

THE PHOSPHATE CONTENT OF CANE JUICE

By Donald McRae.

On resuming, Mr. D. McRae read the following paper on "The Phosphate Content of Cane Juice."

During recent years considerable attention has been paid to the phosphate content of cane juice. It has been shown to give a very good indication of the response of certain cane soils to phosphatic fertilization, while experiments conducted in Hawaii and Porto Rico have shown that defecation is primarily a function of the concentration of phosphate in the juice.

Relation to Soil Fertilizer Requirements:

The following is an extract from a "Memorandum on the Relationship between Sugar Cane Analysis and Soil Requirements," presented by H. H. Dodds, Director of the Natal Sugar Experiment Station, to the Experiment Station Committee in June, 1924:

"Research recently undertaken in Hawaii appears to indicate that the so-called 'Physiological' method of soil analysis, that is, the determination of the mineral food constituents extracted by the plant, is applicable to the study of sugar cane. (H. Walker, 'Industrial and Engineering Chemistry,' Vol. 12, No. 2, p. 164.) While this method is not so reliable and accurate as carefully controlled field experiments and cannot replace them, it is of considerable promise as a means of affording a rapid preliminary approximate survey of soil requirements.

"It is, in a sense, a modification of the conventional method of soil analysis, by which the soil is extracted by weak acids in the laboratory in the effort to imitate natural processes. Instead of this somewhat unsatisfactory method, the plant is allowed to make its own selection of mineral nutriment, and the amount found to be so taken up appears to be a measure of nutriment available in the soil.

"The difficulty naturally arises in the case of most crops in getting suitable bulk samples of the product which fact has probably prevented the method from coming into general use.

"In the case of sugar, however, the correlation of the cultivation with an elaborate manufacturing

process, enables one to obtain small uniform samples of the product (cane juice in this instance) representative of more or less large areas of the cultivated land."

In this connection it is interesting to note that G. N. Hoffer in a paper on "A Simple Test for Detecting the Nutrient Needs of Corn Plants" (Journal of American Society of Agronomy—January, 1926; p. 29) gives details, as indicated in the following abstract, of the application of this method to corn plants.

"Corn plants during the latter part of the growing season, can be used to determine the relative amounts of nitrates and potassium salts available to them during the season. After the ears are well developed on the plants, the presence of reserve nitrates in the stalks can be detected by a simple test of the tissues when the stalk is cut and split open lengthwise. Several drops of a 1 per cent. solution of diphenylamine in 75 per cent. sulphuric acid applied to the tissues will reveal the presence of nitrates in the juices of the tissue by the development of a blue colour. If the test is negative, and the leaves and stalk tissues are of a yellow green colour, nitrate starvation is indicated.

"The accumulation of iron compounds in the nodal tissues of the plants indicates a lack of potassium, as shown by Hoffer and Frost. The test for iron in these tissues consists of applying an acid 4% solution of potassium thiocyanate to them, and the development of a red colour indicates the presence of iron.

"Reserve potassium can be detected in the stalk tissues by cutting a small thin section of the tissue and adding two or three drops of an acid solution of chloride of platinum. Crystals of the double chloride of potassium will be formed, but it requires a microscope to detect them. In making field tests of plants, this test is omitted. The determination of the potash requirements is based on the relative amounts of iron in the nodal tissues. If traces of iron, or small quantities only are found, the supply of potassium salts has been adequate, whereas if iron is abundant, the need of potassium is indicated."

Practically all of the published results in connection with the application of this physiological method to cane have come from Hawaii. In relation to phosphate requirements under Hawaiian conditions, Walker states that if the phosphate content of a cane juice from a given area is less than 0.01 per cent. P_2O_5 , the land will probably respond to phosphatic fertilization. The results of Walker and Glick (*International Sugar Journal*, September, 1923, p. 478) indicate that in Hawaii a cane juice containing less than 0.05 per cent. K_2O indicates a potash deficiency in the soil.

Effect on Clarification and Removal of Colloids:

McAllep and Bomonti (*Hawaiian Planter's Record*, 26, 139, 1922), as a result of a long series of experiments in Hawaii, have shown that for efficient clarification by the defecation method the phosphate content of the juice should not fall below 0.030 to 0.035 gms. P_2O_5 per 100 c.cs. of juice.

