DETERMINATION OF ACIDITY IN PLANT TISSUES

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For many physiological purposes the determination of the concentration and amount of acids in plant juices is a matter of importance. Acid formation or acid splitting may affect the output of carbon dioxide and thereby alter the gas interchange relations. It is necessary to be informed as to the amount of its rise and decline to evaluate properly the energy-releasing processes connected with respiration. It is known also that the degree of acidity in an imbibed fluid has an important influence on the hydratative capacity of colloidal systems and therefore must affect the colloidal mélange of protoplasm. Besides this the concentration of acids in the vacuole has an important bearing upon the osmotic coefficient of its contents. Without attempting then to enumerate all the ways in which a knowledge of the acid content of plant juices may be of physiological importance, it is evident that the determination of this factor is necessary.

In this very brief communication it is not intended to give even a partial survey of the problem, or to touch upon the variety of methods which have been developed for the isolation of definite acids. For technical purposes many ways have been devised by which the acids in various fruits and other plant parts can be determined and extracted in a manner that is satisfactory for the results required. That these methods may not be applicable to some physiological problems is not a criticism of their technical value; but, at the same time, it is questionable in some instances whether the acids extracted really represent either in quantity or condition the acids originally present in the living cell.

In the first place, the manner of obtaining the plant juices may be considered. The common method is by pressure. As far as concentration is concerned, the juice squeezed out may

represent with some accuracy the maximum concentration of the soluble substances present. Even here, however, there may be room for error. With the ordinary means at hand, pressure is not the easiest thing in the world to standardize and it is possible that identical samples might yield juices of slightly different concentration if subjected to different pressures. The speaker is quite aware that such errors may have crept into his own work, but feels rather confident from somewhat empirical tests that have been made that the error cannot be an important one.

Where, however, what may be termed total acidity is desired, a single pressure no matter how powerful opens the road to serious mistakes. By total acidity I mean the total acid content of a given weight of fresh or air-dry tissue. It is here that it is very difficult to tell in many published accounts how great have been the precautions not to leave a considerable percentage of acid in the rejected pulp. It is to be presumed that in most instances investigators were fully aware of this danger, but rarely is there any mention made of the procedure used to obviate the difficulty.

In his own work the writer has found that what appears to be a very close approximation of the actual total acid content may be obtained by repeated pressure. After the first juice had been expressed and the press released the remaining pulp is collected and copiously moistened with water, which it greedily absorbs. This is then pressed once more and the process repeated until the final expressed water shows by titration a practically negligible amount of acid.

All of the samples so obtained are mixed and made up to a definite volume, an aliquot portion of which is then titrated. In regard to the number of times this process is repeated each tissue will no doubt show its own peculiarities. In some very refractory tissues it might be an exceedingly difficult matter to satisfy oneself that the last trace of acid was extracted. In the tissues with which I have worked, notably the succulents, it has been found that the water which comes from the fourth pressing is nearly acid-free. Here again it is impossible to lay down a rule, for the type of press used might influence the result. Only by actual testing can we be sure that the acid is extracted.

By the method outlined we may obtain first a sample of pure juice to determine concentration and second the total amount of acid contained in a given weight of tissue. Where total acidity alone is required the speaker has used a simple method which is not in any way original but which by repeated test has been shown to be satisfactory and to yield very consistent results. Briefly the process is this. A small sample of the tissue is ground in a mortar with a little water and carefully washed silica sand. This is then strained through glass wool: the pulp and wool are again ground and strained and if there is evidence that the tissue is not finely enough comminuted by this time the process is repeated. The various samples are then mixed together and filtered into a graduated flask, care being taken to wash the filter thoroughly. An aliquot portion of the known volume is titrated as usual. Before finally rejecting the pulp it should be tested to determine if it is acid-free. This process may be carried on with considerable speed and the whole determination may be completed within fifteen minutes of the time of taking the sample. Time may also be gained by the use of a centrifuge in place of the filtering.

One thing, perhaps, is evident in the methods outlined, that is, the quickness with which the processes may be carried on, and it is on this point I wish to lay especial stress. We know that the organic acids with which we are dealing in plants are in many cases highly unstable and that if considerable time elapses between their extraction and estimation changes may occur that will influence the final result. Also the acids are easily affected by any substances that may be added to the solutions. Consequently, it is requisite to titrate the juices as soon as possible and in as nearly their original condition as possible in order to obtain results that are significant from a physiological standpoint. It is in these regards that many of the methods commonly employed, no matter how useful they may be for some purposes, are not always available for the study of the activities of the living organism. For instance, the addition of alcohol, while it may serve to clear the juice for the purposes of titration, must undoubtedly produce the esterification of some part of the acids. Similarly other chemical substances will not be without their effect in altering the original acidity. Besides all this, the time

which must elapse in the many filterings and extractions may allow a chance for partial disorganization of the unstabler acids.

These considerations have influenced the writer in his own work to sacrifice the clearness of the solution to be titrated for rapidity of estimation. It is true that from the standpoint of the chemist the extracts procured are often cloudy and colored so that the end point is not so sharp as it would be in a clearer solution but by the use of rather greater quantities of the indicator than usual and by accustoming the eye to the behavior of the specific plant juice it is probable that the results obtained are more nearly accurate than by a method which in the chemical sense may be more perfect. I am fully aware of the various objections which the chemist may bring to the procedures as outlined, and I admit their inadequacies. For the purposes desired, however, they are more suitable than more elaborate ones.

The two greatest difficulties are, first in the color of the solutions and second in the precipitation of protein substances when the neutrality point is approached. For the first there is at present no very good remedy to be suggested. By the selection of an indicator the color change of which is compatible with observation in an already colored solution, something may be done. The color change of the juice often suggests itself as an indicator and if one were certain of the neutrality of its end point it could be used instead of an indicator. As to the flocculation of colloidal substances on the approach of the neutral point it may be said that the precipitate is usually white and does not interfere as much with the color reaction as might be supposed. Of course in separating out the protein may adsorb some acid, but since the precipitate does not appear until the solution is nearly neutral the amount so occluded cannot be large. The addition of substances like bone-black for clearing and decolorization is tempting but open to various objections, the most important of which is that the bone-black may itself adsorb acids.

The writer would welcome the suggestion of improvements in the procedures outlined, particularly in the matter of a satisfactory method of obtaining a perfectly clear and colorless extract for titration purposes. It so happens that the plants which I have especially been investigating yield juices which are usually fairly

colorless and which contain only a small amount of colloidal substances which flocculate out in a neutral solution. The last method outlined was used, with considerable success, during the summer of 1917 at Carmel, California, when the acidity of a number of types of the local plants was determined in addition to that of the succulent forms which were being investigated in detail. The results were interesting, but too few in number to warrant publication at this time.