Determination of Ascorbic Acid in Wheat Flours, Bread Dough Conditioners, and Commercial Vitamin C Tablets by High-Performance Liquid Chromatography[1]

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ABSTRACT

A high-performance liquid chromatography method was developed to determine ascorbic acid (vitamin C) in wheat flours, bread dough conditioners, and commercial vitamin C tablets. Dithiothreotol (0.7m*M*) stabilized vitamin C without reducing extraction efficiency. Extraction and analysis were complete in less than 30 min. Extraction efficiencies were 73–78% for the flours and 100% for the dough conditioners and vitamin C tablets. Vitamin C contents of dough conditioners were found to be higher than the labeled potency. Over-the-counter vitamin C tablets, stored in capped vials for various periods of time at room temperature, had a shelf life of at least 14 years. The method is over 1,000 times more sensitive than the AOAC method (10 ng vs 50 μg) and is not adversely affected by some metal ions and basic compounds.

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Ascorbic acid, generally recognized for its use as a vitamin, is used in curing meat, as an antioxidant in foodstuffs to prevent rancidity, and to prevent browning of cut apples and other fruit. Ascorbic acid and its reversibly oxidized form, dehydroascorbic acid, exist as a redox couple. That equilibrium allows it to be used as both an oxidant in breadmaking and an antioxidant in other foodstuffs. The importance of ascorbic acid in medicine, food science, and cereal science, and in our contemplated research in cereal chemistry, was the basis for our interest in a method to determine ascorbic acid.

Several methods for determining vitamin C have been reported (Eldawy et al 1975, Pachla and Kissinger 1976, Pelletier and Brassard 1975, Schlack 1974, Sood et al 1976, Wagner et al 1979, White and Fitzgerald 1972, Wills et al 1977). The high-performance liquid chromatography (HPLC) methods (Pachla and Kissinger 1976, Sood et al 1976, Wagner et al 1979, Wills et al 1977) required less sample preparation and allowed analysis of trace amounts of vitamin C in certain systems. However, of the HPLC methods cited above, only the method of Sood et al exhibited k′ values (ratio of elution time of vitamin C to that of the void volume of the column) greater than 2, and it required special ion-pairing reagents not universally available. The other HPLC methods with lower k values did not allow a quantitative measurement of vitamin C when contaminants in wheat flours were present.

The HPLC method described here can be used to quantitate vitamin C in the presence of materials that normally elute near the void volume; it has a k′ value for vitamin C of 2.9. The method was applied to four different wheat flours, two types of bread dough conditioners containing vitamin C, and over-the-counter vitamin C tablets ranging from fresh to more than 14 years old.

MATERIALS AND METHODS

Reagents and Chemicals

The L-ascorbic acid standard, USP grade, and metaphosphoric acid, AR grade, were obtained from Mallinckrodt; sodium monobasic phosphate, AR grade, from Sigma Chemical Co.; and dithiothreotol (DTT), AR grade, from Schwartz-Mann. LC grade acetonitrile and methanol were purchased from Burdick and Jackson Laboratories, Inc. Water was purified by reverse osmosis and then passed through charcoal and deionizing filters.

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The vitamin C tablets, obtained from two pharmacies, had been stored on open shelves at room temperature for various periods of time in screw-cap, light-green or dark-brown bottles.

Cain Food Industries supplied the two dough conditioners, Ascorbo-C and CDC-79. CDC-79 dough conditioner contains wheat and soy flours, lecithin, barley malt, and vitamin C. The Ascorbo-C tablets, similar in composition to most over-the-counter vitamin C tablets, contain vitamin C and a starch filler.

RBS-78, BCS-78, and CS-79 were straight grade flours. Each was a composite of many flours milled from hard winter wheat varieties harvested at several locations throughout the Great Plains in 1978 and 1979. Straight grade flour 79-1212 was milled from a composite of two wheat varieties harvested at Manhattan, KS, in 1974 and 1976.

Extraction

A vitamin C tablet was pulverized with a spatula on glassine paper and quantitatively transferred with 5 ml of 0.7m*M* DTT to a volumetric flask to yield a vitamin C concentration of 0.5 mg/ml, according to the composition on the bottle label. An aliquot of each solution was filtered through a 0.45-μm Gelman filter before analysis or further dilution. The stock was then diluted to quarter-strength (125 μg/ml) and one-tenth strength (50 μg/ml).

