

The growth inhibiting effect of maleic hydrazide on plants continues for many months after the original chemical application. For example, Crafts (3) has reported that Bermuda grass will remain inhibited for over a year. Also, similar results are indicated for other grasses (4), potatoes (8), and sweet potatoes (5). Unfortunately, biological data do not permit differentiation between the actual presence of maleic hydrazide and growth inhibition caused by irreversible changes in the plant, which might persist after the chemical itself has disappeared.

Direct evidence of the persistence of maleic hydrazide in plants can be seen from residue data. By Wood's colorimetric method (10), normal residue levels have been found in potatoes and in the roots of turf grasses as long as 8 months after treatment. The residue method is fairly specific for maleic hydrazide, and preliminary isotope dilution and paper chromatography experiments on extracts of plants treated with carbon-14 maleic hydrazide also show the presence of carbon-14 maleic hydrazide in the extracts. Apparently at least part of the chemical in the plant tissues resists breakdown for long periods, and it seems unlikely that instability of maleic hydrazide in the plant is a major factor limiting performance.

Correlation with Field Results

Conclusions based on the laboratory studies were tested under field conditions in two locations. Radishes were sprayed with several formulations at equivalent rates. After harvest the roots were analyzed for maleic hydrazide. Such a procedure measures over-

Table II. MH Residues

Formulation	Parts per Million	
	24	72
	hours, 60 to 100% RH	hours, 35 to 100% RH
DEA salt	14	21
Choline salt	..	23
Potassium salt + sorbitol	12	16
Potassium salt	10	..
Sodium salt	8	..

all performance, not merely absorption rate. It was felt that if this test ranked the formulations in the same order as the laboratory absorption tests, it would support the idea that absorption was a limiting factor in field performance. As environmental conditions could not be controlled, they were merely recorded. Results are shown in Table II.

These residue data are consistent with the laboratory findings. Further support comes from performance data on potatoes. The diethanolamine formulation (MH-30) performs more consistently than a sodium salt formulation, and sprout inhibition and residue levels correlate very well.

Field results, especially on tobacco, indicate that rapid initial absorption may be desirable. For example, about half of the chemical (applied as the diethanolamine salt) might be expected to be absorbed in 4 days at 50% relative humidity or in 1 day at 100% relative humidity (Figure 2). Even when no rainfall occurs to wash off unabsorbed material, the more rapid absorption appears more efficient. This probably involves many complex variables not considered in the laboratory tests.

Field results on tobacco also support the idea that plants growing in dry soil (presumably under moisture stress) absorb more slowly at a given humidity than plants growing in wet soil under similar conditions.

Thus, while a field testing program is the final means of determining the value of any agricultural chemical or formulation, there are obvious advantages in being able to do the preliminary evaluation under controlled conditions. It is thus possible to examine each factor independently. The general quantitative technique described should be useful for studying factors affecting absorption of other systemic agricultural chemicals.

Literature Cited

- (1) Andreae, W. A., *Arch. Biochem. Biophys.* **55**, 584-6 (1955).
- (2) Baker, J. E., private communication, 1957.
- (3) Crafts, A. S., Currier, H. B., Drever, H. R., *Hilgardia* **27**, 723-57 (1958).
- (4) Escutt, J. R., *J. Sports Turf Research Ind.* **8**, 1-5 (1953).
- (5) Ezell, B. D., Wilcox, M. S., *J. Agr. Food Chem.* **2**, 513-15 (1954).
- (6) Freed, V. H., Montgomery, J., Research Rept., Western Weed Control Conf. p. 99, March 1957.
- (7) Hayward, H. E., "The Structure of Economic Plants," Macmillan, New York, 1938.
- (8) Rao, S. N., Wittwer, S. H., *Am. Potato J.* **32**, 51-9 (1955).
- (9) Towers, G. H. N., Hutchinson, A., Andreae, W. A., *Nature* **181**, 1535-6 (1958).
- (10) Wood, P. R., *Anal. Chem.* **25**, 1779-83 (1953).

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PLANT TISSUE ANALYSIS

Leaf Analysis. Errors Involved in the Preparative Phase

A critical investigation of the washing, drying, grinding, and storage of citrus and pineapple leaf material, prior to chemical analysis for nutrient elements, is presented. All these preparative steps are subject to relatively large errors. Satisfactory procedures for minimizing the errors are suggested.

