HPLC METHOD FOR DETERMINATION OF ASCORBIC ACID IN FRUITS AND VEGETABLES

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Abstract

An HPLC method has been worked out for the determination of ascorbic acid in fruits and vegetables. The method has been used to deep-frozen raspberry cream analysis together with three commonly used chemical methods of vitamin C analysis. The HPLC method has been compared with the chemical methods from several aspects and the superiority of HPLC method has been concluded.

Introduction

The change of vitamin C content indicates the quality change of deep frozen foods during their storage. TTT diagrams, characterizing storage temperature—time—tolerance (quality) relationship are constructed on the basis of this change.

Ascorbic acid (AA) and dehydroascorbic acid (DAA) both occurring in very low concentrations form together the vitamin C content of foods.

Vitamin C is sensitive to light, heat and oxidation. In quantitative determinations one must always count with a certain—lower or higher—systematic error depending on how the above mentioned effects can be reduced to a minimum.

In Hungary chemical methods of vitamin C determination are in general use. Vitamin C of a properly prepared food sample is converted by a multistep and generally not instantaneous chemical reaction to form a sensitively measurable coloured compound. It is obvious that a method (HPLC) eliminating this chemical transformation and limiting the period of determination to the sample preparation and a short analysis time may have considerable advantages compared to chemical methods.

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Table 1

Vitamin C content of some fruits and vegetables
[Tangl H., 1952]

	Sort	Concentration (mg%)
Raspberry	Fertőd 401	41.5
	Lloyd George	23
	Nagymaros	33.6
	Malling Promise	53.2
Strawberry		50
Gooseberry		20
Cherry		15
Sugar pea		50
Garden sorrel		40
Tomato		30

We have worked out an HPLC method for the AA component determination in fruits and vegetables and used it to deep-frozen raspberry cream analysis. (Raspberry has an average vitamin C content in comparison with other vegetables and fruits, see Table 1). At the same time the AA or AA and DAA contents of the samples were determined with three commonly used chemical methods too. The HPLC method has been compared with the chemical methods and the superiority of the HPLC method has been concluded.

AA determination with HPLC

Column separation and sensitive detection together ensure the direct selective analysis of vitamin C components. Unfortunately our LABOR MIM liquid chromatograph is equipped only with UV detector which is insensitive to DAA. Though only the AA component has been measured we have got similar or higher results even compared with chemical methods measuring the total vitamin C content.

Sample preparation

A known amount of a vegetable or a fruit was disintegrated in a mixer continuously rinsed with nitrogen. HPLC eluent was added into the mixer and the pulp was transferred to a volumetric flask. After bubbling through a certain amount of nitrogen the flask was filled up with the eluent and closed with a penetrable film. The flask was placed into a refrigerator and the sample was analysed after clearing up.

HPLC analysis

An RP 18 column was used with methanol—water—glacial acetic acid (30 : 69 : 1) eluent containing 10 g/dm^3 NaCl. Detection wavelength was about 230 nm.

AA was identified with addition of L–AA to the sample. A peak height —L–AA concentration calibration diagram was used for AA quantitation in the sample.

Qualification of the HPLC method

Chemical methods chosen for comparison are:

— spectrophotometric method of Spanyár [Keveiné, 1970, Petro O., 1968]
— enzymatic method of firm Boehringer
— TLC method of Petro [Petro O., 1968]

In the course of deep-frozen raspberry cream analysis the four methods were compared in the following respects:

— absolute value of AA or AA and DAA concentration
— deviation of AA or AA and DAA concentration
— relative error (per cent) (100 × deviation/concentration)
— duration of analysis
— interference by accompanying components
— labour requirements
— need of chemicals and special instruments

Comparison of chemical methods

Analysis is based on the redox properties of AA and DAA. If the aim is the total vitamin C determination DAA must be converted to AA or vice versa in a very gentle manner. E.g. generally DAA is reduced with cistein. (At 37 °C within about 30 minutes).

Spectrophotometric method: Fe (3 +) and α, α'-dipyridyl are added to the sample. A red Fe(2 +)-coordination compound is formed and quantitative determination is made spectrophotometrically. Evolution of colour is not instantaneous, it lasts about 60 min at room temperature. This colour reaction is not specific for AA. Other reducing compounds in foods—reductons—also give this reaction. The error can be eliminated with a reference probe: colour reaction is performed also after the AA component is decomposed in the sample. The amount of AA is calculated from the absorbance difference of

sample and reference probe. (AA decomposition lasts about 30 min at 50 °C and pH 6.)

