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Determination of Ascorbic Acid in Vegetables by Derivative Spectrophotometry

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Determination of ascorbic acid (AA) in garlic, green pepper and chestnut was performed by derivative spectrophotometry without using any pre-separation or background correction techniques. The method is based on the measurement of the distances between two extremum values (peak-to-peak amplitudes) in second and third order derivative spectra of the extracts. Ten percent trichloroacetic acid was found to be the most suitable extraction solution. In the second order derivative spectrum the extrema of 253.2 and 259 nm for garlic, and in the third order derivative spectrum, the extrama of 256.4 and 261.6 nm for green pepper and chestrut samples were used for the determination of AA.

Calibration graphs were linear over the concentration range 2.0–10.0 μg ml^{-1}. The results obtained by this method were compared statistically with those obtained by official methods. Relative standard deviations of vegetables for AA varied from 0.89 to 2.99% (n = 5) depending on the method used. The recovery of AA in the vegetables was 91.66–97.89% by the standard addition method.

Key Words: Ascorbic acid, Vegetables, Derivative spectrophotometry

Introduction

Owing to the wide use of ascorbic acid (AA) in canned fruits, vegetables, animal foods and drugs, to enable the determination of AA in different matrices and at different levels many analytical techniques are available such as the titrimetric[1–3], spectrophotometric[4–6], derivative spectrophotometric[7,8], colorimetric[9,10], kinetic-spectrophotometric[11], flow-injection spectrophotometric [12], high-performance liquid chromatographic[13–18] (HPLC), electroanalytic[19–22], capillary zone electrophoretic[23] (CZE), and micellar electrokinetic chromatographic[24] (MEKC) methods. The quantities of AA in some vegetables obtained by these methods are given in Table 1.

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Table 1. Determination of AA in natural products

Material	AA (mg / 100 g)	Method*	Reference number
Peas	13.7	SP	4
Beans	19.7	SP	4
Cauliflower	58.0	SP	4
Chillies	100.5	SP	4
Sweet peppers	58.0	SP	4
Strawberries	62.0 ± 8.0	SP	5
Lemon	48.0	SP	6
Tomato	12.3	SP	6
Parsley	110.2	SP	6
Green pepper	14.4	SP	6
Cauliflower	29.7	DSP	7
Parsley	94.8	DSP	8
Kiwi	79.9	DSP	8
Grapefruit	42.4	DSP	8
Mushrooms	2.0 – 9.0	HPLC	13
Spinach[a]	80.0	CZE	23
Spinach[b]	19.0–52.0	CZE	23
Turnip[b]	72.0	CZE	23
Parsley[b]	97.0	CZE	23

*SP (spectrophotometry), DSP (derivative spectrophotometry), HPLC (high-performance liquid chromatography), CZE (capillar zone electrophoresis).

[a] Cultivated

[b] Purchased

AA concentrations are frequently determined by the titrimetric Association of Official Analytical Chemists (AOAC) method[1] and titrimetric pharmacopoeial method[2] using 2,6-dichlorophenolindophenol (DCPIP) and iodine as a titrant, respectively. Although the results are rapidly obtained, these methods are not specific or are not very sensitive and the reagent itself is not stable and needs standardization before use. Moreover, if the sample solution is coloured, end point detection will be difficult. Spectrophotometry is a fast and simple method for AA determination, but it cannot be used in samples with complex matrices because of background absorption in the UV region. Derivative spectrophotometry is a useful technique for extracting qualitative and quantitative information from spectra composed of unresolved bands[25] and for eliminating the effect of baseline shifts and baseline tilts[26].

Ozgur et al.[7,8] reported the determination of AA in kiwi, parsley and grapefruit by third order (^{3}D), and orange and cauliflower by first order derivative (^{1}D) spectrophotometry without pre-separation or background correction procedures. In the present work, this method was applied to garlic, green pepper and chestnut samples with some modifications. Recovery experiments were carried out by the standard addition method to determine accuracy and precision.