AFTER HARVESTING, plant material is usually subjected to four different preparative steps before the actual chemical analysis is carried out: washing the material to remove surface contamination, drying to stop enzymatic reactions and prepare the material for grinding, mechanical grinding to reduce the material to a state of subdivision suitable for analysis, and final drying to constant weight to obtain a standard-

ized value on which to base the analytical figures. Two other steps are often necessary: storage of the fresh material prior to washing and drying, and storage of the leaf powder prior to analysis.

A careful survey of the literature has revealed a great deal of variation in plant analysis technique, and the newcomer to this field often has great difficulty in selecting efficient methods to suit his purpose. The author, primarily in-

terested in sampling studies for which accurate analytical procedures were essential (15, 16), carried out a careful investigation of the complete preparative phase involved in the chemical analysis of citrus and pineapple leaves.

Apparatus

All flame photometric work was carried out on an Eel flame photometer, using "bottle gas" and compressed air.

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All colorimetric determinations were carried out on a Hilger Spekker absorptiometer of the latest design, H760.

Fresh plant material was dried in an Analis forced-draft oven with stainless steel interior ($\pm 1^\circ$ C.). Leaf powder was dried in a Gallenkamp convection oven with stainless steel interior.

Plant material was powdered in a Vetter mechanical agate ball mill.

Translucent silica Kjeldahl flasks were used during the wet-ashing stage to minimize contamination.

Analytical Methods

All analytical procedures were carefully standardized and modified where required (16). The methods adopted were based on the following: nitrogen by micro-Kjeldahl (10); phosphorus by molybdenum blue (22); potassium, sodium, and calcium by flame photometer (15); magnesium by complexometric titration (15); iron by *o*-phenanthroline (5); manganese by periodate (4); zinc by dithizone (20); and copper by carbamate (9).

Plant solutions were obtained by a wet-ashing technique employing nitric and perchloric acids (15). Where necessary, acids and alkalis were redistilled from all-borosilicate glass stills. Only high grade chemicals were used, further purified where necessary. Pure water was obtained by deionizing distilled water with "mixed bed" resin, Amberlite MB 3.

Washing of Leaf Material

Citrus leaves are always covered with a thin film of dust which is very difficult to remove by mechanical wiping or brushing. The leaves are often contaminated with spray residues, whether applied for nutritional, insecticidal, or fungicidal purposes. These residues stick tenaciously to the leaf. Soil contamination is considerably more pronounced in the case of the pineapple leaves, where the white basal tissue selected for analysis is practically in continuous direct contact with moist soil. Whereas soil contamination may not affect the values for the major nutrients to any significant degree, it will do so in the case of some trace elements, particularly iron.

Instructions in the literature for the removal of surface contamination vary considerably, though it is probable that different materials behave differently towards washing. Piper (13) maintains that owing to the loss by solution of some of the more soluble inorganic constituents, it is not permissible to wash plant samples to remove surface contamination. Any soil particles should be removed by brushing the plant samples individually with a camel's hair brush. Mann and Wallace

Table I. Losses during Washing

Element	Citrus Leaves			Pineapple Leaves		
	Wiped	Washed	Difference, %	Wiped	Washed	Difference, %
N, %	2.67	2.70	+1	1.27	1.26	-1
P, %	0.113	0.111	-2	0.202	0.205	+1
K, %	0.44	0.45	+2	3.13	3.11	-1
Ca, %	3.53	3.47	-2	0.34	0.33	-3
Na, %	0.43	0.38	-10			
Fe, p.p.m.	144	78	-46	139	53	-60
Mn, p.p.m.	23	23	0	127	131	+3
Cu, p.p.m.	5.6	4.6	-20	15.4	10.5	-33

(17) showed that if apple leaves were leached long enough with distilled water, up to 99% of the total potassium may be lost. Thus workers like Boynton and Burrell (2) and Goodall (6) have wiped the surface of leaves with cloth or cotton wool. Jacobson (8) and Ulrich (19) found it necessary to wash leaf samples, intended for iron determinations, in dilute hydrochloric acid solutions. Bathurst (7) washed citrus leaves in a 2% acetic acid solution, while Chapman and Brown (3) used an Ivory soap solution for the same material. No instructions for treating pineapple leaf samples could be traced in the literature.