Enzymatic method: The principle is similar to the previous method but with some tricks the determination is made faster, more precise and reliable and so the method is declared as AA specific.

— In the reduction of the total reducton amount (AA and other reductive components) a catalyst is utilized and thus the colour reaction proceeds within 15 min at 37 °C.

— In the reference probe AA is decomposed with ascorbatoxydase enzyme at room temperature. Decomposition is fast and AA specific, the conversion of other reductons is negligible. One can assume that the absorbance difference of a probe and the corresponding blank probe is proportional to the true AA amount.

Unfavourably the colour reaction coupled with this method is induced also by ligth, so one must work with careful light exclusion.

The TLC method is also combined with chemical conversion. Here AA is oxidised to DAA with bromine water. From DAA DAA-osazone is formed with 2,4-dinitrophenylhydrazine. AA oxidation lasts about 10 min at room temperature. Osazone formation takes 20 min at 60 °C. Beside DAA-osazone other osazones are also formed and TLC is used for their separation. Before TLC separation the osazone precipitate must be filtered, washed, dehydrated and dissolved in an organic solvent. TLC separation in itself takes about 50 min. The amount of DAA-osazone is visually determined by comparison of spot areas with calibration spots. An advantage of the method is the total vitamin C specifity. Derivatisation is, however, time and labour consuming and has many sources of error. Visual evaluation decreases the reliability of the results.

Experiments

Raspberry cream for deepfreeze storage was prepared from mixed sort of industrial raspberry in the pilot plant of the Institute of Preservation and Livestock Products Technology of the University of Horticulture and Food Industry in Budapest. Cream was treated with an ET 9 type triturator, refrigerated in 200 g portions at −20 °C in plastic holders. Analysis for vitamin C was made within three days immediately after preparation. Raspberry cream was defrosted within 3.5—4 min in a microwave oven. At least five parallels pro method were measured and the sample of each parallel measurement was taken from a separate holder.

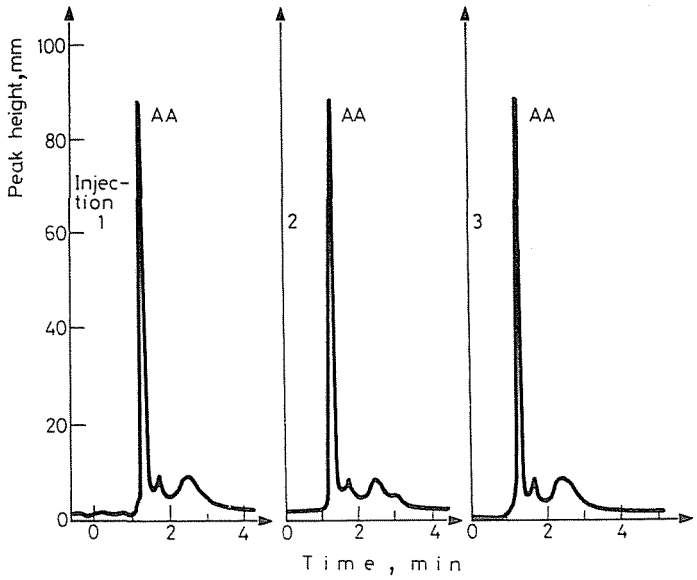

Fig. 1. Three parallel HPLC chromatograms of a sample prepared from raspberry cream Column: RP18, eluent 30 : 69 : 1 methanol-water-glacial acetic acid with 10 g/dm^3 NaCl, flow rate 1 cm^3/min, UV detection at 230 nm)

HPLC analysis

20 g of defrosted pulp was weighed into a 100 cm^3 volumetric flask and filled up with the eluent. Clearing up of the solution lasted about 30 min. A 20 µl volume was injected into the chromatographic column. The chromatogram (Fig. 1) was taken with 1 cm^3/min eluent flow rate at 230 nm wavelength. In Fig. 2 a peak height—concentration calibration curve for quantitative evaluation is shown.

Results

Measured data, calculated deviations and relative errors are summarized in Table 2 and plotted in Fig. 3 where on the X-axis the method is given, on the Y-axis AA or AA and DAA concentration.

