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Turbidimetric Determination of Ascorbic Acid in Foods

A previously reported qualitative test for ascorbic acid has been found to provide a turbidimetric procedure for the determination of this vitamin in foods. Selenious acid reacts only with ascorbic acid and stannous ion among some 50 food ingredients tested to date at low pH and room temperature.

The current direct methods for analysis of ascorbic acid in foods (Freed, 1966; Horwitz, 1970) suffer from nonspecificity. The most widely used method, 2,6-dichlorophenolindophenol titration, gives high results if tannins, reductones, ferrous ion, stannous ion, betanin, and bisulfite are present in the sample. This communication reports a quantitative method for ascorbic acid based on the qualitative test using selenious acid reported by Levine (1936) nearly 40 years ago. The quantitative nature of the reaction between selenious acid (1 mol) and ascorbic acid (2 mol) to form selenium has been reported (Deshmukh and Bapat, 1955). The gravimetric and/or volumetric methods proposed by these workers for the determination of ascorbic acid in vitamin tablets are not useful for analysis of food extracts; many food components will reduce selenious acid to selenium at temperatures of 50–100°.

We have found that stable selenious colloids are formed when food extracts containing ascorbic acid are treated with selenious acid, and the resulting turbidity is proportional to the ascorbic acid content.

MATERIALS AND METHODS

The ascorbic acid is extracted from foods as described in established published methods (Freed, 1966; Horwitz, 1970); it is not necessary to use acetic acid if ferrous ion is expected in the sample. The clarified, strongly acidic solution is diluted with deionized water to give an ascorbic acid level of 0–4 mg/25–50 ml. The ascorbic acid solution is treated with 5 ml of a solution of 1 g of reagent grade selenium dioxide in 80 ml of water and 20 ml of 37% hydrochloric acid. After 5–15 min the reddish suspension is diluted to 100 ml. The optical density of the suspension is measured in a spectrophotometer at 425 nm using the same volume of diluted food extract with 5 ml of dilute hydrochloric acid, and dilution to 100 ml, in the reference cuvette. A calibration curve is prepared using solutions of ascorbic acid in 3% *m*-phosphoric acid at the 1–4 mg/25–50 ml level with 5 ml of the above described selenious acid reagent and dilution to 100 ml after a 5–15 min colloid development time. See Figure 1 for a typical calibration curve.

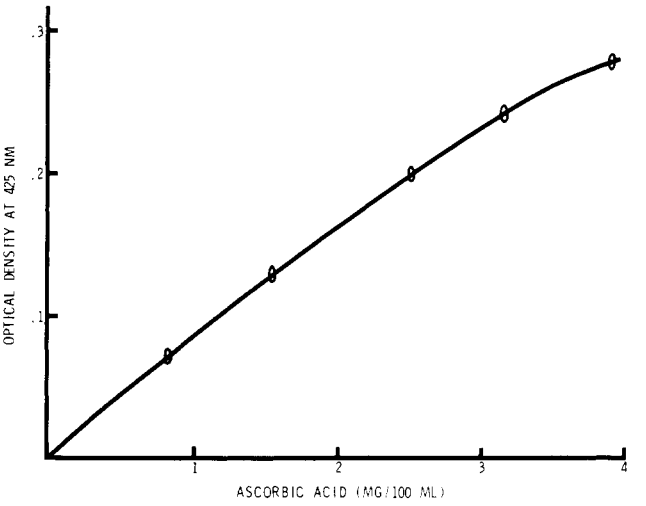

Figure 1. Optical density of selenium colloids prepared from the reaction of ascorbic acid and excess selenious acid.

RESULTS AND DISCUSSION

The method is highly specific for ascorbic acid when conducted at 20–25°. Levine tested 40 commonly occurring organic compounds and found no selenium formation; we find that betanin, sorbic acid, catechol, and acetoin do not reduce selenious acid to metallic selenium. The levels of ferrous ion and bisulfite ion found in food products do not significantly influence the turbidity caused by the ascorbic acid reduction of selenious acid at room temperature. Stannous ion reacts with selenious acid and, if present, must be corrected for by independent analysis and calibration of its reaction with selenious acid (see Figure 2).