In view of the apparently satisfactory results obtained by Chapman and Brown with soap solution (3), it was decided to try the effectiveness of a synthetic detergent, Teepol. Excellent results were finally obtained by first washing each leaf individually in a 0.1% Teepol solution, then in distilled water, followed by rinsing in deionized water. Nicholas, Lloyd-Jones, and Fisher (12) later introduced 0.3% Teepol as washing liquid for leaf tissue testing at the Long Ashton Research Station, England.

To get some idea of the efficiency of this washing procedure as well as to determine possible leaching losses, the following experiment was carried out: A large sample of leaves was picked—for citrus according to the standard sampling techniques of Bathurst (7) and Chapman and Brown (3), and for pineapples according to the procedure of Sideris, Krauss, and Young (14). All the leaves were cleaned by wiping both sides thoroughly with clean pieces of damp cotton wool. The leaves were then well mixed and divided into four lots of 50 leaves each. Two samples were washed according to the Teepol technique; the other two samples were not washed. After treatment, the samples were analyzed for the nutrient elements. The means of the results are recorded in Table I.

The high iron, copper, and sodium losses clearly indicate the inefficiency of the wiping procedure for removing dust and surface contamination. The other losses are not significant.

Drying of Leaf Material

All plant samples should be dried as rapidly as possible after collection, so as to reduce chemical and biological changes to a minimum. If drying is unduly delayed, considerable loss in dry weight may occur, due to respiration, while proteins are also broken down to simpler nitrogenous compounds.

Leaf tissue often contains as much as 90% moisture and the removal of this moisture presents a real problem. To drive the last traces of moisture out of the cells, at a reasonable rate, the temperature must be increased. There is the real danger that the outer drier tissues may undergo thermal decomposition to a considerable extent before the deep-seated moisture is removed. It must be concluded, therefore, that what is commonly termed the "dry weight" of plant material may not at all represent a completely moisture-free condition of the original material in an undecomposed state.

There are two separate requirements that must be satisfied when drying plant material for analysis: a sufficiently high temperature to destroy the enzymes, and the optimum temperature for moisture removal without appreciable thermal decomposition. A careful survey of the literature has revealed that most workers dry leaf material at a relatively low temperature, around 70° C., in order to "kill" the material and prepare it for grinding (1, 3, 13, 18, 19), while leaf powder is dried at 100° to 105° C. to obtain the dry weight basis for the analytical figures (1, 3, 13, 14).

Drying of Fresh Leaves. The rates of moisture losses and thermal decomposition of fresh citrus and pineapple leaves on drying were studied as follows: A large sample of freshly picked leaves was divided into different lots. Using three ovens, set at 50°, 65°, and 105° C. respectively, the percentage loss in weight was determined on triplicate subsamples at different time intervals over a period of 7 days' drying. The samples were then tightly sealed and stored in desiccators. At fortnightly intervals, over a storage period of 2 months, the above drying procedure was re-

Table II. Loss in Weight of Fresh Leaves at Constant Temperature

Time, Hours	Loss in Weight, %					
	Citrus Leaves			Pineapple Leaves:		
	50° C.	65° C.	105° C.	50° C.	65° C.	105° C.
5	41.9 ^a	57.7 ^b	58.5 ^b	24.0 ^a	34.1 ^b	90.5 ^c
10	56.7	57.9	58.5	58.5	90.1	90.7
24	57.2	58.0	58.7 ^c	86.4	90.2	90.7
48	57.5	58.2	58.9	88.7	90.3	90.9
72	57.6	58.2	59.0	89.0	90.4 ^c	91.3
96	57.7	58.2	59.1	89.1	90.6	91.6
168	57.8 ^a	58.2 ^b	59.5 ^c	89.5 ^b	90.9 ^c	92.0 ^c
14 days later	57.8	58.2	59.5	89.7	90.9	92.0
1 month later	58.3	58.2	59.5	91.0	90.9	92.1
2 months later	58.8	58.3	59.5	92.0	90.8	92.1

^a No scorching, fresh leaf color. ^b Slight scorching, dull-brown mottling. ^c Severe scorching, brown throughout. A difference greater than 0.4% is significant.

peated (24 hours' continuous drying at a time). The results are recorded in Table II. Table III records the loss in weight when fresh leaf material was heated to successively greater temperatures, after first being heated to constant weight at 40° C. (24 hours' drying at each temperature).