Experimental

Apparatus

A Shimadzu UV-160A double beam spectrophotometer with 1-cm path length cells and a volume of 3 ml was used under the following operating conditions: scan speed 1500 nm min^{-1}, scan range 200-300 nm, slit width 2 nm and derivation interval ($\Delta\lambda$) 4.2 nm for second (^{2}D) and third order derivative (^{3}D) spectra. Very high smoothing derivative spectra were found at the conditions mentioned above.

A Waring Commercial Blender was used as a mechanical stirrer for samples extraction.

Chemicals

AA was kindly supplied by Roche Pharm. Ind., Turkey, at a purity of 99.8% as determined by titration with DCPIP[1]. All chemicals and solvents were of analytical reagent grade and purchased from Merck Co., Germany. Bidistilled water was used throughout the work.

Solutions

Stock solution of AA (400 μg ml^{-1}): 20 mg of AA was dissolved in 50 ml of 10% (w/v) trichloroacetic acid in a calibrated flask. This solution was prepared freshly and diluted to obtain standard solutions for the preparation of calibration graphs. The same amount of AA was dissolved in 3% (w/v) metaphosphoric acid -8% (v/v) acetic acid solution for DCPIP titration.

10% (w/v) trichloroacetic acid (TCA) solution was used as extraction solution. This solution can be stored at 4°C in the dark for one week.

Preparation of Calibration Graphs

A specified volume (0.05-0.25 ml) of the stock AA solution was diluted to the volume with 10% TCA in a 10 ml calibrated flask to obtain working solutions (2-10 μg ml^{-1}). Then the ^{2}D and ^{3}D spectra of AA solutions were recorded against extraction solution between 200 and 300 nm.

In the ^{2}D, peak-to-peak amplitudes of 253.2 and 259.0 nm were used for the establishment of the calibration graph to analyse garlic samples. In the ^{3}D, 256.4 and 261.6 nm extrema were used to analyse green pepper and chestnut samples for the same purpose.

The concentration of AA in the sample solutions was deduced by means of regression equations of the related calibration graphs.

Sample Preparation

Samples were purchased from local markets. Each sample was analysed on the same day of purchase.

The chestnut and garlic sample pods were cleaned. Green pepper samples were used directly. About 200 g of each sample was weighed as a stock material and cut into small pieces and mixed. Fifteen-gram portions of each sample were homogenized with a mechanical stirrer using 50 ml of 10% TCA solution for about 5 min. After the homogenization of the samples, the extracts were clarified by centrifuging at 5500 rpm (2850 g) for 5 min and then filtered through a filter paper, Schleicher/Schüll 589[3] blue ribbon, using a vacuum pump. The filtrates were analysed directly or after dilution to provide the necessary working

concentration. The same sample solutions were also used in iodine titration method[2]. Then 15 g of each sample was homogenized with 50 ml 3% (w/v) metaphosphoric acid-8% (v/v) acetic acid solution as described above and the same procedure was repeated for the AOAC method[1].

Standard Addition Method

About 100 g of each sample was weighed, cut into small pieces and homogenized. Then 7.5, 15 and 22.5 mg of weighed AA was added to 15 g portions of each material separately in a glass mortar. The mixtures were homogenized again and analysed similarly as described in "Sample Preparation".

Results and Discussion

The UV-spectrophotometric method is not suitable for the determination of AA in fruits and vegetables due to the matrix effect of UV-absorbing substances in the samples. This effect is clearly seen in the absorption spectra of garlic, green pepper and chestnut extracts, together with the spectra of AA solutions prepared in the same solvent and the same concentration as the sample extracts. Absorption and the ^{1}D, ^{2}D and ^{3}D spectra of each sample are shown in Figures 1-3 for each sample, respectively. The derivatization of the absorption spectrum and measurement of the distance between two neighbouring extremum values allow the elimination of matrix effects, because the variable background absorptions overlapping the analyte peaks are smoother in derivative spectra. The ^{2}D spectra of sample and standard solutions in the same solvent and concentration completely overlap each other in the region 253.2-259.0 nm for garlic. The third order derivative spectra of samples and standard solutions in the same condition completely overlap each other in the region 256.4-261.6 nm for green pepper and chestnut.

Ten percent trichloroacetic acid, 5% oxalic acid, 3% metaphosphoric acid, and 8% acetic acid were tested for the extraction and 10% TCA was determined to be the most suitable solvent. The absorption values of the sample solutions used are stable for at least 1 h.

