NOTE

The Determination of Ascorbic Acid in Wheat Flour Suspension by Differential Pulse Polarography

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L-Ascorbic acid (AA) has been recognized as an improving agent in breadmaking since 1935 (Jorgensen 1935). The improving mechanism involves rapid oxidation of AA to L-dehydroascorbic acid (DHAA) (Kuninori and Matsumoto 1963, Grant 1974, Elkassabany et al 1980, Nicolas et al 1980, Cherdkiatgumchai and Grant 1986) and the apparent ability of DHAA to promote sulfhydryl-disulfide interchange reactions of wheat flour proteins (Tsen and Bushuk 1963, Tsen 1965, Meredith 1965, Elkassabany and Hoseney 1980, Elkassabany et al 1980, Nicolas et al 1980).

Studies of the role of AA as an improving agent in flour require reliable methods for its analysis. An extensive review of the techniques available recently appeared (Pachla and Reynolds 1985). Cereal chemists have used a variety of methods including indophenol titration (Carter and Pace 1965), microfluorimetry (Nicolas et al 1980, Elkassabany et al 1980), high-performance liquid chromatography (Lookhart et al 1982), and gas chromatography/mass spectrometry (Pfeilsticker and Marx 1986) for the analysis of AA in wheat flour extracts, suspensions, and doughs. In previous work we used a minor modification of the indophenol-xylene extraction method approved by the AACC (Grant 1974), but this method is tedious and its sensitivity is barely adequate for our requirements.

Differential pulse polarography (DPP) has been recently developed for the determination of AA, and several modifications have been introduced for individual applications (Lindquist and Farroha 1975, Lau et al 1985, Nangniot 1985, Hart 1986). The technique determines AA by examining the anodic wave resulting from the oxidation of the enediol system. The DPP method was found to be more specific and require fewer steps in sample preparation than the classical colorimetric and titration methods.

MATERIALS AND METHODS

Materials

The AA was obtained from Aldrich Chemical Co. Inc., metaphosphoric acid (MPA) from BDH Chemicals, oxalic acid (OA) from Anachemis Ltd., trichloroacetic acid (TCA) from McArthur Chemical Co. Ltd., and mercury (triple distilled) from Fisher Scientific Co. The mercury was further purified to remove base metals by the procedure suggested in the polarographic analyzer manual (EG&G Princeton Applied Research, Princeton, NY). All chemicals were reagent grade or better. Reagent solutions and flour extracts were prepared with glass-distilled water.

A sample of untreated patent flour milled from Canadian hard red spring wheat was obtained from a local commercial mill. The flour contained 10.1% moisture, 0.34% ash, and 13.1% protein (N × 5.7).

Extracting Solution

Four solutions, listed in Table I, were tested as flour extractants to obtain sample solutions suitable for DPP analysis. The eventual choice of an extractant depended on the shape of the polarogram and the recovery of the AA from the wheat flour suspension.

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Assay Procedure for Enzymatic Oxidation of AA by Flour-Water Suspension

Flour (5.00 g) was mixed with a dilute aqueous NaOH solution to give a suspension with a pH of 6.3, the optimum pH for enzymatic oxidation of AA (Grant and Sood 1980). The desired amount of AA was added as an aqueous solution, and volumes and concentrations were calculated to give a final 1:3 w/v flour-water suspension. The suspension was stirred magnetically, and enzyme reaction times were selected so that 30–70% of the added AA was oxidized. The reaction was stopped by acidification. The acid used depended upon the method to be used for AA analysis. When AA analysis was by the indophenol-xylene extraction method (Grant 1974), the reaction was stopped by adding 6% MPA solution to give a final concentration of 3% MPA. When AA analysis was by the DPP method (detailed below), OA at a final concentration of 1.5% was found to be the more suitable acid. Extracts acceptable for the former method were obtained by centrifugation of the suspension at 15,000 × *g* for 5 min. For the DPP method, ultrafiltration of the centrifuged extract was also required. With each assay two control experiments were performed: a zero-time control in which the suspension was acidified before AA was added, and a further control in which a boiled water extract of flour was substituted for the flour. No enzymatic oxidation of AA occurred in the zero-time control, and thus it provided the basis for making the calculation of the percentage of AA that was oxidized in the sample. The latter control permitted correction for nonenzymatic oxidation.