From Table II it can be seen that citrus leaves reached a more or less constant weight after drying for 24 hours at each temperature, though the dry weight at 50° C. was almost 2% greater than at 105° C. The material dried at 105° C. showed severe browning at an early stage, while at 50° C. the leaves maintained a healthy green color, even after 168 hours in the oven. It must be assumed, therefore, that drying at 105° C. and to a certain extent at 65° C. was accompanied by some thermal decomposition of the material. It seems reasonable, also, to assume that the material dried at 50° C. was not completely moisture-free—the continuous slow loss in weight, even after 168 hours in the oven, testified to that. In fact, the same assumption probably holds true for material dried at 105° C.; Willits (27), for example, in drying a sample of potato starch at increasing temperatures, found moisture still being evolved above 150° C. From the losses for citrus leaves recorded in Table III at rising temperatures, it would appear that a predominance of thermal decomposition over moisture losses occurred between 50° and 60° C. and again between 90° and 100° C. Figures recorded in Tables II and III, as well as observation of the scorching effects, showed that losses by thermal decomposition of the leaves became increasingly more important at temperatures above 50° C. However, this is not the end of the problem, because literature (17) indicates that most of the enzymes will be destroyed only above 60° C. It must, therefore, be assumed that at 50° C. the enzymes will only be deactivated and that they will again become active in the presence of moisture. To test this theory the experiment summarized in Table II was carried on for further 2 months. It was reasoned

that the material dried at 50° C., even after 168 hours, would still contain some moisture which may be sufficient to activate decomposition reactions on storage. The figures recorded in Table II show that this theory may be correct where, after 1-month storage under absolute moisture-free conditions, the material dried at 50° C. for 168 hours showed losses which surpassed the extent of thermal decomposition, when the fresh material was dried at 65° C. for the same period. It seems reasonable to assume that the majority of the enzymes were destroyed at 65° C., because no further decomposition could be detected in this material, even after 2 months of storage, and it is certain that this material also had some moisture retained which could not be volatilized at 65° C.

On the basis of these results it was decided to adopt 65° C. as the drying temperature for fresh citrus leaves. It was found to be the minimum temperature required for the destruction of the majority of the enzymes, and at the same time the maximum permissible temperature to exclude excessive thermal decomposition. The fact that the losses reached a more or less constant value after drying for 24 hours at 65° C. is of great importance when handling large numbers of samples, when there is usually a considerable lag during the grinding step. The material may safely be left in the oven at 65° C.

Tables II and III indicate that the drying of fresh pineapple leaves followed much the same pattern as citrus leaves, except that it was more difficult to remove the moisture at 50° C. and that decomposition, although evident from the browning of the material, was more gradual with rising temperature until above 90° C. when the decomposition rate increased. Unlike citrus leaves, pineapple leaves never attained a constant weight when dried at 65° C. even after a week in the oven. In fact, the losses from thermal decomposition increased progressively, the material eventually being darkly colored instead of white.

Here again it was necessary to adopt

Table III. Loss in Weight of Fresh Leaves with Rise in Temperature

(After initial removal of moisture)

Temp., °C. ± 1° C.	Loss in Weight, %	
	Citrus leaves	Pineapple leaves
40	60.8	89.5
50	61.1	89.6
60	62.1	89.7
70	62.5	89.8
80	62.8	89.9
90	63.0	90.0
100	63.5	90.2

Difference greater than 0.2% is significant.

a compromise procedure, because if the material is dried at 50° C. it will retain sufficient moisture to activate the enzymes soon after grinding and storage (Table II). On the other hand, drying the material at 65° C. is unsatisfactory, because of the continuous thermal decomposition which may cause considerable variation when a large number of samples have to be ground. The following procedure was eventually adopted: Fresh pineapple leaves were dried for 72 hours at 50° C. and then ground. After grinding, the leaf powder was placed in a clean bottle and dried for exactly 24 hours at 65° C. Although the material was slightly browned by this treatment, it could safely be stored under sterile conditions for 2 months prior to analysis (Table II), and all the samples could at least be taken to the same state of slight thermal decomposition.

Drying of Leaf Powder. During grinding, the leaf material picks up varying amounts of moisture, which must be removed before weighing out the material for analysis. Because of the difference in physical state between leaf powder and fresh leaves, drying experiments similar to those described above were carried out on leaf powder. The results obtained when citrus and pineapple leaf powders were dried at 65° and 105° C. for different periods are recorded in Table IV.