Linear relationships were obtained between the AA concentration (2.0–10.0 μg ml^{-1}) and the peak-to-peak amplitudes of ^{2}D and ^{3}D spectra of garlic, green pepper and chestnut samples at the wavelengths mentioned above as can be seen in Figures 1 (c), 2 (d) and 3 (d), respectively. The regression equations were as follows:

$^2D_{253.2-259} = 0.0279\ c + 0.0928$ (r = 0.9999) (garlic)

$^3D_{256.4-261.6} = 0.0457\ c + 0.1438$ (r = 0.9999) (green pepper and chestnut)

To compare the results obtained by this method, the same extracts were analysed for AA by the official reference methods, which are based on titration with DCPIP and iodine solutions. The results were statistically compared with those of titrimetric methods by Student's t-test and variance ratio F-test (Table 2). It was determined that there was no significant difference between the three methods in terms of mean values and standard deviations at 95% confidence levels.

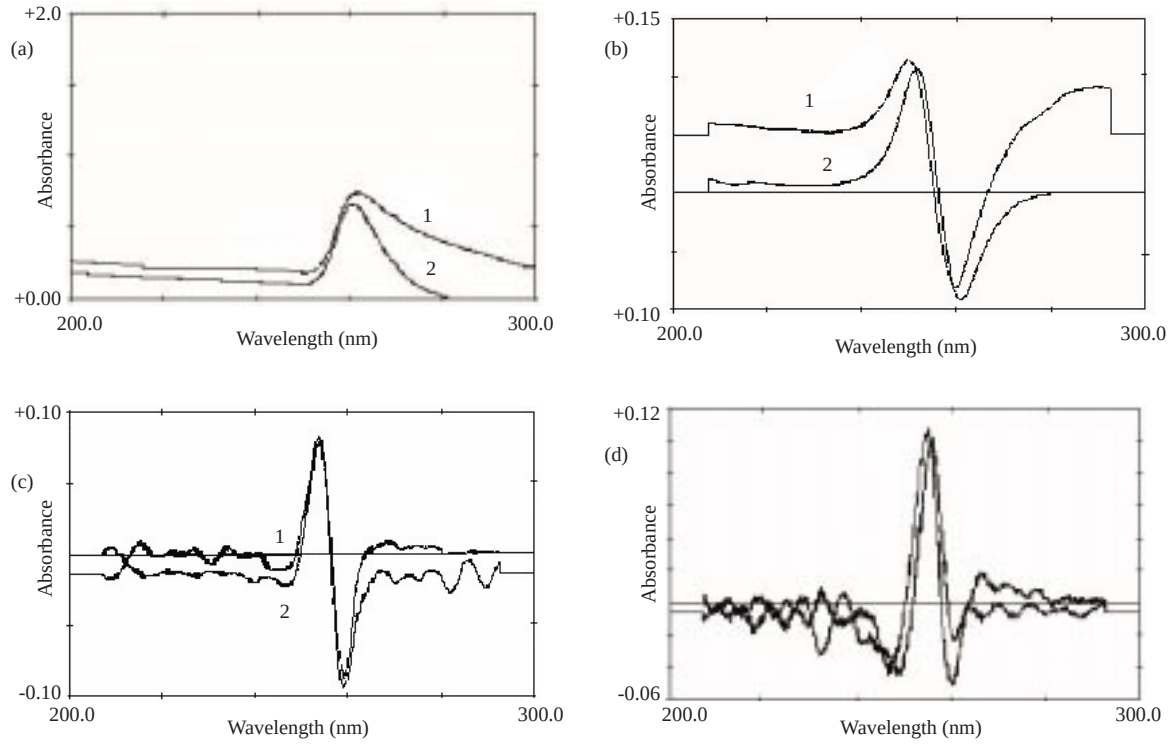

Figure 1. UV-Absorption (a), first derivative (b), second derivative (c), third derivative (d) spectra of garlic (1) and AA (2) (c = 10 μg ml^{-1})