In 1925, J. D. Bond, of the Ewa Plantation Co., Hawaii, published a paper entitled "Defecation in Cane Sugar Manufacture" (*Industrial and Engineering Chemistry*, Vol. 17, No. 5, page 492 (1925)), which has been abstracted as follows:—

"Defecation is primarily a function of the phosphate content of the juice, and is essentially a process for removing coarse dispersoids from the juice by the formation of a flocculent precipitate within the juice. This precipitate not only absorbs colouring and colloidal matter, but, enmeshing the suspended matter, carries it down in settling. Sufficient phosphate must be present in the juice to ensure a precipitate large enough to function efficiently. After sufficient precipitate has been formed very little is gained by additional precipitation, and for this reason the addition of phosphate to juices cannot be justified unless the phosphate content is too low."

More recently, H. O. Paine, J. C. Keane and M. A. McCalip have given results of experiments on "The Influence of Phosphate and Colloid Contents of Cane Juice on Defecation" (*Industrial and Engineering Chemistry*, Vol. 20, No. 3, p. 262 (1928)). The following is an abstract:—

"A series of large-scale laboratory defecation experiments made during the recent sugar season in Porto Rico showed many significant facts concerning cane-juice clarification. The phosphate content of the raw juice, an important constituent from the standpoint of clarification by simple lime defecation, showed an approximately linear relation to the colloid elimination as determined by the dye test. The increased colloid elimination at higher phosphate content was accompanied by an increase in the weight and volume of mud. With increasing phosphate content of raw juice, the rate of increase in volume was greater than the rate of increase in weight of mud.

"The juice at this factory was deficient in phosphates. The raw juice was defecated with and without the addition of phosphate, the juice being limed to the same pH value in each case. A comparison of the two groups showed a decided increase in colloid elimination, as determined by ultra-filtration, an average relative improvement of 28% for total colloids being obtained in the case of addition of phosphate."

No work apparently has been done on the effect of phosphate content on clarification by sulphitation.

Determination of Phosphate:

In view of the importance of the phosphate content of cane juice as outlined above, it is necessary to have a rapid fairly accurate method for its estimation. The standard method, evaporation of a large volume of juice, ashing of the large bulk of carbonaceous material obtained, and determination of the phosphate in the ash by the standard gravimetric method is obviously too cumbersome for the purpose.

Walker has developed the volumetric uranium method of Sutton for use with cane juices. This method, while being fairly rapid has been shown to give low results, and the experience of the author with regard to the method was that with Uba juices at least, the end point was very indistinct. Table 1 consists of the results of a series of tests made by this method, conducted on various samples of crusher juice from Messrs. Natal Estates Factory, Mount Edgecombe, during September, 1926. The results show at once the large fluctuations which can take place in the phosphate content of the crusher juice at any particular mill.

More recently, Springer and Davies have described a modified Pemberton-Neumann Method ("Tropical Agriculture"—Vol. 4, No. 6 (June, 1927), page 108). The method consists essentially of precipitation of phosphate as phospho-molybdate in the boiled and filtered juice, filtration and estimation by titration. It is claimed to be more accurate than the uranium acetate method, but the careful control of conditions necessary during the precipitation of the phospho-molybdate make it rather unsuitable for sugar-house laboratory routine work.

Miss I. Lonstein, of Pretoria, has recently published details of the estimation of phosphate in citric acid soil extracts by Denige's Molybdate Method, and the method employed seemed particularly applicable to work on cane juices. Experiments revealed the fact that the method was admirably suited to the determination of phosphate in boiled and filtered juice, while fairly accurate results can be obtained by using the juice direct. Details of the application of this method are given below.

It was noticed that all the methods evolved so far take no account of the possibility of the presence of organic phosphate in the cane juice, and the effect of boiling and filtering on both the organic and inorganic forms. The author has developed a rapid method for the estimation of the total phosphate in cane juices, described below, and the method has been applied to a large number of juice samples during the recent harvesting season.

