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Spectrophotometric analysis of total ascorbic acid contetnt in various fruits and vegetables

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Abstract: Total ascorbic acid (ascorbic acid + dehydroascorbic acid) has been determined in twenty-one different samples of fruits and vegetables by spectrophotometric method. This method is based on the oxidation ascorbic acid to dehydroascorbic acid by bromine water in the presence of acetic acid. After coupling with 2,4-dinitrophenylhydrazine (DNPH) a red complex was produced and absorbance of that complex was spectrophotometrically measured at 521 nm. A linear concentration range for standard solutions of ascorbic acid was obtained up to 10 μg mL^{-1}, with a correlation coefficient of 0.9929. The contents of ascorbic acid were found between 9 and 49 mg/100 g of fresh fruits, also 3 and 90 mg/100 g of fresh vegetables. The interferences of glucose, fructose and sucrose were also investigated. The limit of detection of ascorbic acid was found to be 0.01 μg mL^{-1} (3σ from 10 measurements of ascorbic acid concentration of 3 μg mL^{-1}), and limit of quantification of ascorbic acid was 0.017 μg mL^{-1}. A relative standard deviation was 2.4 % (n = 10, c = 7 μg mL^{-1}). The content of total ascorbic acid in twenty-one different samples of fruits and vegetables was compared with results of spectrofluorimetric method and literature values.

INTRODUCTION

Vitamin C (L-Ascorbic acid) is water-soluble vitamin with strong reducing action and it is an important co-enzyme for internal hydroxilation reaction. Vitamin C is found in both reduced form (ascorbic acid) and oxidized form (dehydroascorbic acid). It is widely used food additive with many functional roles, many of those are based upon its oxidation-reduction properties. Functional roles include its use as: a nutritition food additive, antioxidant, reducing agent, stabilizer, modifier, colour stabilizer (Eitenmiller *et al.,* 2008). The desire to develop methods with ideal characteristics have resulted a large number of procedures with varying applicability. For the determination of ascorbic acid in food the method should apply for both, ascorbic acid and dehydroascorbic acid, to give a total value of vitamin C (Ball, 1994). Many analytical techniques are mentioning in the literature for the determination of vitamin C in different matrices, such as: titrimetric (Verma *et al.*, 1996), fluorimetric (Xia, 2003), spectrophotometric (Rahman et al., 2006.; Sataya *et al.,* 1998), high-performance liquid chromatography (Nyyssönen *et al.,* 2000), enzymatic (Casella *et al.*, 2006), etc. In this work we used spectrophotometric determination of total ascorbic acid based on coupling with acidic DNPH in twenty-one different samples of fruits and vegetables. DNPH procedure is one of the most simple, accurate and applicable method for determination of total ascorbic acid in fresh food, such as fruits and vegetables.

EXPERIMENTAL

Reagents

Metaphosphoric acid - acetic acid

Fifteen grams of solid metaphosphoric acid were dissolved in mixture of 40 mL glacial acetic acid and 450 mL of distilled water, in a 500 mL volumetric flask. The solution was filtred and collected.

2, 4-dinitrophenylhydrazine solution and thiourea solution

2 g of 2, 4-dinitrophenylhydrazine and 4 g thiourea were dissolved in 100 mL 4,5M H_2SO_4.

Standard vitamin C (Ascorbic acid) solution

A stock solution of 500 ppm of vitamin C was prepare daily.

Samples

Investigated samples were twenty-one different kind of fruits and vegetables obtained from local markets: Black currant, rose hip, parsley, sea buckthorn, date, pepper, orange, red currant, strawberry, red grapefruit, lemon, mandarin, potato, tomato, carrot, banana, cranberry, blueberry, apple, blackberry, cucumber.

Sample preparation

Five grams of sample were homegenized with 25 mL of metaphosphoric acid - acetic acid solution, and it was quantitatively transferred into a 50 mL volumetric flask and shaken gently to homegenize solution. Then it was diluete up to the mark by the metaphosphoric acid - acetic acid solution. The obtained solution is filtred and centrifuged at 4000 rpm for 15 minutes, after what the supernatant solution is used for spectrophotometric determination (Perkin Elmer spectrophotometer Lambda 25) of vitamin C content in 21 samples of different fruits and vegetables.

Estimation of Vitamin C

Procedure**:** 0,23 mL of 3% bromine water were added into 4 mL of centrifuged sample solution to oxidize the ascorbic acid to dehydroascorbic acid and after that 0,13 mL of 10 % thiourea to remove the excess of bromine. Then 1 ml of 2, 4-DNPH solution was added to form osazone. All standards, samples and blank solution were kept at 37 °C temperature for 3 hours in a thermostatic bath. After it all were cooled in ice bath for 30 minutes and treated with 5 mL chilled 85 % H_2SO_4, with constant stirring. As a result, a colored solution's absorbance was taken at 521 nm.

