THE ANALYST
FULL PAPER
www.rsc.org/analyst

Determination of ascorbyl 6-palmitate in food matrices by amperometric flow injection analysis

Susanna Buratti, Maria Stella Cosio, Simona Benedetti and Saverio Mannino*

Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy. E-mail: saverio.mannino@unimi.it; Fax: +39 02 58356632; Tel: +39 02 58356626

Received 10th April 2001, Accepted 22nd May 2001
First published as an Advance Article on the web 25th June 2001

In this paper a rapid method based on a FIA (flow injection analysis) system with amperometric detection for the evaluation of ascorbyl 6-palmitate in foods is described. The selectivity of the proposed method is related to the low anodic potential applied to the working glassy carbon electrode (+0.1 V *vs.* Ag/AgCl) that leaves out interferences from ascorbic acid and phenolic compounds. By flow injection analysis, under optimised conditions, the calibration curve was linear in the range 0–20 mg l^{-1} and the detection limit was 0.2 mg l^{-1}.

Introduction

Ascorbyl 6-palmitate is often used as an antioxidant, particularly in closed systems, to remove oxygen in the head space and in solution. Ascorbyl 6-palmitate is considered to be physiologically acceptable, although it is not found in nature; both the ascorbic acid and palmitic acid moieties produced on hydrolysis are natural compounds.

In the United States ascorbyl 6-palmitate is listed in the Code of Federal Regulation under Section 182.3149, Title 21, as a chemical preservative and is recognised as safe (GRAS) with no specific limitation or restriction. In Europe ascorbyl 6-palmitate is considered an antioxidant and its use is regulated by the Directive No. 95/2/EC of 20 February 1995. A number of methods are reported in the literature for the determination of ascorbyl 6-palmitate and most of them are based on colourimetric,[1] TLC[2,3] or HPLC procedures.[4–7] At present the chromatographic separation of the analyte and its subsequent monitoring by UV[8,9] or electrochemical detection[10] is the preferred technique for the determination of ascorbyl 6-palmitate in a wide range of foods. This paper reports a procedure for the rapid and accurate determination of ascorbyl 6-palmitate in food based on a FIA (flow injection analysis) system with electrochemical detection operating under amperometric conditions at a rather low anodic potential of +0.1 V (*vs.* Ag/AgCl).

Experimental

Reagents

Ascorbyl 6-palmitate (AP) and ascorbic acid (AA) were purchased from Sigma-Aldrich (Milan, Italy); methanol was obtained from BDH (Poole, England); sodium perchlorate monohydrate and sodium acetate were obtained from Merck (Darmstadt, Germany); acetic acid was purchased from Riedel-de Haën (D-30926 Seelze, Germany); dimethyl sulfoxide (DMSO) and metaphosphoric acid were purchased from Sigma-Aldrich.

FIA apparatus

The FIA apparatus consisted of a Jasco 880 PU pump (Tokyo, Japan) and an EG&G PAR Model 400 thin layer electrochemical detector (Princeton, NJ, USA) equipped with a single glassy carbon electrode (surface area 8 mm^2) operating at a potential of +0.1 V, a reference (Ag/AgCl, saturated) electrode and a platinum counter electrode. Data were recorded using a Philips PM 8252 recorder. The connecting tubes were made of PEEK (1.5 mm od × 0.5 mm id), the length of the mixing coil was 300 mm. A schematic configuration of the complete FIA system is shown in Fig. 1. Flow injection experiments were performed under amperometric conditions at room temperature and the carrier solution was methanol 70%, sodium acetate–acetic acid buffer (0.1 M, pH 4.5) 28%, sodium perchlorate monohydrate 2%, ascorbic acid 20 mg l^{-1}. The flow rate was 1 ml min^{-1}.

Voltammetry

Cyclic voltammetric experiments were performed in methanol 70%, sodium acetate–acetic acid buffer (0.1 M, pH 4.5) 28%, sodium perchlorate monohydrate 2%. The concentration of ascorbyl 6-palmitate and ascorbic acid was 2.8×10^{-5} M. Standard solutions were submitted to analysis immediately after their preparation. Voltammetric measurements were carried out using a conventional three-electrode system consisting of a glassy carbon working electrode (surface area 8 mm^2), platinum auxiliary electrode and Ag/AgCl reference electrode. The scan rate was set at 100 mV s^{-1} and cyclic voltammograms were acquired with a Model 270 Electrochemical Analysis System.

Hydrodynamic voltammograms were obtained using the FIA apparatus by running a series of experiments in which the potential was stepped incrementally from 0.0V to +0.8 V (*vs.* Ag/AgCl) and the current responses were recorded.