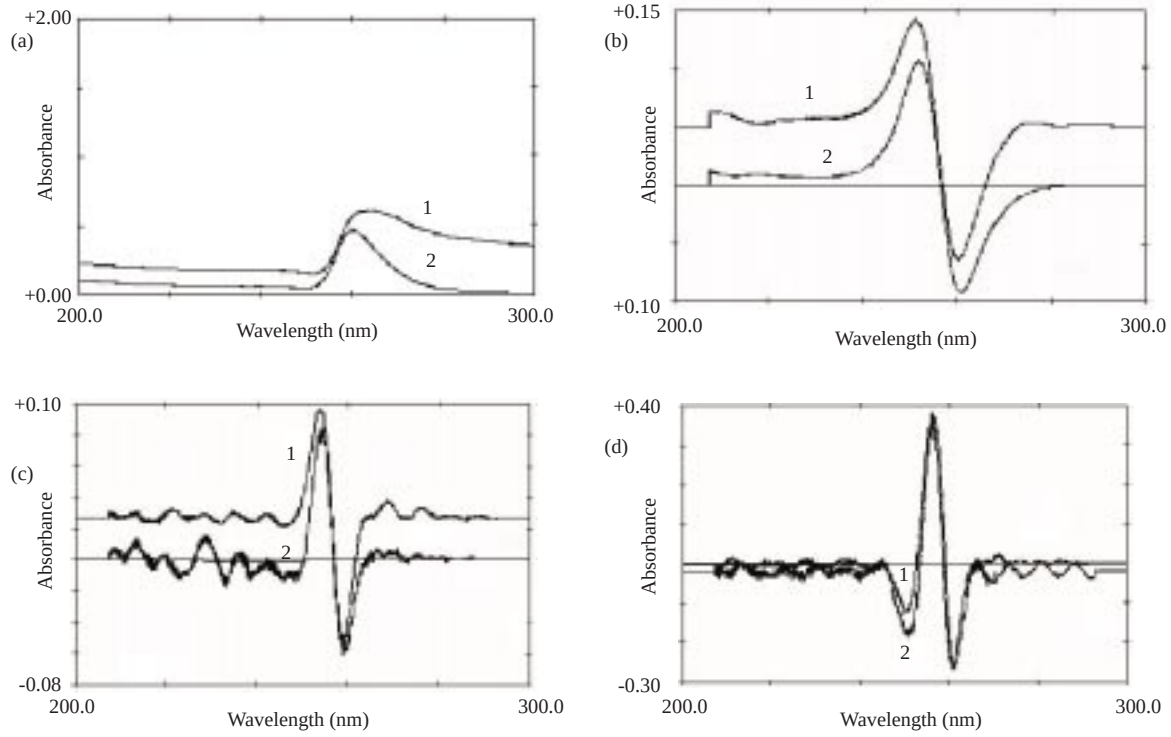

Figure 2. UV-Absorption (a), first derivative (b), second derivative (c), third derivative (d) spectra of green pepper (1) and AA (2) (c = 10 μg ml^{-1})