Results have indicated that the actual percentage of organic phosphate in a juice is comparatively low, often being nil; but in some cases, where the total phosphate content is low, the organic phosphate may amount to approximately 50 per cent. of the total.

Table II. contains the results of a series of tests on 20 samples of crusher juice, supplied through the courtesy of Messrs. Natal Estates, Ltd., Mount Edgecombe.

Table III. gives the results of a series of analyses conducted in connection with a Phosphatic Fertilizer Experiment harvested at the Natal Sugar Experiment Station in July, 1928. The results of this experiment showed that the soil under test responded very well to phosphatic fertilizers, which in this test were applied at the rate of 90 lbs. P_2O_5 per acre. The average results indicate an increase of the total phosphate content of the juice from the fertilized sections over the controls of from 51 to 91 per cent.

Table IV. contains the results of a series of tests conducted in connection with a Cane Variety Experiment harvested at the Natal Sugar Experiment Station in July, 1928. It illustrates well the differences in total and inorganic phosphate obtained from variety canes grown in the same field, and the variations in the phosphate content of the cane juice from different parts of the field.

APPLICATION OF THE COLORIMETRIC METHOD.

Determination of Total Phosphate:

The method consists essentially of evaporating to dryness, 1 c.c. of juice in the presence of calcium acetate, ashing to destroy the organic matter, and extraction with acid.

The following method was developed as being most suitable for work in the laboratory of the Experiment Station; various modifications may be necessary to meet local conditions.

A 5.5 cm. circle of Whatman No. 41 Filter Paper is suitably supported at 4 to 5 points at a convenient height above an electric hot plate with an open element. One c.c. of juice, in which a little calcium acetate has previously been dissolved is measured in a pipette and dropped on to the paper, so that the whole paper is kept moist until all the juice has

been transferred. The paper is dried, placed in an electric muffle furnace and ashed. When completely ashed, it is transferred to a 50 c.c. graduated flask, dissolved in acid (it dissolves completely giving a colourless solution), the volume completed to 50 c.c.s. and the contents of the flask well shaken. The phosphate content in this diluted solution is then determined by the method detailed below.

Determination of Inorganic Phosphate in Boiled and Filtered Juice:

This is determined directly on the boiled and filtered juice. One c.c. is diluted to 50 c.c.s. and the phosphate determined as detailed below, the colour of the juice having very little effect on the determination.

The technique of the colorimetric method described below is that of the Division of Veterinary Education and Research, Pretoria. (See paper by Miss Lonstein, South African Journal of Science, 1926.)

Reagents:

Reagent A. consists of 100 c.c.s. of 10% ammonium molybdate mixed with 300 c.c.s. of 50 per cent. sulphuric acid (by volume) and should be stored away from direct light. If desired the two components can be made up separately and used individually in the colour reaction.

Reagent B. is stannous chloride freshly prepared each day by using 0.1 gm. of tin, one drop of dilute copper sulphate (about 4%) as catalyst, and 2 c.c.s. of pure concentrated hydrochloric acid, warming in a test-tube to hasten the reaction.

These reagents when added to solutions of phosphates give a blue colour which, in the cold, reaches its maximum intensity in five minutes, and thereafter slowly fades. If the colour readings are to be taken in a Dubosc colorimeter with a 50 mm. scale, a suitable standard solution is represented by 0.1 mg. P_2O_5 diluted to about 90 c.c.s. before adding the reagents, and finally to 100 c.c.s. for transferring to the instrument. If ordinary measuring cylinders are used, the greater depth of solution allows of a still lower standard being used. A convenient standard is a solution of microcosmic salt equivalent to 0.5 mg. P_2O_5 per 100 c.c.s. Two c.c.s. of this standard are diluted to 20 c.c.s. in preparing the colour standard.