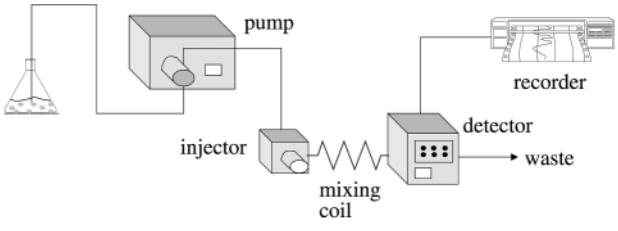

Fig. 1 Schematic configuration of the complete flow system.

 DOI: 10.1039/b103192a

Samples

Commercial samples of vegetable oils (soybean oil, sunflower oil, peanut oil), dried milk formulae for infants, potato purée, flour, and industrial oil for frying, were analysed.

Sample preparation

All samples, except potato purée, were prepared by weighing accurately 0.5–1 g portions of sample into a 10 ml glass-stoppered centrifuge; after the addition of 5 ml methanol, the samples were extracted by mixing for 5 min in a vortex mixer and then centrifuged for 10 min at 5000 rpm. The resultant clear methanol extract was diluted 1:10 with the carrier solution and filtered through a 0.22 μm Millipore filter prior to injection into the FIA system.

Potato purée was extracted with a solution of 90% dimethyl sulfoxide containing 0.12% metaphosphoric acid and 0.02% ascorbic acid. About 1 g of sample was weighed into a 10 ml beaker 5 ml of the extracting solvent was added and the mixture was stirred on a magnetic stirring plate at 20 °C for 60 min. After the extraction period an aliquot of 2 ml was pipetted into a 10 ml glass-stoppered centrifuge tube and 8 ml of methanol was added. The addition of 4 volumes of methanol to the DMSO–acid extract precipitated starch and other polymers. Centrifugation (10 min at 5000 rpm) gave a clear alcoholic supernatant in which AP was present. The clear methanol extract was diluted 1:10 with the carrier solution and filtered through a 0.22 μm Millipore filter prior to injection into the FIA system.

Standard preparation

Accurately weighed 0.01 g portions of AP were dissolved in 10 ml of methanol and then diluted with carrier solution to a range of concentrations of 0.1–20 mg l^{-1}.

Results and discussion

In a preliminary study the best electrochemical condition for the sensitive and selective detection of AP was evaluated. Fig. 2(A) shows the cyclic voltammogram (CV) of ascorbyl 6-palmitate together with that of ascorbic acid which is considered a possible interferent due to its diffusion in many food products. The CV shows the low oxidation potential of the two analytes (oxidation peak of AP is at −0.012 V, oxidation peak of AA is at +0.275 V) and consequently their excellent reducing capacity; furthermore from the hydrodynamic voltammetry reported in Fig. 2(B) it can be seen that by operating at potentials of 0.0, +0.1V (*vs.* Ag/AgCl) AP can be detected without the interference of AA whose oxidation potential is higher.

In previous work[11,12] it has been shown that other antioxidant compounds, such as phenolics or carotenoids, normally require a higher potential for their oxidation, therefore they should not

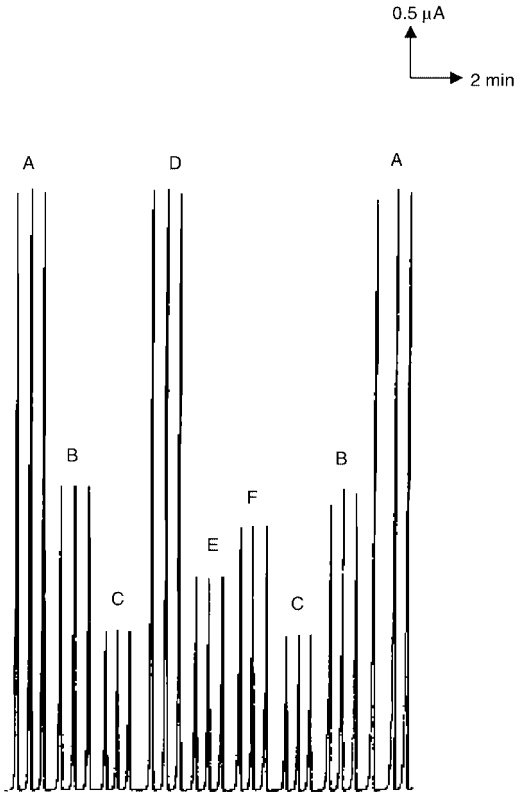

Fig. 3 Transient amperometric signals for increasing concentration of AP standard solutions and for food samples (A: 10 mg l^{-1}, B: 5 mg l^{-1}, C: 2.5 mg l^{-1}, D: industrial oil for frying; E: potato purée; F: dried milk formula for infants). Working potential, +0.1 V (*vs.* Ag/AgCl saturated); flow rate, 1 ml min^{-1}; carrier solution, methanol 70%, sodium acetate–acetic acid buffer (0.1 M, pH 4.5) 28%, sodium perchlorate monohydrate 2%, ascorbic acid (20 mg l^{-1}); injection volume 20 μl.

