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Estimation of Ascorbic Acid in Citrus Juices

An Iodine Titration Method

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THIS iodine titration method for estimation of ascorbic acid was first described before the Food and Nutrition Section of the American Public Health Association in Pasadena, Calif., September 6, 1934, in a paper by A. J. Lorenz, R. W. Reynolds, and J. W. Stevens. Since that time the method has had extensive and satisfactory use by the California Fruit Growers Exchange in the estimation of the ascorbic acid content of citrus juices and various citrus juice products. It also has been used by Mack, Fellers, Maclinn, and Bean (*12*), and Roberts (*16*) in studies on citrus beverages and juices.

In the chemical estimation of ascorbic acid (vitamin C) in various biological materials the two reagents most commonly employed are 2,6-dichlorophenolindophenol and iodine. Titrations with the former reagent are made either in slightly acid solution as originally recommended by Tillmans, Hirsch, and Hirsch (*19*), or in relatively strong acid solution as described by Birch, Harris, and Ray (*2*). The iodine titration is restricted to use in relatively strong acid solution.

An important consideration in the chemical estimation of ascorbic acid is the specificity of the reagent employed. Many plant materials contain in addition to ascorbic acid various reducing substances such as glutathione and certain phenolic compounds which may titrate along with the vitamin. Since iodine is a strong oxidizing agent it may react with these interfering substances and hence the results obtained with the reagent may not be specific for vitamin C. The 2,6-dichlorophenolindophenol, on the other hand, is a relatively weak oxidizing agent and thus does not react so readily with these nonvitamin reducing substances. Because of the greater specificity of this reagent for vitamin C, many workers have preferred it to iodine.

Although the specificity of the method is of vital concern, certain other factors are important: standardization of the reagents employed, stability of the reagents, ease and rapidity of the titration procedure, sharpness of the end point, accuracy or reproducibility of the results, and, for extensive routine testing, the cost of the reagents.

The iodine method has certain advantages and except for its lack of specificity for vitamin C and the indefinite end point it would undoubtedly be used to a greater extent. Careful study of the various factors involved have shown that the most serious objections to its use may be largely eliminated. The interference of nonvitamin reducing substances may be lessened and the end point improved by proper adjustment of the acidity of the titration mixture by the addition of a strong mineral acid as disclosed by Tillmans, Hirsch, and Hirsch (*19*). The end point may be improved further by the use of a double back-titration, which gives better results than the back-titration employed by Tillmans and his associates. The following procedure was adopted for obtaining these improvements in the iodine method for citrus juices.

Method

Twenty milliliters of the natural-strength juice are transferred to a 250-ml. Erlenmeyer flask and 4 ml. of 12 *N* sulfuric acid are added. The added acid lowers the pH of the sample to about 0.02 to 0.08 by the hydrogen electrode. Freshly standardized 0.01 *N* iodine solution is then added until an excess of 1 or 2 ml. is present. Excess iodine may be detected by color change in the sample or by the addition of a drop of starch solution. The test solution is allowed to stand for about 0.5 minute for the reaction to go to completion. Standardized 0.01 *N* thiosulfate solution is now added to give an excess of about 1 ml., with 3 ml. of 0.5 per cent starch solution added as the indicator. A trial titration may be run to determine the amounts of iodine and thiosulfate solutions needed to obtain the desired excess values. Finally, more of the 0.01 *N* iodine solution is added slowly until the well-known starch-iodine end point is reached. The total volume of the iodine solution added minus the volume of thiosulfate solution used (on the iodine equivalent basis) equals the volume of iodine solution consumed by the reducing substances in the sample. One milliliter of 0.01 *N* iodine solution is equivalent to 0.88 mg. of ascorbic acid.

Preparation and Standardization of Reagents

IODINE SOLUTION. An approximately 0.1 *N* stock solution is prepared by dissolving 25 grams of potassium iodide in as little distilled water as possible and then adding about 12.7 grams of resublimed iodine. After the iodine has dissolved, the solution is diluted to 1 liter with distilled water. The solution is protected from light by storing in a dark or wrapped bottle. From this stock solution the 0.01 *N* solution is prepared as needed for use in the ascorbic acid titration by diluting about 100 ml., together with 22.5 grams of potassium iodide, to 1 liter.