Matching Cylinders:

Two ordinary 25 c.c.s. cylinders are chosen of uniform base and bore so that the 20 c.c.s. mark on each stands at the same height. These are converted into matching cylinders by painting the sides with Japan Black so as to exclude horizontal light. On placing over white paper in a good light (preferably artificial light in a dark room) and tilting to secure good reflection, a perfect match should be readily obtained between duplicate standard colours corresponding to 0.01 mg. in 20 c.c.s. The

depth of liquid in the cylinders, and hence the depth of colour, is most easily varied by simply pouring out from either, a few drops at a time, into a clean spare cylinder. Pouring out half a c.c. is sufficient to produce a distinct variation of shade, and hence the reading error is not greater than 0.5 c.cs. in 20 c.cs., or 2.5 per cent. With a good eye and a little experience the matching error may be taken as about 1 per cent., and therefore negligible for all determinations in which only two "significant figures" are required. The total working errors can easily be kept within 2 per cent.

Method:

To a suitable aliquot (1—10 c.cs.) of the unknown, neutralised with ammonia, add exactly 0.5 c.cs. of Reagent A. in an ordinary 25 c.cs. measuring cylinder, and dilute with water to 19.8 c.cs. Add **four drops** (0.2 c.cs.) of Reagent B., so bringing to the 20 c.c. mark, invert over a clean thumb, to mix. Simultaneously treat an aliquot of the standard phosphate solution, equivalent to 0.01 mg. P_2O_5 in **exactly the same way**, and allow both to stand for five minutes for full development of colour. Then transfer to the matching cylinders, pour back from the darker until the colours are identical, and note the amount discarded.

$\frac{\text{Reading depth of Standard}}{\text{Reading depth of Unknown}} \times 0.1 \text{ mg.}$ then gives mgms. P_2O_5 in the aliquot used.

For best results the two readings should not vary more widely than 14 c.cs. and 20 c.cs., and should be completed within 15 minutes after standard colour development in order to guard against slight variations in the rate of fading, due to presence of other salts.

It is, however, perhaps not out of place to mention the pitfalls which may be experienced by anyone unaccustomed to microtechnique.

Experiment Station,
South African Sugar Association,
Mount Edgecombe, Natal.
March, 1929.

(1) Scrupulous cleanliness of vessels, and **careful blank** on all reagents used. Since the final determination is conducted on one hundredth of a milligram P_2O_5 it is obvious that the merest trace of alien phosphorus will upset the results. In particular the sulphuric acid and calcium acetate should be carefully tested for impurities giving a colour reaction, and ammonia be used for neutralisations owing to the difficulty of getting phosphorus-free caustic soda or potash. If working in a general laboratory it is safest for each analyst to retain his own very simple set of apparatus and reagents in his own bench cupboard, and not share with other workers. One slip of a pipette into the wrong bottle may involve making up a fresh reagent. A separate pipette or burette should be kept for each reagent, and pipette of 1 c.c. or less be calibrated with respect to the personal delivering equation of the individual analyst.

(2) Too little stannous chloride, or stannous chloride prepared the day before, may not give full development of colour. Too much freshly prepared stannous chloride confers a greenish tint very difficult to read against the standard blue. Five drops is the permissible maximum, seven drops introduces distinct error, and ten drops gives an unreadable tint.

(3) As in all colorimetric methods, precisely parallel treatment of unknown and standard is essential (especially in regard to the time factor) unless the precise effect or minor deviations is fully known. Thus while differences of temperature as great as 10°C . between standard and unknown during colour development affect the result relatively little, the difference of colour development at 15 c.cs. dilution and 20 c.cs. dilution may introduce an error of 20 per cent. This is because colour varies directly with the concentration of acid contributed by Reagent A. For the same reason preliminary neutralisation must be reasonably precise, and the presence of appreciable amounts of other acids (particularly nitric acid) avoided.



**PHOSPHATE CONTENT OF SAMPLES OF CRUSHER JUICE FROM CANE FROM
VARIOUS DISTRICTS IN NATAL.**

(Determination by Uranium Acetate Volumetric Method.)