Ultrafiltration was performed with Diaflow membranes (pM10, 43 mm, molecular weight cutoff 10,000) and a snap-opening, magnetically stirred ultrafiltration cell (model 52), both from Amicon Corp., Danvers, MA. The membranes were reused after treatment with 1% proteolytic enzyme to remove sorbed protein as recommended by the manufacturer.

Protein Analysis

The amounts of protein in the extracts were determined by the Lowry method as modified by Peterson (1977).

Instrument and Operating Conditions

The DPP experiments were carried out with an EG&G Princeton Applied Research model 364 polarographic analyzer and static mercury drop electrode (model 303 SMDE) equipped with a Ag/AgCl reference electrode and a platinum wire counter electrode. Potential-current curves were recorded with a Cole-Parmer model 8036 X-Y/t recorder.

The experiments were performed at 25° C. Instrumental conditions used in AA determination are shown in Table II. Dissolved air was removed from the solution by bubbling oxygen-free nitrogen through the supporting electrolyte for 8 min and passing it over the solution after adding sample for ½ min before the analysis.

The peak potential (Ep) was used to identify the AA peak. The peak height (current, μA) was measured from the highest to the lowest part of the curve, using as the baseline a blank containing no AA but otherwise identical to the sample (Fig. 1, curve B).

Calibration Curve

A stock solution of anhydrous AA (100 mg/100 ml) was

TABLE I
Effect of Acidic Components on the Polarogram Characteristics of Solutions Containing 10 ppm of Ascorbic Acid[a]

Extracting Solution[b]	Peak Potential (V) in		Peak Heights (μA) in		Protein Content of Extract (μg/ml)	% Recovery of Ascorbic Acid from Flour Extract[c]
	Extracting Solution	Flour Extract	Extracting Solution	Flour Extract		
3% MPA	0.139	0.095	0.975	0.476	37.2	48.8
3% OA	0.0975	0.0875	0.403	0.308	39.2	76.4
1.5% OA	0.0875	0.0875	0.450	0.405	69.2	90.0
OA + TCA	0.105	0.0875	0.404	0.206	31.6	51.0

[a] Results are the average of duplicate analyses.
[b] Extracting solutions: 3% MPA = 3% metaphosphoric acid; 3% OA = 3% oxalic acid; OA + TCA = 1.5% oxalic acid plus 2% trichloroacetic acid.
[c] % Recovery = peak height in flour extract × 100/peak height in extracting solution.

TABLE II
Polarographic Setting for Ascorbic Acid Analysis

Instrumental Variable	Setting
Electrode	Dropping mercury electrode
Display direction	Negative
Initial potential	0.1 V
Drop time	1 sec
Drop size	Medium (surface area 0.0765 cm^2)
Current range	2–5 μA
Scan rate	5 mV/sec
Scan direction	+
Modulation amplitude	50 mV
Mode	DPP
Supporting electrolyte	10 ml of equal volumes of 2% oxalic acid and pH 4.6 acetate buffer (2*M* acetic acid and 2*M* sodium acetate)[a]
Sample injection volume	1.0 ml

[a] From the method of Lau et al 1985.