The normality of the dilute solution is checked, at the time of use, by titration of 20- or 25-ml. portions with standardized 0.01 *N* thiosulfate solutions, using starch solution as the indicator. The starch solution, about 3 ml., is not added until the titration is almost complete.

STARCH SOLUTION. The 0.5 per cent starch solution is prepared according to the procedure outlined by Treadwell and Hall (*20*). Five grams of powdered potato starch are triturated into a paste with a little water and poured slowly into a liter of boiling distilled water. Boiling is continued 1 or 2 minutes to obtain a nearly clear solution. The solution is cooled and allowed to stand several hours and is then filtered and transferred to 50-ml. bottles. After heating for about 2 hours in a steamer, or water bath, the bottles are closed with cork stoppers that have been dipped in hot paraffin. Starch solution prepared in this manner will give a good color reaction and retain its sensitivity for several months and is preferred to most of the soluble starch preparations. The solution may lose its sensitivity within a few days after the bottle is opened, usually because of mold growth.

THIOSULFATE SOLUTION. The convenience of the iodine titration method depends to a considerable extent upon the stability of the thiosulfate solution used as the standardizing agent, and consequently particular attention should be given to its preparation.

The deterioration of thiosulfate solutions has been attributed to a number of causes and several methods have been advanced for stabilizing the reagent. Kolthoff (*8*), Mayr (*13*), Schulek (*17*), Winkler (*21*), Hahn and Clos (*5*), Kolliker (*7*), and others are of the opinion that the deterioration of the reagent is due largely to the action of certain types of bacteria. Hahn and Windisch (*6*), Mayr and Kerschbaum (*14*), and Law (*9*) have pointed out the significance of carbon dioxide in the deterioration processes. Traces of copper may catalyze the decomposition of the solution as shown by Abel (*1*), Skrabal (*18*), and Hahn and Clos (*5*). Atmospheric oxidation and the catalytic effect of light are also recognized as factors.

The stability of the solution prepared by the procedure outlined below is probably due to the substantial exclusion of bacteria, carbon dioxide, and light as deterioration factors.

The approximately 0.1 *N* thiosulfate stock solution is prepared as follows:

The distilled water for the solution is placed in a Florence flask, or some other glass container that will stand boiling over a flame. A rubber stopper, with suitable connection for a buret and with soda-lime tube attached, is inserted loosely in the mouth of the flask. The flask is then placed over a gauze-covered flame and the water boiled for about 15 minutes. During this operation the soda lime should be protected from the steam. After boiling has stopped the thiosulfate crystals, 25 grams per liter of solution, are added and the stopper of the flask is pressed down firmly and secured. The connection for the buret should also be closed, so that any air drawn into the flask upon cooling will enter through the soda-lime tube, which should contain a cotton pad on either side of the soda lime. The thiosulfate crystals are dissolved by agitation and the solution is cooled. The buret, also fitted with a soda-lime tube, is attached and the solution is protected from light.

The thiosulfate solution is standardized essentially as described by Bray and Miller (*3*). The procedure is as follows:

A 0.1 *N* solution of potassium dichromate is prepared by dissolving 4.9035 grams of potassium dichromate, which has been recrystallized 2 or 3 times from water and dried for 48 hours at 110° C., in distilled water and diluting to 1 liter. Twenty-five milliliters of the dichromate solution are transferred to a 1-liter flask containing 2 grams of potassium iodide dissolved in 70 ml. of water, with 3.4 ml. of 6 *N* hydrochloric acid added for acidification. After standing in the dark for about 10 minutes, the solution is diluted to 500 to 600 ml. and titrated with the thiosulfate solution, with about 3 ml. of starch solution added as the indicator very near the end of the titration. The solution turns from blue to green in color at the end point. The normality of thiosulfate solution is calculated on the basis of the dichromate solution as exactly 0.1 *N*.

Other reliable methods of standardizing the thiosulfate solution are available. Before adopting a method at least two of the recognized methods should be employed in parallel. This comparison will enable the operator to prove the accuracy of the method preferred for continued use.