DISTRICT.	Purity of Juice.	Phosphate Content.	DISTRICT.	Purity of Juice.	Phosphate Content.
Umhlali.. .. .	92.4	0.003	Effingham	80.0	0.040
Umfolozi Flats	87.4	0.075	New Guelderland.. .. .	87.7	0.020
Umfolozi Flats	87.3	0.065	Tugela	85.8	0.015
Umfolozi Flats	82.0	0.070	Sinkwazi	92.5	0.010
Kwambonambi	81.9	0.025	Umfolozi Hills	84.1	0.005
New Guelderland	90.8	0.005	Umfolozi Hills	83.7	0.005
Unknown	87.5	0.005	Umfolozi Hills	81.3	0.003
Umfolozi Flats	79.7	0.025	Umfolozi Hills	84.4	0.003
Mount Edgecombe	88.5	0.020	Umfolozi Hills	82.8	0.003
Mount Edgecombe	92.0	0.010	Tugela	88.4	0.005
Eshowe	89.3	0.010	Eshowe	91.9	0.030
Umhlali.. .. .	92.3	0.005	Gingindhlovu	87.8	0.003
New Guelderland	86.8	0.010	Eshowe	91.7	0.010
Umfolozi Flats	82.1	0.050	Eshowe	92.6	0.030
Umfolozi Flats	83.2	0.050	Umgeni	86.7	0.008
Tugela	83.5	0.020	Eshowe	92.7	0.025
Mount Edgecombe	90.0	0.020	Eshowe	75.8	0.005
Umgeni.. .. .	88.4	0.005	Tugela	87.0	0.040
Unknown	84.0	0.005	Tugela	90.6	0.015
Umfolozi Flats	83.7	0.065	Tugela	84.8	0.040
Umfolozi Flats	84.5	0.055	Umfolozi Hills	83.1	0.030
Mount Edgecombe	86.6	0.040	Umfolozi Hills	83.4	0.070

Phosphate content in this table is expressed as grams P_2O_5 per 100 c.c.'s. of Juice.

**PHOSPHATE CONTENT OF SAMPLES OF CRUSHER JUICE FROM CANE FROM
VARIOUS DISTRICTS IN NATAL.**

JUNE, 1928.

(Determination by Colormetric method.)

	Brix.	Sucrose.	Purity.	P ₂ O ₅ A.	P ₂ O ₅ B.	P ₂ O ₅ C.
Effingham	20.3	15.53	76.5	43.0	44.0	41.0
Kwambonambi	19.0	16.18	85.2	38.0	32.5	33.7
Kwambonambi	18.9	16.76	88.7	11.1	7.8	6.8
Kwambonambi	18.7	15.68	83.9	6.8	5.0	4.8
Eshowe	19.5	17.68	90.7	12.1	11.1	9.0
Darnall	19.0	16.45	86.6	11.4	10.5	10.0
Umgeni	20.5	17.61	85.9	45.0	41.0	41.0
New Guelderland.. ..	19.9	17.22	86.5	25.0	23.1	23.1
Duffs Road.. ..	20.5	16.90	82.4	59.0	63.0	57.0
Duffs Road.. ..	20.3	15.71	77.4	56.0	56.0	54.0
Duffs Road.. ..	22.2	18.48	83.2	19.6	16.9	16.9
Duffs Road.. ..	21.4	18.17	84.9	18.1	18.1	17.5
Phoenix	21.3	17.54	82.4	30.3	29.4	27.0
Duffs Road.. ..	21.3	18.91	88.8	51.0	50.0	49.0
Duffs Road.. ..	21.3	18.94	88.9	50.0	43.0	44.0
Duffs Road.. ..	21.5	18.17	84.5	32.5	31.2	31.2
Phoenix	20.7	18.22	88.0	13.2	13.2	12.1
Frasers	21.0	18.84	89.7	12.5	10.0	10.0
Frasers	20.8	18.84	90.6	10.5	9.8	8.5
Umhlali	20.0	16.05	80.3	25.0	25.0	25.0

P₂O₅ **A.** = Total phosphate (as mgs. P₂O₅ per 100 gms.) in expressed juice.

P₂O₅ **B.** = Total phosphate (as mgs. P₂O₅ per 100 gms.) in boiled and filtered juice.

P₂O₅ **C.** = Inorganic phosphate (as mgs. P₂O₅ per 100 gms.) in boiled and filtered juice.

TABLE III.