Comparison of methods

In the course of classification each method got a note from one to four from aspect to aspect listed in page 3. One was given to the best, four to the worst method (see Table 3).

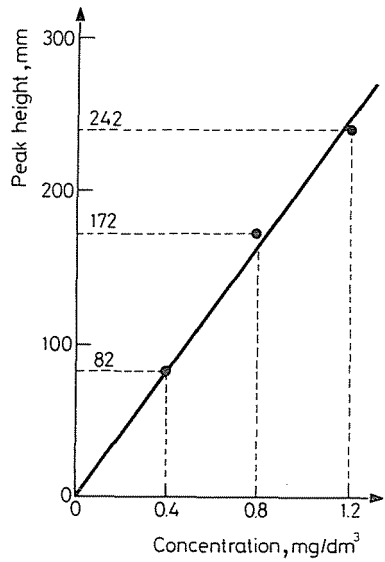

Fig. 2. Peak height-concentration calibration curve (Sample L-Ascorbic acid, UV detection at 230 nm

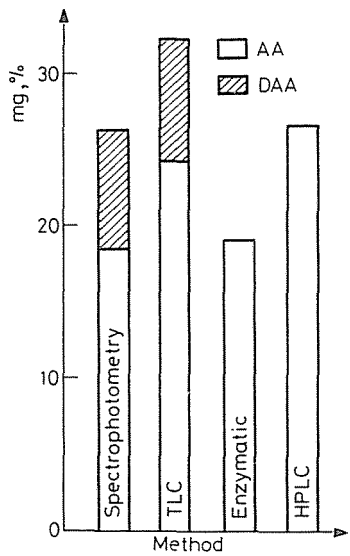

Fig. 3. Vitamin C content of raspberry cream

Table 2

Results of raspberry cream measurements

1. Vitamin C content (mg%) Method/compound	Spectrophotometry	TLC	Enzymatic	HPLC
AA	18.87	24.46	9.21	26.94
DAA	7.89	7.82	—	—
Reducton	21.26	—	—	—
Total vit. C	26.77	32.39	—	—
2. Relative standard deviation, %/compound	**Spectrophotometry**	**TLC**	**Enzymatic**	**HPLC**
AA	1.47	3.96	2.31	0.02
DAA	2.80	0.25	—	—
Reducton	1.85	—	—	—
Total vit. C	3.21	4.15	—	—
3. Relative error %/compound	**Spectrophotometry**	**TLC**	**Enzymatic**	**HPLC**
AA	7.82	15.97	12.02	0.084
DAA	35.51	3.22	—	—
Reducton	8.72	—	—	—
Total vit. C	12.01	12.87	—	—

In HPLC and TLC the number of parallels was high enough to a mathematical statistical evaluation of the validity of results. It was established with T-probe that the two methods gave with 95% confidency the same concentration. In TLC visual evaluation was reliable if the number of estimations was five or more and thus the uncertainty characteristic of the semiquantitative method was reduced. Consequently if one has to choose from these two methods, the reliability is not an adequate basis for decision.

The sum of the notes gave the following order:

The spectrophotometric method is the less favourable. Though it has small standard deviation and relative error, AA concentrations are too small probably due to the interference by reductons.

TLC is on the third place. It gives reliable results like HPLC but the standard deviation is very high. To obtain exact results one must increase the number of parallel measurements. This is very disadvantageous with such a slow method. The method is not suitable for serial analysis though it needs no special instrument.

The second place is given to the enzymatic method. It is AA specific, fast and not expensive but it has relatively high standard deviation and relative

Table 3

Aspects and notes in the comparison of HPLC and chemical methods

Method/Aspect	Spectrophotometry	TLC	Enzymatic	HPLC
St. deviation	2	4	3	1
Specificity	4	2.5	1	2.5
Simplicity	3	4	2	1
Effect of reductons	4	1.5	3	1.5
Duration	3	4	2	1
Costs	2	1	3	4
Sum of notes	18	17	14	11

error. This is due to the light sensitivity of the colour reaction. Much care should be taken during measurements.

The best results were obtained with HPLC. The method is fast, reliable, needs no special chemicals. If one has a liquid chromatograph this method should be chosen. Though the method in the described form is only AA specific, DAA could probably be determined beside AA with a suitable second detector. This is the aim of our future work.

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