The thiosulfate solution is diluted to 0.01 *N* strength, preferably with freshly boiled and cooled water, for use in standardizing the iodine solution and in the ascorbic acid titration procedure. The 0.01 *N* solution deteriorates relatively fast and hence should be prepared fresh each day. The stock solution maintains its strength for several months.

Discussion

The iodine titration procedure outlined above differs from the ordinary iodine technic in two essential respects—namely, the high acidity under which the titration is carried out and the double back-titration.

Sufficient sulfuric acid must be added to lower the pH to about 0.02 to 0.08 to obtain a sharp end point. Lack of acid causes a sluggish titration and an indefinite end point. Furthermore, in the presence of the high acidity, iodine is more nearly specific for vitamin C. The amount of acid recommended is near the upper limit for obtaining a satisfactory titration.

The work of Fujita and Iwatake (*4*), Musulin and King (*15*), Mack and Tressler (*11*), Lorenz (*10*), and others on the use of metaphosphoric acid in the chemical estimation of vitamin C indicates that the acid might be a satisfactory substitute for sulfuric acid in the iodine method.

Sulfuric acid in excessive concentrations may liberate free iodine from the potassium iodide present in the iodine solution, but the quantity of acid recommended will not liberate sufficient iodine in 10 minutes to give a color with starch. The titration will, of course, be complete in considerably less time.

The procedure of adding an excess of iodine, then an excess of thiosulfate, followed by titration of the excess thiosulfate, was adopted to improve the end point. If the titration is carried out directly with iodine, the reaction proceeds too slowly near the end, giving irreproducible results. By adding an excess of iodine all substances capable of being oxidized by the reagent under the existing conditions are oxidized quickly and completely. The added thiosulfate then reacts quickly and quantitatively with the excess iodine. Excess thiosulfate is used, then back-titrated with more iodine to the end point because in titrating solutions containing large amounts of colored substances, such as orange juice or tomato juice, the end point can be detected better by the appearance of the blue color than by its disappearance. The addition of a few extra drops of iodine solution after the reading has been taken shows definitely that the end point has been passed, which is not possible when the end point is shown by the disappearance of color.

A large excess of iodine will result in a high titer, but this is not serious provided the excess does not exceed about 3 ml. The time the excess iodine is allowed to react is likewise not of particular importance if the reaction time does not exceed 3 minutes. The temperature of the solution is not important within the range of about 18° to 30° C.

The method must be used with caution in the estimation of ascorbic acid in canned juices. Higher than true values may be obtained, possibly because of the presence of ferrous iron and stannous tin. Misleading results may therefore be encountered in any canned juice when corrosion of the tin plate has occurred.

The essential oils of citrus fruits may interfere with the chemical estimation of vitamin C in juice products, causing slightly high results, but this source of error may usually be neglected.

The iodine method has the same limitation in the estimation of ascorbic acid in old oxidized products as the other chemical methods. Reversibly oxidized ascorbic acid, which is still biologically active, is not detected directly by the chemical methods. Various procedures have been offered for the application of chemical methods to the estimation of this form of the vitamin, but the methods are rather complicated for routine work and the uncertain evaluation of the results leaves much room for improvement in this direction.

The 2,6-dichlorophenolindophenol method and the improved iodine method give, on the average, nearly identical results with citrus juices. The iodine method is thus satisfactory for use in following the retention of vitamin C during the manufacture and storage of various citrus juice products. Its advantages are as follows: (1) It has a nonfading end point with a deep blue color that can be detected in the presence of any but the darkest colors. (2) Reproducibility of results is ensured by the definite nonfading end point. (3) Only common, inexpensive chemicals, available in almost any laboratory, are used. (4) The reagents employed can easily be standardized by well-known methods. (5) The reagents

employed are relatively stable and can hence be made up in large quantities, thus saving much time.

The 2,6-dichlorophenolindophenol method, with one of the standard procedures, should be employed for vitamin C exploratory work. For more or less routine control work, however, where it has been shown by trial that the modified iodine method gives substantially the same results as the 2,6-dichlorophenolindophenol method, the advantages of the iodine method commend its use.

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