THE RECOMMENDED MODIFICATIONS

CROP (LEAVES)	NITRATE NITROGEN			DIFFERENCE
	GILBERT METHOD	MODIFIED METHOD	DIFFERENCE	
	ppm.	*ppm.*	*ppm.*	*per cent.*
	690	667	− 23	− 3.3
	641	757	+116	+18.1
Beet (midribs removed)	116	92	− 24	−20.7
	95	103	+ 8	+ 8.4
	135	95	− 40	−29.6
	148	112	− 36	−24.3
Cabbage (midribs removed)	67	105	+ 38	+57.0
Celery	183	183	0	0.0
Tomato	628	600	− 28	− 4.5
	637	607	− 30	− 4.7

by freezing and pressing the tissue has considerably different physical properties from that secured by grinding the tissue and extracting the juice under variable pressure.

In order to demonstrate that the amount of nitrate nitrogen found in the juice obtained by the grinding process was not comparable with that in the juice obtained when the tissue was frozen, samples of tomato leaves and beet leaves (without midribs) were ground as recommended by GILBERT. After extracting the juice which could be expressed by hand pressure, and centrifuging, the nitrate nitrogen was determined by the modified method. The ground residue remaining in the cloth was immediately frozen with solid CO_2, and allowed to stand overnight in the frozen state. Sixteen hours later this residue was thawed, pressed at once in the hydraulic press, centrifuged, and the nitrate nitrogen determined in the juice in the same manner. The averages of two closely agreeing determinations are given in each case:

CROP	LEAVES		DIFFERENCE
	GROUND NO_3–N	FROZEN NO_3–N	
	ppm.	*ppm.*	*per cent.*
Tomato	341	435	47.8
"	400	472	36.3
"	537	576	9.5
Beet (midribs removed)	310	411	37.7

It is evident that the discrepancies noted in table VIII may be attributed in part, at least, to the differences which may exist in the nitrate nitrogen content of the juices obtained by the two procedures. The juice obtained when the plant tissue is ground does not seem to be a true aliquot of the juice as it exists in the plant, and it is evident that the values obtained when the juice is secured by freezing will be more nearly correct. It is to be noted, however, that in the above experiments the differences between the two results are exaggerated, since the removal of a less concentrated aliquot tends to concentrate any fractions which may be removed subsequently. Hence the data shown indicate too high values for the frozen aliquot.

Summary

A considerable number of alterations have been made in the technic of estimating nitrate nitrogen in the juice of crop plants as previously recommended from this laboratory. It is recommended that the plant tissue be frozen and pressed after thawing rather than ground and squeezed through cloth by hand. The carbon black for decolorization has been omitted, and the quantities of reagents used for clearing the juice have been changed. A study of the recovery of nitrate nitrogen added to plant juices by the new method indicate rather wide variations, with the average of 25 determinations at 90.05 per cent.

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