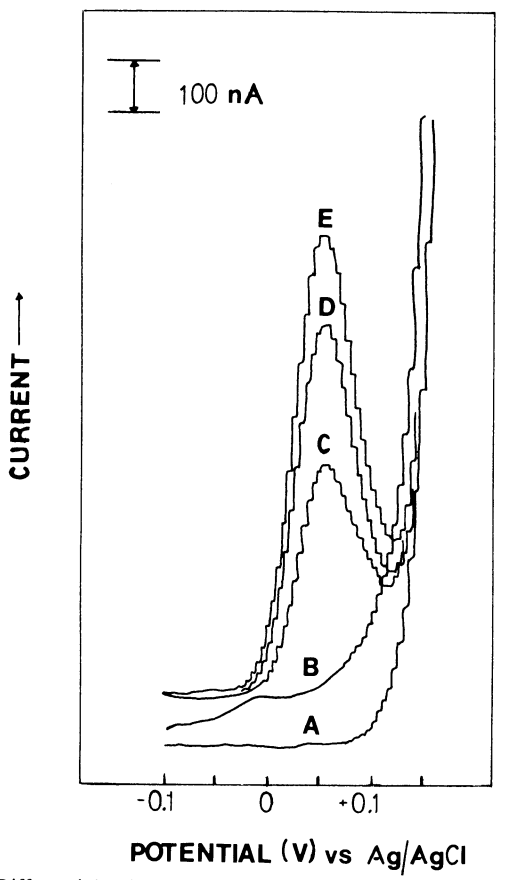

Fig. 1. Differential pulse polarogram of (**A**) supporting electrolyte (Table II); (**B**) wheat flour extract in 1.5% oxalic acid containing no ascorbic acid; (**C**) 10 ppm ascorbic acid in 1.5% oxalic acid wheat flour extract; (**D**) 15 ppm ascorbic acid in 1.5% oxalic acid wheat flour extract; and (**E**) 20 ppm ascorbic acid in 1.5% oxalic acid wheat flour extract.

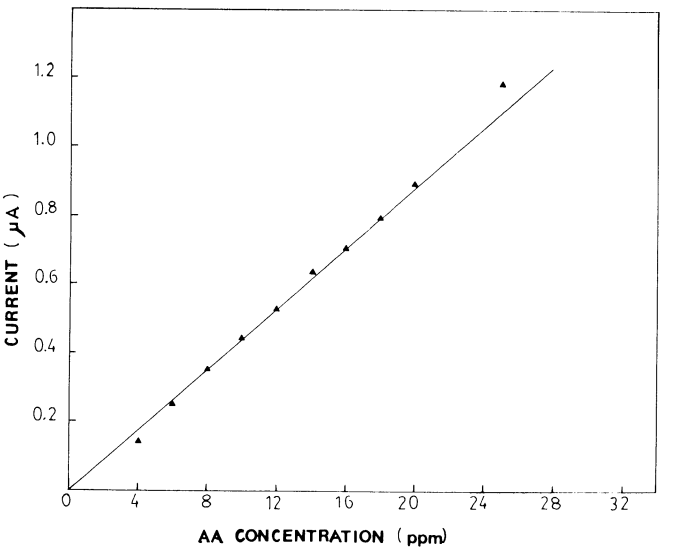

Fig. 2. Calibration curve of ascorbic acid.

prepared in 1.5% OA. Calibration standards (0.4–2.5 mg/100 ml) were prepared by diluting the stock solution with 1.5% OA. The peak heights at Ep of 0.0875 V versus concentration of AA were plotted to obtain the calibration curve (Fig. 2).

RESULTS AND DISCUSSION

The detection limit of the DPP technique was 0.7 ppm, 10-fold more sensitive than the indophenol-xylene extraction method (AACC 1983). We were unable to match the sensitivity of Lindquist and Farroha (1975), who reported detection of as little as 0.025 ppm AA in solution. The reproducibility of the determination as represented by the relative standard deviation of 10 replicates of 10 ppm AA was 2.45%. The calibration curve was linear in the range from 0 to 25 ppm AA solution (Fig. 2) with a correlation coefficient of 0.998. Recovery of AA added to a wheat flour suspension ranged from 90% (for 10 ppm AA) to 96% (for 15, 20, and 25 ppm AA). A standard solution of 10 ppm AA in 1.5% OA solution did not show any change in the peak height when stored at room temperature (25° C) for 12 hr. On the other hand, there was a 5.7% loss of AA from wheat flour extract containing 1.5% OA as the extracting solution when it was left at room temperature for 2 hr after the ultrafiltration. Thus, it is essential that the DPP analysis be performed without delay after the extract was prepared.