These results showed that much greater thermal decomposition occurred when leaf powder was dried at the higher temperatures than when the plant material was dried in its original physical state at the same temperatures. This is probably due to the finely divided state of the powder as compared to the original fresh material. Consequently, the prevailing tendency of workers to dry citrus and pineapple leaf powder at 105° C. prior to analysis, irrespective of the drying period, must be questioned.

From Table IV it is apparent that a difference of almost 2% would be obtained in the dry weight of citrus leaf powder between 65° and 105° C. over a 24-hour period. In the case of pineapple leaf powder this difference

Table IV. Loss in Weight of Leaf Powder at Constant Temperature

Time, Hours	Loss in Weight, %			
	Citrus Leaf Powder		Pineapple Leaf Powder	
	65° C.	105° C.	65° C.	105° C.
2	4.09	5.79 ^a
4	4.25	5.92	2.33	4.81 ^a
8	4.35	5.94	2.40	6.03
16	4.40	6.30	2.40	7.00
24	4.42	6.35	2.42	7.99
48	4.47	6.48	3.01 ^a	9.35 ^b
96	4.64	6.68
168	4.64	7.16 ^b	3.08	12.30

^a Severely scorched. ^b Blackish brown. Difference greater than 0.2% is significant.

amounted to more than 5.5% in a 24-hour period. Whereas a certain degree of variation in temperature for the fresh material may be permissible, the temperature must be strictly controlled for leaf powder, which is more susceptible to thermal decomposition.

In view of the constancy of the dry weight obtained after drying both materials for 24 hours at 65° C., it was decided to adopt this procedure when drying citrus and pineapple leaf powder prior to analysis. It was considered that a negligible amount of moisture was retained by the material when treated in this way, while entailing the least possible amount of thermal decomposition under the circumstances.

Grinding of Leaf Material

It is customary to grind the dried material before analysis, partly for greater ease in manipulation, partly to ensure greater uniformity in composition. Because of the laborious nature of hand grinding, particularly when the samples are large, mechanical grinding in mills is favored by most workers. When selecting a mill it is of the utmost importance to consider the possibility of contamination of the sample, particularly if the trace elements are to be determined.

Hood and his coworkers (7) carried out an intensive study on the mineral contamination during the grinding of plant samples. The types of grinding equipment tested were: the Wiley mill, hammer mill, and a jar mill with flint, porcelain, and mullite balls. They concluded that all the mechanical grinding methods tested resulted in serious contamination with one or more elements, and they warned that particularly large errors would be involved if the common mills are used for grinding plant tissue, intended for trace element analysis.

The author carried out a similar investigation using a Vetter all-agate ball mill. A composite citrus leaf sample was washed, dried, and divided into two sets of triplicate subsamples. One set of samples was broken down, using

Table V. Comparison between Hand Grinding and Mechanical Grinding in Agate

Element	Hand Grinding	Mechanical Grinding
	N, %	2.46
P, %	0.121	0.122
K, %	0.56	0.56
Ca, %	3.67	3.66
Mg, %	0.58	0.57
Na, %	0.32	0.32
Fe, p.p.m.	65	64
Mn, p.p.m.	32	33
Zn, p.p.m.	15	15
Cu, p.p.m.	3.4	3.3

an agate pestle and mortar, and the other set was ground for 2 hours in the agate ball mill. Each sample was analyzed separately (Table V).

No significant contamination occurred and it must be concluded that agate grinding is to be preferred to other methods, particularly for trace analyses. Citrus leaves could be ground to a fine powder within half an hour; pineapple leaves, because of their fibrous nature, took somewhat longer.

Keeping Quality of Leaf Material

Two aspects concerning the storage of leaf material were investigated: decomposition of fresh leaves prior to drying, and decomposition of dried leaf powder on storage.

Fresh Leaf Material. In dealing with large numbers of leaf samples a considerable lag may occur before all the samples can be washed and dried. The question arises as to the extent of the respiratory losses and as to a satisfactory method of storing freshly picked leaves to minimize such losses.

The following experiment was carried out. A large composite sample of leaves, freshly picked, was divided into separate lots and stored, in triplicate, under the following conditions: in sealed polyethylene bags for 2, 4, 7, and 14 days; in the open laboratory atmosphere for 2, 4, 7, and 14 days; and in sealed polyethylene bags in a refrigerator set at -5° C. for 7 and 14 days. After the lapse of the appropriate period, the percentage loss in dry weight (65° C. for 24 hours) was determined for each sample. The means of the results (for citrus leaves) are recorded in Table VI.