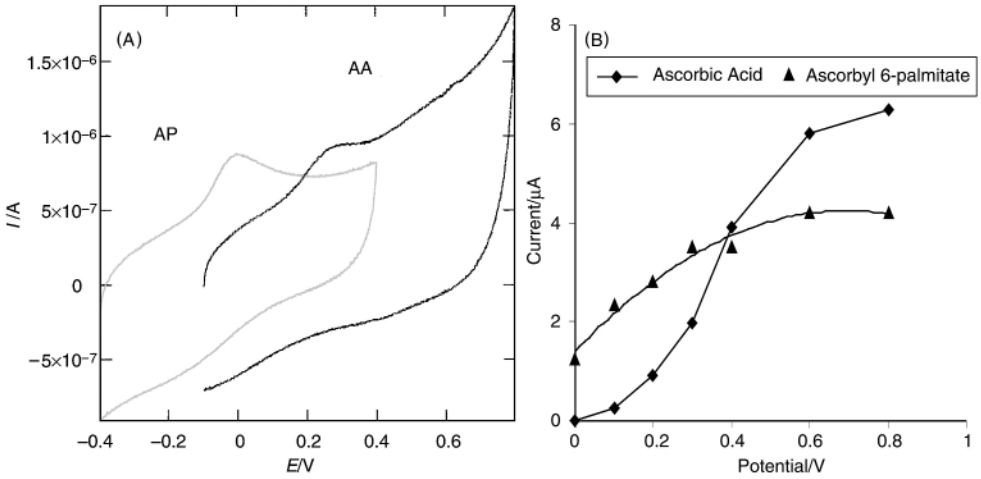

Fig. 2 Ascorbyl 6-palmitate and ascorbic acid electrochemical behaviour. (A): Cyclic voltammograms of ascorbyl 6-palmitate (AP) and ascorbic acid (AA) in methanol 70%, sodium acetate–acetic acid buffer (0.1 M, pH 4.5) 28%, sodium perchlorate monohydrate 2%; standard concentrations, 2.8×10^{-5} M. (B): Hydrodynamic voltammograms of ascorbic acid and ascorbyl 6-palmitate. Flow rate, 1 ml min^{-1}; carrier solution, methanol 70%, sodium acetate–acetic acid buffer (0.1 M pH 4.5) 28%, sodium perchlorate monohydrate 2%; standard concentrations, 2.8×10^{-5} M; injection volume, 20 μl.

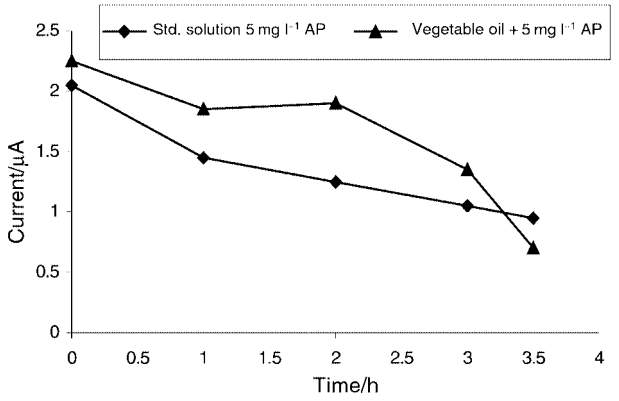

Fig. 4 Reduction degree of ascorbyl 6-palmitate responses in relation with time at the established potential of +0.1V (*vs.* Ag/AgCl). Flow rate, 1 ml min^{-1}; carrier solution: methanol 70%, sodium acetate–acetic acid buffer (0.1 M, pH 4.5) 28%, sodium perchlorate monohydrate 2%; injection volume 20 μl.

Table 1 Ascorbyl 6-palmitate contents in food determined by the proposed method compared with an HPLC method; (average ± *s*) (*n* = 3)

	Ascorbyl 6-palmitate/mg kg^{-1}	
Sample	Electrochemical method	HPLC method
Vegetable oil:		
soybean	nd[a]	nd
sunflower	nd	nd
peanut	nd	nd
Industrial oil for frying	1973 ± 85	1930 ± 76
Potato purée 1	162 ± 7	173 ± 6
Potato purée 2	197 ± 9	190 ± 7
Dried milk formula for infants 1	210 ± 7	200 ± 10
Dried milk formula for infants 2	100 ± 3	96 ± 1
Dried milk formula for infants 3	108 ± 4	110 ± 3
Flour	nd	nd

[a] nd: not detectable

cause interference problems in the analysis of AP under the established conditions.