The precision of the turbidimetric determination is illustrated by the data in Table I. The selenium colloid is stable for several hours if no more than 1 mg of selenium/100 ml of solution is formed. Naturally occurring polymeric materials in the commodities tested appear to

Table I. Optical Density of Selenium Metal Colloids in Treated Food Extracts

Commodity	Reaction time before dilution, min	Sample no.	Optical density	Time from reagent addition to optical scan, min
Orange drink	5	OD-2	0.162	30
	5	OD-3	0.158	30
	5	OD-4	0.164	30
	10	OD-5	0.160	30
	10	OD-6	0.162	30
	10	OD-7	0.160	30
	15	OD-8	0.158	30
	15	OD-9	0.164	30
	15	OD-10	0.162	30
	15	OD-2	0.160	45
	5	OD-2	0.162	180
Tomato juice	15	TJ-1	0.100	30
	15	TJ-2	0.102	35
	15	TJ-3	0.099	40
Cran-apple drink	15	CA-1	0.152	30
	15	CA-2	0.158	35
	15	CA-3	0.164	40
Fruit punch	15	FP-1	0.128	30
	15	FP-2	0.133	35
	15	FP-3	0.130	40

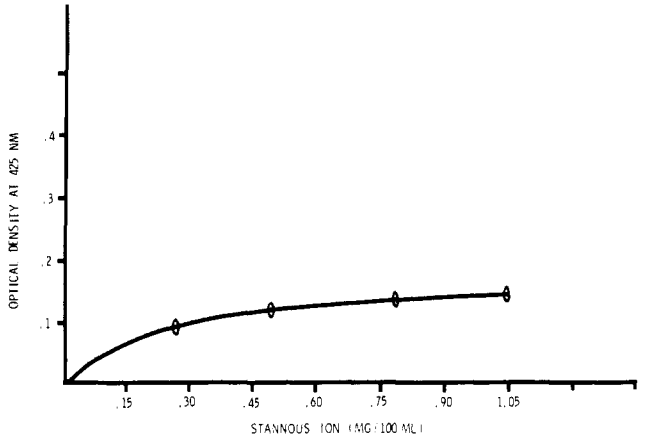

Figure 2. Optical density of selenium colloids prepared from the reaction of stannous chloride and excess selenious acid.

act as colloid stabilizers. Due to the lack of reduction of selenious acid by many food components which reduce the reagents used in current direct titration methods, the turbidimetric determination of ascorbic acid will give results closer to the actual content. Highly pigmented extracts (such as those from beets or berries) can be measured directly by turbidimetric determination. The only disadvantage of the turbidimetric determination is the reduction of selenious acid by stannous ion.

A comparison of the ascorbic acid content of several nutritionally significant sources of vitamin C (Table II) shows a generally lower result by the turbidimetric method as compared to the 2,6-dichlorophenolindophenol (titration) method.

Table II. Content of Ascorbic Acid in Certain Foods

Product[a]	Ascorbic acid, mg/100 g: Titration	Ascorbic acid, mg/100 g: Turbidimetric
Tomato paste	28	25
Green peas	6	4
Tomato juice	17	11
Pineapple–grapefruit juice	24	18
Orange drink	65	68
Green asparagus	19	18
Fruit punch	27	28
Vegetable juice cocktail	19	15
Cran-apple drink	42	34
Grapefruit juice	28	16

[a] Packed in glass or fully enameled cans; no stannous ion present.

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Correction

DETERMINATION OF TRACE METALS IN FOODS USING CHELATING ION EXCHANGE CONCENTRATION

In this article by Richard A. Baetz and Charles T. Kenner [*J. Agric. Food Chem.* **23**(1), 41 (1975)], on page 41, column 2, paragraph 2 under Experimental Section, line 10, the sentence beginning "The ammonium sulfate solution . . . " should be changed to read: "Ammonium sulfate solution (5% w/v). HNO_3 (J. T. Baker No. 5-9603; suitable for mercury determination) and H_2SO_4 (J. T. Baker No. 5-9685; suitable for mercury determination)."