Among the four solutions that were tested, OA at 1.5% concentration was found to be the most suitable flour extractant. Except for MPA, it gave the highest peak height. It also gave the highest recovery of AA (Figs. 1 and 3, Table I). A combination of 1.5% OA and 2% TCA caused more proteins to precipitate, but it did not improve the peak height or the recovery of AA. MPA, which has long been a recommended AA extractant for plant material (Ponting 1943), was found to be unsuitable, as it gave a high background signal, an unsymmetrical peak, and low recovery of AA.

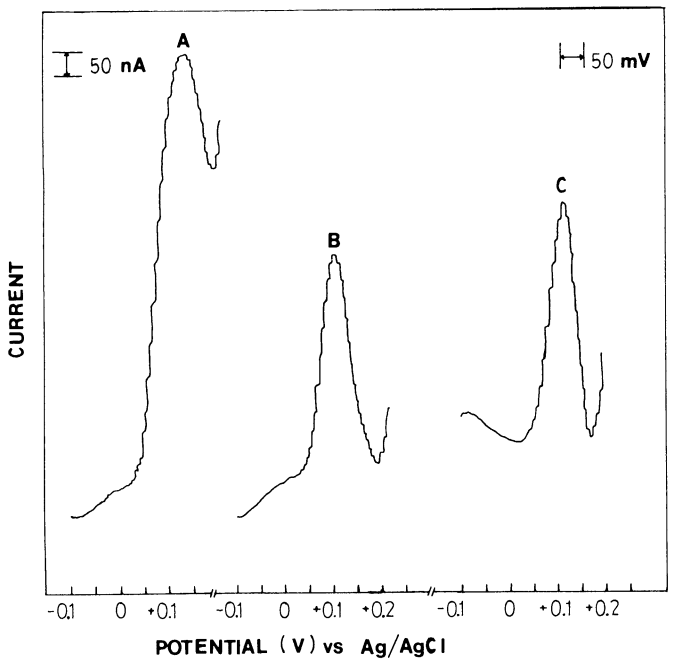

Fig. 3. Differential pulse polarogram of 10 ppm ascorbic acid in (**A**) 3% metaphosphoric acid; (**B**) 1.5% oxalic acid; and (**C**) 1.5% oxalic acid and 2% trichloroacetic acid.

Ultrafiltration of the wheat extract supernatant after centrifugation was found to be necessary. It markedly improved the shape of the DPP signal in a way that could not be obtained with the other clarification methods tested (centrifugation and vacuum filtration through Whatman filter paper No. 42). The ultrafiltration also eliminated foaming that occurred during bubbling the solution with nitrogen. This foaming caused some loss of AA from the assay solution.

The use of a zero time control as the basis for calculating the extent of enzymatic oxidation of AA in the flour-water suspensions made allowance for the fact that recovery of added AA from acidified flour suspensions or extracts was less than 100%. Incomplete recovery may have been caused, at least in part, by components present in the flour in minute amounts that were able to rapidly oxidize a small fraction of the added AA despite the acid conditions.

Very little oxidation of AA occurred in the boiled extract controls. This is in accord with previous observations that there are components in flour that inhibit nonenzymatic oxidation (Sandstedt and Hites 1945, Grant 1974).

The rate of enzymatic oxidation in a suspension of the flour sample as measured by the DPP method was 0.038 ± 0.0025 μmol of AA per minute per gram of flour compared to 0.041 ± 0.002 by the indophenol-xylene extraction method (based on two replicates, 10 injections, with 20 ppm AA solution). The difference was within experimental error.

The DPP method has not yet been applied to the analysis of AA in dough. However, we have determined AA in dough by the indophenol-xylene extraction method after dispersing it in 3% MPA solution with a Waring Blendor. It would appear that similar dispersion of dough in OA solution would result in an extract suitable for AA analysis by the DPP method.

In summary, the DPP method was more sensitive, required less extract, and was less time-consuming than the indophenol-xylene extraction method approved by the AACC. The requirement for ultrafiltration before DPP analysis added somewhat to its complexity, but because a very small volume of sample is required, the ultrafiltration step required very little time.

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