Reactions

Ascorbic acid is oxidized to dehydroascorbic acid by adding bromine water. After that L-dehydroascorbic acid reacts with 2,4-DNPH and produces an osazone, which treated with 85 % H_2SO_4 forms red colored solution. A typical calibration plot was made and used to determine the concentration of ascorbic acid in the investigated samples.

RESULTS AND DISCUSSION

Apsorption maximum of ascorbic acid

To determine the apsorption maximum, standard solutions of ascorbic acid in concentration of 3 mg mL^{-1} were prepared. Spectrum of 2,4-DNPH solution was measured according to this procedure, in the wavelength interval 490-530 nm (Fig. 1).

Calibration curve

After determination of the λ_{max} of colored complex (521 nm) using a Perkin Elmer spectrophotometer, the absorbances of all standards (converted to coloured complex) were taken to construct a calibration curve. The calibration curve was constructed by plotting the concentration versus the corresponding absorbance (Fig. 2). The limit od detection (LOD) of ascorbic acis is 0.01 μg/mL (3σ of 10 measurements of standard solution of ascorbic acis, concentration of 3 mg/mL). The limit of quantification (LOQ) of ascorbic acis is 0.017 μg/ml. The relative standard deviation was 2.4 % for 10 measurments of standard solution of ascorbic acid concentration of 7 μg/mL.

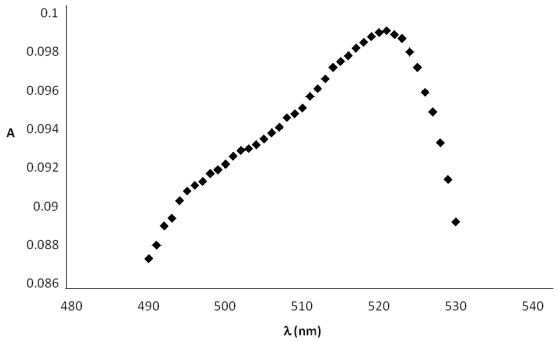

Figure 1. Apsorption maximun

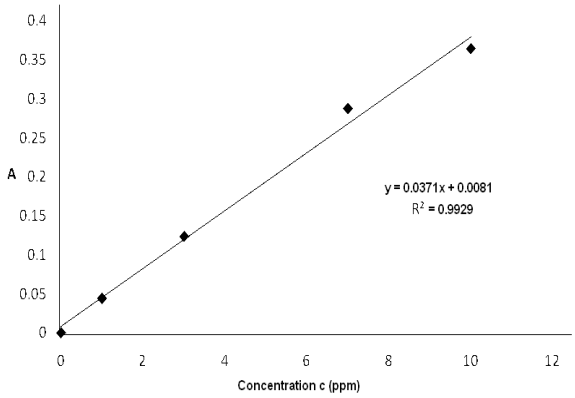

Figure 2: Calibration curve for determination of vitamin C

Interferences of fructose, glucose and sucrose

Using this method can interfere compounds that can provide products with 2, 4-DNPH. Interferences of fructose, glucose and sucrose on total ascorbic acid determination were examined over the apsorption spectrum of 2, 4-DNPH sugar complex, under the same conditions as those for standard solutions and samples. Concentation of investigated interferences were 5 μg/mL.

It has been determined that fructose and sucrose have no response in the interval of 490-530 nm, while glucose had 15 times lower apsorption than ascorbic acid under the same conditions and concentration.

Determination of vitamin C in samples

Samples of different fruits and vegetables were prepared according to previously written procedure. Results of total content of AA in the investigated samples obtained by

spectrophotometric and spectrofluorimetric (Čopra - Janićijević *et al.,* 2011) methods as well as literature value are shown in Table 1. Spectrofluorimetric method was based on the condensation reaction between AA and *o*-phenylendiamine (OPDA) in the absence of the oxidant.

The highest content of total ascorbic acid obtained by spectrophotometric method was found in samples of: black currant (184.36 μg mL^{-1}), rose hip (168.44 μg mL^{-1}), and parsley (90.53 μg mL^{-1}). The lowest content of total ascorbic acid was found in samples of: blackberries (5.18 μg mL^{-1}) and cucumber (3.64 μg mL^{-1}).