Based on the labeled claim for each dough conditioner, 0.4167 g of CDC-79 and 0.0183 g of Ascorbo-C were each extracted with 100 ml of 0.7m*M* DTT to yield vitamin C concentrations of 0.025 and 0.10 mg/ml, respectively. Each solution was blended with a Tekmar homogenizer at 1,000 rpm for 2 min. The extract was then filtered through a 0.45-μm Gelman filter and analyzed.

Wheat flour (50 g) was extracted with 100 ml of 0.7m*M* DTT solution for 2 min at 1,000 rpm on a Tekmar homogenizer and then centrifuged for 10 min at 4,550 $\times g$. The supernatant was filtered through a 0.45-μm Gelman filter and analyzed immediately.

HPLC

The HPLC system consisted of a Varian 5020 pump, a Rheodyne 7120 injector (100-μl loop), a Tracor variable wavelength detector (set at 254 nm), two Waters Associates μ-Bondapak-carbohydrate 10-μm particle columns (30 cm $\times$ 3.9 mm i.d.) in tandem, and a Hewlett-Packard 3388 printer-plotter automation system. Injections of 100 μl were made, and vitamin C was eluted isocratically at room temperature in 9.80 $\pm$ 0.20 min at a flow rate of 2.0 ml/min with a solvent consisting of 30% 0.01*M* NaH_2PO_4 (pH 4.46) and 35% each of methanol and acetonitrile. Those conditions resulted in a back-pressure on the column of 230 atm and gave a k' value for vitamin C of 2.9.

Vitamin C concentration in an extract was determined by comparing the area corresponding to the vitamin C of the extract with the area-concentration relationship from a standard curve prepared with vitamin C. The standard curve was linear over the range from 0.5 μg/ml to 0.5 mg/ml. All extractions and analyses were replicated at least twice.

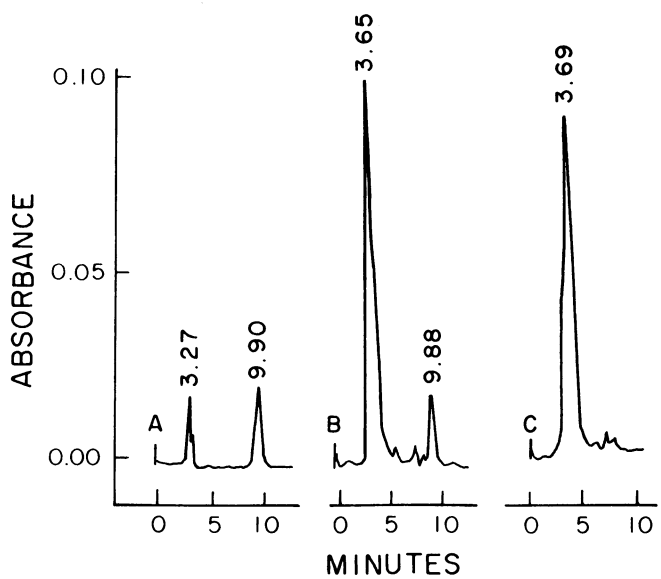

Fig. 1. Chromatogram for vitamin C (9.90 min) in RBS-78 wheat flour. **A**, 5 μg/ml (500 ng) of vitamin C standard; **B**, RBS-78 flour containing 5 μg of vitamin C added per gram of flour; **C**, RBS-78 flour containing no added vitamin C.

Extraction Efficiency

Extraction efficiency (recovery) was determined for vitamin C tablets, each dough conditioner, and each flour by comparing the vitamin C peak areas of known standard solutions (5, 10, 25, and 50 μg/ml) with the increase in peak areas of the extracts of individual samples when known amounts (5, 10, 25, and 50 μg/ml) of vitamin C were added.

RESULTS AND DISCUSSION

Stability of Standard and Sample Extracts

Degradation of the standard and sample extracts was 0, 7, and 15% per day when 0.7m*M* DTT, 3% metaphosphoric acid, and methanol, respectively, were the extraction solvents. Therefore, 0.7m*M* DTT was used for all subsequent studies.

HPLC Analyses

Figure 1 shows vitamin C analyses conducted on three different samples; A consisted of a 5-μg/ml (500-ng) vitamin C standard, B was an extract of RBS-78 flour that had 5 μg of vitamin C added per gram of flour, and C was an extract of RBS-78 flour.