The procedure developed is fast and can be applied to various food matrices. However for potato purée a more efficient extraction procedure should be used since AP is partially complexed with amylose. The solvent system used (90% dimethyl sulfoxide solution containing metaphosphoric acid and ascorbic acid) was effective since dimethyl sulfoxide is a powerful solvent for starch, either in gelatinised or granular form,[13] and it forms strong complexes with helical amylose[14] dissociating the AP–starch complex.[10] Metaphosphoric acid works as a metal ion chelating agent, while ascorbic acid ensures that AP remains in its reduced form.

Transient amperometric signals for increasing concentrations of AP standard solutions and for food samples are shown in Fig. 3. As can be seen, the detector responds rapidly to the dynamic changes in the analyte concentration allowing about 60 determinations per h.

The precision of the overall method was determined at a working potential of +0.1 V (*vs.* Ag/AgCl) for twenty replicate injections of a 10 mg l^{-1} AP standard solution performed over approximately 4 h; a relative standard deviation of 3.3% was obtained. The addition of ascorbic acid to the carrier solution was mandatory in order to recover all the AP, in fact during the optimisation of experimental conditions it was noticed that AP was unstable even though preserved from light and heat. Fig. 4 shows the reduction degree of AP response with time; it is possible to notice that the signal reduction after 2 h of preservation is about 39% for a standard solution and about 16% for a vegetable oil sample protected by natural antioxidants.

A linear calibration plot was obtained within the range of 0–20 mg l^{-1} (r^2 = 0.9994), the slope and the intercept were 0.6968 μA l mg^{-1} and 0.0205 μA, respectively.

The estimated detection limit, calculated using the linear regression technique from Miller and Miller[15] was 0.2 mg l^{-1}.

The proposed procedure was applied in the determination of the AP content in several food samples and the results obtained are reported in Table 1. As can be seen AP was not found in commercial samples of vegetable oil and flour, while it has been used as an oxygen scavenger to extend the stability of industrial oil for frying and potato purée; AP was found in dried milk formulae for infants even if it was not declared on the label; it was probably used to stabilise one or more of the ingredients. The results, obtained with the proposed procedure were compared with those obtained by a recently developed HPLC method with UV detection.[9] The agreement was generally good, and a paired *t*-test showed no statistical difference at the 5% level of significance between the two methods. In order to evaluate the accuracy of the proposed method, a series of recovery experiments in which AP was added directly to food samples was performed. Recovery results were satisfactory and ranged from 103% for flour to 89% for potato purée whose extraction procedure is more complex.

Since some of the techniques actually used for analysis of AP in foods are poorly selective, time consuming and require skilled personnel as well as expensive equipment, the proposed electrochemical method seems to be a significant improvement in terms of sensitivity, selectivity and simplicity.

Conclusions

The FIA system with electrochemical detection provides a less expensive and more versatile system with considerably reduced analysis times compared with chromatographic methods, making it useful for the routine determination of AP in foods. Furthermore, interferences from ascorbic acid and products bearing a phenolic group are eliminated by applying a rather low working potential of +0.1 V.

References

1 J. Budslawski and K. Pogorzelski, *Le Lait*, 1964, **44**, 378.
2 C. H. Van Peteghem and D. A. Dekeyser, *J. Assoc. Off. Anal. Chem.*, 1981, **64**, 1331.
3 M. P. Forn, *Grasas Aceites (Seville)*, 1980, **31**, 187.
4 T. S. Vicente, E. H. Waysek and W. M. Cort, *JAOCS*, 1985, **62**, 745.
5 D. C. Woolard, *N.Z. J. Dairy Sci. Technol.*, 1983, **18**, 55.
6 A. Dieffenbacher and M. J. Trisconi, *Mitt. Gebiete Lebensm. Hyg.*, 1991, **82**, 475.
7 A. Dieffenbacher, *Dtsch. Lebensm.-Rundsch.*, 1998, **94**, 381.
8 I. Rustan, M. A. Damiano and G. Lesgards, *Ann. Fals. Exp. Chim.*, 1993, **86**, 201.
9 A. Dieffenbacher, *Dtsch. Lebensm.-Rundsch.*, 1998, **94**, 381.
10 T. H. Tracy Hung, P. A. Seib and K. J. Kramer, *J. Food Sci.*, 1987, **52**, 948.
11 S. Mannino, O. Brenna, S. Buratti and M. S. Cosio, *Electroanalysis*, 1998, **10**, 908.
12 S. Mannino, S. Buratti, M. S. Cosio and N. Pellegrini, *Analyst*, 1999, **124**, 1115.
13 P. J. Killion and J. F. Foster, *J. Polymer Sci.*, 1960, **46**, 65.
14 T. D. Simpson, F. R. Dintzis and N. W. Taylor, *Biopolymers*, 1972, **11**, 2591.
15 J. C. Miller and J. N. Miller, *Statistics for Analytical Chemistry*, Ellis Horwood, Chichester, 3rd edn., 1991.