Table 1: Content of total ascorbic acid (AA) in the investigated samples

Samples	Spectrophotometric method [a] (AA mg/ 100g)	Spectrofluorimetric method [a] (AA mg/ 100g)	Literature value (AA mg/ 100g)
Black currant	184.36 ± 0.37	25.28 ± 0.26	181 - 215
Rose hip	168.44 ± 0.94	59.56 ± 0.26	426 - 2500
Parsley	90.53 ± 0.61	41.32 ± 0.22	133 - 150
Sea buckthorn	64.60 ± 0.56	37.6 ± 0.67	114 - 1550
Date	60.34 ± 0.37	2.63 ± 0.32	0.4 - 3
Pepper	50.27 ± 0.41	50.54 ± 0.17	44.9 - 80
Orange	49.42 ± 0.33	39.76 ± 0.29	38.9 - 53.2
Red currant	39.94 ± 0.55	20.99 ± 0.37	41 - 81
Strawberry	33.33 ± 0.98	24.32 ± 0.08	41.2 - 60
Red grapefruit	21.43 ± 0.62	28.7 ± 0.30	4 - 34.4
Lemon	21.23 ± 0.15	31.08 ± 0.26	25 - 53
Mandarin	19.90 ± 0.46	57.44 ± 0.22	20 - 26.7
Potato	19.58 ± 0.09	20.89 ± 0.14	5 - 20
Tomato	15.90 ± 0.68	25.51 ± 0.29	12.7 - 39
Carrot	14.93 ± 0.23	17.81 ± 0.24	5.9 - 15
Banana	13.10 ± 0.72	20.19 ± 0.40	8.7 - 18
Cranberry	12.05 ± 0.89	12.18 ± 0.47	10 - 13.3
Blueberry	9.77 ± 0.85	7.13 ± 0.27	2.5 - 16.4
Apple	9.55 ± 0.82	8.21 ± 0.17	4.6 - 6
Blackberry	5.18 ± 0.33	7.73 ± 0.35	3.1 - 7
Cucumber	3.64 ± 0.28	7.68 ± 0.37	2.8 - 4

[a] Average of three determinations ± SD

Agreement for spectrophotometric and spectrofluorimetric methods were the best for the inestigated samples of pepper, potato, cranberry and apple. The biggest differenses of obtained results between two methods and literature value was for the investigated sample of date. The reason could be that date conists a lot of glucose which could interfere. As ascorbic acid is largely similar to the glucose by structure, some of the glucose may be extracted from the sample. Glucose may also form the colored complex with DNPH as ascorbic acid.

Also the differences between obtained results and literature value for some investigated samples may also be explained on the basis that the ascorbic acid concentration vary with conditions such as temperature and the storage period on preservation. Vitamin C content slowly decreases with temperature and storage period of vegetables. Rhman et al has published that the inicial concentration of ascorbic acid were decrease by 76% at 5 °C and 64% at – 10°C during two months storage time (Rhman *et al*., 2007).

From the Table 1 we can see that most results obtained by spectrophotometric method are well comparable with results of spectrofluorimetric method and literature values.

CONCLUSIONS

Spectrophotometric method for determination of total ascorbic acid in fruits and vegetables with 2,4-DNPH is a simple and reliable method. Comparison of results obtained by spectrophotometric method is in a good agreement with results obtained by spectrofluorimetric method and literature values.

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Summary/Sažetak

Sadržaj ukupne askorbinske kiseline (askorbinska kiselina + dehidroaskorbinska kiselina) je određen u dvadeset i jednom uzorku voća i povrća spektrofotometrijskom metodom. Metoda se zasniva na oksidaciji askorbinske kiseline do dehidroaskorbinske kiseline bromnom vodom u prisustvu acetatne kiseline. Nakon kuplovanja sa 2, 4-dinitrofenilhidrazinom (DNFH), stvara se kompleks crvene boje čija se apsorbansa spektrofotometrijski mjeri na 521 nm. Dobiveno je linearno područje za standardne rastvore askorbinske kiseline do 10 μg/mL s koeficijentom korelacije od 0,9929. Sadržaj askorbinske kiseline se kretao između 9 i 49 mg/100 g svježeg voća i između 3 i 90 mg/100 g svježeg povrća. Ispitan je uticaj mogućih interferenci kao što su glukoza, fruktoza i saharoza. Granica detekcije askorbinske kiseline je 0.01 μg/mL (3σ od 10 mjerenja standardnog rastvora askorbinske kiseline koncentracije 3 μg/mL), dok je granica kvantifikacije askorbinske kiseline 0.017 μg/mL. Relativna standardna devijacija (RSD) je 2.4 % (n = 10, c = 7μg/mL). Sadržaj ukupne askorbinske kiseline u ispitivanom dvadeset i jednom uzorku voća i povrća poređen je s rezultatima dobijenim spektrofluorimetrijskom metodom kao i sa literaturnim vrijednostima.