From Table VI it is clear that citrus leaves decomposed fairly rapidly in open air up to the second day, the loss being more gradual thereafter, amounting to a total loss in dry weight of only about 3% after 14 days. When the leaves were kept in a sealed polyethylene bag, however, the decomposition was very rapid and constant, amounting to a loss of about 10% after 14 days of storage. This must be ascribed to the

Table VI. Decomposition of Fresh Citrus Leaves on Storage

Storage Period, Days	Loss in Dry Weight, %		
	Sealed polyethylene	Open atmosphere	Refrigerator at -5° C.
2	1.8	1.4	0
4	2.7	1.7	0
7	5.5	2.0	0.3
14	9.7	2.8	0.4

fact that the moisture could not evaporate and the leaves turned moldy after the fourth day. The material stored in the refrigerator showed virtually no loss in dry weight, and the leaves still had a perfectly fresh green appearance after 14 days of storage. It can be concluded, therefore, that leaves should not be transported from the field to the laboratory, or sent through the mail, in sealed packages. The samples should preferably be open to the atmosphere and should be transported to the laboratory as quickly as possible, where they should be stored under refrigerated conditions until they can be washed and dried. Exactly similar trends were obtained with fresh pineapple leaves.

Storage of Leaf Powder. To determine the keeping quality of leaf powder on storage, powdered samples of citrus and pineapple leaves, dried for 24 hours at 65° C. were divided into two sets. One set was stored in a sterilized, sealed bottle on the laboratory shelf, while the other set was stored in a sealed, sterilized bottle in a refrigerator set at -5° C. Periodic nitrogen determinations were carried out on these samples. The results for citrus leaf powder are recorded in Table VII.

Citrus leaf powder, dried at 65° C. should not be stored for longer than 2 months prior to chemical analysis. On the other hand, it may be stored for long periods under refrigerated conditions, without apparent loss. Similar results were obtained with pineapple leaf.

Conclusion

Each step in the preparative phase of leaf analysis is subject to error; admittedly the errors are not large if careful control is exercised throughout. It is probable that from an agricultural point of view, the tendency would be to neglect these errors in view of the normally larger error associated with sampling. However, these errors are cumulative and if they are neglected they may easily, as a whole, cause an error of 10% or more in the final result. The whole process is so highly susceptible to error that unless care is taken throughout, the analytical result may be of little value.

Different plant materials react differently during the preparative phase, particularly toward drying and washing, and no standard techniques can be laid down. Each type of plant material

Table VII. Decomposition of Citrus Leaf Powder on Storage

Storage Period, Months	Loss in Nitrogen, %	
	Sealed bottle on shelf	Sealed bottle in refrigerator at -5° C.
1	0	0
2	1.2	0
3	3.6	0.2
4	7.0	0.1
5	10.0	0.2

must be carefully studied and a satisfactory treatment program must be worked out. As a result of this study, the following procedure is recommended for the preparation of citrus and pineapple leaves.

Procedure for Citrus Leaves. After picking, the leaf samples should be transported to the laboratory as quickly as possible, transferred to polyethylene bags, and stored in a refrigerator. One sample is removed at a time from the refrigerator, and each individual leaf is washed by first thoroughly sponging both sides with cotton wool in a 0.1% Teepol solution, and then rinsing well with different amounts of pure water. When the whole sample is washed, the water is drained off, and the midribs of the leaves are cut out with stainless steel scissors, to facilitate grinding and drying, as well as to give a more representative sample of the lamina. The leaf halves are then placed in a clean muslin bag and suspended inside a forced-draft oven, set at 65° C. After drying for 48

hours at this temperature, the sample is ground in an all-agate mechanical ball mill. After grinding, to remove the moisture picked up during this step, the leaf powder is placed in a clean bottle and dried for a further 24 hours at 65° C. A subsample may then be weighed out for chemical analysis, or the bottle may be sealed and stored under refrigerated conditions until such time as the analysis can be carried out.

Procedure for Pineapple Leaves. Pineapple leaves are washed as described above. After washing, the middle third of the white, meristematic basal tissue is cut out (16) and placed in a clean muslin bag. The sample is dried as above, but at 50° C., and the drying is carried on for 72 hours. The grinding and final drying of the leaf powder are exactly the same as for citrus leaves.