The analysis shown in Fig. 1A is 100-fold more sensitive than the AOAC method (1980), and the HPLC method can reliably determine vitamin C in samples 100-fold smaller than shown, down to the 10-ng range. The AOAC standard microfluorometry method will only quantitate down to 50 μg of vitamin C, and its reliability is adversely affected by the presence of some metal ions and of basic compounds.

The peaks eluting before the vitamin C peak in Fig. 1B represent extraneous materials extracted from wheat flour. The absence of peaks in the 9–10-min range of Fig. 1C attests to the ability of the HPLC method to resolve contaminants from vitamin C and shows the stability of the baseline. The HPLC method provides better resolution of vitamin C from contaminants in wheat flour extracts than other published methods do.

Wheat Flours

Solvent extraction efficiencies for the four wheat flours BCS-78, RBS-78, 79-1212, and CS-79 were 78, 77, 74, and 73%, respectively. Flours BCS-78 and CS-79 contained 0.6 $\pm$ 0.1 and 0.8 $\pm$ 0.1 μg of vitamin C per gram of flour, respectively. Indiginous levels of vitamin C in flour apparently have not been reported before. RBS-78 and 79-1212 flours contained no detectable levels of vitamin C. BCS-78 and CS-79 differed from RBS-78 and 79-1212 by being bleached with 35 μg of benzoyl peroxide per gram of flour. Treatment of RBS-78 and 79-1212 with 35 μg of benzoyl per gram of flour had no effect on vitamin C concentration or analysis. Therefore, one or more of the flours in the BCS-78 and CS-79 blends apparently contained native vitamin C.

TABLE I
Vitamin C Analysis of Dough Conditioners

Brand Name	Potency		
	Labeled[a] (mg/g)	Analyzed[a] (mg/g)	Analyzed as Percent of Labeled
Ascorbo-C	545	580 $\pm$ 2	106
CDC-79	6.0	7.1 $\pm$ 0.2	118

[a] Mean and SD of analyses on duplicate extractions.

TABLE II
Effect of Age on Potency of Vitamin C Tablets

Age (years)	Ascorbic Acid (μg/ml)[a] Analyzed in Concentration of			Potency of Tablet		
	Stock[b]	1/4 Stock	1/10 Stock	Labeled (mg)	Analyzed (mg)	Analyzed as Percent of Labeled
Fresh	529 ± 6	132 ± 1	53.1 ± 0.2	250	264	106
3	517 ± 12	124 ± 2	52.9 ± 0.4	100	103	103
5	482 ± 1	120 ± 1	49.7 ± 0.3	50	48.6	97.2
	492 ± 8	122 ± 1	48.7 ± 1.1	500	489	97.8
14	490 ± 2	124 ± 1	50.4 ± 2.1	25	24.8	99.3

[a] Mean and SD of analyses on duplicate extractions.
[b] 500 μg/ml according to labeled potency; one-quarter stock, 125 μg/ml; one-tenth stock, 50 μg/ml.

Dough Conditioners

Dough conditioners increase loaf volume of bread by furnishing an oxidizer to fulfill the oxidation requirement of the particular flour. The bromates and iodates long have been useful as oxidizers, but they are less desirable than vitamin C because they are not on the "Generally Recognized as Safe" list and they can overoxidize flours that have low oxidation requirements, resulting in decreased loaf volumes and undesirably bucky doughs.

Solvent extraction efficiency for each dough conditioner was 100% when the vitamin C concentration in the extract was 0.50 mg/ml or less. Our analyses (Table I) showed that the two commercial dough conditioners contained vitamin C at 106 and 118% of the labeled potencies.

Stability of Vitamin C Tablets

Our interest in the stability of ascorbic acid as an oxidizer or dough conditioner led us to analyze vitamin C tablets for shelf-life stability (Table II). Solvent extraction efficiency for the commercial vitamin tablets was 100% when the vitamin C concentration in the extract was 0.50 mg/ml or less. Sample reproducibility within 4% was found for the complete analysis, which included extraction, dilution, and quantitation. The vitamin C concentration in each tablet stored in capped vials was within government specifications (95–115%) even after 14 years. That finding contradicts Wilk's (1976) report of a 50% loss in potency in one year. The degradation effect reported by Wilk might have stemmed from storage conditions or sample preparation, because vitamin C is easily oxidized.

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