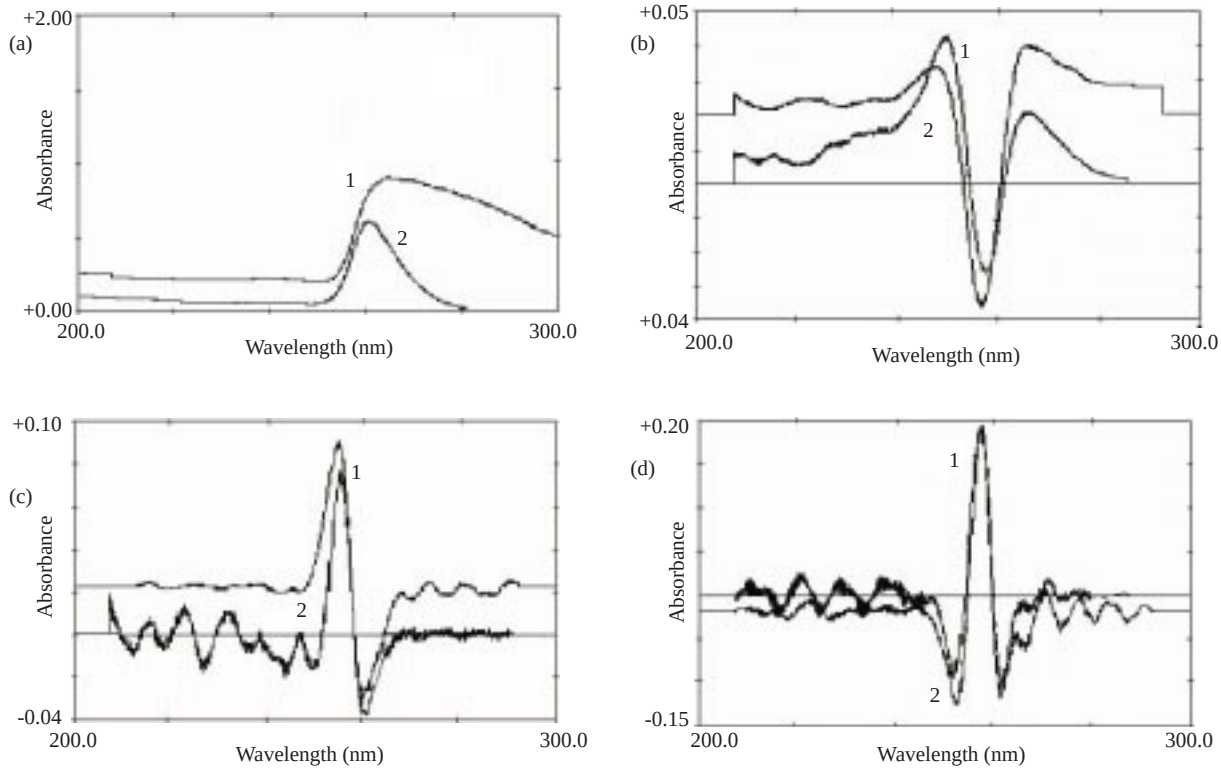

Figure 3. UV-Absorption (a), first derivative (b), second derivative (c), third derivative (d) spectra of chestnut (1) and AA (2) (c = 10 μg ml^{-1})

Table 2. Determination of AA in some vegetables

Material	Deriv. Spectroptroph.		Method A†		Method B††
Garlic					
Mean* ± SD	21.69 ± 0.61		21.38 ± 0.64		21.03 ± 0.22
RSD	2.81%		2.99%		1.05%
		t = 0.79		t = 2.28	
		F = 1.04		F = 2.79	
Green pepper					
Mean* ± SD	67.66 ± 1.47		66.29 ± 1.60		66.85 ± 1.17
RSD	2.41%		2.17%		1.75%
		t = 1.39		t = 1.07	
		F =1.18		F = 1.58	
Chestnut					
Mean* ± SD	16.96 ± 0.25		16.57 ± 0.41		15.79 ± 0.14
RSD	1.47%		2.47%		0.89%
		t = 1.83		t = 2.26	
		F = 2.66		F = 3.18	
		t_{theor} = 2.31 (P = 0.05)			
		F_{theor} = 6.39 (P = 0.05)			

*mg / 100 g of the sample (n = 5)

†Iodine titration, ††DCPIP titration.

The average recovery of AA from garlic, green pepper and chestnut samples was found to be 95.48% from the analysis of each samples added to AA at three different concentrations. The results are shown in Table 3.

Table 3. Recovery of AA added to vegetables

Material	Added Found* (mg / 100 g)	Recovery (%) (mg / 100 g)	
Garlic	-	21.69	-
	50	69.02	94.66
	100	118.69	97.00
	150	167.19	97.00
Green pepper	-	67.66	-
	50	115.99	96.66
	100	164.33	96.67
	150	209.33	94.45
Chestnut	-	16.96	-
	50	62.79	91.66
	100	110.29	93.33
	150	163.79	97.89

*Three separate workups were performed and the mean calculated.

In conclusion, the derivative spectrophotometric method is easy, fast and cheap for the quantitative determination of the AA content of garlic, green pepper and chestnut. Because it does not require expensive solvents and reagents, it may be recommended for the rapid, precise and sensitive quantification of AA in these products. The method can be extended to AA determination in various vegetables provided that the proper wavelengths for peak-to-peak measurements are accurately selected.

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