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Literature Cited

- (1) Bathurst, A. C., D.Sc. thesis, University of Stellenbosch, S. Africa, 1948.
- (2) Boynton, D., Burrell, A. B., *Proc. Am. Soc. Hort. Sci.* **44**, 25 (1944).
- (3) Chapman, H. D., Brown, S. M. *Hilgardia* **19**, 501 (1950).
- (4) Cooper, M. D., *Anal. Chem.* **25**, 411 (1953).
- (5) Fortune, W. B., Mellon, M. G. *Ind. Eng. Chem., Anal. Ed.* **10**, 60 (1938).

- (6) Goodall, D. W., *J. Pomol. Hort. Sci.* **20**, 136 (1943).
- (7) Hood, S. L., Parks, R. Q., Hurwitz, C., *Ind. Eng. Chem., Anal. Ed.* **16**, 202 (1944).
- (8) Jacobson, L., *Plant Physiol.* **20**, 233 (1945).
- (9) Lundblad, K., Svanberg, O., Ekman, P., *Plant and Soil* **1**, 277 (1949).
- (10) McKenzie, H. A., Wallace, H. S., *Australian J. Chem.* **7**, 55 (1954).
- (11) Mann, C. E. T., Wallace, T., *J. Pomol. Hort. Sci.* **4**, 146 (1925).
- (12) Nicholas, D. J. D., Lloyd-Jones, C. P., Fisher, D. J., *Nature* **177**, 336 (1956).
- (13) Piper, C. S., "Soil and Plant Analysis," p. 253, Hassell Press, Adelaide, Australia, 1942.
- (14) Sideris, C. P., Krauss, B. H., Young, H. Y., *Plant Physiol.* **13**, 489 (1938).
- (15) Steyn, W. J. A., *J. African Chem. Inst.* **9**, 39 (1956).
- (16) Steyn, W. J. A., Ph.D. thesis, Rhodes University, Grahamstown, S. Africa, 1957.
- (17) Tauber, H., "Chemistry and Technology of Enzymes," p. 4, Wiley, New York, 1949.
- (18) Thomas, W., *Soil Sci.* **59**, 353 (1945).
- (19) Ulrich, A., in "Diagnostic Techniques for Soils and Crops," p. 157, Am. Potash Inst., Washington, D. C., 1948.
- (20) Verdier, E. T., Steyn, W. J. A., Eve, D. J., *J. Agr. Food Chem.* **5**, 354 (1957).
- (21) Willits, C. O., *Anal. Chem.* **23**, 1058 (1951).
- (22) Yuen, S. H., Pollard, A. G., *J. Sci. Food Agr.* **6**, 223 (1955).

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FOOD ADDITIVE EVALUATION

Cloud Point as a Means of Characterizing the Polyglycols of Polyoxyethylene (8) Stearate

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Polyoxyethylene (8) stearate which has been used extensively in yeast-raised baked goods may be characterized by the cloud point of the recovered polyethylene glycols. This test is sensitive to the molecular weight distribution of the polyglycols in the mixture and hence will distinguish between polyethylene glycols which have a Poisson-type distribution and those of the same average molecular weight but having a nonrandom distribution of polyethylene glycols. Cloud point is particularly sensitive to the presence of polyethylene glycols of molecular weight greater than 600.

POLYOXYETHYLENE (8) STEARATE (Atlas Powder Co., MYRJ 45) is made by reaction of ethylene oxide with commercial stearic acid. MYRJ 45 has been used extensively in yeast-raised baked goods, where it acts as a dough conditioner and retards firming. This product contains monoesters, diesters, and unesterified polyglycols. Esterified and free polyol portions of polyoxyethylene (8) stearate are essentially

identical (7), and are mixtures of various molecular weight polyglycols having a Poisson distribution, as predicted by Flory (2), and average molecular weights between 335 and 350. To characterize MYRJ 45, it is desirable to distinguish between its polyol mixture and poly(ethylene glycol) mixtures having substantially different distributions of poly(oxyethylene glycols). Hydroxyl number and other determinations such as oxy-

ethylene content (3) will distinguish between mixtures differing in average molecular weight, but to distinguish between mixtures which by chance or design have the same average molecular weight but a radically different distribution of polyglycols, an additional test, sensitive to poly(ethylene glycol) distribution was sought.

In attempts to obtain such a test, such characteristics as freezing point,