PHOSPHATE DETERMINATIONS IN PHOSPHATIC FERTILIZER EXPERIMENT.

	Control:			Superphosphate.			Rock Phosphate.			Bone Meal.			Basic Slag.			Rhenania Phosphate.			Rock Phosphate plus Superphosphate.		
	P ₂ O ₅ A.	P ₂ O ₅ B.	P ₂ O ₅ C.	P ₂ O ₅ A.	P ₂ O ₅ B.	P ₂ O ₅ C.	P ₂ O ₅ A.	P ₂ O ₅ B.	P ₂ O ₅ C.	P ₂ O ₅ A.	P ₂ O ₅ B.	P ₂ O ₅ C.	P ₂ O ₅ A.	P ₂ O ₅ B.	P ₂ O ₅ C.	P ₂ O ₅ A.	P ₂ O ₅ B.	P ₂ O ₅ C.	P ₂ O ₅ A.	P ₂ O ₅ B.	P ₂ O ₅ C.
I.	6.0	3.5	—	13.1	11.5	8.7	13.0	9.7	7.8	10.2	7.6	6.0	11.5	9.2	7.8	12.2	9.5	9.2	8.7	8.0	5.1
II.	7.2	4.9	4.9	9.2	6.9	4.4	10.8	9.0	6.9	10.2	7.6	5.8	8.6	7.3	5.5	8.3	6.8	4.0	9.7	9.2	7.8
III.	5.1	—	—	9.2	6.4	4.5	15.3	11.3	7.3	12.2	9.9	8.3	13.6	10.5	7.8	10.7	9.5	7.7	12.7	10.8	9.2
IV.	7.3	5.2	3.2	7.7	5.4	3.6	9.5	7.2	5.8	9.8	6.8	5.3	13.0	11.5	8.1	9.6	8.7	6.2	7.8	7.3	4.4
Average	6.4	—	—	9.8	7.6	5.3	12.2	9.3	7.0	10.6	8.0	6.4	11.7	8.6	7.3	10.2	9.6	6.8	9.7	8.8	6.6

I., II., etc., represent results of analyses of juice from hand samples taken from the various sections of the experiment.

P₂O₅ **A.** = Total phosphate (as mgs. P₂O₅ per 100 gms.) in expressed juice.

P₂O₅ **B.** = Total phosphate (as mgs. P₂O₅ per 100 gms.) in boiled and filtered juice.

P₂O₅ **C.** = Inorganic phosphate (as mgs. P₂O₅ per 100 gms.) in boiled and filtered juice.

TABLE IV.

PHOSPHATE DETERMINATIONS IN VARIETY CANE EXPERIMENT.

	1900 Seedling.			Badila.			P.O.J. 213.			D. 1135			Uba.		
	P ₂ O ₅	P ₂ O ₅	P ₂ O ₅	P ₂ O ₅	P ₂ O ₅	P ₂ O ₅	P ₂ O ₅	P ₂ O ₅	P ₂ O ₅	P ₂ O ₅	P ₂ O ₅	P ₂ O ₅	P ₂ O ₅	P ₂ O ₅	P ₂ O ₅
	A.	B.	C.	A.	B.	C.	A.	B.	C.	A.	B.	C.	A.	B.	C.
I. ..	36	34	29	49	41	41	27	26	26	34	32	30	54	50	46
II. ..	29	29	29	55	54	48	30	29	29	29	29	26	47	44	41
III. ..	22	18	18	36	35	35	32	32	26	37	37	36	50	46	41
IV. ..	33	29	29	41	40	40	30	30	30	41	39	36	38	33	31
V.	52	48	46
Average	30	28	26	45	42	41	30	29	28	35	34	32	50	44	41

I., II., etc., represent results of analyses of juice from hand samples taken from the various sections of the experiment.

P₂O₅ **A** = Total phosphate (as mgs. P₂O₅ per 100 gms.) in expressed juice.

P₂O₅ **B** = Total phosphate (as mgs. P₂O₅ per 100 gms.) in boiled and filtered juice.

P₂O₅ **C** = Inorganic phosphate (as mgs. P₂O₅ per 100 gms.) in boiled and filtered juice.